A mathematical model for the efficacy and toxicity of aminoglycoside

Arjen Koop

M. Sc. Thesis

University of Twente, The Netherlands
Faculty of Electrical Engineering, Mathematics,
and Computer Science
Department of Applied Mathematics

Supervising committee:

Dr. S.A. van Gils
Prof. Dr. E. van Groesen
Dr. C. Neef (Medisch Spectrum Twente)

October 2002 - July 2003

Summary

Aminoglycosides are antibiotics that kill bacteria with bactericidal action. This means that the drug has to get inside the bacteria where it causes misreading of mRNA and a decrease of protein synthesis, which ultimately causes cell death. The bactericidal action is concentration dependent. A model and its application is described in [Nee02] where the temperature-dependent killing is compared with the concentration-dependent killing of bacteria. In this manner, the pharmacokinetic relationships are coupled with the pharmacodynamic relationships of the drug.

From this model it appears that the efficacy of antibiotics is greatest when they are given with a continuous infusion. This is not possible with aminoglycosides because of their toxicity. Thus aminoglycosides have to be administered intermittently, such that the drug is able to disappear from the organs where the toxicity occurs: the kidney and the ear.

Unfortunately, it is not entirely clear how this toxicity occurs. The most plausible theory is that the drug enters the organ by active transport. By its presence within the cells of the organ, the drug disturbs the protein synthesis, such that the cell is not able to function properly and dies. Because of the saturable and concentration-dependent uptake into the cells, the speed of uptake decreases at higher concentrations. When the drug is infused continuously, the concentration in the blood maintains at a constant level, causing the uptake in the cell to continue and thus causing the drug to accumulate in the organ.

The toxicity in the kidney, the nephrotoxicity, is reversible, because the proximal tubular cells, where the toxicity occurs, are able to regenerate. This is not the case with the auditory hair cells, because they are not able to regenerate. Thus the hearing loss is permanent and the ototoxicity is irreversible. In figure 6.1 the scheme of the distribution of the aminoglycosides is presented.

In this report a mathematical model is derived, which incorporates the effects of aminoglycoside on bacteria [Nee02], the saturable and active uptake into kidney cells, the reversible nephrotoxicity and the irreversible ototoxicity. For a continuous administration, analytical solutions are calculated for the optimal concentration in the blood for efficacy and the concentration in the blood below which nephrotoxicity does not occur. For this model a numerical program in Matlab is developed to run simulations for suitably chosen parameters.

Samenvatting

Aminoglycosides zijn antibiotica die bacteria doden door een bactericide werking. Dit betekent dat de drug binnenin de bacteria moet komen, waar het een verkeerd lezen van mRNA en een afname in eiwit synthese veroorzaakt, wat uiteindelijk resulteert in de dood van de cel. De bactericide werking is concentratie afhankelijk. Een model en zijn toepassing is beschreven in [Nee02], waar het temperatuur-afhankelijk doden wordt vergeleken met het concentratie-afhankelijk doden van bacteria. Op deze manier worden de pharmacokinetieke relaties gekoppeld aan de pharmacodynamieke relaties van de drug.

Uit dit model wordt duidelijk dat de effectiviteit van antibiotica het grootste is wanneer ze met een continu infuus worden gegeven. Dit is niet mogelijk met aminoglycosides vanwege hun toxiciteit. Aminoglycosides moeten dus intermitterend gegeven worden, zodat de drug kan verdwijnen uit de organen waar de toxiciteit optreedt: de nier en het oor.

Het is echter niet geheel duidelijk hoe deze toxiciteit optreedt. De meest aannemelijk theorie is dat de drug het orgaan binnenkomt door actief transport. Met zijn aanwezigheid binnenin de cellen van het orgaan, verstoort de drug de eiwit synthese, zodat de cel niet meer naar behoren kan functioneren en dood gaat. Vanwege de verzadigbare en concentratie-afhankelijke opname in de cellen, neemt de snelheid van opname af bij hogere concentraties. Als de drug continu toegediend wordt, behoudt de concentratie in het bloed een constant niveau, zodat de opname de cel in voort gaat en dus een accumulatie van de drug in het orgaan veroorzaakt.

De toxiciteit in de nier, de nephrotoxiciteit, is reversibel, omdat de proximale tubulus cellen, waar de toxiciteit optreedt, kunnen regenereren. Dit is niet het geval met de auditieve haar cellen, omdat zij niet kunnen regenereren. Het gehoor verlies is dus permanent en de ototoxiciteit is irreversibel. Het distributieschema van de aminoglycoside is gepresenteerd in figuur 6.1.

In deze scriptie is een wiskundig model ontwikkeld, dat de effecten van aminoglycosides op bacteria [Nee02], de verzadigbare en actieve opname in de niercellen, de reversibele nephrotoxiciteit and de irreversibele ototoxiciteit bevat. Voor een continu infuus, analytische oplossingen zijn berekend voor de optimale concentratie in het bloed voor de effectiviteit en voor de concentratie in het bloed waaronder nephrotoxiciteit niet voorkomt. Voor dit model is een numeriek programma in Matlab ontwikkeld om voor geschikt gekozen waarden voor de parameters, simulaties te kunnen draaien.

Preface

The research described in this thesis is a practical question by Kees Neef of the Medisch Spectrum Twente in Enschede. The work has been conducted at the University of Twente, as the final project of my study Applied Mathematics. I have chosen to do this project at the University to experience an academic environment. Much medical research has been done on the subject, in mathematical research, however, the subject has not been investigated much. For me, its practical application was one of the important reasons to chose this subject and during the last eight months I have been working on the subject with pleasure.

I would like to thank Stephan very much for helping with my work and discussing the mathematical modeling problems. I would like to thank Kees for his time and the interesting conversations we had on the medical side of the project. Afke and Daan from the Leyenburg Hospital in Den Haag, I would like to thank for their help on collecting patient data and for their guide through a hospital environment. I also want to thank everyone else who have supported me, and in particular my parents Henk and Jeanie, for their loving support throughout the years.

Arjen Koop,
Enschede, June 25th, 2003

Contents

Chapter 1

Introduction

After the introduction of streptomycin in 1944, aminoglycosides have maintained a central role in antimicrobial therapeutics. Tobramycin, gentamicin, amikacin and netilmicin are the primary aminoglycosides used in gram-negative infections, such as *Pseudomonas* and *N. meningitidis*, which cause pneumonia and meningitis.

Despite a profilation of new antibiotics in recent years, there is often no substitute for aminoglycosides in life-threatening infections. A number of factors contribute to their successful and continued use including bactericidal efficacy, limited bacterial resistance, a post-antibiotic effect and low cost.

Nonetheless, nephrotoxicity and ototoxicity complicate treatment of patients with these antibiotics. Despite ongoing efforts over the past 30 years, therapeutic monitoring techniques have failed to reduce aminoglycoside-induced nephrotoxicity. In the last couple of years evidence in both human and experimental models confirms, that large aminoglycoside doses given at extended intervals are less toxic than the same cumulative dose administered more frequently.

In this report a mathematical model for the toxicity and efficacy is developed. We will derive the deterministic equations describing the distribution and action of aminoglycosides. For a continuous infusion, analytical solutions are calculated for the optimal concentration in the blood for efficacy and the concentration below which nephrotoxicity does not occur.

With these equations and with the proper values for the parameters a numerical program in Matlab can calculate the concentration in the compartments and related to these concentrations, the efficacy and nephro- and ototoxicity. With this numerical program insight in the behavior of the model for different dosage regimen, i.e., once-daily, twice-daily and continuous administration can be obtained.

1.1 Reasons to investigate mathematical models in medical science

A mathematical model is a simplified picture of the real world, based on an understanding of the mechanisms of action of the drug. A model is useful if it can explain or predict the outcome of experiments and ultimately the outcome of treatment of infections in patients.

If such predictions are confirmed then the model is validated.

Thus with a mathematical model the outcome of treatment of patients can be predicted. Also the dose, interval and duration of treatments may be optimized in a patient related and economic related manner. In this manner, toxicity in patients may be avoided, resulting in less complications and a decreased hospital time.

A validated model confirms the mechanisms of the antibiotic action, on which the model was based on in the first place, which contributes to an increase in knowledge of antibiotics and pharmacokinetics, which may be of use in other medicine related topics.

1.2 Structure of the report

This thesis is organised as follows. In the following chapter the problem description is stated. In chapter 3 the bactericidal action, the nephro- and ototoxicity of aminoglycosides are explained. The background physiology on cellular membranes, the kidney and the inner ear is discussed in chapter 4. This is necessary for a better understanding of the distribution and action of aminoglycosides. In chapter 5 some aspects of pharmacokinetic/pharmacodynamic modeling are outlined.

In chapter 6 the mathematical model for the efficacy and toxicity is derived. Following in chapter 7 we have calculated analytical solutions for the optimal concentration for efficacy and the concentration in the blood below which no nephrotoxicity occurs. In chapter 8 we comment on the suitability of patient data necessary for deriving the values of the parameters of the model. In chapter 9 we outline our choices of the parameters of the model and we present simulations we have made with a numerical program in Matlab. The thesis concludes with a discussion of the conclusions and recommendations in chapter 10.

Chapter 2

Problem description

Aminoglycoside are antibiotics which kill bacteria with bactericidal action. This means that the drug has to get inside the bacteria where they cause misreading of mRNA and a decrease of protein synthesis, which ultimately causes cell death. The bactericidal action is concentration dependent. This model and its application is described in an article [Nee02] where the temperature-dependent killing is compared with the concentration-dependent killing of bacteria. In this manner, the pharmacokinetic relationships are coupled with the pharmacodynamic relationships of the drug.

From this model it appears that the efficacy of antibiotics is greatest when they are given with a continuous infusion. This is not possible with aminoglycoside because of their toxicity. Thus aminoglycoside have to be administered intermittently, such that the drug is able to disappear from the organ where the toxicity occurs: the kidney and the ear.

Unfortunately, it is not entirely clear how the toxicity arises. The most plausible theory is that the drug enters the organ by active transport, which is saturable and concentration-dependent, meaning that at higher concentrations in the blood, the speed of uptake decreases. This could be some kind of protection mechanism of the cell. By its presence within the cells of the organ, the drug disturbs the protein synthesis, resulting in the death of the cell. When the drug is infused continuously, the concentration in the blood maintains a constant level, causing the uptake in the cell to continue.

The goal of this project is to develop a mathematical model, which incorporates the effects of aminoglycoside on bacteria [Nee02], the saturable and active uptake into kidney cells, the reversible nephrotoxicity and the irreversible ototoxicity. The model should be able to predict at which concentration in the blood, the efficacy is optimal and below which concentration in the blood nephrotoxicity does not occur. The important question is whether an optimal dosage regimen can be found at which efficacy is highest and toxicity is lowest.

Chapter 3

Aminoglycosides

Despite their nephrotoxicity and ototoxicity, aminoglycosides remain one of the antibiotics of choice in the therapy of gram-negative bacteria[1], such as *Pseudomonas* and *N. meningitidis*, which cause pneumonia and meningitis[2]. After administration, aminoglycosides have a volume of distribution that approximates the extracellular space, because they are water-soluble and minimally protein-bound. They undergo glomerular filtration as their primary route of elimination from the body and they appear unchanged in the urine. The pharmacokinetics and pharmacodynamics of aminoglycoside are described in section 3.1.

Aminoglycoside kill bacteria with bactericidal action, which means that the drug has to get inside the bacteria to be effective. This action happens in a concentration-dependent manner and is described in section 3.2.

The major problem of aminoglycosides is their potential for nephro- and ototoxicity. Most cells of animals, thus also of humans, are not able to transport aminoglycosides, with the exception of some eukaryotic cells in the kidney and the ear. Neuromuscular blockade has been reported, but this is sufficiently rare that clinical concern has focused on nephro- and ototoxicity. Toxicity is described in section 3.3.

3.1 Pharmacokinetics/pharmacodynamics of aminoglycosides

The primary aminoglycosides used are tobramycin, gentamicin, amikacin and netilmicin. At physiologic pH, they are polycations with a high degree of polarity and water solubility and have no significant binding to plasma proteins. A number of factors contribute to their continued use including bactericidal efficacy in gram-negative infections, limited bactericidal resistance, synergism with β-lactam agents, a post-antibiotic effect and low cost.

Aminoglycosides are distributed in the extracellular water compartments, because they are excluded from most tissues. They undergo glomerular filtration as their primary route of elimination from the body. They appear unchanged in the urine and the plasma clearance of aminoglycoside is nearly equal to the glomerular filtration rate (GFR). A small proportion ($\leq$ 5 % [Wal94], [Swa97]) of the filtered antibiotic is actively reabsorbed by the proximal tubular cells. The antibiotics bind to negatively charged brush-border membranes within

[1] Bacteria with an additional outer membrane around the cell wall

[2] inflammation of the membranes covering the central nervous system

the tubular lumen, undergo pinocytosis and thus gain access to the inside of the cells. The uptake of aminoglycoside by the brush-border membranes is dose saturable. Within the proximal tubular cells the aminoglycosides accumulate due to their tissue half-lifes of four to five days. After uptake into proximal tubular cells, a number of intracellular processes are disrupted by the presence of aminoglycosides leading to cell death at high drug levels inside the cells.

Besides the uptake in the proximal tubulus in the kidney, the aminoglycosides also bind to the tissues of the perilymph, the endolymph and the organ of Corti located in the inner ear [Pri95]. The uptake in these tissues is rapid; a steady state is reached within 1-3 h. The clearance from the endolymph, the perilymph and the organ of Corti is much slower than the clearance from the plasma, half-lives of several days are reported. In the Organ of Corti the auditory hair cells are located. Aminoglycosides destruct these cells resulting in hearing loss.

3.2 Bactericidal action of aminoglycosides

The killing of gram-negative bacteria by aminoglycoside starts with a passive, concentration-dependent, irreversible ionic binding on the outside of the microorganisms [Pri95], after which the drug is taken inside the bacteria, which is energy- and oxygen-dependent [Eds91]. This means that the transport into the cell costs energy. The rapid early bactericidal phase is therefore drug-concentration dependent: higher drug levels result in more bactericidal action. This also means that the concentration inside the bacteria can be larger than the concentration outside the cell.

Once inside the microorganism the aminoglycosides cause membrane damage, and they bind to the 30S subunit of the ribosomes. This leads to mRNA misreading and a decrease in protein synthesis, which ultimately causes cell death.

The bactericidal action of aminoglycoside, the functional dependence of the killing rate $F(C)$ and the concentration C can be described by the Hill equation according to [Nee02] with,

$$F(C) = \frac{E_{max} C^n}{C_{50}^n + C^n}, \tag{3.1}$$

where E_{max} represents the maximal killing rate of the drug for a certain microorganism, C_{50} the concentration where 50% of the maximal effect is reached, and n is the steepness/shape coefficient of the curve.

3.2.1 Post-antibiotic effect

One of the explanations for the success of intermittent dosing regimes has been the delay in regrowth after the concentration has fallen under the MIC, the so called postantibiotic effect (PAE) [Ode01]. The postantibiotic effect is defined as the suppression of bacterial growth after exposure of the organisms to the drug and is determined by the analysis of regrowth curves [Ger88], [Pri95].

The PAE is defined by the following formula: $PAE = T - TC$, where T is the time

required for the viable counts of the antibiotic exposed cultures to increase by one log_{10} above the counts observed immediately after washing and TC is the corresponding time for the controls.

The mechanism behind this effect is not clear but it has been hypothesized, that when bacteria are exposed to concentrations above the MIC of an antibiotic, the drug will bind covalently to the active sites of the proteins [Ode01] . Synthesis of the proteins is known to be continuous during drug exposure. When excess drug is removed and the concentration reaches a sub-MIC level, most of the proteins are still inactivated and only a low drug concentration is needed to inhibit the newly produced proteins. This results in a prolonged inhibition of cell multiplication until a critical number of free proteins is once more available. In other words, the damage needs to be repaired, before the normal growth rate returns.

The presence or absence of a PAE is likely to be an additional factor contributing to the overall antibacterial effect of a drug, because of the PAE it is not necessary that the serum concentration exceeds the MIC all the time. The PAE is dependent on several factors; the concentration of the antibiotic, the time the bacteria are exposed to the drug, the bacterial species and the antibiotics used to induce the PAE.

In our model for the efficacy of aminoglycosides we do not take the postantibiotic effect into account. In the literature this process has not been investigated enough. We mention the PAE to point out a method to improve the efficacy model in the future.

3.3 Toxicity of aminoglycosides

Two phenomena are important to understand efficacy[3] and toxicity [Cal88]. These phenomena are: binding of aminoglycoside and their transmembrane transport into the interior of the cell. When that is accomplished the result is toxic, for either bacteria or human cells. The problem, therefore, is to find conditions that maximize binding and transport of aminoglycoside in bacteria and to minimize these steps in human cells.

The therapeutic range of aminoglycosides is narrow, toxic concentrations are relatively low. Thus toxicity limits the clinical use of aminoglycoside. Therefore, knowledge of the mechanisms of toxicity and identification of drug- and patient-related factors are necessary for its prevention.

Most cells of animals, thus also of humans, are not able to transport aminoglycosides, with the exception of some eukaryotic cells in the kidney and the ear [WerVr]. Thus aminoglycoside may result in considerable nephrotoxicity and ototoxicity, which are probably related to dosage and duration of therapy and appear to be independent of each other.

3.3.1 Nephrotoxicity

Aminoglycosides are primarily eliminated by glomerular filtration, and appear unchanged in the urine [Pri95], [Swa97]. A small proportion of the antibiotic ($\leq 5\%$) is reabsorbed in the proximal tubulus. This reabsorption starts with binding to the brush border membrane,

[3] bactericidal action, section 3.2

which is a rapid process, dose saturable within clinical achievable concentrations. The rate of binding to membrane phospholipids will be affected by dose administered, tubular luminal concentration and urine flow rate. The aminoglycoside binding causes alterations in the phospholipid composition of the brush border membrane.

Following the binding to the cell membrane, the drug is actively transported inside the cell. There the aminoglycosides accumulate within the lysosomes and they interfere with the function of a number of lysosomal enzymes. As a result of this, the lysosomal activities are depressed, which may eventually lead to the death of the proximal tubular cells. The effect of the aminoglycosides on the kidney function is mostly reversible [WerVr], because of the ability of the tubulus cells to regenerate.

The onset of nephrotoxicity depends on the amount of aminoglycoside attained in the renal cortex [BroWee]. The risk of nephrotoxicity increases as renal cortical concentrations increase. Above a threshold the aminoglycosides may lead to cell death [Min99], below the drug is smoothly released from the renal cortex. Renal failure is reversible in a period of 2 - 6 weeks Bre00].

The definition of aminoglycoside nephrotoxicity is usually based on a change[4] in the patient's baseline serum creatinine [TheMon]. However, due to tubuloglomerular feedback, the process of compensating for nephron loss, the creatinine serum concentration is a belated marker of kidney damage. Better indicators are some kidney cell enzymes found in the urine such as β_2 aminoglobuline and others.

Therapeutic drug monitoring services have failed to reduce aminoglycoside toxicity over the years [Swa97], although two pharmacological parameters are important. The first is that peak aminoglycoside levels correlate with efficacy, as these agents display concentration-dependent bacterial killing. Second, trough levels reflect nephrotoxicity; the kidney is unable to excrete the dose of aminoglycoside within the dosing interval owing to impaired function.

These two points have led to numerous reports evaluating once-daily dosing of aminoglycoside dosing in which the cumulative dose for a 24 hour period would be administered as a single dose. This would take advantage of concentration-dependent killing as well as the postantibiotic effect while minimizing repeated exposure and potential nephrotoxicity.

Predisposing factors for nephrotoxicity are [BroWee], [TheMon], [Pri95]:

- Drug related: dose, duration of treatment, dosage regimen, prolonged high trough levels, prior aminoglycoside treatment, associated drugs.
- Patient related: age, prior renal insufficiency, presence of liver disease (hepatic insufficiency), critically ill patient, sodium-volume depletion, concomitant use of nephrotoxic drugs, shock.

3.3.2 Ototoxicity

The uptake of the aminoglycosides in the tissues of the organ of Corti is rapid [Pri95], a steady state is reached within 1-3 hours. Results from animal studies disagree whether or

[4] a decrease of 50% or more

not this uptake is saturable, i.e. not linear with plasma concentration. It takes more than 6 hours before a plateau phase is reached in the perilymph.

The clearance of the aminoglycosides from perilymph is much slower than the clearance from the plasma, the clearance from the endolymph is even slower. After the end of treatment, the concentration of the aminoglycosides in the outer hair cells can initially increase, due to uptake from peri- and/or endolymph. The clearance from the outer hair cells is extremely slow; after a treatment of 3-4 weeks, the clearance has a half-life of 20-35 days.

Aminoglycosides destruct the hair cells of the organ of Corti, starting with the outer hair cells and going row by row to the inner hair cells [Pri95], [Has97], [Gov91], [Mat87] . Then a degeneration of the sensory hair cells in the cochlea and the vestibular labyrinth takes place. The hair cells are not able to regenerate, so ototoxicity is in principle irreversible. The damage can even take place after the therapy is stopped. There is a cumulative effect of repeated treatments.

In the pathogenesis of aminoglycosides ototoxicity two distinct processes can be distinguished [Pri95]. The first is a rapid electrostatic interaction of the polycationic aminoglycosides with the negatively charged plasma membrane. This results in a displacement of calcium. As the binding of the polycationic aminoglycosides is an electrostatic one, it can be blocked by immediate addition of calcium and increased by forces that make the membrane more negative, for instance noise.

The second step is energy-dependent uptake in the hair cell. Crucial in the development of ototoxicity is binding of the aminoglycosides to phospholipids of the plasma membrane, inhibiting their hydrolysis and resulting in the disturbance of their normal function. This can result in cell death.

Audiometry is used to assess cochlear toxicity [Bru90]. The definition of ototoxicity is an increase in pure-tone threshold from a baseline audiogram $\geq$ 15dB at two or more frequencies, or $\geq$ 20 dB at one or more frequencies.

Predisposing factors for ototoxicity [Pri95]:

- Drug related: Daily dose, duration of treatment, total dose and total area under the curve in plasma and perilymph.

- Other factors: repeated courses, hyperthermia, age, and anemia. Loud noises also aggravate toxicity, and the absence of noise decreases the change of toxicity.

Chapter 4

Physiology

Physiology is the study of the functioning of living organisms, animal or plant, and of the functioning of their tissues or cells [Ber98]. In the healthy human, many variables are maintained within narrow limits. The list of controlled variables includes body temperature, blood pressure, ionic decomposition of the body‘s various fluid compartments, blood glucose levels, and oxygen and carbon dioxide contents of the blood. This ability to maintain the relative constancy of such critical variables is known as homeostasis.

In physiological models, each compartment corresponds to a well defined structure or organ interconnected by blood flow, lymph flow or other material fluxes, with a rapid exchange of the drug between the organs or tissues. Compartments are usually separated by cellular membranes, which are discussed in section 4.1.

Aminoglycosides are transported to the kidney and ear cells by the blood. They are eliminated from the blood by glomerular filtration in the kidney. They have an impact on the kidney and hair cells, resulting in a loss of functioning of these organs. The blood system is discussed in section 4.2, the kidney in section 4.3 and the ear in section 4.4.

4.1 Cellular membranes

Every cell is surrounded by a plasma membrane, that separates it from the extracellular environment. By its lipid composition[1] the membrane acts as a barrier, which allows the maintenance of large concentration differences of many substances between the inside and outside of the cell. The plasma membrane also contains enzymes and receptors that play important roles in the cell's interaction with other cells and with hormones and other substances in the extracellular fluid [Ber98].

The permeability function of membranes, which allows the passage of important molecules across membranes at controlled rates, is central to the life of the cell. Molecules may move from one side of the membrane to another without actually moving through the membrane itself. In other cases, molecules cross a membrane by passing through or between the molecules of the membrane. Thus molecules can enter cells by transport across and transport through the membranes.

[1]The phospholipid bilayer is responsible for the passive permeability properties of the membrane. A phospholipid molecule consists of a polar head group and two non polar fatty acyl chains [Ber98].

4.1.1 Transport across membranes

Transport via protein carriers is called protein-mediated transport. There are two types of mediated transport: active transport and facilitated transport. Although these processes have several properties in common, the principal distinction between them is that active transport is capable of pumping a substance against a gradient of concentration, whereas facilitated transport tends to equilibrate the substance across the membrane. As a consequence active transport requires energy.

Endocytosis is the process that allows material to enter the cell across the membrane, thus without passing through the membrane [Ber98]. In this process, specific membrane receptor proteins recognize and bind to the protein to be taken up. The uptake of solid particles is called phagocytosis ('cell eating'), the uptake of fluids or soluble molecules is called pinocytosis ('cell drinking'). Endocytosis is an active process that requires metabolic energy. Molecules can be ejected from cells by exocytosis a process that resembles endocytosis in reverse.

Endocytosis is an important mechanism for actively reabsorbing macromolecules by the proximal tubule. Active transport is much more rapid than diffusion and the transport rate shows saturation kinetics. The binding of the molecules to receptor proteins and their transduction inside the cells, can be described by Michaelis-Menten kinetics as explained in section 5.4.

4.1.2 Transport through membranes

Some molecules move through membranes simply by diffusing among the molecules that make up the membrane [Ber98]. Diffusion is driven by the concentration difference and eventually results in the uniform distribution of the molecules on both sides of the membrane. The diffusion coefficient D is proportional to the speed with which the diffusing molecule can move in the surrounding medium. The larger the molecule and the more viscous the medium, the smaller is D.

4.2 Blood system

Blood performs many functions in the body [Ber98]. The main function of the circulating blood is to carry oxygen and nutrients to the tissues and to remove carbon dioxide and waste products from the tissues. In addition, blood transports other substances from their sites of production to their sites of action. Blood also contributes to homeostasis, the maintenance of a constant internal body environment.

The most important compartment in pharmacokinetic investigations is the systemic blood circulation, the 'central' compartment [Hol01]. This compartment is easily accessible for continuous measurement. We assume that the concentration of drugs in the blood is homogeneous. The total amount of aminoglycosides in the blood compartment is the sum of the amount coming from administration and from the organs and of the amount transfered out of the blood into the organs and the amount eliminated from the blood.

The drug enters the blood from the site of administration. This can be a instantaneous

influx burst (bolus administration), a constant rate influx or a more complicated influx process. The elimination of many substances from the blood is through the liver and the kidney. The liver cells are not able to transport the aminoglycosides, thus the non renal elimination rate remains constant. The kidney, however, is affected by the antibiotics resulting in a changing renal elimination rate. The pharmacokinetics of antibiotics in the blood is widely investigated. However to describe toxicity a second or even third compartment is needed.

4.3 Kidney

The kidneys are both excretory and regulatory organs [Ber98]. The principle function of the kidney is to filter the body of waste products. The kidney receives about 20-25% (1.25 l/min) of the total output of the heart and it is located in the body along the aorta. It excretes a number of compounds that are no longer needed by the body into the urine. The kidney maintains the composition of the body fluids within narrow boundaries. At the same time, water, sodium ions, glucose, and many other essential substances must be reabsorbed, because they would otherwise be lost for the body.

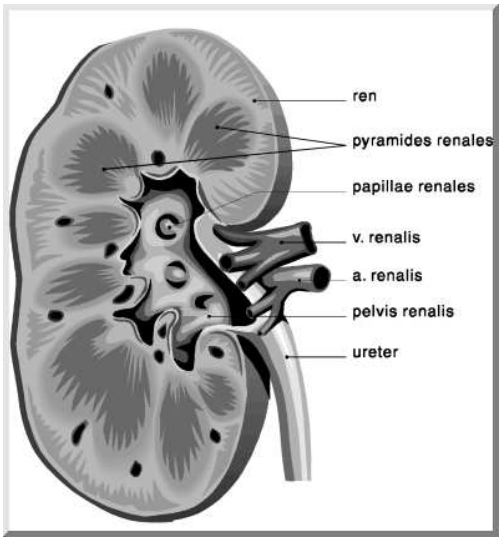

Figure 4.1: *The kidney*

Because of its function, the filtration of waste-products from the blood, the kidney is highly susceptible for toxins. During the process of elimination, toxic substances may easily be taken into the kidney cells. The concentration of the toxic compound or its metabolites may become much higher than those in the blood circulation. Once the toxic substances are inside the kidney cells, they will destruct the cells by inhibiting the protein synthesis, resulting in cell death. The nephrotoxicity is usually defined as the decrease in clearance function of the kidney. However, it may affect any of the functions mentioned above.

4.3.1 Anatomy and function of the kidney

Structure and function are closely linked in the kidneys [Ber98]. The functional unit of the kidney is the nephron. Each human kidney contains approximately 1.2 million nephrons, which are hollow tubes composed of a single cell layer. The coordinated actions of the nephron's segments determine the amount of a substance that appears in the urine. These actions represent three general processes, namely glomerular filtration, absorption and secretion.

Glomerular filtration is the process of filtering the blood. The blood enters the glomerulus and on its way to the tube of the nephron it passes a membrane. This membrane forms a selective filter and acts as a charged barrier, so some molecules are held back on the basis of size and charge. The filtrate is then processed in the tubule to become the ultra filtrate. The concentrations of salts and of organic molecules, such as glucose and amino acids, are similar in the plasma and the ultra filtrate.

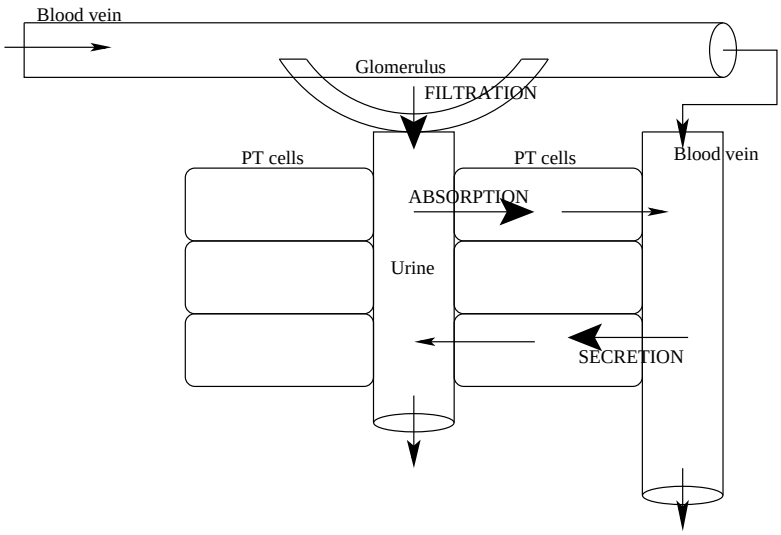

Figure 4.2: *The nephron, shown are the three important processes within the nephron: filtration, absorption and secretion.*

Tubular reabsorption allows the kidneys to retain water and other valuable substances. Although 180 liter of fluid is filtered by the glomerulus each day, less than 1% of the filtered water is excreted in the urine. By the process of reabsorption and secretion the nephrons modulate the volume and the compostion of the urine. Consequently, the nephrons precisely control the volume and composition of the fluid compartments of the body. During reabsorption compounds may accumulate in the proximal tubular (PT) cells in concentrations that exceed plasma levels many times. Thus potential toxicants that were present at non-toxic concentrations in the blood, may reach toxic levels inside the PT cells.

Secretion of substances from the blood into the tubular fluid is a means for excreting various byproducts of metabolism, which are not filtered through the glomerulus. It also eliminates organic anions and bases (e.g. drugs) and pollutants from the body. Many organic compounds are bound to plasma proteins and are therefore unavailable for filtration through the glomerulus. Secretion is thus their major route of excretion in the urine.

Aminoglycosides are freely filtered through the glomerulus, thus no secretion is present. A small portion of the filtered antibiotics is actively reabsorbed into the PT cells.

4.3.2 Assessment of kidney function, the glomerular filtration

Knowledge of the glomerular filtration rate (GFR) is necessary to obtain the severity of kidney disease. The GFR is equal to the sum of the filtration rates of all the functioning nephrons. A fall in GFR means that some nephrons stopped working. Thus, the GFR is an index of kidney function. An increase in GFR generally suggests recovery.

The glomerular filtration rate can be obtained by measuring the clearance by the kidney of a so called marker substance. This marker should have a stable production rate, a stable circulation rate, be freely filtered by the glomerulus and not be reabsorbed or secreted.

In clinical practice the clearance of creatinine is used to estimate the GFR [Pri00]. Creatinine is a byproduct of muscle creatinine metabolism. It is produced at a relatively constant rate and the amount produced is proportional to the muscle mass. The creatinine clearance Cl_{cr} (ml/min) is usually estimated from the serum creatinine concentration according to Cockcroft and Gault [Gau92]

$$Cl_{cr} = F_{sex}\frac{(140 - Y)LBM}{72Cr}, \tag{4.1}$$

where Y is the age, $F_{male} = 1.0$ and $F_{female} = 0.85$, LBM is the lean body weight and Cr is the serum creatinine concentration. In normal adults, the GFR averages 90 to 140 ml/min for males and 80 to 125 ml/min for females. After age 30 the GFR declines with age, but without the loss of the kidneys excretory function or their ability to maintain the fluid balance of the body.

4.3.3 Nephrotoxicity of aminoglycoside antibiotics

Nephrotoxicity is defined as a fall in the creatinine clearance compared to the baseline clearance[2]. The creatinine clearance depends on the number of working nephrons or the 'health' of the kidney. Due to aminoglycoside accumulated in the proximal tubular cells in the kidney, nephrons may die, decreasing the functionality of the kidney.

After the glomerular filtration the aminoglycoside are taken actively into the proximal tubule cells by binding to the brush border[3]. After uptake in the proximal tubulus cells a number of intracellular processes are disrupted by the presence of aminoglycoside.

The killing of kidney cells by the aminoglycoside can be represented by a classic Hill equation [Rou03], which links the aminoglycoside concentration in the proximal tubule to the toxic effect. Thus the creatinine clearance is dependent on the concentration of aminoglycoside in the proximal tubule cells. The nephrons are able to regenerate, but the recovery may be incomplete due to permanent loss of nephrons [Bre00]. Regenerating cells are less susceptible to AG, thus they induce a decrease in uptake of aminoglycoside in the PT cells [Min99].

The kidney is capable of compensation for nephron loss by the process of tubuloglomerular feedback [Van95]. This process regulates the renal blood flow and the glomerular filtration rate by a feedback loop which senses the flow of tubular fluid[4]. Thus changes in rate of filtration caused by for example less nephrons are compensated by an increase in flow in the remaining nephrons. Thus the decrease in creatinine clearance due to nephrotoxicity is not linear related to the number of nephrons. A more appropriate model to describe the tubuloglomerular feedback is an E_{max} model [Rou03].

4.4 Ear

The function of the ear is to transduce and to analyze sound. Sound is produced by waves of compression and decompression that are transmitted in air or other media. Sound is a

[2]The baseline clearance is the creatinine clearance at the start of the treatment.
[3]The brush border is the urine side of the proximal tubule cells.
[4]or some other factor, such as the rate of NaCl reabsorption.

mixture of pure tones, which result from sinusoidal waves at a particular frequency, amplitude and phase. The ear analyzes the composition of sound into a set of pure tones. The human ear is sensitive to pure tones with frequencies that range from 20 to 20000 Hz.

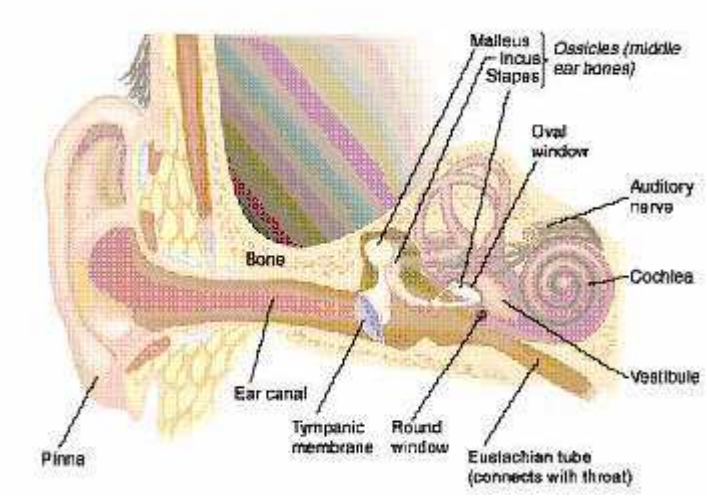

Figure 4.3: *The ear*

The ear can be subdivided into the external ear, middle ear and inner ear. Aminoglycosides only have an impact on the inner ear. The external ear transmits sound waves through the auditory canal to the tympanic membrane. In the middle ear a chain of ossicles connects the tympanic membrane to the oval window, an opening into the inner ear. Beneath the oval window is a fluid-filled component of the cochlea in which the hair cells are located.

The tympanic membrane and the chain of ossicles serve as a matching device. The ear must detect sound waves traveling in air, but the neural transduction mechanism depends on movements established in the fluid column within the cochlea. Thus pressure waves in air must be converted into pressure waves in fluid. The acoustic impedance of water is much higher than that of air. Therefore, without the chain of ossicles most sound reaching the ear would be reflected.

4.4.1 Anatomy and function of the inner ear

The inner ear includes the bony and membranous labyrinths. The cochlea and the vestibular apparatus are formed from these structures. The cochlea is a spiral-shaped organ and includes several chambers filled with fluids, the endolymph and the perilymph. The fluids are separated by membranes, the Reissner's membrane and the cochlear duct.

The neural apparatus responsible for the transduction of sound is the organ of Corti, which is located within the cochlear duct. It consists of three rows of outer hair cells, a single row of inner hair cells and other components. In humans the organ of Corti contains 15000 outer hair cells and 3500 inner hair cells. The inner hair cells provide most of the neural information for hearing. Destruction of hair cells results in deafness.

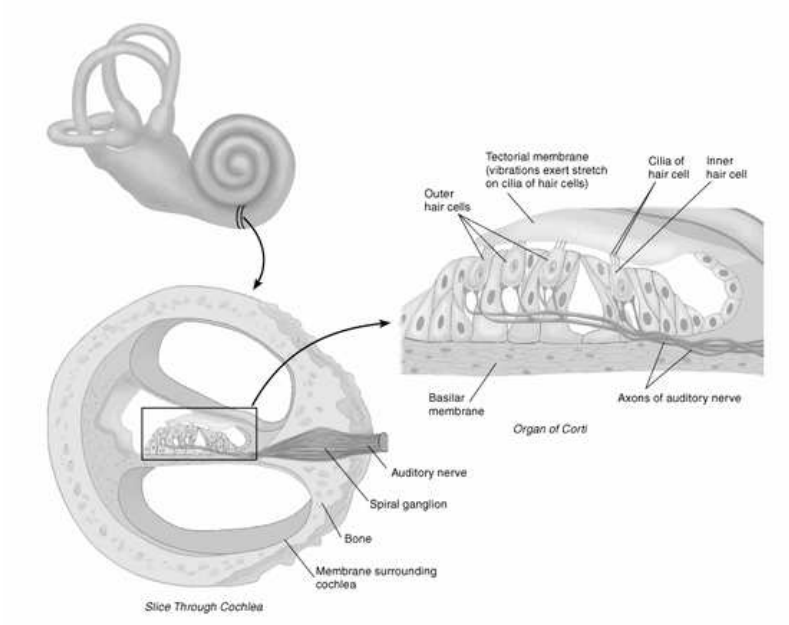

Figure 4.4: *The cochlea, the chambers of the cochlea and the organ of Corti*

Sound waves are transduced by the organ of Corti in the following way. Sound waves that reach the ear cause the tympanic membrane to oscillate. These oscillations result in fluid movements in the cochlea. Part of the energy of these fluid movements is used to displace the basilar membrane, and with it the organ of Corti. The hair cells generate electrical pulses to the brain when moved. The basilar membrane serves as a frequency analyzer, it distributes the waves along the organ of Corti so that different hair cells respond to different frequencies of sound.

4.4.2 Assessment of ear function

A loss of hearing for particular frequencies can result from damage to a part of the organ of Corti or from destruction of hair cells. The degree of deafness can be quantified for different frequencies by audiometry. In audiometry, each ear is presented with tones of different frequencies and intensities. An audiogram is plotted that shows the thresholds of each ear for representative frequencies of sound. High tone audiometry measures the thresholds at the frequencies 8-20 kHz. Comparison with the audiogram of normal individuals, the so called Fausti baseline, shows the auditory deficit. The destruction of hair cells causes a loss in hearing function, so the number of hair cells is a measure for the hearing function. However no relation between the number of hair cells and the hearing function is known.

4.4.3 Ototoxicity

Ototoxicity is defined by a loss of hearing function[5]. In the pathogenesis of aminoglycosides ototoxicity two distinct processes can be distinguished [Pri95]. The first is a rapid

[5]An increase in pure-tone threshold of more than 15 dB at two or more frequencies or more than 20 dB at one or more frequencies from a baseline audiogram.

interaction of the aminoglycosides with the plasma membrane. The second step is energy-dependent uptake in the hair cell.

As is the case in the kidney, once the aminoglycoside is inside the hair cell, it disturbs protein synthesis and other processes resulting in the death of the cell. Thus the aminoglycoside destruct the hair cells of the organ of Corti, starting with the outer hair cells and going row by row to the inner hair cells [Pri95], [Has97]. The hair cells are not able to regenerate, so ototoxicity is in principle irreversible. The damage can even take place after the therapy is stopped, because of the slow elimination from the ear fluids and the organ of Corti.

Chapter 5

Pharmacokinetics and Pharmacodynamics

Two major groups of biological models can be distinguished: mechanistic pharmacokinetic/pharmacodynamic models and physiological models. In the first, the compartments do not necessarily reflect functional entities of the organism. These models are convenient for mathematical modeling, an overview is given by Derendorf [Der95] and they are discussed in sections 5.1 and 5.2.

An additional problem for the modeling of aminoglycoside is the inter patient variation in pharmacokinetics; parameters of the pharmacokinetics of aminoglycoside differ from one patient to the other. Variability of pharmacokinetic/pharmacodynamic models is discussed in section 5.3.

Frequently used equations in pharmacokinetic/pharmacodynamic modeling is the Michaelis-Menten equation and the Hill equation. In section 5.4 we derive these equations. In section 5.5 we comment on the Hill equation.

5.1 Pharmacokinetics and Pharmacodynamics

Pharmacokinetics (PK) describes the quantitative relationships between administered doses and dosing regimens and plasma and/or tissue concentration of the drug. Pharmacodynamics (PD) can be defined as the quantitative relationships between plasma and/or tissue concentration of the drug and the magnitude of the pharmacological effects.

A PK/PD model is a mathematical description of these relationships; the model parameters provide information about intrinsic drug properties. The knowledge of the combined PK/PD model and the parameter estimates allows prediction of concentration versus time (C-t) and effect versus time (E-t) profiles for different dosing regimens.

Different drugs are characterized by different PK/PD models and/or by differences in their model parameter values. For a given drug, the individual parameter values can differ significantly from patient to patient, resulting in population variability in clinical response, i.e., for a given dose, the onset, duration, and magnitude of the pharmacological effect can vary widely between patients.

After administration of the dose, the drug has to reach the systemic circulation; the systemic kinetic processes, i.e., distribution, metabolism, and excretion, determine the C-t profile of the drug. For the drug to cause a PD effect, they have to reach the site of action[1], which is that tissue compartment that contains the specific pharmacological receptors that the active drug will interact with. The extent and rate of distribution of the active drug into the tissue compartment depend on the anatomical location of the target tissue and its physiological perfusion rate, as well as the permeability of the tissue to the active drug in order to reach the receptors.

After reaching the site of action, the interacts with the pharmacological receptors. These receptors are biological macromolecules that bind the drug molecules and induce the observed pharmacological response. In general, the tissue compartment is assumed to be kinetically homogeneous, i.e., the concentration of drug is homogeneous and the drug-receptor interaction is instantaneous.

The pharmacokinetic variables of a drug determine the time course of drug concentration in serum and at the site of infection. Two different categories of pharmacokinetic variables exist. Some variables may be altered by adjusting the dosing regimen, whereas others result from the chemical properties of a particular drug.

Pharmacodynamic parameters of antimicrobials relate drug concentration to the desired effect. In most cases, these parameters are determined in vitro, and then extrapolated to the in vivo situation. The reason for this is that measurements are difficult to perform in vivo during therapy. The majority of these parameters may be fit to a standard sigmoid E_{max} model.

5.2 Pharmacokinetic/Pharmacodynamic models

In this section different aspects and processes of PK/PD models are described within a mathematical approach. These equations will be necessary when the mathematical model for the toxicological action of aminoglycoside is exploited. First we describe the transport of antibiotics, the pharmacokinetics and then we discuss growth and death of bacteria and cells, the pharmacodynamics.

5.2.1 Transport of antibiotics

The simplest model is a zero order efflux process, which supposes a constant mass or concentration elimination rate k from the compartment [Hol01], which can be described by

$$\frac{dC(t)}{dt} = -k, \qquad C(t) > 0. \tag{5.1}$$

In this case the drug concentration decreases linearly with time. Note that an extra restriction has to be posed on this equation, because the concentration can not have a negative value.

[1] also called the biophase.

For many processes it is reasonable to assume first order kinetics. This means that the mass or concentration efflux rate out of a compartment is proportional to the mass or concentration still remaining in the compartment

$$\frac{dC(t)}{dt} = -kC(t). \tag{5.2}$$

The proportionality constant k is called the rate constant of the process. It is related to the half-life of the transfer process by $t_{1/2} = \frac{ln(2)}{k}$. In case of multiple first order effluxes from the compartment the apparent rate constant of the decay of the concentration in the compartment is the sum of the rate constants of the individual elimination processes.

Many biological transport processes are enzyme controlled [Hol01]. In this case the elimination from the compartment might be saturable and its rate could obey Michaelis-Menten kinetics[2] in which the proportionality is not a constant but a function of $C(t)$,

$$\frac{dC(t)}{dt} = \frac{-V_m C(t)}{K_m + C(t)}, \tag{5.3}$$

where V_m is the maximal velocity of elimination and K_m is the concentration remaining to be eliminated if the rate has decreased to 50 % of V_m.

Another transport process is diffusion, which results in the uniform distribution of molecules and tends to equalize the concentrations on the two sides of a barrier [Ber98]. The diffusion rate $\frac{dC(t)}{dt}$ is proportional to the area A of the barrier and to the difference in concentration ΔC of the diffusing substance on the two sides of the barrier. Fick's first law of diffusion states that

$$\frac{dC(t)}{dt} = -DA\frac{\Delta C}{\Delta x}, \tag{5.4}$$

with D the diffusion coefficient. When it is assumed that the membrane is infinitely thin and that the concentration outside the cell is zero, then the rate of diffusion becomes

$$\frac{dC(t)}{dt} = -kC(t). \tag{5.5}$$

5.2.2 Growth and death of bacteria and cells

The rate of change in number of bacteria N as a function of time is the difference between the growth rate λ and killing rate k, where the killing rate is assumed to be proportional to the concentration $C(t)$, can be described by

$$\frac{dN}{dt} = \lambda N - kN(t)C(t). \tag{5.6}$$

For an antibiotic exhibiting saturable drug-receptor interactions, the killing rate is not linearly related with the concentration. The rate of change in number of bacteria has to be altered to

$$\frac{dN}{dt} = \lambda N(t) - \frac{kC(t)}{K_m + C(t)}N(t). \tag{5.7}$$

[2] see section 5.4.

In pharmacokinetic/pharmacodynamic relations a so called Hill equation or E_{max} model is frequently incorporated in equation (5.6) to become

$$\frac{dN(t)}{dt} = (\lambda - \frac{E_{max}C^n}{C^n + EC_{50}^n})N(t), \tag{5.8}$$

where E_{max} is the maximum killing rate, EC_{50} is the concentration at which 50 % of the maximum effect is obtained and n is the Hill factor, a measure of cooperativity of the molecules of the drug, determining the shape of the graph.

In the above mentioned models, the growth rate is uncontrolled. However, because of limitations of space, nutrients and other factors, there should be a maximum number of bacteria N_{max} to which the in vitro culture can grow over time [Vin96]. The N_{max} can be incorporated in the model according to

$$\frac{dN(t)}{dt} = (\lambda(1 - \frac{N(t)}{N_{max}}) - \frac{E_{max}C^n}{C^n + EC_{50}^n})N(t). \tag{5.9}$$

Another factor which has to be taken into account, is the development of resistance [Vin96]. There are, basically, two mechanisms. The first is a gradual increase in resistance over time. This can be accounted for by incorporating an adaption rate α in the model according to [Vin96]:

$$\frac{dN(t)}{dt} = (\lambda(1 - e^{-\alpha t})(1 - \frac{N(t)}{N_{max}}) - \frac{E_{max}C^n}{C^n + EC_{50}^n})N(t), \tag{5.10}$$

or according to [Rou03]

$$\frac{dN(t)}{dt} = (\lambda(1 - \frac{N(t)}{N_{max}}) - \frac{E_{max}e^{-\alpha t}C^n}{C^n + EC_{50}^n})N(t). \tag{5.11}$$

A second mechanism by which the emergence of resistant micro-organisms can be explained is by assuming the presence of bacterial sub-populations with different susceptibility to the drug. One approach to describe this type of emergence of resistance is to use a two-population model. Two different values of the EC_{50} for the two different populations can be incorporated in the model. In addition, there are two different values for the growth rates and the killing rates. The two-population model does not contain the adaptation-rate, but a N_{max} can be included.

$$\frac{dN}{dt} = \frac{dN_1}{dt} + \frac{dN_2}{dt}, \tag{5.12}$$

$$= \Big(\lambda_1(1 - \frac{N_1(t)}{N_{max}}) - \frac{E_{max_1}C^n}{C^n + EC_{50_1}^n}\Big)N_1 + \Big(\lambda_2(1 - \frac{N_2}{N_{max}}) - \frac{E_{max_2}C^n}{C^n + EC_{50_2}^n}\Big)N_2. \tag{5.13}$$

5.3 Variability of PK/PD models

A major problem of pharmacokinetic/pharmacodynamic models is the variability in the dose-effect relationship [Box92]. If variability among different individuals was negligible then major questions of pharmacokinetics and pharmacodynamics could be answered by just six people - one young, one old, one male, one female, one with renal failure and one with liver failure - instead of the thousand or more who are exposed to a drug before it is

marketed. Because of the overwhelming influence of variability in defining the dose-effect relationship it is worth considering the sources of variation. The components of the dose-effect relationship that contribute to variability are dose-concentration (PK) factors and concentration-effect (PD) factors.

5.3.1 Dose-concentration factors

Compliance includes all misinformation about the actual dose a patient has taken. It may be due to the patient taking too much or too little or it may be due to failure of the investigator to record the information accurately.

All drugs which are metabolized to an important extent and have a high extraction ratio will unavoidably have variable **biovariability**. As molecules become more complicated this problem will become more severe.

Tissue distribution is probably a relatively minor component of variability. Reduced organ perfusion, especially to the eliminating organ, is likely to be more important in severe ill patients. Rapid, large changes in volume of distribution are recognized in critically ill patients treated with aminoglycosides.

Variations in **metabolism** and/or renal **excretion** due to body size, age, other drugs, disease states or genetic causes are important determinants of clearance. Drugs eliminated mainly by the kidney have largely predictable differences based on estimates of renal function, so their typical unanticipated variability is smaller.

5.3.2 Concentration-effect factors

The contribution of variability due to **distribution** from the blood to the site of action will depend largely on changes in perfusion of the target tissue. It is only likely to be an important determinant of variability for drugs which act within minutes after administration, e.g., intravenous anaesthetic agents. Variation in the size of the target organ is a theoretical cause of changes in distribution time.

The **sensitivity** of a receptor, defined in terms of affinity for binding or potency relative to another agent, may be an important source of variability in response when typical concentrations produce effects which are less than 80% of maximum.

The **maximum achievable response** from a drug will vary directly with the factors which control it, such as receptor density. Inter-individual differences in efficacy are poorly defined in continuous sense, but many patients are classified as responders or non-responders which may be due to differences in efficacy.

5.4 Occupation theory and Michaelis-Menten kinetics

The *affinity* of a drug is the tenacity with which it binds to its biological receptor on the cell membrane [Ken97]. In statistical terms, the affinity is the probability of a drug molecule binding to a free drug receptor at any given instant. The *intrinsic efficacy* of a drug is that property that imparts the biological signal to the drug receptor (and thus to the cell)

to result in a biological response. Thus the affinity gets the drug to the receptor and the intrinsic efficacy determines what it does when it gets there.

The first proposed theory from the point of view of attempting to describe drug-receptor interaction has been occupation theory. In this theory a response only appears from a receptor when it is occupied by an appropriate drug molecule. Assuming that a concentration of drugs A is in the receptor compartment such that free diffusion controls access to the receptor R, the binding of A to the receptor can be described by the equation

$$[A] + [R] \underset{k_2}{\overset{k_1}{\rightleftharpoons}} [AR], \tag{5.14}$$

where k_1 and k_2 are the association and dissociation rate constants, $[AR]$, $[A]$ and $[R]$ are the concentrations of drug-receptor complex, the drug A and the receptor R. This physical-chemical model can be extended with so called Michaelis-Menten kinetics [Mur89]. For this we assume that the receptor combines directly with the drug molecule and that the receptor is not consumed in the process, i.e., the receptor acts as a true catalyst. Equation (5.14) can now be extended to

$$[R] + [A] \underset{k_2}{\overset{k_1}{\rightleftharpoons}} [AR] \overset{k_3}{\rightarrow} [R] + [P], \tag{5.15}$$

where k_3 is the transducing rate constant and $[P]$ is the concentration of the resulting product receptor inside the cell. The overall mechanism is a conversion of the drug via the receptor R into a product P.

The law of mass action states that the rate of a reaction is proportional to the product of the concentrations of the reactants. Then applying the law of mass action to equation (5.15) leads to a system of reaction equations for each reactant:

$$\frac{\partial[A]}{\partial t} = -k_1[A][R] + k_2[AR] \tag{5.16}$$

$$\frac{\partial[R]}{\partial t} = -k_1[A][R] + (k_2 + k_3)[AR] \tag{5.17}$$

$$\frac{\partial[AR]}{\partial t} = k_1[A][R] - (k_2 + k_3)[AR] \tag{5.18}$$

$$\frac{\partial[P]}{\partial t} = k_3[AR]. \tag{5.19}$$

Now define the total number of receptors R_t, as the sum of the free R and combined AR receptors, as $[R_t] = [R] + [AR]$. Then by adding equations (5.17) and (5.18) we find that

$$\frac{\partial[R_t]}{\partial t} = \frac{\partial[R]}{\partial t} + \frac{\partial[AR]}{\partial t} = 0. \tag{5.20}$$

Thus the total number of receptors R_t is a constant. At equilibrium the number of occupied receptors remains constant, thus $\frac{\partial[AR]}{\partial t} = 0$ and

$$k_1[A][R] = (k_2 + k_3)[AR]. \tag{5.21}$$

Substituting $[R_t] = [R] + [AR]$ and rearranging gives

$$[AR] = \frac{[R_t][A]}{[A] + \frac{k_2+k_3}{k_1}}. \tag{5.22}$$

Equation (5.22) shows that the fraction of receptors occupied by drug molecules depends on the drug concentration and the equilibrium dissociation constant $K_A = \frac{k_2+k_3}{k_1}$ of the drug-receptor complex. Assuming that k_3 is much less than k_2, K_A is equal to the dissociation constant of the AR complex (k_2/k_1). As such, K_A is a measure of the binding affinity of the drug. A high K_A indicates a low binding affinity and vice versa. K_A corresponds to the concentration of drug that binds to 50% of the available receptor population, thus it can be seen as a drug constant with a unique value for each type of pharmacologic receptor.

Now the rate of change of the resulting product inside the cell, equation (5.19) can be expressed as

$$\frac{\partial[P]}{\partial t} = k_3 \frac{[R_t][A]}{[A] + K_A}, \tag{5.23}$$

or as

$$\frac{\partial[P]}{\partial t} = V_{max} \frac{[A]}{[A] + K_A}. \tag{5.24}$$

According to [Mau00] a slightly different equation compared to (5.14) can be used. This new equation assumes that more molecules can bind to one receptor:

$$\gamma[A] + [R] \overset{k_1}{\underset{k_2}{\rightleftharpoons}} [A_\gamma R] \overset{k_3}{\rightarrow} [R] + \gamma[P], \tag{5.25}$$

with γ the number of molecules binding to the receptor. The new equation for the rate of change of the resulting product inside the cell becomes

$$\frac{\partial[P]}{\partial t} = \frac{V_{max}[A]^\gamma}{K_A + [A]^\gamma}. \tag{5.26}$$

According to [Mur89] equation (5.26) can be used for a cooperative phenomenon. This equation may be a reasonable quantitative form to describe a reaction's velocity in a Michaelis-Menten sense, the detailed reactions (5.25) are not realistic. However, equation (5.26) is a useful empirical relation, which is often used in pharmacodynamics.

In the mechanism (5.15) one receptor molecule binds to one drug molecule, that is the receptor has one binding site. However, there are receptors which have more than one binding site, which is called cooperative binding. After binding to a drug molecule at one site, the receptor can then bind to another drug molecule at another site. As an example we consider the case where a receptor has 2 binding sites. Following [Mur89], a reaction mechanism for this model is then

$$[A] + [R] \overset{k_1}{\underset{k_2}{\rightleftharpoons}} [AR] \overset{\rightarrow}{k_3} [R] + [P], \tag{5.27}$$

$$[A] + [AR] \overset{k_4}{\underset{k_5}{\rightleftharpoons}} [A_2R] \overset{\rightarrow}{k_6} [AR] + [P], \tag{5.28}$$

where the k's are the rate constants indicated. The mass action law applied to (5.27) gives

$$\frac{d[A]}{dt} = -k_1[A][R] + (k_2 - k_4[A])[AR] + k_5[A_2R],$$

$$\begin{aligned}
\frac{d[AR]}{dt} &= k_1[A][R] - (k_2 + k_3 + k_4)[AR] + (k_5 + k_6)[A_2R], \\
\frac{d[A_2R]}{dt} &= k_4[A][AR] - (k_5 + k_6[A_2R], \\
\frac{d[R]}{dt} &= -k_1[A][R] + (k_2 + k_3)[AR], \\
\frac{d[P]}{dt} &= k_3[AR] + k_6[A_2R].
\end{aligned} \tag{5.29}$$

The conservation of total receptors is obtained by adding the 2nd, 3rd and 4th equations:

$$\frac{d[R_t]}{dt} = \frac{d[R]}{dt} + \frac{d[AR]}{dt} + \frac{d[A_2R]}{dt} = 0. \tag{5.30}$$

At equilibrium $\frac{d[AR]}{dt} = \frac{d[A_2R]}{dt} = 0$, which leads to the rate of change for the product $[P]$

$$\frac{d[P]}{dt} = \frac{k_1[A][R_t](k_3(k_4 + k_6) + k_4k_6[A])}{(k_2 + k_3 + k_4)(k_5 + k_6) - (k_5 + k_6)(k_1 - k_4)[A] + k_1k_4[A]^2}, \tag{5.31}$$

$$= \frac{[R_t][A](k_3K'_m + k_6[A])}{K_mK'_m + K'_m[A] + [A]^2}, \tag{5.32}$$

with $K_m = \frac{k_3+k_2}{k_1}$ and $K'_m = \frac{k_6+k_5}{k_4}$.

For the uptake of drugs into the kidney and ear compartments we will assume that we are allowed to use equation (5.24). For the killing rate of bacteria and cells, we assume that cooperativity has to be taken into account, thus for the killing rates we use equation (5.26).

5.5 Hill equation, relating concentration to effect

There is only one widely used pharmacodynamic model relating concentration to effect [Box92]. As discussed in section 5.4, this theoretical model is based on the law of mass action and Michaelis-Menten kinetics [Ken97]. The active and saturable uptake of a drug into cells can be described by this same equation [Ber98]. The model is called E_{max} model and the rate of uptake is equal to

$$\frac{dC_{in}(t)}{dt} = \frac{E_{max}C(t)}{K_m + C(t)}, \tag{5.33}$$

where E_{max} is the maximal change in effect the drug can produce, $C_{in}(t)$ is the concentration of the drug inside the cell, $C(t)$ is the concentration of the transported substance in the compartment from which it is being removed, and K_m is the apparent Michaelis constant for the transporter, which is the concentration of the transported compound required for half-maximal transport.

The E_{max} model may often need to be extended to describe concentration-effect curves which are either steeper of shallower. This extension was proposed by Hill and it is quite empirical and there is not theoretical basis for the parameter n [Box92], [Mur89],

$$E = \frac{E_{max}C(t)^n}{K_m^n + C(t)^n}. \tag{5.34}$$

The parameter n is a measure of cooperativity, it makes the curve steeper when it is greater than 1 and shallower when less than 1. Values of n greater than 5 approximates responses which behave as if there is a threshold concentration below which no drug effect is seen, but almost full response is observed at concentration just over the threshold.

Besides setting n large in equation (5.34), a threshold can be incorporated in a different manner [Box92] by setting the effect equal to

$$E(t) = \begin{cases} 0 & \text{if } C(t) < C_{th} \\ \dfrac{E_{max}(C(t) - C_{th})^{\gamma}}{(K_m - C_{th})^{\gamma} + (C(t) - C_{th})^{\gamma}} & \text{if } C(t) > C_{th}, \end{cases} \tag{5.35}$$

where C_{th} is the value of the threshold concentration.

In the model of [Rou03] the threshold is incorporated in a different manner, namely

$$E(t) = \begin{cases} 0 & \text{if } C(t) < C_{min} \\ \dfrac{E_{max}C(t)^{\gamma}}{C_{50}^{\gamma} + C(t)^{\gamma}} & \text{if } C(t) > C_{min}. \end{cases} \tag{5.36}$$

Now the effect is set to be zero below a certain value for the concentration of drug in the kidney cells. Above this value it is assumed that the full effect is present instead of setting the effect proportional to the difference between the concentration in the PT cells and the threshold concentration as in equation (5.35). This assumption results in a discontinuous function for the effect and thus for the creatinine clearance. We have chosen to use the model of equation (5.34) to avoid discontinuity as in [Rou03]. In figure 5.1 the concentration-effect curves are shown for different values of γ. Also shown are the effect curves with the threshold and the effect curve used by Rougier.

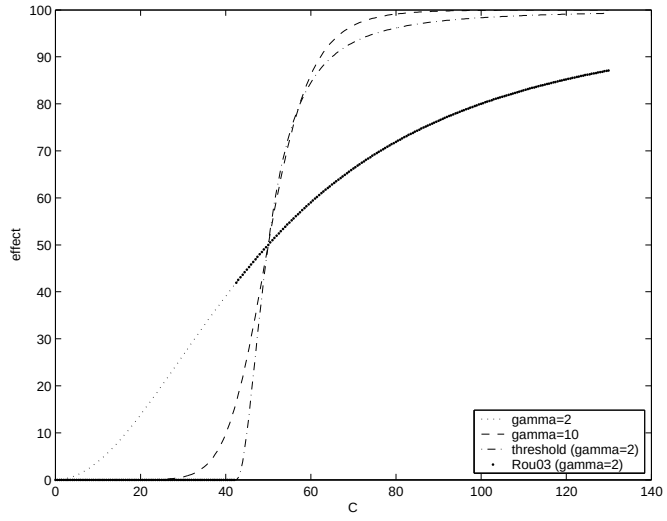

Figure 5.1: *Effect as a function of the concentration of drug $C(t)$ for different values of γ. C_{50} has the value 50 mg/l and E_{max} is 100. Shown are the concentration effect curves for γ is equal to 2 ($\cdots$) and 10 ($- - -$). The third curve ($-.-.$) is the effect as described by equation (5.35). With the thick dots the effect-concentration curve used by [Rou03], equation (5.36) is shown. This curve has a discontinuity at the threshold value of C_{min} = 42.5 mg/l.*

Chapter 6

Mathematical model of aminoglycosides

In the previous chapters the background on the action of aminoglycosides, the physiology and PK/PD modeling is outlined. In this chapter a mathematical model is developed based on these factors. The focus of interest of the model are the concentrations inside the blood, the kidney cells, the ear fluids and the organ of Corti. These concentrations are related to each other by influx and efflux processes such that the rate of change is equal to "what comes in" minus "what goes out".

In figure 6.1 the scheme of distribution of the aminoglycosides in the human body is presented. The aminoglycosides are infused into the blood, from which it is filtered into the urine by the kidney. From the blood the drug is also taken into the ear fluids and the organ of Corti. The model for the concentration in the blood is outlined in section 6.1.

A part of the filtered drugs is taken actively into the kidney cells, where they interfere with certain processes inside the cells resulting in the death of the cells. This process is explained in section 6.2. From the kidney cells the drug is eliminated into the blood and into the urine. The model for the concentration inside the kidney cells is derived in section 6.3.

The nephrotoxicity is related to the concentration of aminoglycosides in the proximal tubular cells as explained in section 6.4. The efficacy of the drugs (i.e. the rate of killing of bacteria) is related to the concentration in the blood and is explained in section 6.5. The concentration in the ear fluids and the related ototoxicity is presented in section 6.6.

Finally in section 6.7 we compare our model with the model described in [Rou03]. In this article a model for the nephrotoxicity is developed. We will comment on some of the modeling choices made in [Rou03] and we compare them with our choices.

6.1 The concentration in the blood

In this section we derive the equation for the rate of change of the concentration of drug in the blood. Related to the concentration in the blood is the killing rate of the bacteria, which is explained in section 6.5.

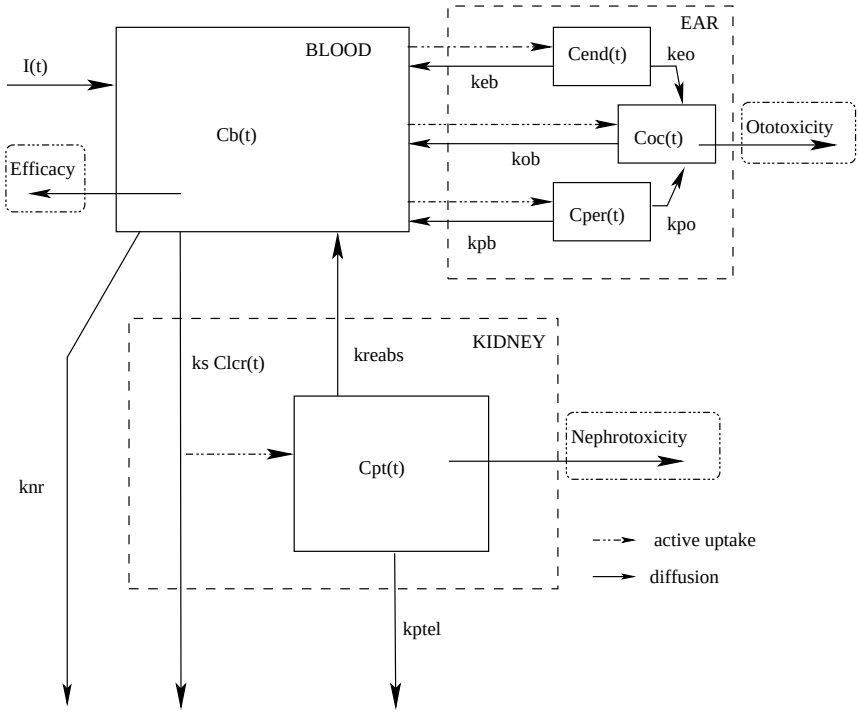

Figure 6.1: *Scheme of the distribution of Aminoglycoside. From the blood compartment the aminoglycoside is distributed into the proximal tubular cells in the kidney and into the inner ear fluids, the endolymph and the perilymph, and to the organ of Corti. The efficacy of the drugs is related to the concentration in the blood. The nephrotoxicity is related to the concentration of aminoglycosides in the kidney cells and the ototoxicity is related to the concentration of drugs in the organ of Corti.*

The total amount[1] of aminoglycoside in the blood compartment is the sum of the amount of aminoglycoside coming from administration (the dose), the ear fluids and from renal tubular reabsorption [Bro88] minus the amount transfered out of the blood into the ear fluids and the amount eliminated from the blood.

The total elimination from the blood can be divided into a renal elimination and a non-renal elimination pathway. Aminoglycosides are freely filtered through the glomerulus, see figure 4.2, thus their renal clearance is linearly proportional to the creatinine clearance $Cl_{cr}(t)$. The elimination coefficient k_{el} can thus be written as

$$k_{el} = k_{nr} + k_s Cl_{cr}(t), \tag{6.1}$$

with k_{nr} the non-renal elimination rate and k_s the renal clearance coefficient.

The renal tubular reabsorption is the process in the kidney of absorbing the aminoglycoside into the kidney cells and from there the aminoglycosides are eliminated into the blood [Bro88]. The rate of elimination to the blood is dependent on the concentration of

[1]The amount Q_b is equal to wV_dC_b with w the weight, V_d the volume of distribution and C_b the concentration in the blood.

the drug inside the kidney cells.

The rate of change of the concentration in the blood $C_b(t)$ can now be expressed as:

$$\frac{dC_b(t)}{dt} = I(t) - \Big(k_{nr} + k_s Cl_{cr}(t)\Big)C_b(t) + k_{ear}C_b(t) + k_{reabs}C_{pt}(t) + K_{ear}C_{ear}(t), \quad (6.2)$$

where $I(t)$ is the infusion rate (mg/l/h) of aminoglycoside, either by continuous infusion or intermittent infusion, k_{ear} the active transfer rate (1/h) from the blood to the ear fluids, which is dependent on the concentration in the blood, k_{reabs} the renal tubular reabsorption constant (1/h), $C_{pt}(t)$ the concentration (mg/l) of aminoglycoside in the proximal tubular cells of the kidney , K_{ear} the transfer rate (1/h) from the ear fluids to the blood, and $C_{ear}(t)$ the concentration of aminoglycoside in the ear fluids (mg/l).

In [Rou03] the transfer rates to and from the ear compartment are assumed to be linear. However, the uptake of aminoglycoside in the ear fluids is active and saturable. In section 6.6.1 the proper transfer rates to and from the ear fluids will be taken into account.

6.2 The number of kidney cells

The aminoglycosides are taken into the kidney cells, where they interfere with a number of intracellular processes, such that the cell is not able to function properly and dies. The process of killing of the renewable kidney cells can be described with a model similar to a model for harvesting a single renewable population [Mur89]. The kidney cells have a growth rate, depending on the population, which more or less maintains a constant population equal to the maximum size of the kidney, which is called the environment's carrying capacity M_{max}. This means that the growth and death rates are about equal. Killing the cells affects the mortality rate and the number of kidney cells adjusts and settles down to a new equilibrium state.

The killing rate of the kidney cells is in direct relation with the concentration of aminoglycosides inside the proximal tubular cells [Min99] and it can be described by an E_{max} model. The change in number of living kidney cells $M(t)$ as a function of the logistic regeneration rate and the killing rate can thus be expressed as

$$\frac{dM(t)}{dt} = \Big(\lambda_k(1 - \frac{M(t)}{M_{max}}) - \frac{E_{max}C_{pt}(t)^\gamma}{Q_{50}^\gamma + C_{pt}(t)^\gamma}\Big)M(t), \quad (6.3)$$

with $M(0) = M_{max}$, and with λ_k the regeneration rate, E_{max} the maximal killing rate, $C_{pt}(t)$ the concentration of aminoglycosides in the proximal tubulus cells, γ the Hill sigmoidity coefficient and Q_{50} the concentration in the kidney cells for which half the maximal killing rate is obtained. After the end of the killing, the recovery time T is of order $O(\frac{1}{\lambda_k})$, namely the time scale of reproductive growth.

6.3 The concentration in the kidney

In this section we will derive the equation for the rate of change of the concentration of drug in the kidney cells. The transport of aminoglycoside through the kidney cells starts with binding to the brush border membrane. Thereafter the aminoglycosides are taken

actively and saturably [Bro88] into the cells, which can be described by a Michaelis-Menten equation [Der95], [Ber98]. The aminoglycosides accumulate within the kidney cells. In the absence of tissue damage, the drugs is eliminated to the blood by diffusion with rate k_{reabs}. However, when cells die, drug release is instantaneous, since it follows the extrusion of the remaining of the dead cells into the urine [Bro88].

Assume that at time t the number of kidney cells is $M(t)$ and the concentration inside the kidney cell is $C_{pt}(t)$. Then at time t, the total amount $Q_{tot}(t)$ of aminoglycosides inside the kidney cells is equal to $Q_{tot}(t) = M(t)C_{pt}(t)$.

The total amount of aminoglycoside in the kidney cells at time $t+\Delta t$ is the total amount at time t, plus the amount taken up from the blood during the interval Δt minus the amount eliminated by living cells, minus the amount lost from cells killed during the interval Δt.

The amount taken up from the blood is the number of cells times the active uptake rate per cell $\frac{V_{max}C_b(t)}{K_m+C_b(t)}$ times the length of the interval. Thus the amount taken up from the blood is equal to $\frac{V_{max}C_b(t)}{K_m+C_b(t)}M(t)\Delta t$.

The amount eliminated by living cells during the interval is the elimination rate $k_{reabs}C_{pt}(t)$ per cell times the number of living cells $M(t)$ times the length of the interval.[2] The amount eliminated by living cells is equal to $k_{reabs}C_{pt}(t)M(t)\Delta t$.

The amount lost from cells killed during the interval Δt is equal to the concentration $C_{pt}(t)$ times the number of cells killed. Thus the amount lost is equal to $C_{pt}(t)\frac{E_{max}C_{pt}(t)^\gamma}{Q_{50}^\gamma+C_{pt}(t)^\gamma}M(t)\Delta t$.

Then the total amount inside the kidney cells at time $t+\Delta t$ is equal to

$$\begin{aligned} Q_{tot}(t+\Delta t) &= Q_{tot}(t) + M(t)\frac{V_{max}C_b(t)}{K_m+C_b(t)}\Delta t - M(t)k_{reabs}C_{pt}(t)\Delta t \\ &\quad -\frac{E_{max}C_{pt}(t)^{\gamma+1}}{Q_{50}^\gamma+C_{pt}(t)^\gamma}M(t)\Delta t. \end{aligned} \tag{6.4}$$

The rate of change of the total amount inside the living kidney cells is then equal to

$$\frac{dQ_{tot}}{dt} = M(t)\frac{V_{max}C_b(t)}{K_m+C_b(t)} - M(t)k_{reabs}C_{pt}(t) - \frac{E_{max}C_{pt}(t)^{\gamma+1}}{Q_{50}^\gamma+C_{pt}(t)^\gamma}M(t), \tag{6.5}$$

with $\frac{dQ_{tot}(t)}{dt} = \frac{dM(t)}{dt}C_{pt}(t) + \frac{dC_{pt}(t)}{dt}M(t)$, this gives

$$\frac{dC_{pt}(t)}{dt} = -\frac{dM(t)}{dt}\frac{C_{pt}(t)}{M(t)} + \frac{V_{max}C_b(t)}{K_m+C_b(t)} - k_{reabs}C_{pt}(t) - \frac{E_{max}C_{pt}(t)^{\gamma+1}}{Q_{50}^\gamma+C_{pt}(t)^\gamma}.$$

$\frac{dM(t)}{dt}$ is given by equation (6.3), which leads to the rate of change of the concentration in the kidney cells

$$\frac{dC_{pt}(t)}{dt} = -\lambda_k(1-\frac{M(t)}{M_{max}})C_{pt}(t) + \frac{V_{max}C_b(t)}{K_m+C_b(t)} - k_{reabs}C_{pt}(t). \tag{6.6}$$

[2]The elimination process is a diffusion process, which is driven by the concentration difference between the exterior and interior of the cell. The structure of the nephrons is such that the concentration in the blood outside the proximal tubular cells is zero, because the drugs is filtered from the blood at an earlier stage in the blood stream of the kidney.

6.4 Nephrotoxicity, the creatinine clearance and the serum creatinine concentration

Nephrotoxicity is defined as a fall[3] in the creatinine clearance compared to the baseline creatinine clearance[4]. Under influence of the decrease in number of kidney cells the glomerular filtration may change. The kidney is capable of compensating for nephron loss by the process of tubuloglomerular feedback, which increases the flow through the remaining nephrons when a fraction of cells die [Van95]. The fraction of dead cells $E(t)$ is equal to

$$E(t) = \frac{M_{max} - M(t)}{M_{max}}. \tag{6.7}$$

The tubuloglomerular feedback can be described by a Hill equation [Rou03], where the decrease in clearance is related to the fraction of dead cells $E(t)$ [Rou03],

$$Cl_{cr}(t) = Cl_{cr0} - \frac{Cl_{cr0}E(t)^{\delta}}{E_{50}^{\delta} + E(t)^{\delta}} + Var(t), \tag{6.8}$$

where Cl_{cr0} is the creatinine clearance at the start of the treatment, E_{50} the fraction of dead cells for which the clearance is decreased by 50%, δ the Hill sigmoidity coefficient and $Var(t)$ the circadian variation, which represents the daily variance in clearance. According to [Koo89], the most widespread value for this variance is 10%. We choose to neglect the circadian variation. Rewriting equation (6.8) leads to

$$Cl_{cr}(t) = Cl_{cr0}\frac{E_{50}^{\delta}}{E_{50}^{\delta} + E(t)^{\delta}}. \tag{6.9}$$

Creatinine is freely filtered through the glomerulus, thus a decrease in renal function involves a rise[5] in the concentration of serum creatinine[6] which depends on the daily muscular production rate k_2 (mg/l/h) and the daily renal elimination. The serum creatinine concentration $C_{scr}(t)$ (mg/l) varies as follows [Rou03]

$$\frac{dC_{scr}(t)}{dt} = -Cl_{cr}(t)C_{scr}(t) + k_2. \tag{6.10}$$

6.5 Efficacy

Because it is not possible to count the total number of bacteria in the body in clinical situations, the efficacy of a treatment with antibiotics can be measured by so called shadow parameters, such as body temperature or the time needed for a blood sample to sedimentate. Many methods and parameters to describe the efficacy of treatment with antibiotics are used in the literature. Examples are the time above the minimum inhibitory concentration ($T_{>MIC}$), the area under the concentration-time curve (AUC), total lethality [Nee02] and the number of bacteria (or increase or decrease in number of bacteria) [Nee02].

To describe the efficacy of treatment with antibiotic drugs we calculate the number of bacteria as a function of time and concentration in the blood compartment. Following [Nee02]

[3] a decrease in creatinine clearance of 50% or more compared to the baseline creatinine clearance
[4] the baseline creatinine clearance is the creatinine clearance at the start of the treatment
[5] nephrotoxicity is also defined as a rise of 0.5 mg/dl or 45μmol/l of the serum creatinine concentration
[6] the serum creatinine is the creatinine in the blood

and [Vin96] a PK/PD model can be developed to characterize the bactericidal killing rate as a function of the concentration of the antibiotic in the blood. The objective is to create a germ killing effect, i.e. reduction in number of microorganisms of 10^{-12}.

Aminoglycosides act by bactericidal action, which means that the drug has to get inside the cells of the bacteria to be effective [Pri95]. This action is concentration-dependent and saturable, meaning that higher drug levels result in more bactericidal action and that the transport into the cell costs energy. The rate of change in number of bacteria $N(t)$ can be described by a growth rate λ_b and a killing rate $F(C_b)$ related to the concentration in the blood $C_b(t)$,

$$\frac{dN(t)}{dt} = \Big(\lambda_b - F(C_b(t))\Big)N(t). \tag{6.11}$$

The bactericidal action of aminoglycosides, the functional dependence of the killing rate $F(C_b)$ and the concentration $C_b(t)$ can be described by a Hill equation [Nee02],

$$F(C_b) = \frac{F_{max}C_b^{\gamma_k}}{C_{50}^{\gamma_k} + C_b^{\gamma_k}}, \tag{6.12}$$

where F_{max} represents the maximal killing rate of the drug for a certain microorganism, and γ_k is the Hill factor for the killing process, which is a measure of cooperativity of the molecules of the drug determining the steepness/shape of the graph. C_{50} is the concentration at which 50% of the maximum effect is reached.

The sensitivity of the organisms is usually expressed as the minimal inhibitory concentration C_{mic}, which is the lowest concentration at which no growth is observed. No growth of the bacteria population can be expressed as $dN/dt = 0$, from which it follows that

$$C_{50} = \Big(\frac{F_{max} - \lambda_b}{\lambda_b}\Big)^{\frac{1}{\gamma_k}} C_{mic}. \tag{6.13}$$

Combining equations (6.11) and (6.12) gives the following equation for the rate of change in number of bacteria

$$\frac{dN(t)}{dt} = \Big(\lambda_b - \frac{F_{max}C_b(t)^{\gamma_k}}{C_{50}^{\gamma_k} + C_b(t)^{\gamma_k}}\Big)N(t), \tag{6.14}$$

Equation (6.14) has the following solution for the number of microorganisms

$$N(t) = N_0 \mathrm{e}^{\int_{t_0}^{t} (\lambda_b - F(C_b(\tau)))d\tau}, \tag{6.15}$$

with N_0 the initial number of the microorganisms at time t_0.

In equation (6.11) the growth is uncontrolled, if $\lambda_b > F(C_b(t))$. However, because of limitations of space, nutrients and other factors, a maximum number of bacteria N_{max} to which the bacteria can grow over time can be incorporated [Vin96] and [Mou97]. Another factor that can be taken into account, is the development of resistance as described by [Vin96], [Mou97] and [Rou03].

To determine the efficacy of the treatment we use equation (6.14). We neglect the resistance of bacteria to aminoglycosides and we assume that the blood compartment is large enough to avoid the use of a maximum number of bacteria.

6.6 Ototoxicity

Besides the nephrotoxicity and efficacy we want to incorporate the ototoxicity of aminoglycosides. Although the ototoxicity progresses slowly an is thus mainly found in patients treated for long periods, its irreversibility is a major concern for the use of aminoglycosides.

Aminoglycosides are transported actively and saturably into the endolymph and the perilymph[7] and into the organ of Corti[8] [Bro88]. From the endolymph and the perilymph the aminoglycosides are eliminated by diffusion to the blood and to the organ of Corti, which is driven by the concentration difference. The diffusion rate is zero if the concentration outside is greater than the concentration inside.

The rate of change of the concentration of aminoglycosides in the perilymph, $C_{per}(t)$, and the endolymph, $C_{end}(t)$ involves the uptake from the blood and the elimination to the blood and to the organ of Corti:

$$\frac{dC_{end}(t)}{dt} = \frac{J_{be}C_b(t)}{K_{end}+C_b(t)} - k_{eb}(C_{end}(t)-C_b(t))_+ - k_{eo}(C_{end}(t)-C_{oc}(t))_+, \quad (6.16)$$

$$\frac{dC_{per}(t)}{dt} = \frac{J_{bp}C_b(t)}{K_{per}+C_b(t)} - k_{pb}(C_{per}(t)-C_b(t))_+ - k_{po}(C_{per}(t)-C_{oc}(t))_+, \quad (6.17)$$

where J_{be} and J_{bp} are the maximum uptake rates from the blood to the endolymph and the perilymph, K_{end} and K_{per} the concentrations at which half the maximum uptake rate is reached, $C_b(t)$ is the concentration in the blood, k_{eb} and k_{pb} are the elimination rates from the endolymph and perilymph to the blood and k_{eo} and k_{po} are the elimination rates to the organ of Corti. The last two terms in equations (6.16) and (6.17) are equal to zero when $C_{oc}(t)$ and $C_b(t)$ are greater than $C_{per}(t)$ and $C_{end}(t)$.

The rate of change of the concentration of aminoglycosides in the organ of Corti $C_{oc}(t)$ is related to the uptake from the blood, the diffusion from the endolymph and the perilymph and the elimination to the blood,

$$\frac{dC_{oc}(t)}{dt} = \frac{J_{bo}C_b(t)}{K_{oc}+C_b(t)} + k_{eo}(C_{end}(t)-C_{oc}(t))_+ + k_{po}(C_{per}(t)-C_{oc}(t))_+ - k_{ob}(C_{oc}(t)-C_b(t))_+, \quad (6.18)$$

with J_{bo} the maximum uptake rate from the blood, K_{oc} the concentration in the organ of Corti at which half the maximum uptake rate is reached and k_{ob} the elimination rate to the blood. The diffusion terms are equal to zero when $C_{oc}(t)$ is greater than $C_{end}(t)$ and $C_{per}(t)$ and the last term is equal to zero when $C_b(t)$ is greater than $C_{oc}(t)$.

The organ of Corti consists of hair cells[9], which transduce the sound waves into electrical waves to the brain. Aminoglycosides inside the organ of Corti results in the destruction of the hair cells, which results in deafness [Bro88]. The hair cells are not capable of regenerating [Bro88]. To describe the killing of the hair cells, a Hill equation is used, relating

[7] The inner ear fluids of the cochlea, which is spiral-shaped organ and includes several chambers of fluid, the organ of Corti and the hair cells.

[8] the neural apparatus responsible for transduction of sound.

[9] 15000 outer and 3500 inner hair cells

the killing of the hair cells to the concentration in the organ of Corti. The rate of change of the number of hair cells $N_{hc}(t)$ is thus equal to

$$\frac{dN_{hc}(t)}{dt} = -\frac{Fhc_{max}C_{oc}(t)^n}{F_{50}^n + C_{oc}(t)^n}N_{hc}(t), \tag{6.19}$$

where Fhc_{max} is the maximum killing rate for the hair cells, F_{50} is the concentration at which half the maximal killing rate is achieved and n the Hill sigmoidity coefficient.

6.6.1 The concentration of aminoglycosides in the blood

Taking transfer rates to and from the ear fluid compartments and the organ of Corti into account, the equation for the concentration of aminoglycosides in the blood compartment, i.e. equation (6.2), can be rewritten as:

$$\begin{aligned}\frac{dC_b(t)}{dt} &= I(t) - k_{nr}C_b(t) - k_sCl_{cr}(t)C_b(t) + k_{reabs}C_{pt}(t) \\ &+k_{eb}\Big(C_{end}(t) - C_b(t)\Big)_+ + k_{pb}\Big(C_{per}(t) - C_b(t)\Big)_+ + k_{ob}\Big(C_{oc}(t) - C_b(t)\Big)_+ \\ &-\frac{J_{be}C_b(t)}{K_{end} + C_b(t)} - \frac{J_{bp}C_b(t)}{K_{per} + C_b(t)} - \frac{J_{bo}C_b(t)}{K_{oc} + C_b(t)}.\end{aligned} \tag{6.20}$$

Again the diffusion terms are equal to zero when $C_b(t)$ is greater than $C_{end}(t)$, $C_{per}(t)$ and $C_{oc}(t)$.

6.7 Comparison with the model of Rougier

In [Rou03] a deterministic model is described for the nephrotoxicity. In that article the efficacy and ototoxicity are not included. Their model for nephrotoxicity differs from our model in the choice of reabsorption in the kidney cells and in the choice for the effects of aminoglycosides on the kidney cells. Also the regeneration of kidney cells is not incorporated.

Throughout the article of Rougier the amount of aminoglycosides is used in the equations instead of the concentration. Effects on cells and uptake into cells of aminoglycosides are dependent on the concentration, because this reflects a 'density'. The amount does not say anything about the amount per cell or per liter.

The rate of reabsorption of drugs into the blood from the kidney cells is dependent on the concentration of drugs in the kidney cells. In [Rou03] the reabsorption is directly substracted from the filtration rate, thus setting the reabsorption rate dependent on the amount in the blood.

In [Rou03] the aminoglycosides kill cells of the kidney by a so called Effect. This effect is related to the amount of drug in the kidney cells and represents, in fact, the killing rate of the cells. Related to this effect is the creatinine clearance, which should be related on the number of kidney cells and not on the killing rate.

The effect in [Rou03] is chosen discontinuously, it only occurs when a threshold is reached, below which no killing occurs. As a result the creatinine clearance is also a discontinuous

function, which can not be the case. We have chosen a continuous function for the creatinine clearance by relating it to the number of kidney cells.

In [Rou03], the regeneration of kidney cells is not incorporated. Their creatinine clearance returns to normal, because its coupled with the killing rate, which returns to zero.

Chapter 7

Analytical results for a continuous infusion

The mathematical model for the distribution and action of aminoglycosides is derived in the previous chapter. The model consists of a coupled system of differential equations. For a continuous infusion the system reaches a steady state, for an intermittent administration the model is a dynamic system changing over time, driven by the dose and duration of interval.
In this chapter we present analytical solutions for the optimal concentration in the blood for efficacy and for the concentration in the blood above which nephrotoxicity occurs. Both solutions are calculated for a constant infusion, because then the system approaches a steady state. The optimal concentration for efficacy is presented in section 7.1. The concentration in the blood above which nephrotoxicity occurs is presented in section 7.2.

7.1 Optimal concentration in the blood for efficacy

7.1.1 Optimal concentration using the model of [Nee02]

To calculate the optimal concentration for efficacy, we first follow [Nee02], in which an one compartment model is assumed, thus without the kidney cells and the ear fluids. The equation for the rate of change of the concentration in the blood is then

$$\frac{dC_b(t)}{dt} = I(t) - k_{el}C_b(t), \tag{7.1}$$

with $k_{el} = k_{nr} + k_s Cl_{cr}$ the elimination rate from the blood. In case of a constant infusion, the concentration at steady state is a constant, so $\frac{dC_b}{dt} = 0$ and $I = k_{el}C_b$ and thus is the concentration in the blood equal to

$$C_b = \frac{I}{k_{el}}. \tag{7.2}$$

The total amount of drug P per liter administered during total treatment T is equal to

$$P = \int_0^T I dt = k_{el}C_b T. \tag{7.3}$$

So there is a relation between the total treatment time and the resulting concentration C_b if the total dose is a constant. The number of bacteria at the end of treatment is equal to

$$\begin{aligned} N(T) &= N_0 \mathrm{e}^{(\lambda_b - F(C_b))T}, \\ &= N_0 \mathrm{e}^{\dfrac{(\lambda_b - F(C_b))P}{k_{el} C_b}}. \end{aligned} \tag{7.4}$$

The optimal concentration can be found by minimizing the function

$$C_b \to N_0 \mathrm{e}^{\dfrac{(\lambda_b - F(C_b))P}{k_{el} C_b}}. \tag{7.5}$$

The exponent is an increasing function, so the minimum will be reached when $(\lambda_b - F(C_b))/C_b$ is minimal, i.e., $(F(C_b) - \lambda_b)/C_b$ is maximal. Differentiating with respect to C_b, setting this derivative equal to zero and solving for the concentration C_b leads to the optimal concentration C_{opt}. With the use of equation (6.13) C_{opt} becomes

$$C_{opt} = C_{mic}\Big(\frac{\lambda_b}{2}(F_{max}(\gamma_k - 1) + 2\lambda_b + \sqrt{F_{max}}\sqrt{\gamma_k^2 F_{max} - 2\gamma_k F_{max} + 4\gamma_k \lambda_b + F_{max}})\Big)^{\frac{1}{\gamma_k}}, \tag{7.6}$$

which corresponds to the optimal concentration found in [Nee02], thus an optimal concentration can be found for efficacy when an one compartment model is assumed.

Graphically this optimal value is reached when the line through the origin and the point $(C_b, F(C_b) - \lambda_b)$ is tangent to the graph of $F(C_b) - \lambda_b$ as shown in figure 7.1.

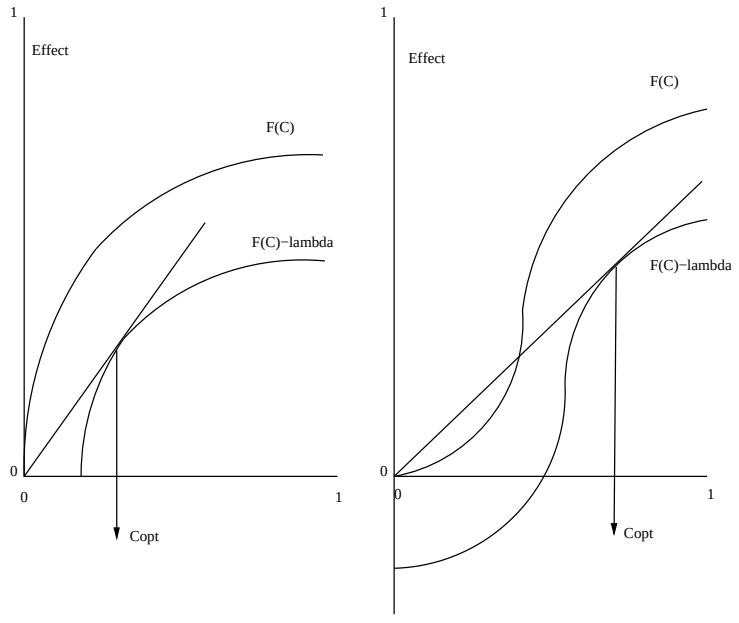

Figure 7.1: *The killing rate versus concentration curve for $0 < \gamma < 1$ (left) and for $\gamma > 1$ (right). On the x-axis, the concentration of the drug is shown in arbitrary units. The y-axis represents the relative killing effect. The curve $F(C)$ represents the killing rate as a function of the concentration. The curve $F(C) - \lambda$ is the curve where the killing rate depends on the difference between growth and killing. The optimal concentration C_{opt} is the point where the tangent through the origin hits this curve.*

7.1.2 Optimal concentration for efficacy with the mathematical model

To calculate the optimal concentration for efficacy for the model with the kidney and ear compartments as described in chapter 6, we assume that no nephrotoxicity occurs, thus that $M(t) = M_{max}$ and $\frac{dM(t)}{dt} = 0$ in equation (6.3).

The concentration of aminoglycosides in the blood is given by equation (6.20). In case of a constant infusion, the concentration at steady state is a constant, so $\frac{dC_b(t)}{dt} = 0$. This also applies to the concentrations in the ear fluids and the organ of Corti, thus $\frac{dC_{end}(t)}{dt} = \frac{dC_{per}(t)}{dt} = \frac{dC_{oc}(t)}{dt} = 0$. Substituting this into equations (6.16)-(6.18) and into (6.20) we obtain

$$I(t) - (k_{nr} + k_s Cl_{cr}(t))C_b(t) + k_{reabs}C_{pt}(t) = 0. \tag{7.7}$$

At steady state and without nephrotoxicity, it follows from equation (6.6), that

$$C_{pt}(t) = \frac{V_{max}C_b(t)}{k_{reabs}(K_m + C_b(t))}, \tag{7.8}$$

and thus that

$$I(t) = (k_{nr} + k_s Cl_{cr}(t))C_b(t) - \frac{V_{max}C_b(t)}{K_m + C_b(t)}. \tag{7.9}$$

Writing $k_{el} = k_{nr} + k_s Cl_{cr}$ we find that

$$C_b = \frac{I}{k_{el}} + \frac{V_{max}C_b(t)}{k_{el}(K_m + C_b(t))}, \tag{7.10}$$

with the last term greater than zero, which represents the portion of drugs taken up after filtration into the kidney cells. Thus with the same infusion rate a higher steady state concentration is reached, than with the one compartment model used by [Nee02]. Thus taking the kidney compartment in consideration, results in a higher concentration in the blood, thus it can be concluded that the kidney delays the elimination of drugs from the blood.

The total amount of drug P per liter administered during total treatment time T is now equal to

$$P = \int_0^T I(t)dt = \Big(k_{el}C_b - \frac{V_{max}C_b}{K_m + C_b}\Big)T. \tag{7.11}$$

The number of bacteria is given by equation (6.15). At the end of treatment $N(T)$ is equal to

$$N(T) = N_0 \mathrm{e}^{(\lambda_b - F(C_b))T}, \tag{7.12}$$

or

$$N(T) = N_0 \mathrm{e}^{\frac{(\lambda_b - F(C_b))P}{k_{el}C_b - \frac{V_{max}C_b}{K_m + C_b}}}. \tag{7.13}$$

The optimal concentration can be found by minimizing the function

$$C_b \to N_0 \mathrm{e}^{\frac{(\lambda_b - F(C_b))P}{k_{el}C_b - \frac{V_{max}C_b}{K_m + C_b}}}. \tag{7.14}$$

Because of the higher concentration in the blood (7.10) than with the model of [Nee02] (7.2), the killing rate $F(C_b)$ is larger than with the one-compartment model. Also $k_{el}C_b > k_{el}C_b - \frac{V_{max}C_b}{K_m+C_b}$ and thus $1/k_{el}C_b < 1/(k_{el}C_b - \frac{V_{max}C_b}{K_m+C_b})$. Combining this with the fact that the exponent in this case is a decreasing function, we can conclude that the number of bacteria in this model is less with the same infusion rate than with the one-compartment model of [Nee02] and thus is the decrease in number of bacteria higher than in the one-compartment model.

7.2 The maximal concentration in the blood avoiding nephrotoxicity

Besides an optimal concentration in the blood for efficacy, a concentration in the blood can be determined below which no nephrotoxicity occurs. At steady state the rate of change of C_b, C_{pt} and M are equal to zero, thus we obtain the following equations

$$I - \Big(k_{nr} + k_s Cl_{cr}\Big)C_b + k_{reabs}C_{pt} = 0 \tag{7.15}$$

$$\lambda_k(1 - \frac{M}{M_{max}}) - \frac{E_{max}C_{pt}^{\gamma}}{Q_{50}^{\gamma} + C_{pt}^{\gamma}} = 0 \tag{7.16}$$

$$-\lambda_k(1 - \frac{M}{M_{max}})C_{pt} + \frac{V_{max}C_b}{K_m + C_b} - k_{reabs}C_{pt} = 0 \tag{7.17}$$

with $Cl_{cr} = \frac{E_{50}^{\delta}}{E_{50}^{\delta}+E^{\delta}}$ and $E = \frac{M_{max}-M}{M_{max}}$. From equation (7.16) the number of kidney cells at steady state is equal to

$$M = M_{max}(1 - \frac{E_{max}C_{pt}^{\gamma}}{\lambda_k(Q_{50}^{\gamma} + C_{pt}^{\gamma})}), \tag{7.18}$$

or the concentration in the kidney cells at steady state is equal to

$$C_{ptss} = (\frac{\lambda_k(M - M_{max})}{M_{max}(\lambda_k - E_{max}) - \lambda_k M})^{1/\gamma}Q_{50}. \tag{7.19}$$

or using the definition for E (6.7)

$$C_{ptss} = (\frac{\lambda_k E}{E_{max} - E\lambda_k})^{1/\gamma}Q_{50}. \tag{7.20}$$

Substituting this into equation (7.17) and solving for C_b we find the steady state value of the concentration in the blood

$$C_{bss} = -\frac{(\lambda_k E)^{1/\gamma}Q_{50}K_m(\lambda_k E + k_{reabs})}{(\lambda_k E + k_{reabs})(\lambda_k E)^{1/\gamma}Q_{50} - V_{max}(E_{max} - \lambda_k E)^{1/\gamma}}. \tag{7.21}$$

Substituting C_{ptss} and C_{bss} in equation (7.15) we find a relation for the fraction of dead cells E and the infusion rate I:

$$\begin{aligned} I &= -\frac{(E^{\delta}k_{nr} + (k_{nr} + k_s)E_{50}^{\delta})(\lambda_k E)^{1/\gamma}Q_{50}(\lambda_k E + k_{reabs})}{(E_{50}^{\delta} + E^{\delta})(\lambda_k E + k_{reabs})(\lambda_k E)^{1/\gamma}Q_{50} - V_{max}(E_{max} - \lambda_k E)^{1/\gamma})} \\ &- k_{reabs}(\frac{\lambda_k E}{E_{max} - \lambda_k E})^{1/\gamma}Q_{50}. \end{aligned} \tag{7.22}$$

Now we want to investigate the stability of the steady state of the number of kidney cells, which is presented in appendix A. From this analysis we conclude that for M equal to zero or M equal to M_{max} the system is stable.

We now want to know if the concentration in the blood at which nephrotoxicity occurs is higher or lower than the optimal concentration for efficacy. This optimal efficacy concentration is presented in equation (7.6) and the concentration at which nephrotoxicity occurs is presented in equation (7.21) when $E = E_{50}$. However both the concentrations are expressed in different parameters, which are independ of each other, so no analytical comparison between the two can be made.

Adopting the values as chosen in section 9.1 we find for the optimal concentration for efficacy a value of 3.1 mg/l, which is equal to the value found in [Nee02]. For the concentration in the blood at which nephrotoxicity occurs we find a value of 2.8 mg/l and for the toxic concentration in the kidney we find a value of 17.2 mg/l. Note, however, that these concentrations are for a steady state situation. Adopting the values of section 9.1 the infusion rate can be plotted as a function of the fraction of dead cells E. This is shown in figure 7.2.

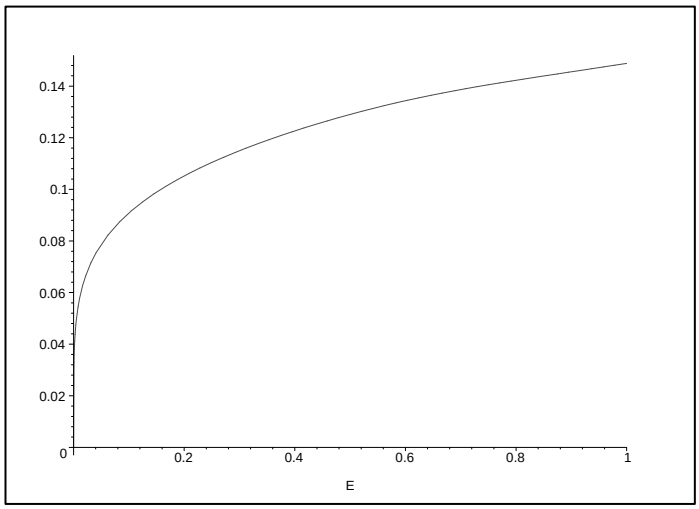

Figure 7.2: *The infusion rate in mg/l/kg/h as a function of fraction of dead kidney cells E for the parameter set of section 9.1. Nephrotoxicity occurs when E is equal to E_{50}. The infusion rate necessary to achieve this steady state value is 0.14 mg/l/kg/h. The toxic concentration in the kidney is 17.2 mg/l and the toxic concentration in the blood is 2.8 mg/l.*

We would like to compare the optimal concentration for efficacy with the concentration below which no nephrotoxicity occurs. However, both the concentrations are expressed in different parameters, where the parameters for the toxicity are not generally known. Adopting the parameter values of section 9.1 results in a lower toxic concentration in the blood than the optimal concentration for efficacy. However, we can not conclude at this stage, that the toxic concentration is always lower than the optimal concentration.

7.3 Conclusions

In this chapter we have analysed the mathematical model for an optimal concentration for efficacy, when an one compartment model is assumed and when the kidney and ear compartments are taken into account. Also we have analysed the model for a concentration below which no nephrotoxicity occurs.

We have found an analytical solution for the optimal concentration in the blood for efficacy using an one compartment model as done by [Nee02]. Then we have incorporated the kidney and ear compartments to compare the concentration in the blood for this model with the concentration in the blood for the one compartment model, when the same infusion rate is used.

We have found that in the kidney and ear compartment model the concentration in the blood reaches a higher level due to reabsorption of filtered drugs in the kidney cells, this is when no nephrotoxicity is assumed. We can say that the kidney delays the elimination of the antibiotics from the blood. As a consequence the decrease in number of bacteria will be higher in the kidney and ear compartment model.

The optimal concentration in the blood in the kidney and ear compartment model can be found optimising the object function in equation (7.14).

Assuming a constant infusion, we have calculated the steady state values for the concentration in the blood, the concentration in the kidney and the number of kidney cells. Also we have calculated the infusion rate to achieve a steady state as a function of the number of kidney cells.

Setting the number of kidney cells equal to the toxic value, an analytical solution can be found for the concentration in the blood above which nephrotoxicity occurs. Also analytical solutions for the toxic concentration in the kidney and the necessary infusion rate to maintain this steady state are calculated.

Using the parameter values as described in section 9.1 we have found, that for this parameter set, the concentrations in the blood are nephrotoxic before the optimal concentration for efficacy is reached.

Chapter 8

Patient data

A model of antibiotic action is a simplified picture of the real world, based on a certain understanding of the mechanisms of action of the drug and of its pharmacokinetics. In the preceding of this report the mechanisms of action, chapters 3 and 4, and the mathematical model, chapters 5 and 6, are explained. The model is useful if it can explain or predict the outcome of experiments and ultimately predict the outcome of treatments in patients. If such predictions are confirmed then the model is validated.

To be able to explain or predict the outcome of experiments, the parameters of the model have to be chosen in such a way that the outcome of the model fits either patient data or findings in the literature. Unfortunately, suitable patient data are not easily found. In section 8.1 we outline the suitability of patient data and our search for suitable patient data. In section 8.2 we present the findings and conclusions from the data obtained from the Cystic Fybrosis patient data base of the Leyenburg hospital in Den Haag. Unfortunately, we are not able to validate our mathematical model with the patient data.

In the future, the department of pharmacy of the Leyenburg hospital will start with a project to statistically investigate the occurrence of ototoxicity within the patient data base of CF patients. In section 8.3 we outline a possible protocol to be followed for this research and we also explain how these findings could be incorporated within our mathematical model to find the parameters needed. In the last section of this chapter we present the conclusions and recommendations concerning patient data.

8.1 Patient data, suitability and method of calculation of parameters

As explained before, to choose the parameters of the mathematical model suitable patient data are needed. Our model calculates the concentration of aminoglycosides in the blood, ear fluids and kidney cells, given the baseline creatinine clearance, the dose, interval and duration of treatment. The efficacy is related to the concentration of drugs in the blood, the nephro- and ototoxicity are related to the concentration in the kidney and in the hair cells. Suitable patient data will have to include measurements of all these factors.

For efficacy, some parameter sets are known as described in [Nee02]. Nephrotoxicity occurs usually after approximately 6-14 days of treatment. After the end of treatment the kidney

function returns to normal after a few weeks. So for nephrotoxicity patients are needed, who are treated for longer periods and whose serum creatinine concentration is measured at the start, during and at the end of treatment. Ototoxicity usually progresses relatively slowly and at patients treated for longer periods[1]. Audiograms should be made before and after the treatments.

With these measurements and with the knowledge of the dose, the interval, the duration of treatment and the baseline creatinine clearance, the parameters of the model can be calculated using the method of the least squares or χ-squared test [Ago86]. Then the parameters have to be calculated such that the object function χ^2 is minimal with

$$\chi^2 = \sum_{i=1}^{m} \frac{(Q_{calc}(i) - Q_{meas}(i))^2}{Q_{meas(i)}}, \tag{8.1}$$

where m is the number of measurements, $Q_{calc}(i)$ is the calculated quantitiy, and $Q_{meas}(i)$ is the measured quantity [Baa01].

The parameters necessary for the nephrotoxicity should be drawn from such a calculation with the measured creatinine serum concentrations. The parameters for the ototoxicity should be drawn from the measurements in the audiograms.

8.1.1 Patient data at the Medisch Spectrum Twente in Enschede

First we have looked at the patients treated at the Medisch Spectrum Twente in Enschede. Unfortunately, most patients were treated for short periods of 2-8 days. This period is too short for nephrotoxicity to occur. Audiograms are not made, with one exception, thus ototoxicity can not be derived. Thus we have decided not to use this database.

8.1.2 Patient data at the Leyenburg Hospital in Den Haag

The database at the Leyenburg hospital in Den Haag consists of 180 patients. These patients are treated with tobramycin, an aminoglycoside, in treatments of three weeks and usually they are treated for a couple of periods during a few years. To detect hearing loss, audiograms are made[2] at each treatment and a treatment is stopped, when a sustainable hearing loss, which is 15 dB or more at one or more frequencies, compared to an earlier audiogram is detected. Unfortunately a quantification of hearing loss or a relation between hearing loss and the number of hair cells is not known.

The dosage data and the audiograms are recorded from 1995 onwards, history of an earlier date is not known. The serum creatinine concentration is only recorded when a patient is released from the hospital. Thus there are no baseline values known and the serum creatinine concentration measurements during the treatment are not always recorded.

8.2 Results from the patient data of the Leyenburg hospital

From the 180 CF patients we have selected a number of 25 patients and 25 extra patients of whom enough audiograms are made. From these first 25 patients we have selected 9

[1] for example CF patients, which are treated for infections on a regular basis for periods of years.
[2] The treatment of tobramycin is the reason for measuring the hearing loss with the audiograms

patients of whom the dosage history was known and recorded. Most patients were treated twice daily, with an exception of a few, who were treated either once or three times daily.

From these 9 selected patients we have calculated the cumulative administered dose since 1995. There is no quantification method known for the total hearing loss We propose to quantify the total hearing loss with

$$CH = \sum_{i=8}^{20} MT(i) - FB(i), \tag{8.2}$$

with CH the measure for the total hearing loss, $MT(i)$ is the measured hearing threshold at frequency i kHz and $FB(i)$ is the threshold value of Fausti[3] at frequency i kHz. In this manner we compare the hearing function of the patient to the hearing function of a healthy person.

In table 8.2 the total administered dose during the treatments, the decrease in hearing function of the left (o) and right (x) ear and the total decrease in hearing function of the 9 patients are shown. The values shown are the differences between the last en first measured audiogram[4].

Patient	Tobramycin (mg)	amikacin (mg)	gentamicin (mg)	x (dB)	o (dB)	total (dB)
1	47 500			300	-30	270
2	110 210			90	60	150
3	131 620			150	160	310
4	134 720			85	280	365
5	67 740	56 000		65	55	120
6	51 600			95	380	475
7	75 700		22 840	145	325	470
8	47 120			380	410	790
9	32 440			105	-10	75

Table 8.1: *For each patient the total administered dose of tobramycin (mg), amikacin (mg) and gentamicin (mg), the decrease in hearing function in the right (x) and left (o) ear and the total decrease in hearing function n both ears are shown. The decrease values shown are the difference between the last en first measured audiogram.*

A decrease of 130 dB at one ear or 260 dB at two ears corresponds to an average decrease in hearing function of 10 dB at each measured frequency. As can be seen from table 8.2 a decrease of 130 dB or more at one ear occurs in 3 patients. A decrease of 130 dB or more at both ears occurs in 3 other patients. A total decrease of 260 dB or more occurs in 6 patients. Thus 6 out of 9 patients have an ototoxic change in hearing function.

In figure 8.1 the progression of the change in hearing function during the aminoglycoside treatments of the 9 patients is shown. Shown are the CH-values as defined by (8.2) for the right (x), left (o) and both (total) ears. From figure 8.1 it can be concluded that all patients

[3]The Fausti baseline are the threshold values for a healthy ear.

[4]High tone audiograms measure the hearing threshold of a patient at the frequencies 8-20 kHz compared to the Fausti baseline.

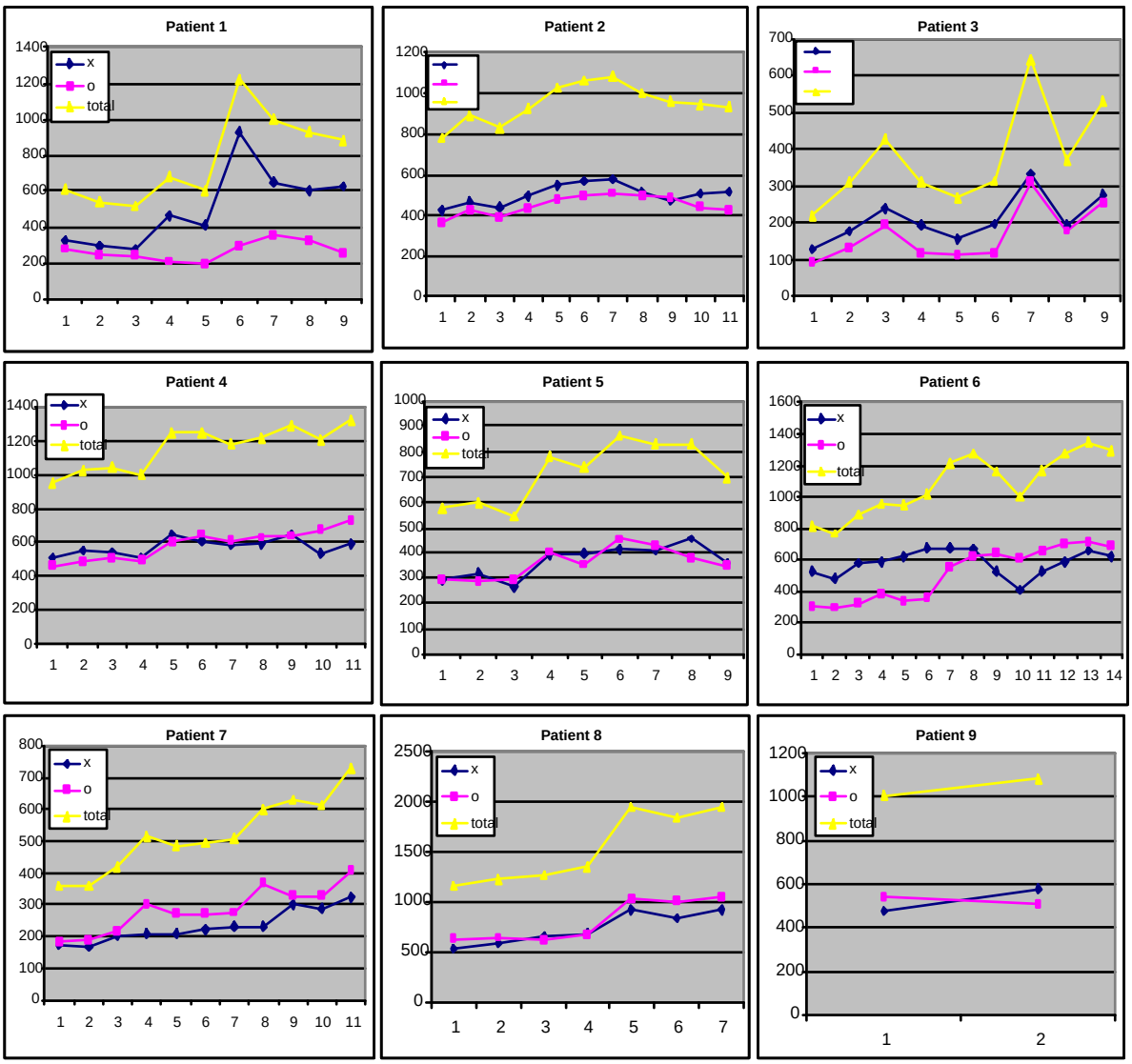

Figure 8.1: *Results of the audiograms of the CF patients of the Leyenburg hospital in Den Haag. Shown are the CH-values (y-axis) as defined by (8.2) for the right (x), left (o) and both (total) ears for each audiogram (x-axis) made of a patient. We have used this quantification of hearing loss, because no other method is known.*

have a gradual total decrease in hearing function during the treatment with tobramycin. Although we have not applied a statistical test to confirm a relation between the use of tobramycin and hearing loss, the gradual progression of hearing loss in all patients as shown in figure 8.1 suggest that treatments with tobramycin do indeed decrease the hearing function. Unfortunately no relation between the number of hair cells and the hearing thresholds are known, thus these results do not help in finding values for the parameters of the model.

8.3 Recommendation for patient data research

The model described in this report is a deterministic model. When investigating patient data different parameters should be collected than when a statistical research is done. More important, the research questions asked in the two types of research differ from each other.

In the deterministic model we assume that the processes behind the development in time of the concentrations of aminoglycosides in the organs can be described by the equations of chapter 6. Data research should then be aimed at collecting the data of these variables, the serum creatinine concentrations and the hearing loss, such that the parameters in the equations of the model can be calculated using the object function of equation (8.1).

To investigate the data statistically, research questions such as "Is there a relation between cumulative administered dose and ototoxicity?" or "Is there a difference in toxicity when patients are treated once or twice daily?" are very important. The data to be collected should give information on the two related variables, thus the dosage regimen and a quantification of toxicity or the cumulative dose and hearing loss.

Our model is a deterministic model and in the following sections we will outline a possible protocol to collect proper patient data, such that the necessary parameters can be derived. Additionally, we will comment in section 8.3.3 on the choice of technique for a possible statistical research.

8.3.1 Selection of patients

As explained before patients must be selected, who are treated with aminoglycosides for longer periods, longer than 7 days to detect nephrotoxicity and over a period of a few years to detect hearing loss. During the treatments the administered doses, dose interval and duration should be recorded. Patient characteristics such as age, weight, volume of distribution[5] and daily creatinine production should be recorded.

To calculate the parameters of the mathematical model a large number of serum creatinine levels at the start, during and after the end of treatment should be measured. Over the years audiograms should be made in the high frequency range of 8-20 kHz, because ototoxicity first occurs at high frequencies.

The patients should be selected on the use of no other nephro- and ototoxic drugs during the aminoglycoside treatments. Other exclusion criteria are: evidence of abnormal renal and auditory function (creatinine serum concentration > 120 mg/l, hearing loss > 50 dB at two frequencies in 0.25-8 kHz frequency range), evidence of urinary tract or middle ear infection and consistency of patient response.

8.3.2 Selection of variables and parameters

When suitable patients are selected the proper parameters and variables have to be recorded. In general this means that the input variables of a treatment (dose related variables), the patient characteristics and the result variables (efficacy, kidney and ear function variables)

[5] CF patients for example have a different volume of distribution than normal patients

have to be collected.

Important variables are: age, weight, the total dose, mean daily dose, dosage interval, duration of treatment, (extrapolated) peak and trough concentration, half life, alterations in creatinine clearance or serum creatinine concentration, volume of distribution, daily creatinine production, auditory alterations in 0.25-8 kHz and 8-20 kHz frequency range.

8.3.3 Selection of a statistical technique

To answer the question which statistical technique should be used, the research question is the starting point [Baa01]. If the research question is for example "Is there a relation between administered dose and ototoxicity?", then the question is about coherence. Other types of questions could be about frequency ("How often does ototoxicity occur?") or differences ("Is there a difference in ototoxicity between once daily or twice daily dosage?"). The three different types of questions request a different statistical technique.

The data can be nominal or ordinal, continuous or discrete, which also requires a different statistical technique. At last it is important to know if the data represent a sample or an entire population.

Thus a clear answer on which statistical technique to use cannot be given at forehand. The factors mentioned above will have to be taken into account. Some examples of statistical tests, which we will not explain further, are: the student's t-test (continuous), the Mann-Whithney test (continuous), the Chi-squared test (discrete) and the Fisher test (discrete).

8.4 Conclusions on patient data

The goal of the patient data research was to acquire the parameter values. However, as explained in this chapter, we have not been able to obtain suitable patient data, with which the parameters could be calculated.

In the next chapter we will run simulations with chosen parameters from the literature. These simulations will show the behavior of the mathematical model. However the simulation results can not be validated with patient data.

In this chapter we have commented on what suitable data are and what criteria should be met. Summarized we conclude that data of patients are needed, who are treated for longer periods over a few years and of whom enough measurements are made on serum creatinine concentrations and audiograms. Also enough patient related variables should be collected as well as treatment related variables.

Lastly we conclude that a quantification of hearing loss or a relation between hearing loss and the number of hair cells should be developed.

Chapter 9

Numerical simulations

To run simulations on the mathematical model and to investigate the behavior of the model, we have developed a numerical program in Matlab. In this numerical program the parameter values have to be chosen, as well as the treatment and patient properties from which then the concentration in the blood, the number of bacteria, the concentration in the kidney cells, the number of kidney cells, the creatinine clearance, the concentration in the ear compartments and the number of hair cells are calculated.

We have chosen the parameter values according to findings in the literature as presented in section 9.1. With these parameter values the different quantities can be calculated with the numerical program.

In section 9.2 we investigate the differences in efficacy and toxicity between an once-daily, a twice-daily and a continuous administration. In section 9.3 we discuss the maximal continuous infusion to avoid toxicity and a intermittent infusion, with the same total daily dose to verify the analytical solutions of section 7.2.

To compare our model with the model used in [Rou03], we have also developed a Matlab version of the model of [Rou03]. In section 9.4 we will comment on the results we find using this Matlab model. Finally in section 9.5 we present the conclusions of the simulations of this chapter.

9.1 Choice of parameters

In the mathematical model for aminoglycosides a large number of parameters have to be chosen to be able to simulate the efficacy, the nephro- and ototoxicity. The parameters for the efficacy are described in [Nee02] and [Cor98]. The parameters for the nephro- and ototoxicity should be calculated using the method described in section 8.1. However, as explained in that same section, we were not able to perform such a calculation due to the lack of suitable patient data.

In the literature, case studies on patients and experiments on animals with aminoglycosides are described. From these investigations we draw, as good as possible, the values needed to run simulations. In this section we present the choices for parameter values. In appendix B an example of the input screen of our Matlab program is shown.

For the treatment properties we choose a fictitious patient, who has a weight of 80 kg, an age of 30 years and a volume of distribution of 0.25 l/kg, which is a common value for aminoglycosides in healthy patients [Nee02], [Cor98]. For the dosis we adopt a dosage of 7 mg/kg/day corresponding with [Nic95]. The number of treatment days and tf, the factor of the treatment time for which the simulation is run, can be chosen freely.

The non-renal clearance coefficient k_{nr} is chosen as 0.006932 1/h to have a non-renal half-life of 100 hours [Hur87]. The half-life of aminoglycosides in the blood is 2-4 hours[1], following [Pri95] we choose a value of 3 hours. Specific values for a normal creatinine clearance are around 100 ml/min [Pri95]. Using $k_s = (k_{el} - k_{nr})/Cl_{cr}$, we find that k_s has the value 0.0022.

According to [Nee02] the growth rate λ_b of the bacteria *Pseudomonas aeruginosa* is set to 0.995 1/h [Cor98], F_{max} is 7.115 1/h [Cor98], γ_k is 0.416 [Cor98] and C_{mic} is 1 mg/l [Mon96]. With equation (6.13) C_{50} is found to have a value of 78.8 mg.

For the uptake and elimination into and from the kidney cells, we refer to studies done by de Broe, Giuliano and Verpooten [Ver86], [Ver89], [Giu86], [Bro88] and [Bro91]. We thus set K_m equal to 15 mg/l and V_{max} to 1 mg/l/h. Healthy kidney cells eliminate the aminoglycosides with a diffusion rate of 0.006932 1/h corresponding with a half-life of 100 hours [Tul88].

To a large extent, the nephron's of the kidney are able to compensate for the loss of nephrons. A comparison with the liver can be made, where the liver cells still function as normal, when only 20% of the cells are alive. A precise value for the kidney is not known, but based on this argument we chose a value of 0.8 for E_{50} meaning that when 20% of the cells are alive, the creatinine clearance is still half the baseline value. We also assume that this process also occurs in a 'threshold' manner, so we adopt a value of 6 for δ. The daily production rate of creatinine is 12 mg/l/h [Rou03].

For the number of kidney cells parameter values are not known, we thus derive the values from the literature. Kidney's have approximately $2 * 10^6$ nephrons [Ber98], thus $M_{max} = 2 * 10^6$. Kidney's show a slow (2-5 weeks) recovery of kidney function after aminoglycosides nephrotoxicity [Bre00], we choose a recovery time T_r of 2 weeks. According to [Mur89], the regeneration rate λ_k is of order $O(1/T_r)$, thus we set λ_k equal to 0.003 1/h. In [Rou03] a value for Q_{50} is derived, we adopt this value, because their model for killing rate in relation with the amount of drugs inside the kidney shows resemblance with our model. Thus we set Q_{50} equal to 50 mg/l. The process of killing of the kidney cells shows 'threshold behavior' [Min99], thus we adopt a value of 5 for γ. In [Nic95] an once daily program is evaluated. As described in section 9.1.1 we choose a value of 0.5 for E_{max} such that with a once-daily dosage nephrotoxicity occurs after 14 days.

The uptake rates for the ear fluids and the organ of Corti are not known and are based on rat studies or data from patients. The half-lifes of the aminoglycosides in the ear fluids and the organ of Corti are 100 hours [Pri95], which corresponds to a value of 0.00693 1/h for k_{ob}. For k_{eb}, k_{eo}, k_{pb} and k_{po} we choose half this value to have a half-life of 100 hours

[1] actually this value depends on the clearance rate by the kidney

for the drugs inside the endolymph and perilymph. It is known that the uptake is slower than in the kidney cells, thus that J_{be}, J_{bp} and J_{bo} are smaller than 1. The levels in the ear fluids and organ of Corti are lower than in the kidney cells, thus the affinity of the drugs is lower and thus K_{end}, K_{per} and K_{oc} are higher than K_m. We also know that the uptake in the endolymph is faster than in the perilymph, which is in its turn faster than the uptake in the organ of Corti, thus we choose a value of 0.7 for J_{be}, 0.5 for J_{bp} and 0.1 for J_{bo} and a value of 20 for K_{end}, K_{per} and K_{oc}.

Lastly the parameters for the hair cells, the killing rate Fhc_{max}, the concentration with half maximal killing F_{50} and the Hill sigmoidity coefficient n are based on a case study by [Car80], as described in section 9.1.1. Thus we set Fhc_{max}, F_{50} and n equal to 0.1, 60 and 8.

For most of the parameters discussed above the results obtained by the numerical program depend highly on the choice of the parameter values. However a different choice for one parameter can be compensated for by a different choice for another parameter. For example the killing and regeneration rate of the kidney cells can be chosen such that the growth rate of the kidney cells are still the same. We have tried to choose each parameter to agree with findings in the literature.

9.1.1 Simulation results to chose the killing rate parameters

In [Nic95] an once-daily administration program is evaluated. They have found that after 14 days of treatment with 7 mg/kg/day, nephrotoxicity had developed in 50% of the patients. To meet the criterium of a decrease of 50% in creatinine clearance after 14 days with this administration we choose a value of 0.5 for E_{max}. The result for the number of kidney cells and the creatinine clearance is shown in figures 9.1a and b.

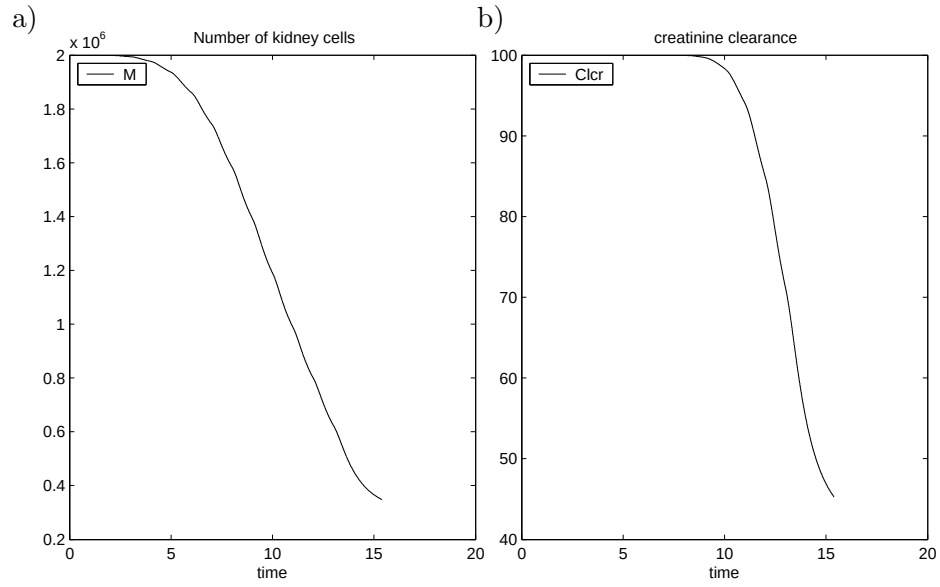

Figure 9.1: *a) The number of kidney cells and b) the creatinine clearance for an once-daily administration with 7 mg/kg/day to achieve nephrotoxicity after 14 days of treatment [Nic95].*

In [Car80] a case of a patient with a complete loss of hearing function is described. This

patient was treated for 80 days with 302.6 mg/kg. Thus we choose values of 0.1 for Fhc_{max}, 60 for F_{50} and 8 for n, such that after 80 days of treatment of a twice-daily administration of 302.6 mg/day, all the hair cells are killed. This result for the hair cells is shown in figure 9.2.

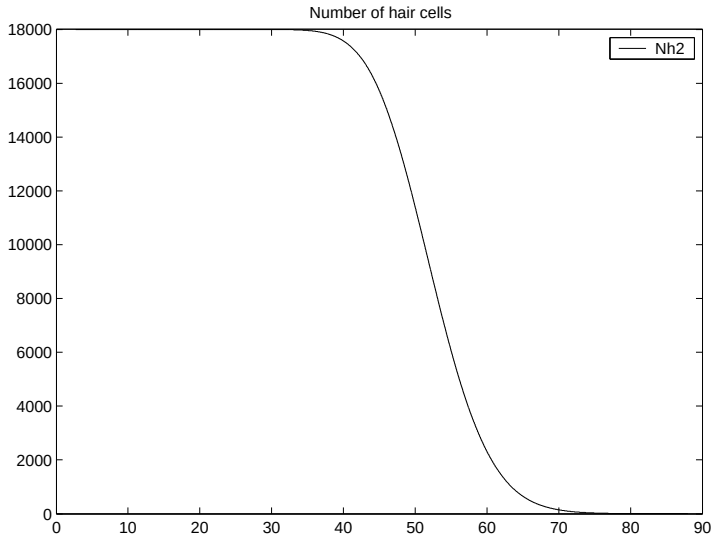

Figure 9.2: *The number of hair cells for a twice-daily administration with 302.6 mg/day, such that after 80 days of treatment all the hair cells are killed [Car80].*

9.2 Once-daily, twice-daily and continuous administration

In this section we will investigate the concentration in the blood, the efficacy, the uptake of the aminoglycosides into the kidney cells, the number of kidney cells, the creatinine clearance, the uptake of the drug into the ear compartments and the number of hair cells for an once-daily (OD), a twice-daily (TD) and a continuous administration of aminoglycosides.

9.2.1 The concentration in the blood and efficacy

To investigate the efficacy for the three different dosage regimen we simulate a treatment of 4 consecutive days with a dose of 7 mg/kg/day. We adopt the values for the parameters of section 9.1 and we assume the treatment to be effective when a reduction of 10^{-12} in number of bacteria is achieved at the end of treatment. In figure 9.3a the concentration of aminoglycosides in the blood for OD (solid line), TD (dotted line) and a continuous (dash-dotted line) administration is shown. Also shown (dashed line) is the common target for treatment of 2 mg/l for through concentrations. Both the OD and the TD administration have a trough level lower than this target. The continuous administration results in a higher level in the blood than 2 mg/l.

In figure 9.3b the number of bacteria for the different dosage regimen are presented. All regimen achieve the limit for efficacy at the end of treatment of 4 days. However the OD administration is clearly less effective than the other two regimen, it reaches the efficacy limit in the fourth day of treatment where the TD and continuous administration reach this

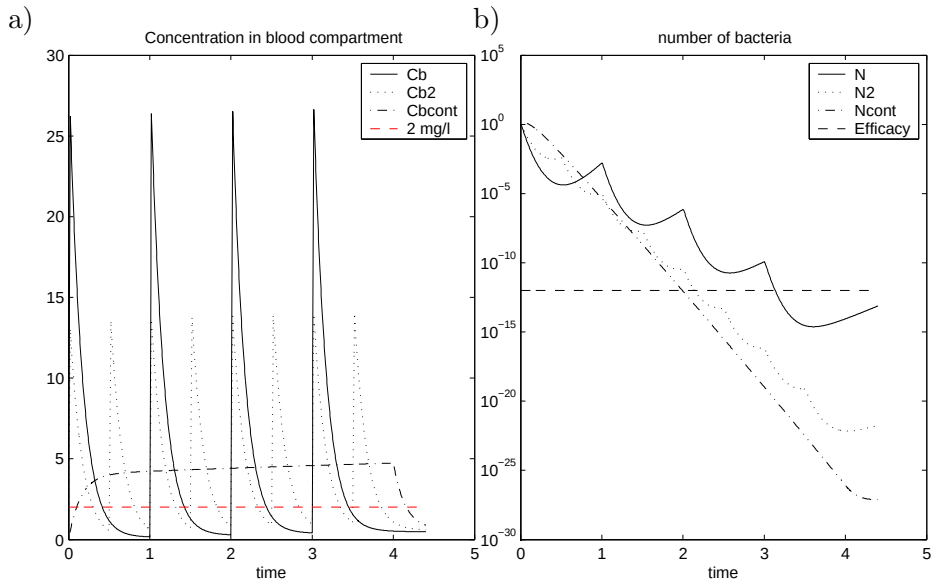

Figure 9.3: *a) The concentration in the blood compartment in mg/l for OD (solid line), a TD (dotted line) and a continuous infusion (dash-dotted line) versus the time in days. Also shown is the target of treatment of 2 mg/l for trough levels. b) The number of bacteria for the different dosage regimen versus the time in days. The limit for efficacy, a reduction of* 10^{-12} *in number of bacteria, is shown with the dashed line.*

limit in the third day of treatment. The TD administration is almost as effective as the continuous infusion. This is mainly caused by the fact that for a continuous administration it takes some time for the concentration in the blood to reach a plateau level, while the concentration in the blood for a TD administration reaches a peak level immediately after the end of infusion.

From figure 9.3 it can be concluded that in this case the continuous infusion is more effective than a TD administration, which is more effective than a OD administration. However, all three dosage regimen reach the efficacy limit within four days.

9.2.2 Concentration in the proximal tubular cells

To investigate the uptake of aminoglycosides in the proximal tubular cells for the different dosage regimen, we again administer a dose of 7 mg/kg/day with an infusion time of 30 minutes. We treat the patient for 11 consecutive days after which we let the simulation run for 5 more days.

In figure 9.4a the concentration of aminoglycosides in the blood for the different dosage regimen is shown. The trough levels of the OD and the TD administration are both below this concentration level in the first eight days of treatment. After these eight days the through level of the TD regimen starts to exceed the 2 mg/l level. The peak concentrations also gradually increase for the once and twice daily administration. The concentration in the blood for the continuous administration gradually increases until the 9th day of treatment, after which a steeper increase can be seen. After the end of treatment the concentration in the blood returns gradually to zero.

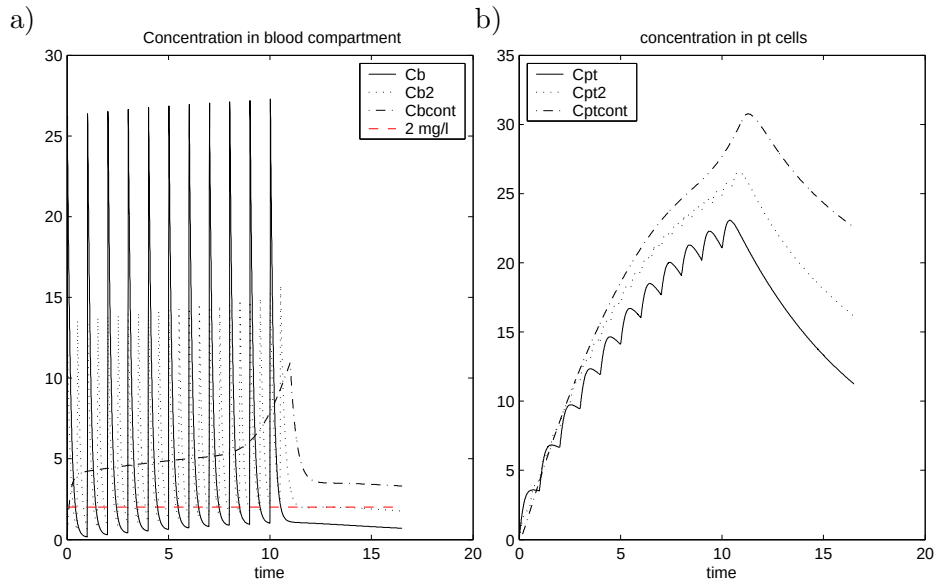

Figure 9.4: *a) The concentration in the blood compartment in mg/l for the different dosage regimen. Also shown is the target of 2 mg/l for trough levels. b) The concentration of aminoglycosides in the proximal tubular cells in mg/l for the different dosage regimen.*

In figure 9.4b the concentration of aminoglycosides in the proximal tubular cells for the different dosage regimen is presented. All dosage regimen show an increasing level of the concentration, apparently a 'steady' level in the PT cells is not yet reached after 11 days of treatment. The OD administration clearly causes the aminoglycosides to be taken up more slowly and at a lower level[2], than the TD[3] and continuous administration. The level in the PT cells with the continuous infusion shows a sharp rise in the uptake after 9 days of treatment, because of an increase in the concentration in the blood compartment, which is caused by a decrease in creatinine clearance. After the end of treatment the concentration in the kidney cells returns gradually to zero for all regimen.

From figure 9.4 it can be concluded that OD administration results in a slower uptake and a lower level of the concentration of aminoglycosides in the kidney cells than the TD and continuous administration. Also it can be concluded that with a continuous administration of 7 mg/kg/day the concentration in the kidney cells reaches the level for nephrotoxicity after 9 days of treatment for these parameter values.

9.2.3 Number of kidney cells and creatinine clearance

To investigate the differences for the different dosage regimen in the number of kidney cells and in the creatinine clearance due to the killing of the kidney cells by the aminoglycosides in the proximal tubular cells, we simulate a treatment of eleven consecutive days and monitor the patient for five more days. We again administer a dose of 7 mg/kg/day with an infusion time of 30 minutes. We assume that the patient has a normal renal function at the start of the treatment, i.e., the patient has a creatinine clearance of 100 ml/min.

[2]respectively 88% and 74% compared to the TD and continuous administration
[3]83% compared to the continuous administration

Nephrotoxicity is defined as a decrease of 50% in creatinine clearance[4].

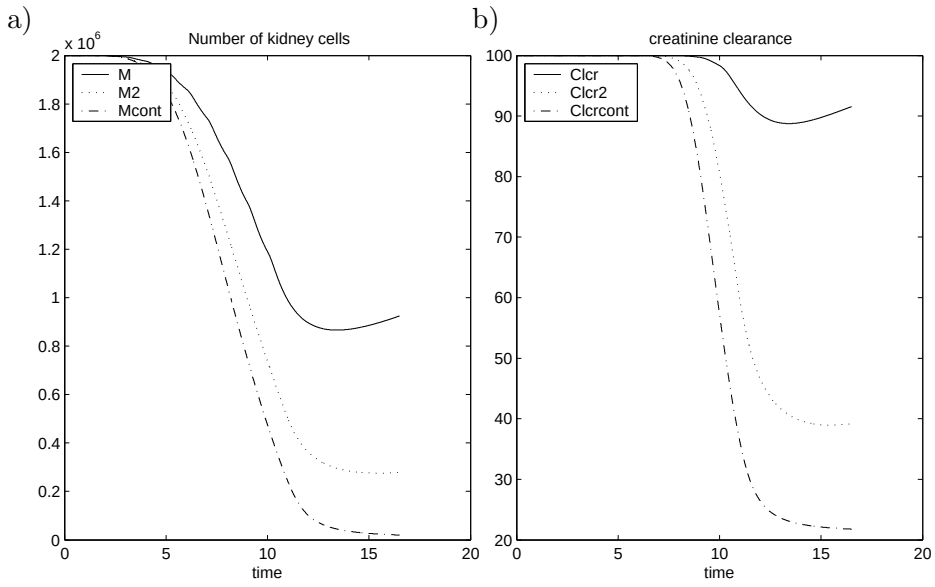

Figure 9.5: *a) The number of kidney cells for the different dosage regimen. b) The creatinine clearance for the different dosage regimen.*

In figure 9.5a the number of kidney cells for the different dosage regimen is shown. 20% of the maximum amount of kidney cells corresponds to a value of $0.4 * 10^6$. This number is reached after 10 days of treatment for the continuous administration, after 12 days for the TD administration and after 14 days for the OD administration, see figure 9.1. The treatment is stopped after 11 days, but for all regimen the number of kidney cells still decreases after that due to the concentration of aminoglycosides still present in the kidney cells. The number of kidney cells with the OD administration shows a recovery after two days after the end of treatment. The number of kidney cells with TD administration shows signs of recovery, whereas for the continuous administration the number of kidney cells is to low to recover.

In figure 9.5b the creatinine clearance for the different dosage regimen with the defined toxicity limit is presented. With the continuous administration, nephrotoxicity occurs after ten days of treatment. For the TD regimen the toxicity occurs two days later, where the OD administration does not reach the nephrotoxicity level. Recovery occurs for the OD administration, where for the continuous administration no recovery takes place.

From figures 9.5a and 9.5b the relation between the rise in concentration in the blood in figure 9.4a and the creatinine clearance is shown clearly. A rise in the concentration in the blood was shown after 9 days of treatment. From figure 9.5b it can be concluded that at that moment the creatinine clearance had decreased significantly causing the renal clearance to decrease.

[4]Recall that a value of 0.8 is chosen for E_{50}, such that when 20% of the kidney cells are alive the creatinine clearance is still 50% of the baseline value.

From figure 9.5 it can be concluded that OD administration results in slower rate of killing of the kidney cells, which in its turn causes a later decrease in creatinine clearance than TD and continuous infusion. Also it can be concluded that the return to baseline clearance appears faster for OD administration compared to the other two dosage regimen.

From figure 9.5 it can also be seen that the creatinine clearance is a belated marker of the renal function due to the tubuloglomerular feedback, the mechanism to compensate for loss of kidney cells. The number of kidney cells in figure 9.5a reduces before the creatinine clearance in figure 9.5b.

9.2.4 Uptake in organ of Corti and ototoxicity

To investigate the difference in uptake in the organ of Corti and decrease of number of hair cells of the different dosage regimen, we simulate a treatment of 7 days and monitor the patient for 21 more days. We administer a dose of 7 mg/kg/day. Unfortunately no quantification of ototoxicity is known, thus we present the decrease in number of hair cells as a measure for the ototoxicity.

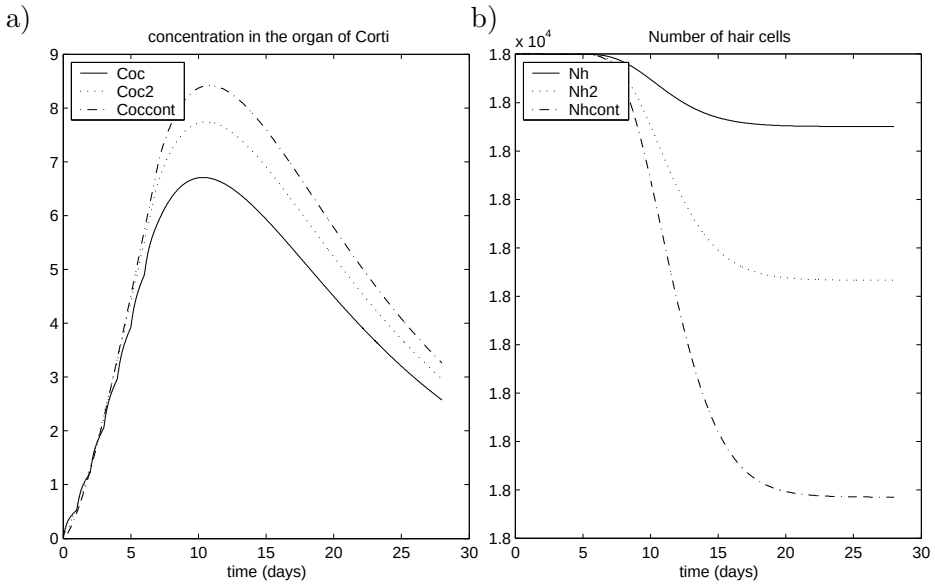

Figure 9.6: *a) The concentration of aminoglycosides in the organ of Corti for the different dosage regimen. b) The number of hair cells for the different dosage regimen.*

In figure 9.6a the concentration in the organ of Corti is shown. The release from the organ of Corti is slow, resulting in a slow decrease of the concentration after the end of treatment. Shown is the concentration for the different dosage regimen and again the concentration for continuous administration is higher in the organ of Corti than for TD administration, which is higher than for OD concentration. Due to the uptake from the endolymph and perilymph into the organ of Corti, the level in the organ of Corti continues to rise after the end of treatment.

In figure 9.6b the number of hair cells for the different dosage regimen are shown. The killing of the hair cells continues after the end of treatment, due to the uptake from the endolymph and perilymph and the slow release from the organ of Corti. This explains

the findings of progressing ototoxicity in the literature after the end of treatment. The decrease in number of hair cells is greater with the continuous administration than with TD administration, which has a greater decrease than with OD administration.

9.3 Comparison in efficacy of a nephrotoxic continuous infusion with an intermittent administration

In section 7.1 the optimal concentration in the blood for efficacy with an one compartment model is analytically derived. Adopting the parameter values of section 9.1 this concentration is found to be 3.1 mg/l. In section 7.2 the nephrotoxic concentration in the blood and in the kidney cells are derived. Adopting the parameter values of section 9.1, these concentrations are found to be 2.8 mg/l for the blood and 17.2 mg/l for the kidney cells. Thus we find that for these choices of the parameters, the optimal concentration in the blood for efficacy is higher than the nephrotoxic concentration in the blood.

The infusion rate to be given to reach a certain steady state for the number of kidney cells is presented in equation (7.22). When nephrotoxicity occurs, the number of kidney cells is equal to $0.2 * M_{max}$. Using equation (7.22) this corresponds to a total daily dose of 68.3 mg. After 10 days of treatment with a continuous infusion of a total daily dose of 68.3 mg, the number of bacteria is found to be equal to $8.2 * 10^{18}$, thus this dosage does not result in an efficient therapy.

From section 9.2.3 we know that with an OD administration the concentration in the kidney cells and the decrease in creatinine clearance is lower than with a continuous infusion. From section 9.2.1 we know that the number of bacteria with an OD administration is higher than with a continuous infusion.

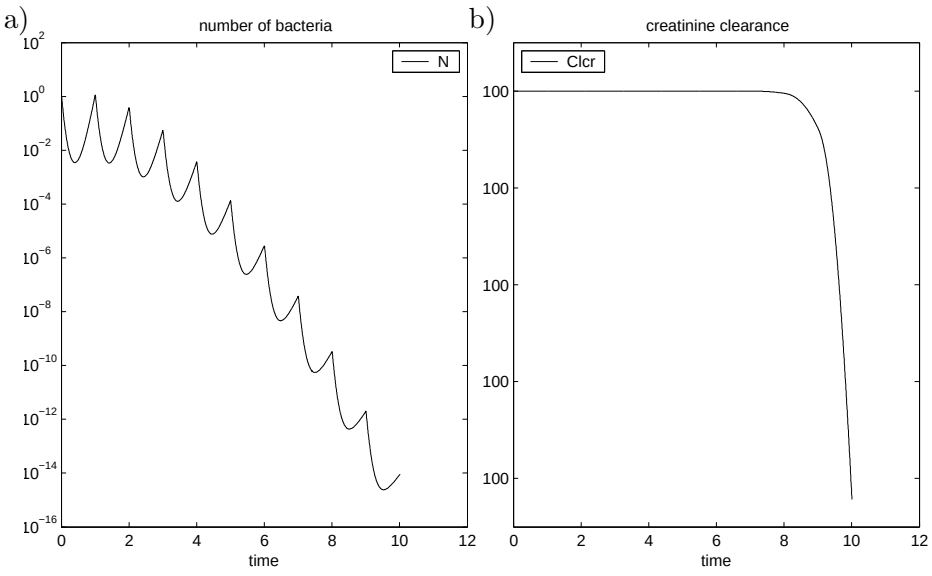

Figure 9.7: *a) The number of bacteria and b) the creatinine clearance for a total daily dose of 273.2 mg given once-daily*

With a total daily dose of 68.3 mg the OD administration does not reach the nephrotoxic level for the number of kidney cells. Thus we can increase the total daily dose. We simulate a treatment of 10 days with a total daily dose of four times 68.3 mg OD, thus 273.2 mg OD. In figure 9.7 the result for the efficacy and the creatinine clearance is shown. After 10 days of treatment the number of bacteria is found to be equal to $8.8 * 10^{-15}$ and the creatinine clearance is equal to 100 ml/min. Thus nephrotoxicity does not occur for this dosage and the treatment is effective.

Thus with these values for the parameters an OD administration of four times the nephrotoxic dosage results in an effective and non nephrotoxic treatment, whereas a continuous administration of the nephrotoxic dosage results in an ineffective and toxic treatment.

9.4 Comparison with the model of Rougier

We have also modeled the model of Rougier with Matlab to investigate their model. The results of the model of Rougier for a OD administration for the concentration in the blood, the amount of aminoglycosides in the kidney cells and the creatinine clearance are shown in figures 9.8a-c.

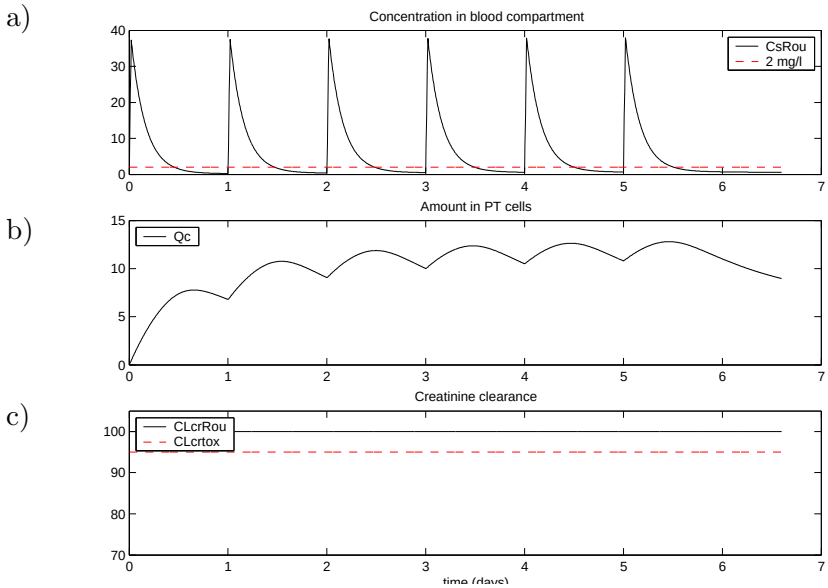

Figure 9.8: *Results of the model of Rougier for a OD administration a) The concentration of aminoglycosides in the blood. b) The amount of aminoglycosides in the kidney cells. c) The creatinine clearance.*

Administering a dose of 800 mg OD, we notice that the amount of aminoglycosides in the kidney does not agree with the plots presented in [Rou03], where the amount in the kidney is approximately equal to 45 mg after 6 days of treatment. In figure 9.8 the amount in the kidney is only about 12 mg and with a threshold of 42.5 mg for the effect, no decrease in creatinine clearance can be seen. In [Rou03] a value of 0.06 1/h for the rate of elimination from the kidney cells is used. This corresponds to a half-life of 11.5 hours. In the literature a half-life of 100 hours is commonly used and we assume that this should be the case.

Thus we adopt a half-life of 100 hours for the elimination rate from the kidney cells, which corresponds to an elimination rate of 0.006932 1/h. Using this value in the model of [Rou03], we find the results presented in figure 9.9 for the concentration in the blood, the amount in the kidney and the creatinine clearance. Now after six days of treatment the amount in the kidney cells is approximately 45 mg, which corresponds to the results presented in [Rou03]. We thus conclude that we should take the latter choice for the elimination rate.

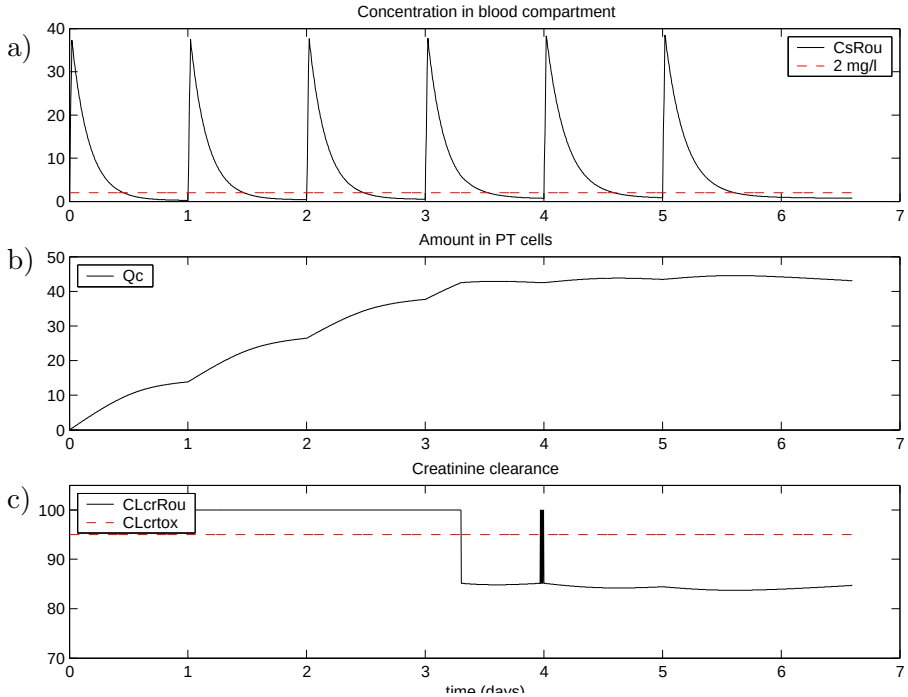

Figure 9.9: *Results of the model of [Rou03] with the adjustment for the elimination rate from the kidney cells. a) The concentration in mg/l of aminoglycosides in the blood. b) The amount in mg of aminoglycosides in the kidney cells. c) The creatinine clearance in ml/min.*

In figure 9.9c the creatinine clearance is presented. In [Rou03] the effect of aminoglycosides is chosen discontinuously in relation with the amount of aminoglycosides in the kidney. The creatinine clearance is directly related to the effect, which results in a discontinuous creatinine clearance. After approximately 3.3 days the amount of aminoglycosides in the proximal tubular cells is equal to 42.5 mg, which is the threshold value below which no effect is seen. In [Rou03] the effect is modeled in such a way that with higher amounts in the kidney cells the full effect is present. This results in the jump, seen in figure 9.9c, in the creatinine clearance.

At the end of the fourth day the amount of aminoglycosides in the kidney cells reaches again the threshold level, resulting again in a discontinuous creatinine clearance, which jumps back and forth from the baseline value to a lower value. However, in [Rou03] this discontinuity is not shown, which we can not explain. We do feel however that this jumping should be avoided, thus in our model we have incorporated the killing of kidney cells, which results in a different and continuous choice for the effect.

In this section we have presented the results we have found by simulating the model of Rougier. We have discussed that a different value for the elimination rate from the kidney should be chosen. We have also mentioned that the effect should be chosen as a continuous function in relation with the amount in the kidney cells.

9.5 Conclusions

To be able to run simulations on the mathematical model we have developed a numerical program in Matlab. For this numerical program we have chosen the parameter values according to findings in the literature. With these parameter values and with the treatment and patient properties, the program calculates the concentration in the blood, the number of bacteria, the concentration in the kidney cells, the number of kidney cells, the creatinine clearance, the concentration in the ear compartments and the number of hair cells.

With the numerical program we have investigated the differences in efficacy and toxicity between an OD, a TD and a continuous administration.

We have found that a continuous administration is more effective, reduces the number of bacteria more, than a TD administration, which results in a lower number of bacteria than an OD administration. All three regimen reach the effective limit of a reduction of 10^{-12} within 4 days of treatment with a 7 mg/kg/day administration.

We then have found that an OD administration results in a lower concentration in the kidney cells, than a TD administration, which results in a lower concentration in the kidney cells than a continuous administration. The killing rate is lower for lower concentrations in the kidney and thus the decrease in creatinine clearance is lower for OD administration than for a TD administration, which in its turn results in a lower decrease in creatinine clearance than a continuous administration.

We have also found that with a continuous infusion nephrotoxicity appears more rapidly, induces a higher decrease of the renal function and is more prolonged than with a TD or an OD administration. Also the same is valid for a TD administration compared to an OD administration.

The simulations also show clearly that the creatinine clearance is a belated marker of the renal function due to the tubuloglomerular feedback, the mechanism to compensate for loss of kidney cells. The number of kidney cells reduces before the creatinine clearance.

The concentration in the organ of Corti is higher for a continuous administration, resulting in a higher killing rate of hair cells, than with TD and OD administration. Thus the ototoxicity is greater for continuous administration than with the other two regimen. Also the killing of hair cells continues after the end of treatment due to the uptake of drugs from the endolymph and perilymph to the organ of Corti and due to the slow elimination from the organ of Corti.

Adopting the parameter values of section 9.1, we find that the optimal concentration in the blood for efficacy is higher than the nephrotoxic concentration in the blood. With the

assumptions on the parameter values we find an optimal concentration in the blood for efficacy of 3.1 mg/l, a nephrotoxic concentration in the blood of 2.8 mg/l and a nephrotoxic concentration in the kidney cells of 17.2 mg/l.

With our choices of the parameters we have found that an OD administration of four times the nephrotoxic dosage results in an effective and non nephrotoxic treatment, whereas a continuous administration of the nephrotoxic dosage results in an ineffective and toxic treatment.

Chapter 10

Conclusions and recommendations

10.1 Conclusions

In this report a mathematical model for the toxicity and efficacy is developed. We have derived the deterministic equations describing the distribution and action of aminoglycosides. The model incorporates the efficacy, the saturable and active uptake into the kidney cells, the reversible nephrotoxicity and the irreversible ototoxicity.

In the case of a continuous infusion, analytical solutions are calculated for the optimal concentration in the blood for efficacy, the concentration in the blood below which nephrotoxicity does not occur and the toxic concentration in the kidney cells.

Adopting suitably chosen values for the parameters, we have found that, for these parameter values, the optimal concentration in the blood is higher than the concentration in the blood below which no nephrotoxicity occurs.

We have chosen the parameter values, such that they agree with findings in the literature. The parameter values should be drawn from patient data. However, we were not able to find suitable data and as a consequence our model is not validated with patient data.

A numerical program in Matlab is developed, which can calculate, with suitably chosen values for the parameters, the concentration in the compartments and related to these concentrations, the efficacy and nephro- and ototoxicity. With this numerical program insight in the behavior of the model for different dosage regimen, i.e., once-daily, twice-daily and continuous administration can be obtained.

With the simulations we have found that a continuous administration is more effective, reduces the number of bacteria more, than a TD administration, which results in a lower number of bacteria than an OD administration.

We have also found that with a continuous infusion nephrotoxicity appears more rapidly, induces a higher decrease of the renal function and is more prolonged than with a TD or an OD administration. Also the same is valid for a TD administration compared to an OD administration.

10.2 Recommendations

Analytical calculations for the concentration in the blood for optimal efficacy and below which nephrotoxicity occurs, are calculated for a continuous infusion. The same concentrations should be derived for intermittent infusion, i.e. once or twice daily administration.

We have presented an example with the simulation program in which a higher efficacy and lower toxicity with the use of an once-daily administration was found, than with the use of a continuous administration. In this example, the once-daily administration received a four times as high a total daily dose than the continuous administration. Instead of one example for a suitably chosen parameter set, analytical solutions for the best dosage regimen should be found.

The results found with the simulation program are dependent on the choice of the parameters. Especially the choice of γ, the parameter defining the steepness of the killing curve of the kidney cells ,is very important for the outcome of the simulations. The choice of this parameter and the sensitivity for this parameter should be investigated more thoroughly.

To be able to draw the parameter values from patient data, patients are needed, who are treated for longer periods over a few years and of whom enough measurements are made on serum creatinine concentrations and audiograms. Also, the model should be validated with suitable patient data.

In the literature no quantification method of hearing loss or a relation between hearing loss and the number of hair cells is known. To incorporate ototoxicity in the model, such a quantification or relation should be developed.

Lastly we mention that our model is a deterministic description of the action and distribution of aminoglycosides. Another approach to derive the equations is the stochastic modeling of the effects of the drug on the cells. Also a method with system control could be considered.

Appendix A

Stability investigation of the steady state

To investigate the stability of the system for the concentration in the blood, the concentration in the kidney and the number of the kidney cells we calculate the Jacobian matrix, which is

$$Jac = \begin{pmatrix} \frac{\partial f_1}{\partial C_b} & \frac{\partial f_1}{\partial C_{pt}} & \frac{\partial f_1}{\partial M} \\ \frac{\partial f_2}{\partial C_b} & \frac{\partial f_2}{\partial C_{pt}} & \frac{\partial f_2}{\partial M} \\ \frac{\partial f_3}{\partial C_b} & \frac{\partial f_3}{\partial C_{pt}} & \frac{\partial f_3}{\partial M} \end{pmatrix}$$

which becomes

$$Jac = \begin{pmatrix} -k_{nr} - ksCl_{cr} & k_{reabs} & 0 \\ \frac{K_m V_{max}}{(K_m + C_b)^2} & -\lambda_k(1 - \frac{M}{M_{max}}) - k_{reabs} & \frac{\lambda_k C_{pt}}{M_{max}} \\ 0 & \frac{M\gamma Q_{50}^{\gamma} E_{max} C_{pt}^{\gamma-1}}{(Q_{50}^{\gamma} + C_{pt}^{\gamma})^2} & \lambda_k(1 - \frac{2M}{M_{max}}) - \frac{E_{max} C_{pt}^{\gamma}}{Q_{50}^{\gamma} + C_{pt}^{\gamma}} \end{pmatrix}$$

The characteristic polynomial can be defined by $det(xI - Jac)$ where x are the eigenvalues of Jac. Substituting the steady state values for C_b, C_{pt} and M the characteristic polynomial $f(x)$ becomes

$$f(x) = a_0 x^3 + a_1 x^2 + a_2 x + a_3$$

with

$$a_0 = 1 \tag{A.1}$$
$$a_1 = a + b + c \tag{A.2}$$
$$a_2 = ab + ac + bc - k_3 k_4 - k_1 k_2 \tag{A.3}$$
$$a_3 = abc - a k_3 k_4 - k_1 k_2 c \tag{A.4}$$

with

$$a = k_{nr} + k_s Cl_{cr} \tag{A.5}$$
$$b = \lambda_k(1 - \frac{M}{M_{max}}) + k_{reabs} \tag{A.6}$$
$$c = -\lambda_k(1 - \frac{2M}{M_{max}}) + \frac{E_{max} C_{pt}^{\gamma}}{Q_{50}^{\gamma} + C_{pt}^{\gamma}} \tag{A.7}$$

$$k_1 = k_{reabs} \tag{A.8}$$

$$k_2 = \frac{K_m V_{max}}{(K_m + C_b)^2} \tag{A.9}$$

$$k_3 = \frac{\lambda_k C_{pt}}{M_{max}} \tag{A.10}$$

$$k_4 = \frac{M\gamma Q_{50}^{\gamma} E_{max} C_{pt}^{\gamma-1}}{(Q_{50}^{\gamma} + C_{pt}^{\gamma})^2} \tag{A.11}$$

According to [Ser00] this polynomial and thus the system is stable if and only if $a_1, a_2, a_3 > 0$ and $a_1 a_2 > a_3$. Substituting the steady state values for C_b, C_{pt} and M we find that

$$a_1 = k_{nr} + k_s Cl_{cr} + k_{reabs} + \lambda_k,$$

which is always positive. For a_2 we find

$$\begin{aligned} a_2 = {} & (k_{nr} + k_s Cl_{cr})(\lambda_k + k_{reabs}) + (\lambda_k E + k_{reabs})\lambda_k(1 - E) \\ & -\gamma(\lambda_k - E)\left(\frac{\lambda_k E}{E_{max} - \lambda_k E}\right)^{\frac{\gamma-1}{\gamma}} \\ & -\frac{k_{reabs} K_m V_{max}}{\left(K_m - \frac{(E\lambda_k)^{1/\gamma} Q_{50} K_m (E\lambda_k + k_{reabs})}{(E\lambda_k)^{1/\gamma} Q_{50}(E\lambda_k + k_{reabs}) - V_{max}(E_{max} - \lambda_k)^{1/\gamma}}\right)^2} \end{aligned}$$

and for a_3 we find

$$\begin{aligned} a_3 = {} & (k_{nr} + k_s Cl_{cr})(\lambda_k E + k_{reabs})(\lambda_k - E) \\ & -\lambda_k\left(\frac{\lambda_k E}{E_{max} - \lambda_k E}\right)^{\frac{\gamma-1}{\gamma}}(1 - E)\gamma E_{max} \\ & -\frac{k_{reabs}\lambda_k Q_{50}}{M_{max}}\left(\frac{\lambda_k E}{E_{max} - \lambda_k}\right)^{1/\gamma}(\lambda_k - E) \end{aligned}$$

Thus a_2 and a_3 are dependent on E. With equation (7.22) in mind, E is dependent on the infusion rate, thus the stability of the system is dependent on the infusion rate. Unfortunately we can not solve $a_2, a_3 > 0$ for E so we can not make a valid statement about the stability of the system. For E equal to 0, thus M equal to M_{max}, we find that

$$a_2 = (k_{nr} + k_s Cl_{cr})(k_{reabs} + \lambda_k) + k_{reabs}\lambda_k - \frac{k_{reabs}}{K_m V_{max}} \tag{A.12}$$

$$a_3 = (k_{nr} + k_s Cl_{cr}) k_{reabs} \lambda_k \tag{A.13}$$

which are both greater than zero. For $E = 0$, $a_1 a_2 > a_3$. Thus the system is stable for $M = M_{max}$. For $E = 1$, thus $M = 0$, a_2 and a_3 are greater than zero and $a_1 a_2 > a_3$, thus then system is also stable.

Appendix B

Numerical program in Matlab

To run simulations on the mathematical model and to investigate the behaviour of the model, we have developed a numerical program in Matlab. In this numerical program the parameter values have to be chosen, as well as the treatment and patient properties from which then the concentration in the blood, the number of bacteria, the concentration in the kidney cells, the number of kidney cells, the creatinine clearance, the concentration in the ear compartments and the number of hair cells are calculated.

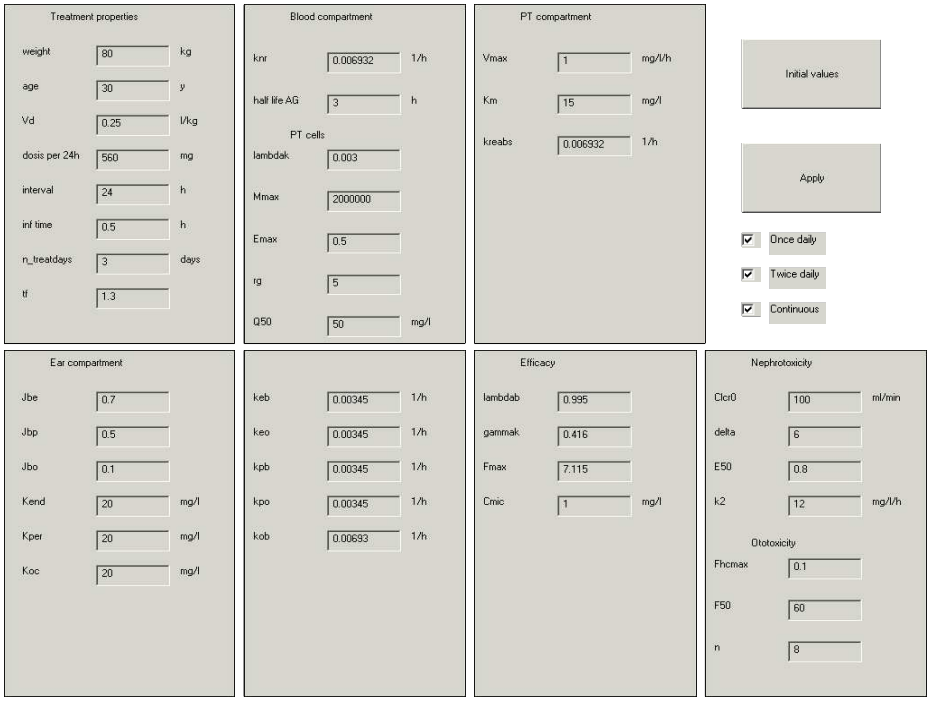

Figure B.1: *The input screen for the numerical program in Matlab. The parameter values, the patient and treatment properties have to be chosen. Also a dosage regimen has to be chosen, which can be once-daily, twice-daily or continuous administration, or any combination. The program automatically loads default values, other values can be chosen in this input screen. With 'apply' the simulation is started.*

References

[Ago86] D'Agostino, R.B., Stephens M.A., *Goodness-of-fit techniques*, Statistics: textbooks and monographs, volume 68, Marcel Dekker, Inc. New York and Basel, 1986

[Baa01] Baarda, D.B., de Goede, M.P.M., *Basisboek Methoden en technieken*, derde herziene druk, Wolters-Noordhoff bv Groningen/Houten, 2001

[Ber98] Berne, R.M., Levy, M.N., Physiology, 4th edition, Mosby, Inc. St.Louis, 1998

[Boo90] Boogaard, P.J., *Development of proximal tubular cell systems to study nephrotoxicity in vitro*, proefschrift, Offsetdrukkery Kanters B.V. Alblasserdam, 1990

[Box92] Van Boxtel, C.J., Holford, N.H.G., Danhof, M., *The In Vivo Study Of Drug Action, Principles And Applications Of Kinetic-Dynamic Modelling*, Elsevier, 1992

[Bre00] Brenner, B.M., Rector, F.C., *The kidney*, 6th edition, Philadelphia, Pa.: Saunders, 2000

[Bro88] De Broe, M.E., Giuliano, R.A., Verpooten, G.A., Insights into the renal handling of aminoglycosides: a guideline for prevention of nephrotoxicity, *J. Drug Dev.*, vol. 1, suppl. 3, p. 83-92, 1988

[Bro91] De Broe, M.E., Verbist, L. Verpooten, G.A., Influence of dosage schedule on renal cortical accumulation of amikacin and tobramycin in man, *J. Antimicrob. Chemother.*, vol. 27, suppl. C, p. 41-47, 1991

[BroWee] De Broe, M.E., De Weerdt, D.L., *Why once daily Netilmicin? Pharmacological, toxicological, clinical arguments*, Department of Nephrology-Hypertension, University of Antwerp, Belgium

[Bru90] Brummett, R.E., Morrisson, R.B., The Incidence of Aminoglycoside antibiotic-Induced Hearing Loss, *Arch Otolaryngol Head Neck Surg*, Vol 116, pg 406-410, 1990

[Cal88] Caldwell, D. (editor), Journal of drug development, the impact of novel dosing regimen on the safety and efficacy of aminoglycosides, vol. 1, supp. 3, ISSN 0267-5323, Gardner-Caldwell Communications, Ltd., 1988

[Car80] Carriere, E.G.J., *Gehoorbeschadiging door gentamicine, een klinisch en dierexperimenteel onderzoek*, proefschrift, W.D. Meinema B.V., Delft, 1980

[Cor98] Corvaisier, S., Maire, P.H., Bouvier d'Yvoire, M.Y., Barbaut, X., Bleyzac, N., Jelliffe, R.W., Comparisons between antimicrobial pharmacodynamic indices and bactericidal killing as described by using the Zhi Model, *Antimicrobial Agents and Chemotherapy*, vol. 42, no. 7, p. 1731-1737, 1998

[Der95] Derendorf, H., *Handbook of pharmacokinetic/pharmacodynamic correlation*, G. Hochhaus, ISBN 0-8493-8303-X, 1995

[Eds91] Edson, R.S., Terrell, C.L., The Aminoglycosides, Division of infectious Diseases and Internal Medicine, *Mayo Clin Proc 66*, p. 1158-1164, 1991

[Ger88] Gerber, A.U., Comparison of once-daily versus thrice-daily human equivalent dosing of aminoglycosides: basic considerations and experimental approach, *Journal of drug development*, vol. 1 p. 17-23, 1988

[Giu86] Giuliano, R.A., Verpooten, G.A., Verbist, L., Wedeen, R.P., De Broe, M.E., In vivo uptake kinetics of aminoglycosides in the kidney cortex of rats, *J Pharmacol Exp Ther*, vol. 236, no. 2, p. 470-475, 1986

[Gov91] Govaerts,P.J., Claes,J., van de Heyning, P.H., Derde, M.P., Kaufman, L., Marquet, J.F.E., de Broe, M.E., Effect of Isepamicin Dosing Scheme on Concentration in Coclear Tissue, *antimicrobial Agents and Chemotherapy*, Nov. 1991, p. 2401- 2406

[Has97] Hashino, E., Shero, M., Salvi, R.J., Lysosomal targeting and accumulation of aminoglycoside antibiotics in sensory hair cells, *Brain Research*, vol. 777, p. 75-85, 1997

[Hol01] Holz, M., Fahr, A., Compartment Modeling, *Advanced Drug Delivery Reviews*, vol. 48, p. 249-264, 2001

[Hur87] Hurst, A.K., Iseri, K.T., Gill, M.A., Nogushi, J.K., Gilman, T.M., Jelliffe, R.W., Comparison of four methods for predicting serum gentamicin concentrations in surgical patients with perforated or gangrenous appendicitis, *Clin. Pharmacy*, Vol. 6, p. 234-238, 1987

[Koo89] Koopman, M.G., Koomen, G.C., Krediet, R.T., de Moor, E.A., Hoek, F.G., Arisz, L., Circadian rythm of glomerular filtration rate in normal individuals, *Clin. Sci.*, vol. 77, no. 1, p. 105-111, 1989

[Mat87] Mathews, A., Bailie, G.R., Clinical pharmacokinetics, toxicity and cost effectiveness analysis of aminoglycosides and aminoglycoside dosing services, *Journal of Clinical Pharmacy and Therapeutic*, vol. 12, p. 273-291, 1987

[Mau00] Maurin, M., Rougier, F., Maire, P., *Note de calcul sur les lois de hill, aspects probabilistes, deterministes et epistemologiques*, Rapport INRETS-LTE no. 2026, 2000

[Min99] Mingeot-Leclercq, M.P., Tulkens, P.M., Aminoglycosides: Nephrotoxicity, *Antimicrobial Agents and Chemotherapy*, vol. 43, no. 5, p. 1003-1012, 1999

[Mon96] Montgomery, W., Gottlieb, T., Weekes, L., NSW Therapeutic Assessment Group Inc., Aminoglycoside dosage regimens, A position statement of the NSW Therapeutic Assessment Group Inc., December 1996

[Mou97] Mouton, J.W., Vinks, A.A.T.M.M., Punt, N.C., Pharmacokinetic-Pharmacodynamic Modeling of Activity of Ceftazidime during Continuous and Intermittant Infusion, *Antimicrobial Agents and Chemotherapy*, Vol. 41., No. 4, p. 733-738, 1997

[Mur89] Murray, J.D., *Mathematical Biology*, Springer-Verlag, Berlin, Heidelberg, New York, London, Paris, Tokyo, 1989

[Nee02] Neef, C., van Gils, S.A., IJzerman, W.L., Analogy between temperature-dependent and concentration-dependent bacterial killing, *Computers in Biology and Medicine*, Vol. 32, p. 529-549, 2002

[Nic95] Nicolau, D.P., Freeman, C.D., Belliveau, P.P., Nightingale C.H., Ross, J.W., Quintilliani, R., Experience with a Once-daily Aminoglycoside Program administered to 2184 Adult Patients, *Antimicrobial Agents and Chemotherapy*, vol. 39, no. 3, p. 650-655, 1995

[Ode01] Odenholt, I., Pharmacodynamic effects of subinhibitory antibiotic concentrations, review, *International journal of Antimicrobial Agents*, vol 17, p. 1-8, 2001

[Pri95] Prins, J.M., *Antimicrobial therapy of severe gram-negative infections, Dosing of aminoglycoside and studies on endotoxin release*, Academisch proefschrift, De Meern: Tessel offset, 1995

[Pri00] Price, P., Finney, H., Developments in the assessment of glomerular filtration rate, *Clinica Chimica Acta*, vol. 297, p. 55-66, 2000

[Rou03] Rougier, F., Claude, D., Maurin, M., Sedoglavic, A., Ducher, M., Corvaisier, S., Jelliffe, R., Maire, P., Aminoglycoside Nephrotoxicity: Modeling, Simulation, and Control, *Antimicrob Agents Chemother*, vol. 47, no. 3, p. 1010-1016, 2003

[Ser00] Seroul, R., *Programming for mathematicians*, Springer, Berlin, 2000

[Swa97] Swan, S.K., Aminoglycoside nephrotoxicity, *Seminars in Nephrology*, vol. 17, no. 1, p. 27-33, 1997

[TheMon] *Therapeutic drug monitoring*, ch 9, p. 248-294

[Van95] Vander, A.J., *Renal physiology*, 5th edition, McGraw-Hill, Inc., Health professions division, 1995

[Ver86] Verpooten, G.A., Giuliano, R.A., Pattyn, V.M., Scharpe, S.L., De Broe, M.E., Renal cortical uptake kinetics of gentamicin in rats with impaired renal function, *Am J Kidney Dis*, vol 8, no 5, p. 304-307, 1986

[Ver89] Verpooten, G.A., Giuliano, R.A., Verbist, L., Eestermans, G., De Broe, M.E.,

Once-daily dosing decreases renal accumulation of gentamicin and netilmicin, *Clin Pharmacol Ther*, vol. 45, no. 1, p. 22-27, 1989

[Vin96] Vinks, A.A.T.M.M., *Strategies for Pharmacokinetic Optimization of Continuous Infusion Therapy of Ceftazidime and Aztreonam in Patients with Cystic Fibrosis*, proefschrift, CIP-DATA Den Haag, 1996

[WerVre] Werdmuller,B.F.M., Vreede, R.W., Aminoglycosiden, *Capita selecta over keuze en toepassing van antibiotica*, pages 28-35

List of Symbols

The following is a list of the symbols and abbreviations used in this report.

Symbols

$C(t)$	concentration of drugs at time t
$C_b(t)$	concentration of aminoglycosides in the blood
$C_{ear}(t)$	concentration of aminoglycosides in the ear fluids
$C_{end}(t)$	concentration in the endolymph
C_{mic}	minimal inhibitory concentration
$C_{oc}(t)$	concentration in the organ of corti
$C_{pt}(t)$	concentration of aminoglycosides in the PT cells
$C_{per}(t)$	concentration in the perilymph
$C_{scr}(t)$	concentration of serum creatinine
$Cl_{cr}(t)$	creatinine clearance
Cl_{cr0}	creatinine clearance of a healthy person
$Cr(t)$	serum creatinine concentration
C_{50}	concentration at which 50 % of the maximal effect is reached
D	diffusion coefficient
$E(t)$	fraction of dead kidney cells compared to the maximum number of cells
E_{max}	maximum killing rate of the kidney cells
E_{50}	fraction of dead kidney cells at which the clearance is decreased by 50%
F_{max}	maximum killing rate for the bacteria
F_{50}	concentration at which half the maximal killing rate is achieved
$I(t)$	Infusion rate
J_{be}	maximum uptake rate for the endolymph
J_{bo}	maximum uptake rate for the organ of corti
J_{bp}	maximum uptake rate for the perilymph
k	elimination rate
k_{ear}	transfer rate from the blood to the ear
K_{ear}	elimination rate from the ear fluids to the blood
k_{eb}	elimination rate from the endolymph to the blood
k_{el}	elimination rate
K_{end}	concentration at which half the maximum uptake rate is reached for the endolymph
k_{eo}	elimination rate from the endolymph to the organ of Corti
K_m	Michaelis-Menten constant for uptake in the kidney
k_{nr}	non-renal elimination rate
k_{ob}	elimination rate from the organ of Corti to the blood

K_{oc}	concentration at which half the maximum uptake rate is reached for the organ of corti
k_{pb}	elimination rate from the perilymph to the blood
K_{per}	concentration at which half the maximum uptake rate is reached for the perilymph
k_{po}	elimination rate from the perilymph to the organ of Corti
k_{reabs}	the reabsorption rate from the PT cells to the blood
k_s	renal clearance coefficient
k_2	daily muscular production rate of creatinine
$N(t)$	number of bacteria
$N_{hc}(t)$	number of hair cells
N_0	initial number of microorganisms
$M_l(t)$	number of living kidney cells
M_{max}	maximum number of kidney cells
N_{max}	maximum number of bacteria
T	recovery time
$t_{1/2}$	half-life
$Q_b(t)$	amount of aminoglycosides in the blood
$Q_{tot}(t)$	total amount of aminoglycosides in the kidney
Q_{50}	concentration in the kidney cells at which half the maximal killing rate is obtained
V_d	volume of distribution
V_{max}	maximum rate of uptake into the kidney cells
w	weight
α	adaptation rate
γ	hill sigmoidity coefficient for the killing of kidney cells
γ_k	Hill factor for the killing process
δ	hill sigmoidity coefficient for the creatinine clearance
λ	growth rate
λ_b	growth rate for bacteria
λ_k	regrowth rate of the kidney cell

Abbreviations

AG	Aminoglycoside
CF	Cystic Fybrosis
GFR	Glomerular Filtration Rate
MIC	Minimum Inhibitory Concentration
OD	Once-daily
PAE	Postantibiotic effect
PD	Pharmacodynamics
PK	Pharmacokinetics
PT	Proximal Tubulus
TD	Twice-daily