Influence of pharmaceutical-grade albumin infusions and plasma albumin concentration on protein binding of drugs

Thesis for the degree Ph.D.

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2 List of Papers

(I) Reine PA, Kongsgaard UE, Andersen A, Thogersen AK, Olsen H. Infusion of albumin attenuates changes in serum protein binding of drugs in surgical patients compared with volume replacement with HAES. Acta Anaesthesiol Scand 2008 March;52(3):406-12.

(II) Reine PA, Kongsgaard UE, Andersen A, Thogersen AK, Olsen H. Infusions of albumin increase free fraction of naproxen in healthy volunteers: a randomized crossover study. Acta Anaesthesiol Scand 2010 April;54(4):430-4.

(III) Reine PA, Vethe NT, Kongsgaard UE, Andersen AM, Line PD, Ali AM, Bergan S. Mycophenolate pharmacokinetics and inosine monophosphate dehydrogenase activity in liver transplant recipients with an emphasis on therapeutic drug monitoring. Not yet published.

3 Abbreviations

AAG	alpha-1 acid glycoprotei
AcMPAG	mycophenolic acid acyl glucuronide
AUC	area under the curve
AUEC	area under the effect curve
CsA	cyclosporine A
HPLC	high-performance liquid chromatography
IMPDH	inosine 5′-monophosphate dehydrogenase
MMF	mycophenolate mofetil
MPA	mycophenolic acid
MPAG	mycophenolic acid 7-O-glucuronide
PD	parmacodynamics
PK	pharmakokinetics
TDM	therapeutic drug monitoring
TRL	tacrolimus
UGT	uridine diphosphate–glucuronosyltransferase

4 Erratum

p. 49 line 4: «restrictively» should be replaced with «nonrestrictively»

5 Introduction

Albumin is the most abundant plasma protein in the human body. It has a number of important physiological functions and binds a number of drugs. Hypoalbuminemia is a marker of disease and a predictor of outcome. Albumin solutions have been available in clinical practice since the Second World War. Despite our widespread experience with and extensive research into albumin solutions, there is still widespread controversy regarding their place in modern clinical medicine.

5.1 Intravenous fluids

The intravenous administration of fluids is necessary in a number of situations to replace lost fluid or blood and to maintain the fluid balance in critically ill patients. It is common to divide the available intravenous fluids into two groups—crystalloids and colloids—based on their oncotic properties. Crystalloid solutions are aqueous solutions of low-molecular-weight ions (salts) with or without glucose, whereas colloids also contain high-molecular-weight substances, such as proteins or large glucose polymers (1).

A wide range of crystalloid solutions is available, varying in their compositions and concentrations of salts. The most common crystalloids in clinical practice are normal saline (isotonic NaCl), Ringer's lactate solution (isotonic

physiological solution), and 5% glucose (hypotonic). A number of hypertonic solutions are also available for electrolyte replacement and rapid volume resuscitation.

Colloid solutions contain high-molecular-weight substances, derived from plasma proteins or synthetic glucose polymers, suspended in an electrolyte solution. The high-molecular-weight substances present in colloid solutions exert an osmotic activity that tends to maintain these solutions intravascularly. The only blood-derived colloids commonly available in Europe are albumin solutions with variable albumin contents, typically in the range of 5%–25%. Albumin solutions will be discussed in more detail later in this thesis. Synthetic colloids include gelatins and dextrose starches. Haemaccel® is a gelatinous solution derived from bovine material, but because of histamine-mediated allergic reactions and fear of disease transmission, gelatin-derived solutions have fallen from favor in many countries. Dextran and hetastarches are examples of dextrose-starch-derived colloids. Dextran is a complex glucan composed of chains of variable length. Dextran solutions are described on the basis of the molecular weight of the dextran present in the solution. Dextran 40 (Rheomacrodex, MW 40,000 Da) and dextran 70 (Macrodex, MW 70,000 Da) are used in clinical practice. Dextran 70 is the better volume expander, but dextran 40 is utilized for its advantageous effects on the microcirculation. A number of different hydroxyethyl starch solutions are available, with different

molecular weights and degrees of molar substitution (the proportion of glucose units in the starch molecule that have been replaced with hydroxyethyl units). The effects and adverse effects of these solutions are dependent on their composition (2). Hydroxyethyl starches are the synthetic colloids most commonly used in clinical practice.

There are extensive controversies in the medical community regarding the use of colloids and crystalloids, and the appropriate types of colloids and crystalloids that should be used are debated. However, there is agreement about several characteristics of these solutions, and several generalizations can be made (1).

1. *Crystalloids, when given in sufficient amounts, can be just as effective as colloids in restoring the intravascular volume.*
2. *Replacing an intravascular volume deficit with crystalloids requires three to four times the volume required when using colloids.*
3. *Most surgical patients have an extracellular fluid deficit that exceeds their intravascular deficit.*
4. *A severe intravascular fluid deficit can be more rapidly corrected using colloid solutions.*
5. *The rapid administration of large amounts of crystalloids is more frequently associated with tissue edema.*

Despite these differences, it has been difficult to prove the superiority of one type of fluid over the other in the clinical context. There have been many

attempts to produce convincing evidence in this field using meta-analyses of the available research. However, the heterogeneity and diversity of the available trials and the complex clinical situation have made it difficult to draw firm conclusions.

5.2 Pharmacotherapy

Pharmacotherapy is the use of drugs to prevent, treat, or cure a disease or alleviate its symptoms. Modern pharmacotherapy includes an array of different substances, ranging from natural products from plants and animals to synthetic chemical compounds. Biotechnology has also supplied us with therapeutic agents in the form of various proteins, antibodies, and enzymes. In recent years, gene therapy has emerged as a promising new prospect. The science or art of pharmacotherapy is undoubtedly very complex. The sheer number of different drugs, the variability of these drugs, and the intrapatient variability makes this a difficult science from which to generalize or to comprehend fully. The literature in this field of medicine is immense.

To better describe pharmacotherapy, it is traditionally separated into two distinct parts: pharmacokinetics (PK), or what the body does to the drug, and pharmacodynamics (PD), or what the drug does to the body.

5.2.1 Pharmacokinetics

The pharmacokinetic processes that drugs undergo are absorption, distribution, metabolism, and excretion. The absorption of a drug is influenced by several variables, such as the route of administration (oral, intravenous, intramuscular, subcutaneous, transdermal, inhalation, etc.), its solubility, and its lipophilicity. After the drug is absorbed, the fraction of the dose that escapes metabolism and the efflux transporters in the gut wall and enters the systemic circulation is distributed about the body. The degree and rate of distribution is dependent on the size, the solubility, and the protein binding of the drug molecules. The distribution of a drug in the body is described by the apparent volume of distribution. This is a variable that relates the total amount of drug in the body to the plasma concentration. Drug metabolism is the process whereby the body transforms the drug from its original form, often to a water-soluble form that can be excreted. Metabolism can occur in many organs, such as the liver, kidney, plasma, and intestine, with the liver the predominant site of metabolism. When drugs are metabolized, they can change into either an inactive drug that can be excreted or an active metabolite that has its own pharmacological effect. An example of the latter is morphine, which is converted into morphine-6-glucuronide, a potent analgesic in its own right. Some drugs are administered in the form of a prodrug, and the metabolite is the active drug. Mycophenolate mofetil (MMF) is one such inactive prodrug and is only active when metabolized into mycophenolic acid (MPA). A drug can be excreted from the

body via the liver, kidney, saliva, respiratory tract, etc. The rate of excretion is described as the drug's half-life, which is the time it takes for 50% of the drug to be eliminated. The pharmacokinetic properties of a drug are patient dependent and may vary with age, weight, sex, and ethnicity. These properties may also be altered by disease.

5.2.2 Pharmacodynamics

"Pharmacodynamics" describes the biological and physiological effects of a drug on the body. The term refers to the mechanisms of the drug action and the relationship between the drug's concentration and its effect. For the drug to have an effect, it must bind to an effect site or receptor. When a drug binds to a receptor, it either elicits a response (agonist) or blocks the receptor (antagonist), preventing the normal physiological action at the receptor site. Desired effects are termed "responses" or "effects", whereas undesired effects are termed "side effects" or "adverse effects". The amount of drug required to achieve a particular therapeutic response varies among individuals, and this is described by a dose–response curve and expresses the potency of the drug. The therapeutic index describes the relationship between the desired effect and the toxic effect of a drug. The therapeutic window is the dosage range between the minimum effective dose and the minimum toxic dose.

The PK and PD of a drug are patient dependent and may change in an individual patient over time. Of particular interest are the changes that occur in the critically ill and during surgery.

5.2.3 PK and PD in critically ill patients

The pharmacological effects of some drugs (e.g., sedatives, vasopressors, and antihypertensive drugs) commonly used to treat critically ill patients can be monitored easily and the dose titrated according to their responses (3). However, the effects of many other drugs, such as antibiotics, immunosuppressants, anticonvulsants, anti-inflammatory drugs, and anticancer drugs cannot be immediately observed, and clinical dose titration is not possible. Knowledge of how the pathophysiology of critically ill patients changes the PK and PD of drugs is therefore essential to optimize drug doses to achieve the pharmacodynamic targets.

In septic patients, endotoxins may stimulate the production of various endogenous mediators that can affect the vascular endothelium, resulting in either vasoconstriction or vasodilatation, with the maldistribution of the blood flow, endothelial damage, and increased capillary permeability (4). Increased systemic vascular permeability occurs within hours of inflammatory stimuli, such as surgery or sepsis (5). Capillary leakage results in fluid shifts from the

intravascular compartment to the interstitial space (6). The shift of fluids from the intravascular compartment increases the apparent volume of distribution for hydrophilic drugs, which reduces the plasma drug concentration. The apparent volume of distribution of hydrophilic drugs may also be increased by the presence of mechanical ventilation, hypoalbuminemia, extracorporeal circuits, and significant burn injuries (6). These changes in apparent volume of distribution are of little importance for lipophilic drugs.

In critically ill patients, the cardiac output can be either increased as a result of vasodilatation, fluid resuscitation, or stimulation with vasoactive drugs, or reduced as a result of vasoconstriction, hypovolemia, or cardiac dysfunction. Changes in the cardiac output can alter the perfusion of the metabolizing organs, increasing or reducing the clearance of a drug. Enhanced renal elimination of circulating drugs has been described with increasing regularity in the critically ill (7).

The various treatments instituted for critically ill patients have implications for PD. The polypharmacy applied in any such patient entails potential drug–drug interactions. Renal replacement therapy is becoming increasingly common in this patient group and can influence the PK of drugs. The effect of renal replacement therapy on PK is dependent on the drug in question and the modality of the renal replacement used. Extracorporeal membrane oxygenation

can influence the drug PK by increasing the volume of its distribution, and by the possible binding of the drugs to the circuit (8).

The consequences of altered PK depend on the pharmacodynamic properties associated with the optimal activity of an individual drug. β-Lactam antibiotics, for example, are time-dependent antibiotics, and the time at which the serum level exceeds the minimum inhibitory concentration is the major pharmacodynamic index associated with the drug's efficacy, and as such, the goal of dosing is to optimize the duration of the systematic exposure to the drug (9). For such drugs, increased clearance should justify a more frequent dosing regimen or continuous infusion. On the contrary, concentration-dependent killing antibiotics, such as aminoglycosides, are dependent on the peak drug concentration for their maximal bactericidal effect (9). For drugs with such profiles, increased apparent volume of distribution would justify an increased dose.

5.2.4 Pharmacotherapy and drug–protein binding

Albumin and alpha-1 acid glycoprotein (AAG) are the most important drug-binding proteins in the circulation (10;11). Whereas albumin is primarily responsible for the binding of acidic drugs, basic drugs display greater affinity for AAG (12). Albumin is the most abundant plasma protein, with a

physiological concentration in the range of 35–45 g/L. In contrast, AAG has a plasma concentration of only 0.4–1 g/L under normal conditions. The plasma concentration of albumin is frequently reduced in a variety of disease states, whereas AAG is an acute-phase protein and may therefore be elevated in a disease state. Both albumin and AAG are produced in the liver and liver disease may therefore markedly reduce the concentrations of these proteins.

The degree to which they are bound by plasma proteins is one of the parameters that influence the PK of drugs. Changes in protein binding may alter the PK of a drug, thereby also altering its PD.

The apparent volume of distribution of any drug is dependent on the binding properties of the drug in both the plasma and the tissues. The volume of distribution (Vd) may be expressed by the following formula:

$$V_D = V_P + V_T \left(\frac{fu}{fu_t} \right),$$

where V_P represents the plasma volume, V_T the tissue volume, f_u the drug fraction unbound in the plasma, and f_{ut} the drug fraction unbound in the tissues. Therefore, the volume of distribution determines how readily a drug will be displaced from the blood into the tissue compartments. A high free fraction in the blood results in the movement of the drug from the blood/plasma to the tissue compartment, increasing the distribution of the drug. Conversely, a higher

free fraction in the tissue results in the movement of the drug from the tissue to the blood/plasma and a reduction in its volume of distribution (13). High levels of protein–drug binding in the blood/plasma confine the drug to the blood/plasma and restrict its movement to other compartments, resulting in a low apparent volume of distribution.

The other PK parameter that is partly dependent on protein binding is the drug clearance. “Clearance” describes the degree to which an organ of elimination clears the blood of the drug and is dependent on the blood flow to the organ, protein binding, and enzymatic or secretory activity. The clearance (CL) of a drug by a specific organ is understood to be the product of the blood flow to the organ (Q) and the extraction capacity of the organ for the drug (E) (14).

$$CL = Q \times E$$

When the extraction capacity for a drug approaches 1, the blood arriving at the organ is completely cleared of the drug, and the clearance is then sensitive to changes in the blood flow but not to changes in the binding of the drug by plasma proteins or to enzymatic capacity. This pattern of clearance is termed “high-extraction clearance”. When the extraction ratio is lower than 1, the clearance is dependent on other factors, such as protein binding, enzymatic capacity, and membrane permeability, and this is known as “low-extraction clearance” or restrictive clearance. For drugs with a high extraction ratio, the eliminating organ is capable of removing all the drug presented to it,

independent of the binding of the drug to cells or plasma proteins. However, for drugs with a low extraction ratio, protein binding may be a limiting factor in the elimination of the drug.

5.3 Albumin

5.3.1 History

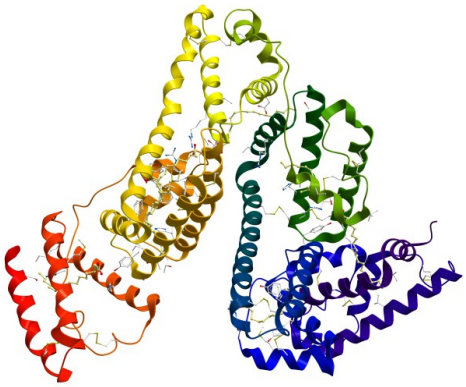
The name “albumin” originates from the Latin word “albus” (white) or “albumen” (whiteness). The name was first used when early medical chemists proved the presence of proteins in various bodily fluids by heat coagulation and salt precipitation. The product resembled egg white and was therefore termed “albumen”. Hippocrates (*c* 460 BC–*c* 370 BC) first mentioned some of albumin’s physiological properties, but albumin was not properly identified, isolated, and named until the early 1800s. Before that time, the term “albumin” was used to describe protein precipitates from bodily fluids. Philippus von Hohenheim, known as Paracelsus (1493–1541) and one of the founders of medical chemistry, considered albumen in the urine (which he precipitated by the addition of acid) an important indicator of disease (15).

The purification of proteins has since come a long way. In 1840, the first recorded dialysis was performed by the French physiologist, C. Denis. He placed

blood serum in a sac of intestine immersed in water and found that some of the protein precipitated as the salt was removed through the sac. Unlike the action of heat, this precipitation was reversible when a small amount of salt was added. The protein that was soluble in water without salt was called “albumin” and the protein that precipitated in little globules was called “globulin”. By the nineteenth century, chemists had refined crystallization, and albumin was one of the earliest proteins to be crystallized. In the 1930s, T. Svedberg and K. O. Petersen in Uppsala studied the proteins in blood serum with the new technique of ultracentrifugation (16). Further advances were made in the Svedberg laboratory when A. Tiselius applied the Schlieren optics developed by Svedberg to the technique of electrophoresis, and albumin was readily identified as the prominent anionic constituent (17). Electrophoresis has since continued to be the usual method for identifying and judging the purity of albumin solutions.

5.3.2 Physiology

Albumin is the most abundant protein in blood plasma, with a normal concentration in the range of 35–45 g/L. Structurally, it is a single peptide chain, consisting of 585 amino acids held in three homologous domains by 17 disulfide bonds, forming a heart-shaped molecule (18;19). It belongs to a multigene family of proteins that includes α-fetoprotein and vitamin D-binding protein.



Albumin molecule

Albumin is produced in the liver (20). Between 6% and 10% of the albumin pool is degraded per day, giving it a relatively long biological half-life of about 20 days. Only ~40% of the total body albumin is located in the vascular compartment. The remainder is located in the tissues. The transfer rate of albumin from the plasma to the interstitial compartment is low in skeletal and cardiac tissues, and relatively high in visceral organs (21).

Albumin is a molecule, with a variety of physiological functions. It is a major contributor to the colloid osmotic pressure and facilitates the transport, distribution, and metabolism of many endogenous substances, such as fatty acids, amino acids, bile, and hormones, and exogenous substances such as drugs. For many hormones and vitamins, albumin acts not only as a carrier, but also as

a reservoir. A number of endogenous and exogenous toxins are sequestered by albumin and, in that way, rendered harmless in the circulation.

Serum albumin accounts for approximately 80% of the colloid osmotic pressure of plasma. This is caused by the molecular effect and by the extra osmotic pressure induced by the Donnan effect, whereby sodium, potassium, and other cations are held in the plasma by the negative charges surrounding the molecules (22).

Many drugs bind reversibly to albumin. The binding of drugs to plasma proteins affects their distribution and rate of metabolism in the body by controlling their free active concentrations and providing reservoirs for them. The drug-binding properties of albumin have been extensively studied. Early binding studies revealed two primary and nonoverlapping drug-binding sites on albumin, with a third binding site quite selective for digitoxin only (23;24). Later crystallographic studies revealed that drug sites 1 and 2 are located on subdomains IIA and IIIA, respectively. The binding sites exhibit variable degrees of specificity, and displacement reactions have been described among drugs with the same binding site. The affinity for one drug at a specific binding site may also be altered as a consequence of the binding of a second drug at a different binding site because of a structural change along a common interface (18), or by the binding of fatty acids. More recent crystallographic work has

identified several other drug pockets on the protein, in addition to the two primary drug-binding sites in subdomains IIA and IIIA. These sites can also be specific binding sites for one or more drugs, acting as secondary binding sites for compounds with another primary binding site (25).

Fatty acids have low solubility in aqueous solutions and therefore require a transporter in both the plasma and the interstitial compartment. Albumin is the primary transporter for fatty acids and plays a major role in fatty acid transfer between the capillary plasma and the cells utilizing them. The mechanism is unclear, but the interplay between plasma and interstitial albumin is probably critical (26). Seven binding sites for fatty acids have been identified on the albumin molecule (27;28). The binding of fatty acids to albumin induces conformational changes in drug site 1, increasing its affinity for some drugs (29). Although this effect appears to be relatively small, it demonstrates the complexity of the binding characteristics of albumin. Drug site 2 directly overlaps one of the high-affinity fatty-acid-binding sites. However, at physiological levels of fatty acids, this competition is not likely to be a problem because the residual concentrations of the drug-binding sites under these conditions are likely to be sufficient for drug binding (25).

5.3.3 Albumin solutions

Human albumin solutions were developed in the United States during the Second World War to provide an alternative to blood and dried plasma for fluid resuscitation in military casualties (30). The first documented clinical use of albumin occurred on the December 8, 1941, when seven burn victims from the Pearl Harbor attack were treated with albumin solutions.

Albumin for therapeutic use is predominantly derived from pooled human plasma, although both time-expired blood and, in some countries, placental material have been used as sources in the past (31). The traditional method for its isolation has been cold ethanol fractionation, first described by Cohn and colleagues in 1946 (32). In later years, some manufacturers have changed to the chromatographic processing of plasma, a process that provides both a purer product and a higher yield (31). Albumin solution has been pasteurized since the 1940s (33), a process that has been proven very effective in preventing the transmission of disease (34). To prevent the denaturation of the albumin molecule during heating to 60 °C, stabilizers are added to the solution. Although the main methods used in the manufacturing process have remained unchanged for the past 60 years, the albumin solution has undergone continuous improvement to reduce the amount of impurities, damaged proteins, and unwanted substances in the end product (31). Different manufacturers use different production methods, which might affect the end product. It has been

demonstrated that albumin from different manufacturers has different levels of metal ions, which could generate damaging oxygen intermediates (35), and that certain batches of albumin solutions influence the expression of endothelial cell adhesion molecules (36). The manufacturing process may also oxidize the albumin molecule to different extents, affecting the properties of the end product (37).

Although albumin solution has been used widely since the Second World War, it has been difficult to demonstrate that it has advantages over alternative fluids. Increasing controversy concerning its use was aroused after the Cochrane Injuries Group Albumin Reviewers reported possible excessive mortality after the administration of albumin to critically ill patients (38). Escalating prices and increasing concern regarding the administration of human products have further discredited albumin solutions. However, a large, multicenter, randomized, controlled trial reestablished the safety of albumin solutions in 2004 (39). Although albumin is considered safe and has been widely used for nearly seven decades, there are no clinical situations in which albumin solutions are indispensable. Despite the many important physiological properties of albumin and the fact that hypoalbuminemia is a predictor of increased morbidity and mortality, correcting hypoalbuminemia has not been proven beneficial; nor has albumin administration to hypovolemic patients been shown to be more favorable than the administration of crystalloids (39;40).

5.4 Mycophenolic acid (MPA)

MPA was first isolated by Gosio in 1896 (41). Through the years, it has been investigated as an antibiotic, antiviral, antifungal, antitumor, and anti-inflammatory agent. However, it is within the field of immunosuppression that MPA has gained a foothold as a therapeutic agent. It was first approved in 1995, with the brand name “CellCept”, as a rejection prophylaxis after renal allograft transplantation.

5.4.1 Mechanism of MPA action

MPA is a potent, selective, and noncompetitive reversible inhibitor of inosine 5′-monophosphate dehydrogenase (IMPDH), the rate-limiting enzyme in the de novo synthesis of guanine nucleotides (42). De novo purine synthesis is critically important for the proliferative responses of human T and B lymphocytes, and a reduction in guanine nucleotides leads to cell-cycle arrest because DNA synthesis is blocked (43). MPA is relatively selective because most other cell lines can utilize a salvage pathway to produce guanine. MPA is also fivefold more potent in its inhibition of the IMPDH type II isoform, which is expressed in activated T and B lymphocytes, than in its inhibition of the type I isoform, which is expressed in most other cell types (44).

5.4.2 Pharmacokinetics of MPA

Mycophenolate mofetil (MMF), the 2-morpholinoethyl ester of MPA, is a prodrug of MPA. The prodrug demonstrates better bioavailability, less between-patient variability, and a shorter time to its maximum concentration (45). After its oral administration, MMF is rapidly absorbed and metabolized to MPA, with a peak in the plasma MPA concentration after approximately 1 h. The bioavailability of MPA from orally administered MMF is estimated to be 94.1% relative to the intravenous route in healthy volunteers (46). However, according to the label of the commercially available product, the absolute bioavailability of MPA is 72% after the oral administration of MMF to renal transplant recipients. MPA undergoes enterohepatic recirculation, which is supported by the presence of mycophenolic acid 7-O-glucuronide (MPAG) in the bile, and is evident as a secondary peak in plasma MPA after 6–12 h (47).

More than 99% of MPA is retained in the plasma compartment (48;49) and the concentration does not decrease in response to hemodialysis, indicating its tight binding to plasma proteins (50). Approximately 98% of MPA is bound to albumin (51). The degree of albumin binding is dependent on the albumin concentration and renal function. Renal impairment reduces the binding of MPA to albumin because of both the direct effect of uremia and the accumulation of the metabolite MPAG, which displaces MPA from albumin (51;52).

The liver is the main metabolizing site for MPA, but the gut wall and kidney are also thought to contribute. The uridine diphosphate–glucuronosyltransferase (UGT) enzyme family is responsible for the metabolism of MPA to its metabolites MPAG and mycophenolic acid acyl glucuronide (AcMPAG) (53). MPAG is the primary metabolite and appears in the plasma at concentrations 20–100-fold higher than that of MPA (54), and 60%–80% of MPAG is bound to albumin (48). AcMPAG is an active metabolite that inhibits IMPDH in vitro (55). MPA is a restrictively cleared drug, which means that its clearance is dependent on the free fraction of the drug (56). The administration of radiolabeled MPA has shown that 93% of the drug is eliminated in the urine, whereas 6% is eliminated in the feces (47).

Drug interactions may influence the pharmacokinetics of MPA. MPA is commonly administered together with either cyclosporine A (CsA) or tacrolimus (TRL). Pharmacokinetic studies have demonstrated that significantly higher MPA concentrations were attained in patients when MMF was coadministered with TRL than when it was administered with CsA (57). It has been established in a rodent model that this difference occurs because CsA interferes with the enterohepatic recirculation of MPA (58). Steroids are another common constituent of immunosuppressive protocols, and these have been demonstrated to influence the pharmacokinetics of MPA (59). Steroids have also been shown to enhance the activity of the enzyme responsible for the metabolism of MPA

(60), thereby reducing its bioavailability. The bioavailability of MPA may also be reduced when it is coadministered with some antibiotics and antacids (61;62). The binding of MPA to plasma proteins is reduced by sodium salicylate and furosemide, which thus increase the free fraction of MPA (48).

The many intrinsic and extrinsic factors that influence the absorption, enterohepatic recirculation, albumin binding, and metabolism of MPA give rise to considerable overall variability in its PK, both between individuals and within the same individual over time. Its unpredictable PK and the narrow therapeutic index of MPA, combined with the serious consequences of therapeutic failure and its potential dangerous adverse effects, constitute a powerful rationale for implementing therapeutic drug monitoring (TDM) for MPA.

5.4.3 Therapeutic drug monitoring of MPA

MMF was approved for clinical use in adult renal transplant recipients at a fixed dose of 2 g per day, and the efficacy of fixed-dose regimens has been established (63). However, because of the variability in the PK of MPA outlined above, the prediction of MPA exposure based solely on the dose can lead to MPA concentrations that are lower or higher than desired in individual patients, and both high inter- and intrapatient variability over time has been established

(64;65). Therefore, there has been increased interest and research into the TDM of MPA to optimize its efficacy and safety.

Significant correlations have been established between the area under the curve (AUC) for the concentration –versus time curve for MPA and clinical outcomes (acute rejection and adverse events) in renal transplant patients (66-69). A relatively small, single-center clinical trial demonstrated that therapeutic MPA monitoring using a limited sampling strategy can reduce the risk of treatment failure and acute rejection in renal allograft recipients 12 months after transplantation, with no increase in adverse events (70). However, two large multicenter, randomized controlled trials comparing fixed-dose with concentration-controlled administration of MPA was less convincing, and both dosing strategies revealed a reluctance among clinicians to adhere to the target MPA levels (71;72). Considerable overlap has also been reported between the MPA AUC values of patients experiencing rejection and the values of those not experiencing rejection (73). This indicates that there is large variability in the pharmacodynamic response to MPA and suggests that a pharmacodynamic marker could be beneficial in optimizing MPA treatments. The pharmacodynamic response to MPA is also variable, depending on the expression of the IMPDH enzyme and possibly also on the induction of other enzymes by MPA treatment (74), and functional gene variants (75). A study that measured IMPDH activity in renal transplant recipients demonstrated that high

pretransplant IMPDH activity was associated with organ transplant rejection (76), and Raggi et al. have reported that IMPDH activity is a reliable predictor of rejection episodes in renal transplant patients (77). Moreover, IMPDH activity can be reliably quantified and offers a way to describe the immunosuppression exerted by MPA.

6 Aims of the thesis

Understanding the drug-binding properties of pharmaceutical-grade albumin and the consequences of changes in plasma albumin concentrations are the aims of this thesis.

The specific objectives of the projects were as follows.

1. To investigate the changes in the albumin-binding capacity and the free fraction of naproxen, warfarin, and digitoxin in patients transfused with pharmaceutical-grade albumin, compared with patients receiving a modified starch solution (Study 1).
2. To investigate the displacement properties of the stabilizers present in pharmaceutical-grade albumin for naproxen in healthy volunteers (Study 2).
3. To explore the changes in plasma albumin in the early period following liver transplantation and to determine how the albumin concentration influences the free fraction of MPA; and to assess whether changes in the free fraction of MPA influence its PD (Study 3).

7 Methods

7.1 Paper 1

This study was a randomized study of 40 surgical patients undergoing major surgery. The patients were randomized to receive either albumin or a starch solution when there was a clinical indication for volume replacement. The randomization was computer based and handled by the hospital's office for clinical research. We performed a block randomization with two blocks of 20 patients. Interim analyses were performed after completion of the first block. No attempt was made to blind either the clinician or the laboratory staff.

The serum samples were collected (i) when anesthesia was induced, (ii) after the total amount of albumin or starch had been administered during the surgical procedure, (iii) 5 h after sample 2, and (iv) on the first postoperative day. Digitoxin, warfarin, and naproxen were added to the serum samples to measure drug binding. The albumin and AAG concentrations were measured immunophelometrically. Tryptophan, N-acetyl-DL-tryptophan, digitoxin, warfarin, and naproxen were determined by high-performance liquid chromatography (HPLC). The free concentrations of the drugs were measured with ultrafiltration.

The percentage of free drug was calculated as the ratio of the ultrafiltrate (free) concentration to the total concentration. The number of specific binding sites and the dissociation constants were calculated according to Scatchard. The Mann–Whitney–Wilcoxon test was used to compare the two groups. The Wilcoxon signed-rank test was used for statistical analyses within groups.

7.2 Paper 2

This study was a randomized crossover study of six healthy volunteers. The study individuals were given three 250 mg naproxen tablets, at 08:00 h (T_0), and were then randomized to receive 100 mL of albumin (200 mg/mL) or 100 mL of Ringer's acetate solution over a period of 15 min, at T_0+2.5 h.

Serum samples were collected at T_0+2 h and every 15 min for 1 h and 45 min, and thereafter every 30 min for the next 3 h. The albumin concentrations were measured immunonephelometrically. The N-acetyl-DL-tryptophan, caprylic acid, and naproxen concentrations were determined by HPLC. The free concentrations of naproxen were measured with ultrafiltration.

The percentage of free drug was calculated as the ratio of the ultrafiltrate (free) to the total concentration. The half-life of N-acetyl-DL-tryptophan was calculated from the elimination rate constant found by determining the slope of the log-

linear part of the elimination curve. Paired-sample *t* test were used to compare the two groups at the different sampling points.

7.3 Paper 3

This study was a prospective observational study of 20 liver transplant recipients treated with MMF. The transplant recipients were enrolled consecutively, depending on the laboratory and its sampling capacity.

A series of blood samples was collected on two occasions from each patient. The first session was in the early posttransplantation period (3rd–4th days posttransplantation), and the second session was when the graft was established, approximately 2 wk after the first session. Each set of samples consisted of 10 different measuring points. The first sample was taken before the intake of the morning dose of MMF at 9:00 h (T_0), and further samples were collected at T_0+30 min, T_0+45 min, and T_0+60 min. Subsequent samples were taken every 30 min for the next 3 h. Total and free MPA (after ultrafiltration) were determined by HPLC. The IMPDH activity in the $CD4^+$ cells was determined by incubating the lysates of the isolated cells with saturating amounts of substrate for 120 min. The xanthine produced was determined quantitatively with liquid chromatography coupled to electrospray ionization and tandem mass

spectrometry. The activity of IMPDH was expressed as the xanthine production rate during the incubation (pmol/10^6 cells/min).

The percentage of free MPA was calculated as the ratio of the ultrafiltrate (free) to the total concentration. The drug exposure and enzymatic activity were expressed as the area under the concentration curve (AUC) and calculated with the trapezoidal method. We used Pearson's product-moment correlation coefficient (r) to test the relationship between albumin and the free fraction of MPA (linear). Spearman's rank order correlation (rho) was used to correlate MPA, free MPA, and free-fraction MPA with IMPDH activity (nonlinear). The related-samples Wilcoxon signed-rank test was used to compare session 1 and session 2.

8 Results

8.1 Paper 1

The groups were evenly balanced with regard to age, height, weight, and sex. The Starch group had a small but statistically significantly lower preoperative albumin concentration compared with that of the Albumin group. During surgery, the albumin concentration decreased by 38% in the Starch group, whereas the albumin concentration in the Albumin group remained unchanged throughout the experiment. The stabilizers in the pharmaceutical-grade albumin, N-acetyl-DL-tryptophan and caprylate, were detected in the sera after the infusion of albumin, decreasing rapidly in subsequent samples. In the Starch group, the percentages of free naproxen, warfarin, and digitoxin increased throughout the observation period. For naproxen and digitoxin, the increased free fraction was combined with an increased number of binding sites. Naproxen also exhibited an increase in its dissociation constant. In the Albumin group, the percentage of free naproxen and the dissociation constant for naproxen increased between sample 1 and sample 2. There was a marginal reduction in the amount of free warfarin in samples 2 and 3, followed by a reduction in the dissociation constant and number of binding sites in sample 3 only. The binding characteristics of digitoxin remained unchanged in this group.

In this experiment, we observed an increase in the percentages of free naproxen, warfarin, and digitoxin. This can be explained by the significant decline in the albumin concentration caused by bleeding, combined with the infusion of colloids and crystalloids. In the Albumin group, we only observed an increase in the percentage of naproxen. This increase coincided with the measured concentrations of the stabilizers. Thus, the presence of stabilizers in pharmaceutical albumin might explain the displacement of naproxen from its binding site, as hypothesized. However, we did not observe any changes in the binding parameters for digitoxin or any increased binding of warfarin. The lack of displacement reactions observed was probably caused by the relatively low and short-lasting concentrations of stabilizers detected in the sera. Furthermore, warfarin has a different binding site on albumin than digitoxin and naproxen. The binding of warfarin to albumin is further complicated by free fatty acids, which may both enhance the binding of warfarin to albumin or displace warfarin from albumin.

This study demonstrates that the infusion of starch solutions results in a reduction in albumin concentration and a significant reduction in the binding parameters of albumin for naproxen, digitoxin, and warfarin. However, the infusion of albumin causes the albumin concentration to be maintained, with sustained binding properties, except for the binding of naproxen in a transient period when the stabilizers were at their peak concentrations. Because the

displacing properties of the stabilizers are short-lived, albumin infusions can have a positive impact on the PK of highly protein-bound drugs in patients with grave hypoalbuminemia.

8.2 Paper 2

This study had a crossover design. Therefore, the two groups were identical with regard to their baseline characteristics. During the infusion of albumin, the albumin concentration increased in all six individuals. In contrast, the infusion of Ringer's acetate solution caused a marginal reduction in the mean albumin concentration. The mean free fraction of naproxen increased by 30% when albumin was infused, returning to baseline during the next 15–30 min. The naproxen values in the Ringer's acetate group followed the expected course throughout our measurements. N-Acetyl-DL-tryptophan and caprylate appeared in our samples after the albumin was infused in the Albumin group. N-Acetyl-DL-tryptophan had a half-life of approximately 30 min, whereas the caprylate half-life was less than 15 min. We detected no N-acetyl-DL-tryptophan or change in the caprylate values in the Ringer's acetate group.

This study has shown that the stabilizers present in pharmaceutical-grade albumin displace naproxen from its binding site on albumin. However, the effect is short-term and probably of little importance for most patients. Nonetheless, in

some patients treated simultaneously with potentially toxic albumin-bound drugs and albumin infusions, the levels of the pharmacologically active unbound drug might transiently reach unintended high concentrations.

8.3 Paper 3

The study included 12 male and eight female liver transplant recipients, 16 of who completed both sampling sessions. The MPA C_{max} occurred at a median of 1 h after dosing, and the minimum IMPDH activity occurred simultaneously. There was no statistically significant difference between session 1 and session 2 with respect to the pharmacological variables, with the exception of free-fraction MPA at C_{max}, which was significantly higher in session 1 than in session 2. The free-fraction MPA $AUC_{0–4h}$ correlated negatively with the albumin value. The IMPDH area under the effect curve 0-4h ($AUEC_{0–4h}$) correlated with the total and free MPA $AUC_{0–4h}$ for session 1, whereas there was no significant correlation for session 2.

There was no statistically significant correlation between IMPDH $AUEC_{0–4h}$ and the free-fraction MPA $AUC_{0–4h}$ or the trough measurements of MPA, free MPA, or free-fraction MPA. The T_0 measurement for IMPDH correlated with IMPDH $AUEC_{0–4h}$ for both sessions. The percentage inhibition of IMPDH correlated with the total MPA $AUC_{0–4h}$ for session 1 and session 2, whereas free MPA only

correlated significantly for session 1. The percentage inhibition of AUC also correlated with the T_0 measurement of IMPDH for session 1 and the MPA C_{max} for both sessions. The IMPDH at MPA C_{max} correlated inversely with both MPA $AUC_{0–4h}$ and free MPA $AUC_{0–4h}$ for session 1 and with C_{max} for total and free MPA and the T_0 measurement of IMPDH for both sessions.

Despite the large variation in free-fraction MPA, which correlated with the albumin level, our study indicates that total MPA is an equivalent or better predictor of the immunosuppressive response exerted by MPA than IMPDH activity in liver transplant recipients. However, the measurement of IMPDH activity is a promising approach to TDM in patients treated with MPA. The IMPDH T_0 activity sample shows particular potential and should be investigated further.

9 Discussion

The three studies included in this thesis deal with questions regarding albumin solutions and protein binding of drugs. The reason for embarking on this topic was the renewed attention directed toward pharmaceutical albumin solutions after the Cochrane Injuries Group Albumin Reviewers reported that the administration of albumin to critically ill patients was, in fact, dangerous and probably increased the mortality of these patients (38). Although the usefulness of albumin solutions has been debated on a regular basis, it has been difficult for many to accept that albumin solutions are in fact harmful. A number of letters were written to the BMJ in response to the original article, criticizing the way the meta-analysis was conducted and the conclusions drawn (78-83). Several explanations were postulated to explain the detrimental effects of albumin solutions (85). At our institution, we took an interest in the drug-binding properties of pharmaceutical-grade albumin.

This interest in the drug-binding capacity of pharmaceutical-grade albumin was evoked by the discovery that an albumin assay (the Vitros “dry chemistry” bromocresol green albumin method) underestimated the albumin concentration in pharmaceutical-grade albumin compared with a manual method. These findings were reproduced in sera taken from patients who had received pharmaceutical-grade albumin (84). This suggested that the in vivo binding

properties and possibly the transport functions of pharmaceutical-grade albumin were less than those of endogenous albumin. One suggested explanation for the reduced binding capacity of pharmaceutical-grade albumin was the presence of stabilizers in the end product. The effect of the stabilizers in pharmaceutical-grade albumin was further investigated by Olsen at al., who demonstrated that caprylic acid and N-acetyl-DL-tryptophan, used as stabilizers in the purification process when pharmaceutical albumin is manufactured, both markedly reduced the binding capacity of pharmaceutical-grade albumin in vitro (86). These findings possibly explain why pharmaceutical-grade albumin was not beneficial when administered to critically ill patients. If pharmaceutical-grade albumin does not exhibit the binding properties of endogenous albumin, albumin solutions are merely highly priced colloids.

9.1 Drug–protein binding

The binding of drugs by proteins is a tremendously complex science. The degree of protein binding of a particular drug depends on a number of variables: the affinity of the drug for the various protein-binding sites, the plasma concentration of the protein, the molar concentration of the drug relative to the molar concentration of the unoccupied plasma-binding sites, and the presence of endogenous or exogenous compounds that may compete with the drug for the binding sites on the protein (87). Furthermore, the effects of altered protein

binding are dependent on the route of administration, the hepatic extraction ratio, and the therapeutic index.

The idea that protein-binding interactions can lead to clinically significant drug interactions emerged in the mid-1960s, with the discovery of markedly increased prothrombin times in patients treated with warfarin and phenylbutazone (88), and severe hypoglycemia in patients treated with sulfonamides and tolbutamide (89). The drugs involved in these interactions are highly protein bound. It was also demonstrated that phenylbutazone displaces warfarin from human serum albumin (90). Realistic theoretical arguments, supported by in vitro evidence and in vivo observations, led to the conclusion that these clinical interactions are caused by the displacement and increased free fraction of the drug. However, pharmacokinetic theory and the confirmation of other mechanisms to explain the observed interactions have since demonstrated that plasma-binding displacement rarely has any clinical significance. Nevertheless, the original description of a clinically significant protein-binding interaction was established and has since been difficult to eradicate. In most cases, displacement reactions and altered protein binding do not lead to any clinically significant interactions, and this has been the subject of a number of articles (87;91-93). However, they might have clinical relevance for some drugs in certain situations, and in TDM settings, they can be of major importance. I will discuss below when displacement interactions might be important, and the implications for TDM.

9.1.1 Displacement reactions

The “free drug hypothesis” maintains that only the free drug is available to cross physiological membranes and to interact with receptors. Based on this notion, it is expected that the effect of a given drug will correlate more closely to measurements of the free drug concentration than to the total drug concentration. A change in the free drug concentration as a result of its altered protein binding is therefore assumed to alter the effect of a given drug. However, the situation is much more complex. A change in the free drug concentration has a number of consequences and the net result for its PD and PK can be difficult to predict. Furthermore, it is not necessarily the drug concentration in the serum (central compartment) that is of interest, but the drug concentration at the site of action, which is in turn dependent on the physicochemical properties of the drug (12).

If drug A is displaced from its protein-binding site by drug B, the free fraction of drug A will increase acutely. The increased free fraction of drug A is then free to act at its receptor site and intuitively, one would expect the effect of drug A to increase. However, the increased concentration of the free drug is not only free to act at its receptor site, but is also available to be redistributed, metabolized, and excreted. So how can we predict the net effect of a displacement reaction?

For any displacement reaction to be clinically relevant, the drug in question must have a relatively narrow therapeutic index, or the range between the

concentration of the drug that achieves the desired response and the concentration that results in toxicity. For drugs with a broad therapeutic index, a moderate change in their free concentration may not lead to any increase in their effect or toxicity.

The redistribution of a displaced drug to a new equilibrium occurs rapidly and will have a buffering effect on the consequences of a displacement reaction. The amount of redistribution is, in turn, dependent on the volume of distribution of the particular drug. For a drug with a large volume of distribution (> 20 L), a displacement reaction would probably have minimal consequences for the free fraction of the drug because of its redistribution. However, for a drug with a relatively small volume of distribution (< 10 L), its redistribution will be modest because most of the drug is located in the central compartment (87).

The consequence of a displacement reaction on the clearance of a drug is dependent on the ability of the clearing organ to remove the drug. If this capacity to clear the drug is limited ("restrictively cleared drug"), only a small fraction of the drug is eliminated on its passage through the clearing organ. For such drugs, the clearance is proportional to the free fraction of the drug, so an increase in the free fraction would lead to a proportional increase in the clearance of the unbound drug and the concentration of the unbound drug would return to the predisplaced value after a transient increase. For a drug that is

nonrestrictively cleared, the eliminating organ removes most of the drug presented to it. Clearance is then dependent on the blood flow to the eliminating organ, and is therefore not altered by an increase in the free fraction. Consequently, an increase in the free fraction of a drug caused by a displacement reaction can be sustained and potentially result in an increased effect (87).

For drugs that have their site of action in the central compartment, the free unbound drug in the plasma is responsible for the interaction with the drug receptor and the clinical response. This is in contrast to drugs that must cross membranes to reach their target receptors, when the physiochemical properties of the drug and the involvement of drug transporters become factors (12). One example of this is the differential effects of morphine and oxycodone, both of which mediate their analgesic effects by binding to the μ-opioid receptor. In vitro, morphine has a 26-fold greater affinity for the receptor than oxycodone. From this, one would assume that morphine would be a much more potent analgesic than oxycodone. However, in vivo, the two analgesics are equipotent (94). The explanation for this finding is that the active transport of oxycodone results in a brain concentration of free oxycodone three times higher than that in the blood, whereas the free morphine concentration is merely 0.56 times higher than that in the blood (95).

For a displacement reaction to be clinically significant, the drug displaced must fulfill a number of criteria. Foremost, it must have a narrow therapeutic index. It must also be highly protein bound (> 80%), exhibit a relatively small volume of distribution, and be restrictively cleared.

9.1.1.1 Pharmaceutical-grade albumin and displacement reactions

The binding sites of the stabilizers in pharmaceutical albumin, N-acetyl-DL-tryptophan and caprylic acid (C8 fatty acid) and their drug displacement properties have been described previously (86;96). The stabilizers have also been demonstrated to displace bilirubin from its binding site on albumin (97). The demonstrated poor binding properties of pharmaceutical-grade albumin in vitro, caused by the stabilizers in the solution, raises a number of questions. Does the displacing effect endure in vivo? Can the stabilizers alter the binding properties of endogenous albumin? Does this have any clinical significance?

Our first study was designed to address these questions. We compared surgical patients transfused with albumin with those transfused with starch colloids. We were able to detect the stabilizers in the sera of the patients who received albumin, but the concentrations detected were relatively low and decreased rapidly in the subsequent samples. We conducted binding studies with three test drugs—naproxen, digitoxin, and warfarin—but only naproxen displayed reduced binding as a consequence of displacement. However, our results for the binding

studies were obscured by the unpredictable surgical setting, with variable transfusion requirements and transfusion rates, large fluid shifts, and hemodilution. To separate the effects of the stabilizers and to study the phenomena in closer detail, we proceeded to a study of healthy volunteers. In that study, we demonstrated the displacing effect on naproxen of pharmaceutical-grade albumin in vivo, but we also established the short half-lives of the stabilizers in vivo and the rapid return to the baseline binding properties of albumin. N-acetyl-DL-tryptophan had a half-life of approximately 30 min and caprylate a half-life of less than 15 min. However, the stabilizers only exerted significant displacing effects at their peak concentrations and had already disappeared in the redistribution phase. The very short-lived displacement effect of the stabilizers in pharmaceutical-grade albumin means that clinically significant drug interactions are unlikely in most clinical settings. Only during infusion and in a very short period thereafter will this effect be detectable. However, critically ill patients treated with highly protein-bound drugs with narrow therapeutic indices, while simultaneously receiving large volumes of pharmaceutical-grade albumin over a prolonged period, might be exposed to enhanced effects of these drugs. Of course, this is a relatively rare occurrence, but relatively large volumes of albumin are transfused into the pediatric intensive-care population, burn patients, patients undergoing ascites drainage, and patients in renal dialysis. If these patients are treated simultaneously with highly albumin-bound drugs with narrow therapeutic

indices, interactions with potentially serious consequences could ensue. Drugs that possibly exhibit such properties include anticancer drugs, such as etoposide, and immunosuppressant drugs, such as MPA. However, unpublished in vitro data from our own research group suggest that MPA is not displaced from albumin by albumin stabilizers. In addition to these drugs, there are several drugs used as sedatives in the intensive-care setting, such as diazepam, fentanyl, and propofol, that might be potentiated by albumin infusions, although this is probably rarely clinically important.

9.1.2 Pharmaceutical-grade albumin drug-binding properties

The study we undertook with a surgical population demonstrated that patients transfused with an albumin solution had a higher drug-binding capacity than patients treated with a starch solution. This result is not very surprising, considering that the median albumin concentration was reduced by 38% in the Starch group and remained unchanged in the Albumin group. However, this indicates that the drug-binding capacity of pharmaceutical albumin is maintained in vivo after transfusion. Whether the conserved binding properties of albumin are something that can be utilized in clinical practice is not yet clear.

9.1.3 Possible therapeutic benefits of pharmaceutical-grade albumin

Albumin plays important roles in various different physiological processes and equilibria, which distinguishes it from most other plasma proteins, which have specific functions in the body. Despite more than 70 years of experience using albumin solutions, there is still no clinical situation in which albumin has been proven to be superior to other fluids. The reason for this may well be that albumin solutions are not exceptionally beneficial or that the appropriate research has not been done. Much of the research has so far focused on the role of albumin as a volume replacement fluid. There has been little emphasis on the physiological functions of the albumin molecule or the opportunity to utilize it in targeted patient populations. Albumin fluids have also changed considerably over time, and the fluids are not uniform in their constituents, but can vary considerably from one manufacturer to another. Of course, this makes comparing different studies from different geographic areas and time periods difficult. Moreover, any potentially beneficial effects for individual patients might be lost in the study material when the research lacks focus.

For research into albumin solutions to be advanced, it is important that albumin solutions are viewed as complex and possibly multifunctional products. The standardization of albumin solutions should not be limited to their protein content, but should include other properties, such as their binding capacities and

antioxidant and anti-inflammatory potentials. The potential of albumin is being evaluated in several areas of medicine.

The utilization of a number of properties of albumin has been attempted in the field of neuroprotection. In the context of experimental ischemic stroke, pharmaceutical albumin has been found to be neuroprotective by reducing brain swelling, preventing postischemic thrombosis, supplying an antioxidant activity, in hemodilution, and increasing the perfusion to the ischemic tissue (98). A large, ongoing, multicenter placebo-controlled trial is currently investigating the efficacy of albumin solutions in this patient group (99). Albumin has also been implicated in Alzheimer's disease because of its amyloid-beta-binding capacity. A clinical trial including 29 patients showed the potential benefit of using a plasma exchange schedule with 5% albumin solution in the treatment of Alzheimer's patients (100). Albumin infusions favorably influence the total plasma oxidant capacity in acute lung injury, and a randomized controlled trial of furosemide with and without albumin demonstrated favorable oxygenation in the Albumin group (101;102). In medical therapeutics, variable albumin levels can pose a challenge with regard to highly albumin-bound drugs with narrow therapeutic indices, as outlined in the text above. For patients undergoing chemotherapy with highly cytotoxic drugs, the use of albumin infusions to maintain stable albumin levels may be beneficial in improving the predictability

and safety of these drugs, because variable albumin levels can influence the PK of the drugs and thereby their effects and adverse effects.

9.2 Protein binding and TDM

When TDM of the plasma or blood concentration of a drug is performed on highly protein-bound drugs with narrow therapeutic indices to adjust the dose, it is important to know whether changes in protein binding might occur. Most routine TDM techniques measure the total drug concentration rather than the unbound concentration. This is not a problem for drugs that display concentration independent binding to plasma proteins; total drug measurements will predict the free drug concentration and the clinical effect. However, drugs that saturate the binding capacity of the protein display concentration-dependent protein binding, and total drug measurements might not accurately predict the clinical effect of the drug.

There are several examples in the literature of how measurements of total drug concentrations might be misleading. Cortisol replacement therapy has been advocated based on the response to the cosyntropin stimulation test, which measures the total cortisol level. In a study that included critically ill patients with hypoalbuminemia, nearly 40% had subnormal total serum cortisol concentrations, despite a normal or elevated serum concentration of free cortisol

(103). Anticonvulsants, such as phenytoin, carbamazepine, and valproic acid, are strongly protein bound, mainly by albumin. The clinical utility of monitoring free phenytoin, free carbamazepine, and free valproic acid is well documented in the literature (104). Immunosuppressant drugs are another group of drugs that commonly combine a high degree of protein binding and narrow therapeutic indices, and MPA has been much debated in this regard.

9.2.1 TDM of MPA

The validity and usefulness of any TDM protocol require that the actual effect of the drug in question is measured. Whether the effect of the drug is best predicted by the total drug concentration, the free drug concentration, or some other measure depends on the properties of the specific drug and the clinical situation. A number of studies have demonstrated a correlation between MPA plasma concentrations and acute rejection episodes in a variety of transplant populations. In the renal transplant population, adjustment of the MPA dose based on the total drug concentration has been shown to be beneficial but difficult to accomplish (71;72). So far, TDM has not proven beneficial for liver transplant patients. Inter- and intrapatient variability over time may be more complex in the liver transplant population due to variable liver and kidney function and changing albumin concentrations. MPA is 98%–99% protein bound (54). An in vitro study demonstrated that increasing concentrations of MPA

were necessary to achieve 50% inhibition of IMPDH isoform II as the concentration of albumin was increased (48). Therefore, any change in protein binding or in the albumin concentration could have a major impact on the concentration of free MPA and consequently on IMPDH suppression. A recent study that included adult liver transplant recipients during the early posttransplantation period demonstrated high variability in the free MPA concentration and a lack of any relationship between the total and free concentrations of MPA. This suggests that the total MPA AUC should not be used to adapt the MMF dosing regimen during the early posttransplantation period (105).

Variations in protein binding are not the only apparent limitation of MPA AUC measurements. There appears to be a considerable overlap in the MAP concentrations that result in rejection and those that do not (73), and different MPA doses seem to be required by African-Americans and Koreans compared with other ethnic groups, despite identical PD profiles (106). Therefore, it would seem advantageous to establish a pharmacodynamic measurement closer to the clinical outcome that would better reflect the net result of the pharmacokinetic and pharmacodynamic variability.

In Study 3 in this thesis, we demonstrated a large variation in the free fraction of MPA and a negative correlation between albumin and the free fraction of MPA.

This significant variation in the free MPA concentration may have, but does not necessarily imply, clinical significance. To determine whether the variation in the free MPA concentration is clinically significant, it should be correlated with a marker of immunosuppression or a "hard" endpoint, such as organ rejection.

IMPDH is the target enzyme for MPA, and IMPDH activity can be reliably quantified, so this offers a method of describing the immunosuppression exerted by MPA (107). Exactly how the measure of IMPDH activity is linked to the immunosuppressive response has not yet been fully established. The IMPDH AUEC value (Figure 1) is the most intuitive parameter, because this could be an absolute pharmacodynamic biomarker reflecting the variations in both IMPDH activity and MPA PK. However, we know that there is great interindividual variability in IMPDH activity (108). Whether this variable, IMPDH activity, should be reduced to an absolute value or reduced to a percentage of the T_0 (predose), pretransplantation, or washout value (measured in a sample from which all MPA is removed in vitro) is at present unclear. Furthermore, we do not know whether the IMPDH activity throughout the dosing interval or its maximum suppression is the best indication of the true immunosuppressive effect exerted by MPA.

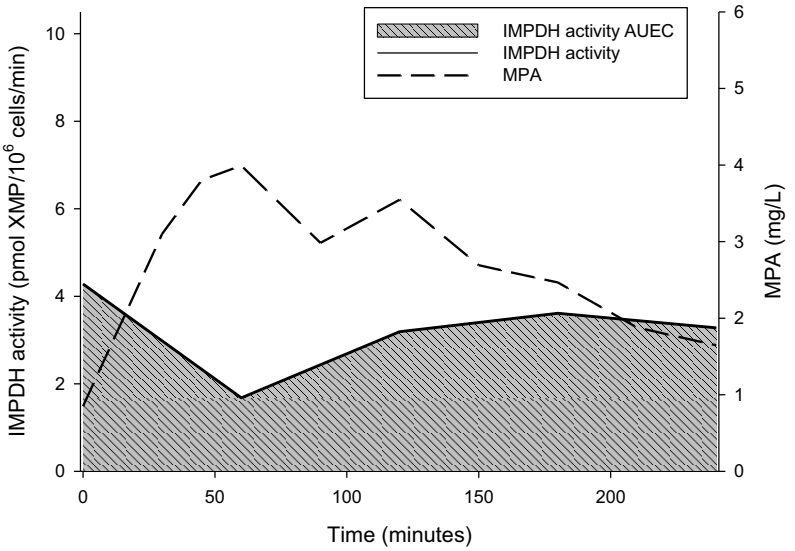


Fig. 1. Example of MPA concentration and IMPDH activity in Liver transplantation patient treated with MPA (from study 3)

In Study 3, we choose IMPDH activity in $CD4^+$ cells as our marker of the MPA-specific response. If the measured increase in the free fraction of MPA is to be clinically relevant, it must affect the IMPDH activity. The free fraction or concentration of MPA should therefore correlate better with the IMPDH activity than would the total MPA concentration. However, despite the large variation in the free fraction of MPA, the total and free MPA concentrations were equally good predictors of the immunosuppressive effect, as expressed by the IMPDH activity in $CD4^+$ cells. Whether total or free MPA is the better predictor of the immunosuppressive effect exerted by MPA is debatable. An explanation for this finding might be that the measurement of the free drug is less robust than the measurement of the total drug because of the very low concentrations of free

drug measured. Therefore, the true significance of the free drug could be lost in the analytical process. This may be reflected in our study, in which 18% of the free MPA measurements were below the lowest limit of quantification. However, the assumption that only the free fraction of a drug is pharmacologically active may be overly simplistic. This theory is based on the hypothesis that only the free drug can act on the target receptors and that protein-bound drugs are inactive. This might be true to a certain extent, but the relationship between the kinetics of the extracellular protein binding and intracellular enzyme binding of a drug may, on the cellular level, produce an equilibrium that differs from the free versus total concentrations that we can measure in the plasma.

We performed a number of correlation analyses in this study. The most robust and consistent correlation was found between T_0 IMPDH (predose activity) and $AUEC_{0–4h}$ IMPDH. For this to be a clinically significant finding, our hypothesis that $AUEC_{0–4h}$ IMPDH is a true description of the net immunosuppression exerted by MPA must be true. Nevertheless, if that assumption is correct, T_0 IMPDH is the ideal parameter to use in the TDM of MPA, because it requires only a single blood sample and it is relatively robust with regard to timing.

10 Conclusions of the thesis

- Pharmaceutical-grade albumin retains it binding capacity for naproxen in vivo. Serum from patients transfused with albumin has a better binding capacity for naproxen, digitoxin, and warfarin than serum from patients transfused with starch colloids.
- The stabilizers present in pharmaceutical-grade albumin, N-acetyl-DL-tryptophan and caprylate, displace naproxen from its binding site on albumin in vivo. The half-lives of these stabilizers in serum are short. Therefore, the clinical effect of the displacement reaction is probably negligible.
- The free fraction of MPA correlates negatively with the albumin value in liver transplant recipients.
- The total and free concentrations of MPA correlate equally well with IMPDH activity in $CD4^+$ cells.
- The T_0 IMPDH measurement appears to be a robust measure of the immunosuppressive effect exerted by MPA in liver transplant recipients.

11 Future perspectives

Albumin solutions undoubtedly have a number of pharmacological properties. The historical studies in which albumin solutions have been used as a universal colloid, with little regard to its pharmacological properties, probably have little relevance in determining the place of albumin solutions in modern medicine. The pharmacological properties of albumin solutions should be explored in a more systematic way. Our work has demonstrated that albumin solutions retain their drug-binding properties in vivo. This might be exploited in some clinical settings in which a predictable and stable pharmacokinetic profile of a highly protein-bound drug is essential for the required outcome. One such setting, which would be interesting to explore, is the administration of highly cytotoxic drugs, such as etoposide, in anticancer protocols. Patients who receive this podophyllotoxin derivative comprise a group with highly variable albumin concentrations, attributable to the disease itself and as a consequence of the treatment. Alteration of the albumin concentration might result in a higher concentration of free drug, with a potentially enhanced effect. Alternatively, the increased free drug concentration might result in its more rapid elimination and a shorter half-life, with resulting treatment failure. Transfusion of these patients with albumin to keep their albumin levels stable during treatment may produce a more predictable pharmacokinetic profile and a consequently better outcome.

IMPDH activity is a pharmacodynamic marker that has shown promise as a parameter with which to tailor immunosuppressive treatments. Our research indicates that the T_0 measurement is particularly robust. However, a clinical study correlating this measure with hard end-points, such as organ rejection and adverse MPA effects, is required to validate our findings.

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