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DRUG ADMINISTRATION AND BLOOD SAMPLING FOR PHARMACOKINETIC STUDIES IN PEDIATRIC CANCER PATIENTS

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To my Parents

"If I would do it all over again?
Why not,
I would do it slightly different"
- Freddy Mercury

ABSTRACT

This thesis focuses on drug administration and blood sampling for pharmacokinetic studies in pediatric cancer patients. Only about one third of the drugs used to treat children have been adequately tested with sufficient information regarding safety and efficacy for pediatric patients. The European regulation has led to a focus on pre-clinical and clinical trials within the pediatric population. A prerequisite for a quality assurance of trials is a standardized drug administration and suitable blood sampling procedures.

We have shown that blood sampling from the central venous access can be used under certain circumstances for therapeutic drug monitoring of methotrexate. However, carefully evaluated standardized instructions regarding rinsing and flushing after drug administration is required if blood samples for drug concentrations should be withdrawn from the central venous access. The importance of minimizing the total discarded blood volume, i.e. waste volume and sampling volume when using the central venous access has to be emphasized.

A standardized routine for intravenous drug administration was developed enabling an exact time point for start and cessation of the intravenous infusion which is crucial when blood sampling is performed solely after the infusion. A standardized short time infusion was used for studying the pharmacokinetics of tobramycin which revealed that dosing of tobramycin based on body surface area appears to be more consistent than dosing based on body weight. Furthermore, our pharmacokinetic findings enable a possibility to adjust the dose to obtain a predetermined target values of systemic drug exposure (AUC) and AUC:MIC ratio. The influence of the infusion time of the maximum serum concentration of tobramycin can be predicted from the determined pharmacokinetic data with the possibility to control the peak concentration without affecting AUC.

The standardized drug administration enabled the possibility to develop a limited sampling strategy for estimation of the tobramycin AUC. One blood sample gives an accurate estimate of the AUC enabling a valuable tool in therapeutic drug monitoring and for pharmacokinetic studies in large groups of pediatric patients. The actual sampling time is of great importance while a minor deviation in the infusion time is of less significance for the estimation of AUC using the developed limited strategy.

Despite the importance of reducing painful procedures capillary blood sampling can be suitable for pharmacokinetic studies of doxorubicin using a limited sampling strategy based on one concentration measurement at each treatment occasion in pediatric patients.

In summary, this thesis has highlighted important areas to be considered when performing pharmacokinetic studies in children.

LIST OF PUBLICATIONS

This thesis is based on the following papers, which will be referred to in the text by their Roman numerals:

I. **Ritzmo C,** Albertioni F, Cosic K, Söderhäll S, Eksborg S. Therapeutic Drug Monitoring of Methotrexate on the Pediatric Oncology Ward: Can Blood Sampling From Central Venous Accesses Substitute for Capillary Finger Punctures? *Ther Drug Monit. 2007;29:447-451.*

II. **Ritzmo C,** Söderhäll S, Eksborg S. Pharmacokinetic Studies in Pediatric Cancer Patients – Standardization of Intravenous Drug Administration. *Manuscript.*

III. **Ritzmo C,** Eksborg S, Kalin M, Söderhäll S, Jakobson Å. Pharmacokinetics of Tobramycin after an Intravenous Short Time Infusion in Paediatric Cancer Patients. *Submitted to Acta Paediatrica.*

IV. **Ritzmo C,** Jakobson Å, Söderhäll S, Eksborg S. Limited sampling strategy for estimation of the tobramycin area under the serum concentration versus time curve after an intravenous short time infusion. *Manuscript.*

V. **Palm C,** Björk O, Björkholm M, Eksborg S. Quantification of doxorubicin in plasma--a comparative study of capillary and venous blood sampling. *Anticancer Drugs. 2001;12:859-64.*

VI. **Ritzmo C,** Söderhäll S, Karlén J, Nygren H, Eksborg S. Pharmacokinetics of doxorubicin and etoposide in a morbidly obese pediatric patient. *Pediatr Hematol Oncol. 2007;24:437-445.*

The previously published papers were reprinted with kind permission from Wolters Kluwer Health (I, V) and Informa Healthcare (VI).

Additional paper

Ritzmo C, Eksborg S and Söderhäll S. Pharmacokinetics of anthraquinone glycosides in childhood acute lymphoblastic leukaemia. *Manuscript in preparation.*

CONTENTS

LIST OF ABBREVIATIONS

95% CI	95% confidence interval
α	distribution rate constant
β	elimination rate constant
ALL	acute lympblastic leukemia
AUC	area under the serum concentration versus time curve
BMI	body mass index
BSA	body surface area
BW	body weight
C	Celsius
C_{bol}	concentration for a bolus injection
C(0)	the anticipated initial plasma drug concentration, given as the intercept on the concentration axis when extrapolated back to time zero
Cl	serum clearance
C_{max}	maximum serum concentration
CV	coefficient of variation
CVAD	central venous access devices
DS	Down's syndrome
GFR	glomerular filtration rate
h	hour
k	rate constant
MPE%	percentage of mean prediction error
MIC	minimal inhibitory concentration
MRD	minimal residual disease
MTD	maximum tolerated dose
r_s	spearman rank correlation coefficient
RMSE%	percentage of root mean square prediction error
t	time
$t_{1/2}$	half-life
TDM	therapeutic drug monitoring
Vd_{area}	apparent volume of distribution

1 INTRODUCTION

1.1 OVERVIEW OF THE THESIS

This thesis focuses on drug administration and blood sampling for pharmacokinetic studies in pediatric cancer patients. Fortunately, the former attitude to protect children against clinical research in drug development has been replaced by the attitude to protect children though research (Rose 2009). Approximately two thirds of the drugs used to treat children have not been adequately tested and information regarding safety and efficacy for pediatric patients is insufficient or absent (Roberts *et al.* 2003; Vassal 2009). Drug dosing in children has therefore been based on a trial and error approach (Roberts *et al.* 2003). However, the experience of the pediatricians and the numerous clinical trials that have been performed have enabled a safe drug treatment despite the lack of information regarding safety and efficacy (Vassal 2009).

The importance and need for age-appropriate pharmacotherapy was already pointed out more than 100 years ago by the American pediatrician Dr. Abraham Jacobi who wrote "Pediatrics does not deal with miniature men and women, with reduced doses and the same class of disease in smaller bodies, but… has its own independent range and horizon" (Kearns *et al.* 2003).

The pediatric population is a vulnerable group with developmental, physiological and psychological differences from adults, which makes age and development related research of drugs particularly important (Kearns *et al.* 2003). The European regulation on medicines for pediatric use (Dunne 2007) has led to a focus on the need for pre-clinical and clinical trials within the pediatric population. A prerequisite for a quality assurance of trials is a standardized drug administration and suitable blood sampling procedures.

The main objective of this research project is to emphasize areas of special importance for pharmacokinetic studies and for therapeutic drug monitoring in pediatric patients including infants.

1.2 CHILDHOOD MALIGNANCIES

Cancer in children is rare and accounts for 1% of all cancers in humans (Vassal 2009). In Sweden approximately 250 children under the age of 15 are diagnosed with cancer each year (Socialstyrelsen 2009).

Cancer in children differs significantly from cancers in adults. Many pediatric tumours arise from embryonal precursor cells while environmental factors may influence the development of adult tumours (McGregor *et al.* 2007; Voute *et al.* 2005) . The most common malignancies in childhood are leukemia and lymphoma (~40%) followed by brain tumours (~30%) and a diversity of other malignancies (Gustafsson *et al.* 2007) whereas the most common tumours occurring in adulthood are carcinomas in the breast, prostate, large intestine and lung (Socialstyrelsen 2009).

Today, more than 75% of the children with cancer are cured if they are treated appropriately (McGregor *et al.* 2007). Despite the high cure rates cancer still remains one of the leading causes of death in children below the age of 15 (Socialstyrelsen 2009). Furthermore, the intensive treatment with the risk of toxicity and late-effects for the survivals are also areas requiring further efforts (McGregor *et al.* 2007).

1.3 CANCER TREATMENT

The prognosis of childhood cancer has improved dramatically since the introduction of chemotherapy for the treatment of childhood leukemia more than 60 years ago (Farber *et al.* 1948).
The successful treatment is mainly due to the multimodality approach, which integrates surgery and radiotherapy to control local disease with chemotherapy to eradicate systemic (metastatic) disease (Hammond 1986). This multimodality approach has become the standard approach for treatment of most childhood cancers today.

1.3.1 Chemotherapy

Chemotherapy plays a major role in the treatment of childhood malignancies due to the high proliferation rate and the high ability of the malignant cells to become apoptotic (Voute *et al.* 2005). Generally, the first goal with chemotherapy based strategy is to obtain complete remission and then eradicating the minimal residual disease (MRD) (Voute *et al.* 2005).

The goal of cancer chemotherapy in pediatric and medical oncology is to cure patients by eradicating all cancer cells and on empiric observations made in early clinical trials involving children with drug-sensitive cancers, such as acute lymphoblastic leukemia (ALL) (Pizzo and Poplack 2006). These basic principles include the use of multidrug combination regimens, the administration of drugs at the maximally tolerated dose rate (i.e. dose intensity) and the administration of chemotherapy before the development of clinically evident metastatic disease.

The primary rationale for using combination chemotherapy is to overcome drug resistance to individual drugs (Goldie and Coldman 1984). It is not feasible to accurately predict whether a particular patient's tumour will respond to a given drug and administering anticancer drugs in combination ensures a greater chance of achieving a response, i.e. exposing the tumour to at least one active agent (Pinkel *et al.* 1971, Pizzo and Poplack 2006). The combination therapy may also prevent or delay the development of acquired resistance in initially responsive tumours (Goldie 2001, Pinkel *et al.* 1971).

Most anticancer drugs interfere at some stages with the synthesis or function of the nucleic acids, DNA and RNA. Anticancer drugs are classified by their mode of action and the combination regimens utilize the substances different cytotoxic mechanisms to achieve additive or synergistic effects to destroy malignant cells (Pizzo and Poplack 2006).

1.3.1.1 Methotrexate

The antimetabolite methotrexate competitively inhibits the enzyme dihydrofolate reductase and interferes with several critical pathways such as synthesis of DNA, RNA and proteins which require folate cofactors (Crom *et al.* 1987).

Intravenous methotrexate therapy is widely used for the treatment of various neoplastic diseases, both in adults and in children. Children with acute lymphoblastic leukaemia and osteogenic sarcoma are treated with methotrexate doses of 5 and 12 g/m^2, respectively (Gustafsson *et al.* 1998; Smeland *et al.* 2003). Methotrexate at such high doses is potentially lethal, but the development of severe toxicity can be minimized by a subsequent rescue with calcium folinate, increased hydration and alkalization of urine (Bleyer 1978). The calcium folinate dose is based on measured methotrexate concentrations in plasma collected after cessation of administration, traditionally obtained by repeated finger lancet punctures (Bleyer 1978).

1.3.1.2 Doxorubicin

Anthraquinone glycosides, i.e. doxorubicin, epirubicin, daunorubicin and idarubicin, is an important class of antineoplastic drugs. The drugs are topoisomeras II inhibitors and acts by interfering with the enzyme topoisomerase II which unfolds the DNA molecule during DNA replication, transcription and repair (Kuffel *et al.* 1992). DNA intercalation and production of reactive oxygen radicals are other mechanisms of action (Kuffel *et al.* 1992).

Doxorubicin, the most frequently used drug within this class, has activity against a large variety of tumours in children as well in adults (Speth *et al.* 1988). The clinical use of doxorubicin is limited by the myelosuppression and irreversible cardiac toxicity (Lipshultz *et al.* 1991; Speth *et al.* 1988).

1.3.1.3 Etoposide

Etoposide is phase-specific drug acting in the late S and early G_2 phases of the cell cycle (Knoester *et al.* 1993). The mechanism of action is to cause breaks in DNA by either stabilization of type II topoisomerase-DNA complexes or by the formation of free radicals (Slevin 1991). Etoposide is active against both hematological and solid tumours (Belani *et al.* 1994).

1.3.2 Side-effects

Most chemotherapeutic drugs acts on all proliferating cells, and hence both malignant cells and normal cells are affected (Hoekman *et al.* 1999). Acute toxicity, e.g. myelosuppression, nausea, vomiting, and hair loss, which occurs hours or weeks after drug administration are usually reversible (Hoekman *et al.* 1999). Myelosuppression induced by chemotherapy affects all three major cell lines, i.e. platelets, erythrocytes and neutrophils, in the bone marrow (Kuhn 2002). Damage to platelets can result in bleeding while damage to erythrocytes may cause fatigue (Kuhn 2002). Decreasing neutrophils may result in an increased risk of infections (Kuhn 2002).

Some drugs have a specific toxicity for one or more organs, e.g. anthracyclines may cause cardiotoxicity (Hoekman *et al.* 1999). Anthracycline induced cardiotoxicity may occur during treatment, weeks or even years after completion of chemotherapy (Raschi *et al.* 2009). Risk factors for cardiotoxicity induced by anthracyclines includes high-cumulative dose, high dose intensity, female gender, young and older age (Lipshultz 2006).

1.3.2.1 Neutropenia

Neutropenia is one of the most important risk factors for infections in children undergoing chemotherapy. Neutropenia, i.e. defined as a neutrophil count <500 cells/mm^3, or a predicted decrease from <1000 cells/mm^3 to 500 cells/mm^3, increases the risk of infections considerably (Hughes *et al.* 2002). The infections may be life-threatening and requires hospitalization, antimicrobial therapy and can lead to postponed chemotherapy which might compromise disease free survival (Kuhn 2002). Prolonged periods of neutropenia, i.e. 10 days or more, and a neutrophil count of less than 100 cells/mm^3 increases the risk of mortality (Pizzo 1999).

Fever might be the only symptom of a severe infection immunocompromised patients and a specific site of infection is generally lacking (Pizzo 1999). Neutropenic fever is defined as oral temperature >38.0 ºC on two occasions 60 minutes apart or >38.5 ºC on one occasion and a neutrophil count less than or equal to 500 cells/mm^3. The risk of rapid progression of an infection in the neutropenic patient requires, after relevant cultures are obtained, a prompt initiation of empirical antibiotic therapy at the onset of fever (Hughes *et al.* 2002).

1.3.3 Supportive care

Supportive care is an important part of the treatment of cancer. Therapeutic approaches to reduce treatment toxicity, e.g. the administration of leucovorin to counteract the toxicity of high-dose methotrexate (Bleyer 1978), the use of antiemetics to block nausea and vomiting (Roila and Del Favero 1997) and antibiotics for infections are examples of ways to make the therapy more tolerable.

1.3.3.1 Empirical antibiotic treatment in patients with neutropenic fever

Aminoglycosides (e.g. tobramycin) have for many years been used in combination therapy as empirical treatment in neutropenic febrile episodes in pediatric cancer patients (Drusano *et al.* 2007). These drugs have a broad antimicrobial spectrum. Moreover, tobramycin is highly active against *Pseudomonas aeruginosa* (Anderson *et al.* 1975). Aminoglycosides exert a concentration-dependent anti-bacterial effect and have a prolonged postantibiotic effect, i.e. the bacterial killing continues after the serum concentration has declined below the minimal inhibitory concentration (MIC) (Burgess 2005; Drusano *et al.* 2007; Turnidge 2003; Zhanel and Craig 1994). Peak concentration as well as the systemic drug exposure (area under the concentration versus time curve) above MIC of the bacterial pathogen, have been related to the efficacy and toxicity (Begg *et al.* 2001; Burgess 2005; Drusano *et al.* 2007; Turnidge 2003).

1.4 DRUG DOSING IN PEDIATRIC ONCOLOGY

The continuous change in body weight and body composition throughout infancy and childhood complicated pediatric drug therapy and especially dose calculations (Rane and Wilson 1976).

1.4.1 Dosing of chemotherapy

Dosing of anticancer drugs based on body surface area (BSA) dates back more than half a century (Crawford *et al.* 1950; Pinkel 1958). The BSA dosing concept is based on a correlation between BSA and some particular patient characteristics such as the glomerular filtration rate (GFR) and blood volume (Felici *et al.* 2002). The aim of normalizing using BSA rather than body weight is to reduce the relative dose as the body size increases (Reilly and Workman 1993) and avoid overdosing in older children (Bartelink *et al.* 2006).

BSA was originally calculated using the formula by DuBois and DuBois using height and weight (DuBois and DuBois 1916). This formula has been questioned since it is based on only nine individuals (Sawyer and Ratain 2001). The Mosteller formula enabled a simplified calculation of BSA and is frequently used within pediatric oncology today (Mosteller 1987). One drawback with the BSA calculation is to accurately assess the patient's height and weight (Sawyer and Ratain 2001).

Despite the fact that dosing of anticancer drugs normally is based on BSA some exceptions exists. In infants, i.e. below one year of age, dosing based on body weight has been recommended since the relationship between body weight and BSA are different as compared to older children (Voute *et al.* 2005). However, it has been pointed out that evaluation of pharmacokinetic characteristics is required, especially in neonates and infants, to allow more precise dosage recommendations for anticancer drugs (McLeod *et al.* 1992).

In obese patients dosing based on ideal body weight is often made, due to an ill-defined assumption that dosing in relation to actual BSA puts obese patients at an increased risk for toxicity (Baker *et al.* 1995; Poikonen *et al.* 2001). However, inappropriate dose reductions may compromise the treatment efficacy (Baker *et al.* 1995).

1.4.2 Dosing of antibiotics

Four main methods, i.e. age-based, bodyweight-based, body surface area-based dosing regimens and allometric scaling have been described for dose calculations (Bartelink *et al.* 2006). Dosing of aminoglycosides is normally based on bodyweight (Bragonier and Brown 1998; Dupuis *et al*. 2004; Turnridge 2003). A need for increasing the doses expressed in mg/kg with decreasing age has previously been reported (Dupuis *et al.* 2004).

1.5 PHARMACOKINETICS

The area under the concentration versus time curve (AUC), i.e. the systemic drug exposure is one important pharmacokinetic parameter (van Warmerdam *et al.* 1994). Other common pharmacokinetic parameters are clearance, i.e. the measure of the

body's ability to remove the drug from the blood or plasma, $t_{1/2}$, i.e. the time period required for the concentration to be reduced by one-half and vd_{area} which refers to the apparent volume required to account for all of the drug in the body if it were present in the same concentration as in plasma (Levy and Bauer 1986, Undevia *et al.* 2005).

The fate of the drug in the body can be described in terms of compartmentalized systems, e.g. one- and two-compartment models (Levy and Bauer 1986). In the two-compartment model there is a visible distribution phase which is not apparent in the one-compartment model (Levy and Bauer 1986).

Despite the goal of a consistent dosing of anticancer drugs using BSA a considerable inter patient variability in the systemic drug exposure exists (Eksborg *et al.* 1985; Frost *et al.* 2002; Ratain *et al.* 1990; Speth *et al.* 1988;). Dose individualization has been considered the best method o§f reducing interindividual variability (Undevia *et al.* 2005). Furthermore, dose adjustments based on drug plasma concentrations or AUC has been suggested to improve the efficacy while reducing toxicity (DeSoize and Robert 1994).

Pharmacokinetic studies form an essential part of the development of new drugs, drug regimens and treatment optimization (Hvidberg 1990). The validity of calculated pharmacokinetic parameters depends in part on an accurate drug administration, the site for blood sampling and correct timing of sampling.

Pharmacokinetic parameters, e.g. clearance and volume of distribution, are usually estimated from plasma/serum concentration time curves which require the withdrawal of blood samples during and after cessation of the infusion (Reed 1999). The accuracy of the estimated parameters is generally increased with increasing number of blood sample (van Warmerdam *et al.* 1994).

1.6 LIMITED SAMPLING STRATEGIES

Limited sampling strategies are an approach to perform pharmacokinetic studies while circumventing problems such as multiple venipuntures which can be inconvenient for both the patient and the medical staff (van Warmerdam *et al.* 1994). Limited sampling models have been developed for several anticancer drugs (Eksborg 1990; Liliemark *et al.* 1996, Ratain and Vogelzang 1987; Ratain *et al.* 1991, Strömgren *et al.*1993).

Population pharmacokinetics has become an increasingly used method and has been especially appealing for pharmacokinetic studies involving infants and children (Finkelstein *et al.* 2009). However, discrepancies in pharmacokinetic analysis results for doxorubicin obtained by using two standard population pharmacokinetic software programs have been reported (Finkelstein *et al.* 2009).

1.7 THERAPEUTIC DRUG MONITORING

Chemotherapy dosing is toxicity-based. Anticancer drugs have the lowest therapeutic index of any class of drugs and produce significant, even life-threatening toxicity at therapeutic doses (Bleyer 1978; Kuhn 2002). However, implementing a significant

dose reduction or delay in therapy to attenuate the toxicity may comprise the therapeutic effect and an increased risk of tumour recurrence (Pizzo and Poplack 2006). Unfortunately, the adjustments in the dose and treatment schedule needed to balance the risks of toxicities from therapy against the risk of tumour recurrence must often be made empirically, since therapeutic drug monitoring (TDM) for most agents is not available (Hon and Evans 1998). An individualized drug dose and schedule based on specific patient characteristics and on plasma drug concentration measurements, i.e. TDM, have been suggested to be a more rational approach (Pizzo and Poplack 2006, Sparreboom 2005).

1.7.1 Methotrexate

Monitoring of methotrexate plasma concentrations is essential in high-dose therapy as measured concentrations specifies when there is a need for an increased hydration, increase of the calcium folinate rescue or when the rescue safely can be discontinued (Bleyer 1978).

1.7.2 Aminoglycosides

TDM based on peak and trough concentrations have been used for aminoglycosides, e.g. tobramycin, due to their narrow therapeutic index with the potential risks for severe nephro- and ototoxicity. Elevated trough concentrations of tobramycin are associated with nephrotoxicity (Knoderer *et al.* 2003; Turnidge 2003) probably due to a delayed elimination resulting in drug accumulation (Begg *et al.* 1995). TDM with a concentration measurement immediately prior to the next administration ("trough concentrations") is often used to identify patients with delayed elimination.

1.8 INTRAVENOUS DRUG ADMINISTRATION

Inappropriate drug administration techniques including inaccurate infusion times, incorrect administration of previous doses, remaining or loss of drug solution in administration devices may produce misleading measured drug concentration (Gould and Roberts 1979; Higashida 1989; Leff and Roberts 1981, Nahata 1988; Roberts 1981).

The intravenous route for drug administration has been considered to result in a prompt drug delivery. However, intravenous drug infusions with antineoplastic drugs are usually administered using an infusion set, i.e. tubing and drip chamber, pre-filled with saline or glucose connected to the infusion bag. Moreover, intravenous infusions are commonly finalized using a flush drip to ensure complete drug delivery. This procedure limits the possibilities to record an exact time point for start and cessation of the infusion and is unsuitable for pharmacokinetic studies without blood sampling during the infusion. Central venous access devices (CVAD) equipped with extension tubing and three-way stop-cocks may also contribute to problems with prompt drug delivery.

Duration of the "bolus" injection must be carefully specified since the peak concentrations are often strongly dependent on the administration time.

1.9 CENTRAL VENOUS ACCESSES DEVICES

CVAD are mandatory in the care of patients undergoing intensive chemotherapy (Johansson *et al.* 2004). The CVAD is used for administration of chemotherapy, blood products, antibiotics and many other supportive medications (Johansson *et al.* 2004). CVAD also provide the ability to obtain blood samples without the pain and anxiety associated with venipuncture or capillary finger pricks (Barton *et al.* 2004; Gyves *et al.* 1984).

There are currently several types of CVAD in clinical use, e.g. tunnelled central venous catheters, subcutaneous port systems and peripherally inserted central venous catheters.

1.9.1 Subcutaneous injection ports

In the early 1980s the totally implanted subcutaneous port system was introduced (Niederhuber *et al.* 1982; Starkhammar and Bengtsson 1985). The system consists of a metal or a plastic reservoir with a silicone membrane (Gyves *et al.* 1984), placed in a subcutaneous pocket usually at the chest below the clavicle. The reservoir is connected to a catheter tunneled under the skin and inserted into a central vein. A Huber-point needle is put through the skin and the silicone membrane into the PORT when the system is to be used, Figure 1.

Figure 1. The subcutaneous injection port, i.e. the metal reservoir with a silicone membrane. A Huber-point needle is put through the membrane.

1.10 BLOOD SAMPLING

Blood sampling are commonly performed by capillary finger pricks or peripheral venous puncture. Most pharmacokinetic investigations require repeated peripheral venous blood sampling (van Warmerdam *et al.* 1994). Venipuncture can be a frightening and painful experience for children but may also result in an excessive blood loss (Fradet *et al.* 1989, Harrison 1991, Kauffman and Kearns 1992). Anxiety and pain is common even in minor procedures (Ljungman *et al.* 2000). Repeated capillary can therefore be unsuitable in some circumstances (Kauffman and Kearns

1992). CVAD provide reliable access to the blood stream for administration of drugs, parental nutrition but are also used for blood sampling (Barton *et al.* 2004).

The importance of limited sampling strategies and population pharmacokinetics for minimizing the inconvenience for children has been emphasized (Cole *et al.* 2006).

1.10.1 Blood sampling from central venous access devices

Three different techniques, i.e. the discarded method, the reinfusion method and the push-pull or mixing method, have been described for blood sampling from central venous accesses (Frey 2003).

1.10.1.1 The discarded method

The discarded method have been reported to be the most frequently used method in pediatric bone marrow transplant units (Keller 1994). The purpose is to discard an appropriate volume of blood to clear the catheter of potential contaminates such as saline or heparin to obtain an accurate blood sample for analysis (Frey 2003). Discarded volumes of 0.5 to 10 mL have raised concern of a significant risk of blood loss which is a disadvantages (Keller 1994). A discarded volume of 5 mL has been considered as a standard volume to be discarded but a volume of 3 mL has been suggested to sufficient (Cole *et al.* 2006).

1.10.1.2 The reinfusion method

The reinfusion method minimizes the blood loss associated with diagnostic blood sampling (Adlard 2008). Instead of discarding the blood volume needed to clear the catheter from contaminates the discarded blood volume is reinfused after the blood sample for analysis is obtained (Adlard 2008). Due to concerns for introducing blood clots (Cosca *et al.* 1998) or possible contamination of the blood being reinfused (Frey 2003) the method has not gain wide acceptance in the clinical practice (Adlard 2008).

1.10.1.3 The push-pull method

The push-pull method limits the blood loss as well as the potential risk of introducing pathogens (Adlard 2008). The blood volume needed to clear the catheter is withdrawn and reinfused 3 to 4 times without removal of the syringe followed by using a second syringe and obtaining the blood sample for analysis (Adlard 2008). Disadvantages have been expressed due to risk for hemolysis of the blood and possible difficulties in obtaining enough blood for the push-pull technique.

1.10.2 Blood sampling site dependence

Because of the concern that blood samples for drug concentration measurements obtained from CVAD may provide spurious results, it has been suggested that blood samples should be drawn from peripheral veins or from a catheter lumen not used for drug administration (Busca *et al.* 1994; Carreras *et al.* 1988; Franson *et al.* 1987; McBeth *et al.* 2004; Shulman *et al.* 1998).

Contamination of the specimen by residual drug within the catheter lumen (Busca *et al.* 1994; Claviez *et al.* 2002; Shulman *et al.* 1998) can cause divergent observed drug

concentrations. The importance of an adequately saline volume for flushing the CVAD prior to withdrawal of blood samples for tobramycin has been reported (Mogayzel *et al.* 2008).

Differences between capillary and venous blood samples have been reported (Bömelburg 1987, Chiou 1989a; Chiou 1989b Murphy *et al.* 1990). Unappreciated differences in drug concentration from different sampling sites sometimes result in an entirely different interpretation of pharmacokinetic parameters (Chiou 1989a; Chiou 1989b).

2 AIMS OF THE THESIS

2.1 GENERAL AIM

The primary aim of this thesis is to standardize routines for drug administration and blood sampling for use in pharmacokinetic studies in pediatric cancer patients.

2.2 SPECIFIC AIMS

The specific aims of the thesis were to:

- Evaluate the possibility to substitute capillary blood sampling with blood samples drawn from subcutaneous injection port for the analysis of methotrexate concentrations for regulation of the subsequent leucovorin rescue (Paper I).

- Obtain exact time points for start and cessation of the intravenous infusion while ensuring that the scheduled dose is administrated to the patient by the use of proper technique for drug administration (Paper II).

- Use a standardized short time intravenous infusion for studying the pharmacokinetics of tobramycin (Nebcina®) (Paper III).

- Develop a limited sampling strategy for estimation of the systemic drug exposure of tobramycin and to investigate the impact of the infusion time and a deviation in the actual time for blood sampling using this strategy (Paper IV).

- Evaluate the possibility to substitute venous blood samples with capillary sampling for therapeutic drug monitoring of the anthraquinone glycoside doxorubicin (Paper V).

- Demonstrate the usefulness of a limited sampling strategy for doxorubicin in children (Paper VI and preliminary results).

3 MATERIALS AND METHODS

The methods used in this thesis are briefly described below. Detailed information is found in the individual papers.

3.1 PATIENTS

All patients included in the papers are patients suffering from malignancies with the majority being children or adolescents expect in paper V which also includes fourteen adults. The pediatric diagnosis includes acute lymphoblastic and myeloblastic leukemia, osteogenic sarcoma, ewings sarcoma, rhabodomyosarcoma, hodgkin disease, non-Hodgkin lymphoma and neuroblastoma The adult malignancies includes hodgkin's disease, non-Hodgkin lymphoma, multiple myeloma, and chronic lymphoblastic leukemia.

In paper I nine pediatric patients with a median age of 15 years were included. Twenty-three patients with a median age of 9 years were included in paper III. The pharmacokinetic data from those patients were utilized in study IV.

Sixteen patients with a median age of 37 years were included in study V. Paper VI consisted from the being of solely one patient (age: 14 year; BMI=46.3 kg/m^2). However, during the preparation of the manuscript one additional patient female patient (age: 15 year; BMI=42.6 kg/m^2) was sampled for therapeutic drug monitoring in the clinical routine care.

The presented preliminary results include a total of 33 patients (12 standard risk, 14 intermediate risk and 7 high risk patients) with acute lympblastic leukemia treated.

3.2 ETHICS

All patients and/or their parents gave their informed consent to participate in the studies. Study I was primarily classified as a quality assurance project not considered to require an ethical approval. However, later on the study was reviewed by the local ethics committee whom had no ethical considerations regarding the project.

Study III was approved by the regional ethics committee in Stockholm and the Swedish Medical Products Agency. The regional ethics Committee at Karolinska Hospital in Stockholm approved study V and VI.

Study II and IV were not considered to require an ethical approval since study II was a laboratory experiment and study IV utilizing the pharmacokinetic data obtained in study III.

3.3 DRUG ADMINISTRATION

3.3.1 Methotrexate

Four patients were treated with methotrexate (12 g/m^2) as a 4 hour constant infusion according to the SSG VIII Osteosarcoma protocol (Smeland *et al.* 2003).

Five patients, including the lymphoma patient, were treated with methotrexate (5 g/m^2) as a 24 h hour constant infusion according to the 1992 ALL treatment protocol of the Nordic Society for Paediatric Haematology and Oncology (NOPHO) (Gustafsson *et al.* 1998). Ten percent of the dose was administered over 1 h and the remaining 90% as a constant infusion for 23 hours, using an IVAC Model 561 infusion pump (Medical Systems Scandinavia, Täby, Sweden).

All drug infusions were followed by rinsing the catheter with 25 mL of saline.

3.3.2 Tobramycin

Tobramycin, 8 mg/kg, was diluted to a final volume of 10 mL and administration as a standardized constant rate intravenous infusion during 5.0 minutes using the subcutaneous access port. After drug administration the CVAD was carefully rinsed with 15 mL of saline.

3.3.3 Doxorubicin and etoposide

In paper V, the median doxorubicin dose was 33.6 mg/m^2. All patients were treated according to their individual treatment protocol depending on their diagnosis which is not outlined in this thesis. The adult patients were given an intravenous infusion during 2 hours while the two of the pediatric patients received the infusion over a period of 4 and 6 h, respectively. The remaining four pediatric patients including the patients in the preliminary results reported were given doxorubicin according to the NOPHO ALL-92 protocol, i.e. as a 24 h intravenous infusion (Gustafsson *et al.* 1998).

In paper VI, the male obese patient received doxorubicin and etoposide over a 4 and 2 hour period, respectively, according to the OEPA course in the GPOH-HD 2002 pilot protocol (Graubner *et al.*). In addition, one female obese patient was treated with doxorubicin (4 hour infusion) according to the 2000 ALL treatment protocol of the Nordic Society for Paediatric Haematology and Oncology. The doses of doxorubicin and etoposide were based on an adjusted BSA. The height of the patient was plotted in a growth curve for the appropriate gender and age, giving the expected body weight with a span of -2 to +2 standard deviations. The upper limit of the expected body weight was used to calculate the adjusted BSA.

3.4 BLOOD SAMPLING

In study I, methotrexate concentration measurements in capillary blood samples were compared with blood samples drawn from the subcutaneous access port. Capillary and venous blood samples were obtained within 3 minutes of each other at a total of 71 occasions. The samples were collected every 6th hour beginning 24 hours and 36 hours after start of the 4 h and the 24 h methotrexate infusions, respectively, until the methotrexate concentrations were repeatedly below 0.2 μmole/L.

In study III blood sampling for tobramycin concentrations were obtained from the subcutaneous injection port during an 8 hour period with a total number of eleven blood samples per patient using the reinfusion technique, Figure 2.

Doxorubicin and doxorubicinol concentration measurements from capillary and venous blood samples were compared in study V. The capillary and venous blood samples were collected simultaneously, i.e. with a time difference of less than 30 seconds (11 patients) or less than 5 minutes (five patients).

In study VI as well as in the presented preliminary results in this thesis, capillary blood sampling was used in combination with a previously developed limited sampling procedure (Eksborg 1990), i.e. with blood sampling 15 minutes and 1 hour prior to cessation of the 2 to 6 and 24 h doxorubicin infusion, respectively.

Blood samples for etoposide concentrations were obtained immediately prior to start and 1, 2, 3, 6, 12 h after the end of infusion. Blood sampling was performed using the subcutaneous access port.

3.4.1 Sampling from central venous access

The sampling procedure followed the clinical routine, i.e. flushing the catheter with 10 mL of saline and discarding 5 mL prior to withdrawal of the blood sample (2 mL) for concentration measurement of methotrexate (Paper I).

In paper III, VI (etoposide), the blood samples where drawn from the subcutaneous access port using the distal three-way stopcock. Blood sampling was preceded by flushing the catheter with 10 mL of saline and withdrawal of 10 mL of waste blood into a syringe firmly attached to the stopcock, Figure 2. A second syringe was attached to the remaining position in the stopcock and the blood sample was withdrawn followed by reinjection of the waste blood thereby minimizing the discarded blood volume,

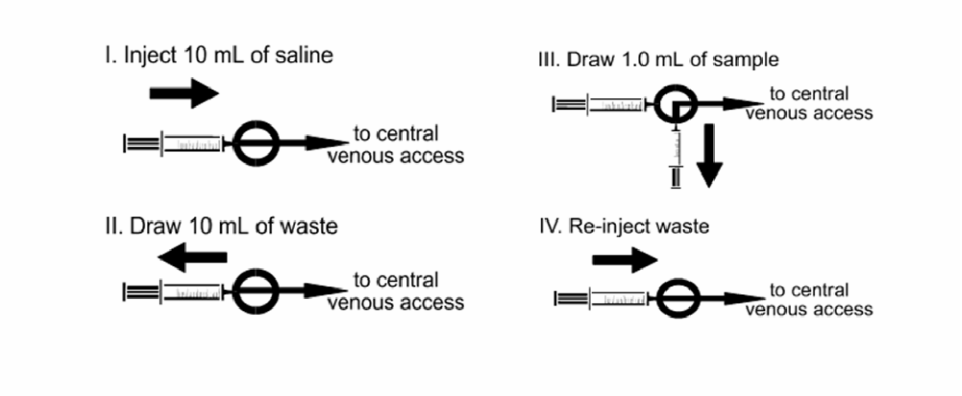

Figure 2. Blood sampling from the subcutaneous injection port using the reinfusion technique.

3.4.2 Venous sampling

The venous blood samples (Paper I, III,VI) were drawn from the subcutaneous injection port or obtained from a peripheral vein (Paper V) and collected in Vacutainer® tubes (Becton Dickinson, Franklin Lakes, NJ, U.S.A).

The children and adolescents in paper V had at the time for blood sampling a peripheral catheter present, for diagnostic procedures, which were used to avoid additional needle punctures.

3.4.3 Capillary sampling

The capillary blood (0.5 mL) was collected, from a finger tip using a Minilancet (CCS Clean Chemical Sweden, Borlänge, Sweden), in Microtainer® tubes (Becton Dickinson, Franklin Lakes, NJ, U.S.A) containing lithium heparin (Paper I) or into micro hematocrit capillary tubes (id 1.2 mm; length 75 mm) treated with ammonium heparin (Kebo Lab AB, Stockholm, Sweden) (Paper V, VI and preliminary results).

3.5 ANALYTICAL METHODS

3.5.1 Methotrexate and tobramycin concentrations (Paper I and III)

Methotrexate and tobramycin concentrations were analyzed at the Department of Clinical Pharmacology and the Department of Clinical Microbiology (Karolinska University Laboratory, Solna, Sweden), respectively, as part of the clinical routine. The concentration measurements were analyzed by fluorescence polarisation immunoassay (FPIA) on an FLx TDx analyzer (Abbott Scandinavia AB, Diagnostics Division, Stockholm, Sweden).

Briefly, the competitive binding immunoassay allows an antigen labeled with fluorescein (tracer-antigen complex) and the patient's antigen to compete for binding sites on the drug-specific antibody. The relationship between polarization and concentration of the unlabeled drug in the sample is established by measuring polarization values of calibrators with known concentrations of the drug.

The lower limit of quantification was 0.050 μmol/L and 0.5 μg/mL for methotrexate and tobramycin, respectively. The intra- and inter-day precisions, i.e. coefficient of variation (CV) were less than 5%.

3.5.2 Spectrophotometry (Paper II)

In paper II, an *in vitro* study was initially performed to establish the volume needed to be withdrawn from the infusion set to obtain an exact time point for start of the intravenous drug infusion by avoiding the concentration gradient and minimize the delay in drug delivery.

IVAC Signature Edition™ infusion sets (IVAC®; IVAC Medical Systems, Inc., San Diego, CA, U.S.A) were connected to infusion bags containing 100 mL of 0.9% saline (Baxter Medical AB; Kista, Sweden) and 1 mL of Giemsa stock solution (Merck kGaA; Darmstadt, Germany) was injected into the infusion bags followed by carefully mixing of the bags. The tubing and drip chamber of the infusion sets were prefilled with saline. A three-way stopcock (Connecta™ Plus 3; Becton Dickinson Infusion Therapy AB, Helsingborg, Sweden) and a syringe (Codan Medical ApS; Rødby, Denmark) were connected to the infusion sets, Figure 3A.

Fractions of 5 mL were repeatedly withdrawn from the infusion set. The absorbance in the withdrawn fractions were measured photometrically at 630 nm (Shimadzu UV-160 Spectrophotometer, Lambda Instrument AB, Stockholm, Sweden) and compared with the absorbance in a sample drawn directly from the infusion bag.

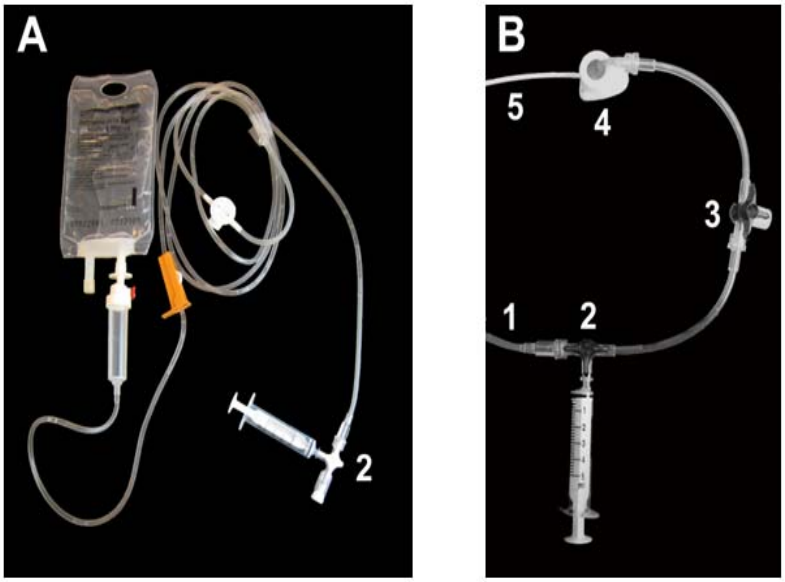

Figure 3. The infusion system. **A:** The infusion system used in the present *in vitro* study. 2. The connected three-way stopcock equivalent to the distal three-way stopcock in Figure 3B. **B:** Equipment used in the proposed routines for intravenous drug administration. 1. Tubing to be connected to the infusion pump and infusion bag. 2. Distal three-way stopcock. 3. Proximal three-way stopcock. 4. Subcutaneous access port. 5. Central venous line.

3.5.3 Standardized routines for intravenous drug administration (Paper II)

To simulate the clinical setting an infusion bag attached to an infusion set was coupled to two three-way stopcocks in series coupled to a subcutaneous access port, Figure 3B. The drip chamber and tubing contained 19 mL and the two three-way stopcocks including 10 centimeter of tubing and a central venous subcutaneous injection port contained 1.5 mL of fluid. The setting was then used for development of standardized routines for intravenous drug administration for pharmacokinetic studies in pediatric patients.

The actual time point for start of infusion dependent on the concentration gradient within the subcutaneous access port and the tubing between the port and the distal stopcock, Figure 3B, was estimated using the developed standardized routines.

The accuracy of the estimated concentration C(0) (i.e. the anticipated initial plasma drug concentration, given as the intercept on the concentration axis when extrapolated back to time zero) for an over-estimation of the infusion time were compared to actual C(0) for various infusion times (0.1 up to 24 hours) and half-live (0.1 to 12 hours) using the equations given by Eksborg *et al* (Eksborg *et al.* 1985). For simplicity reasons our calculation were based on a one compartment model ($C=C(0)*e^{-kt}$) (Rowland and Tozer 1994).

3.5.4 Doxorubicin and doxorubicinol concentrations (Paper V, VI and preliminary results)

Plasma levels of doxorubicin and doxorubicinol were assayed by a modified analytical method based on extraction and reversed-phase liquid chromatography, cf. (Eksborg *et al.* 1979).

Briefly, 100 µL of plasma sample was mixed with the internal standard (daunorubicin or idarubicin) dissolved in 0.1 M phosphoric acid and transferred into a SepPak C18 extraction column (Waters, Milford, MA). After rinsing with 5 mL of phosphate buffer (pH 7.0) the antraquinone glycosides were eluted with 4 ml of methanol. The elute was evaporated, redissolved in 0.1 M phosphoric acid and injected into a Nova-Pak Phenyl Radial-Pak cartridge (Waters, Milford, MA, USA). Acetonitrile, typically 32%, in 0.01 M phosphoric acid was used as the mobile phase. Liquid chromatography carried out with an LC pump Model 10-AVVP and a fluorometric detector (Model RF-551; Shimadzu, Kyoto, Japan) which was operated at 501/600 nm. Chromatographic data was collected and processed using Datamonitor version 3.0 extra (Watrex, Prague, Czechoslovakia) integration system. All plasma concentrations reported are mean values of duplicate analysis.

3.5.5 Etoposide concentrations (Paper VI)

Plasma levels of etoposide were quantified by a modified method based on reversed-phase liquid chromatography with fluorometric detection, cf. (Liliemark *et al.* 1995). Briefly, 0.5 mL plasma was mixed with 2 mL chloroform containing the internal standard, teniposide. After evaporation of the organic phase under nitrogen, the residue was redissolved in 0.5 mL methanol, sonication for 5 minutes and mixed with water (0.5 mL). The elute was introduced to a Zorbax SB-Phenyl column (4.6*150 mm) on a reversed-phase liquid chromatographic system equipped with a LλMDA LC-pump Model LC-10AD and a fluorometric detector Model RF-10AXL (Shimadzu, Kyoto, Japan) operating at 290/320 nm. The mobile phase consisted of water-methanol-acetonitrile-acetic acid (50:43:6:1) at a flow rate of 1.0 mL/min.

3.6 PHARMACOKINETIC EVALUATION

The pharmacokinetics of tobramycin and etoposide were evaluated by compartment analysis using the PC-nonlin program (version 2) (SCI Software, Lexington, Kentucky, U.S.A). The reciprocal of measured serum concentrations were used as weights in the iterative procedure. The optimal pharmacokinetic models were established by visual inspection of the fitted serum concentration versus time curves and from the weighted squared residuals by using the F-ratio test (Boxenbaum *et al.* 1974).

3.6.1 The pharmacokinetics of tobramycin (Paper III)

The pharmacokinetic estimates i.e. the maximum serum concentration (C_{max}), distribution (α) and elimination (β) rate constants, distribution and elimination half-lives ($t_{\alpha 1/2}$ and $t_{\beta 1/2}$) and the area under the serum concentration versus time curve (AUC) of the pharmacokinetic parameters were obtained from PC-NONLIN.

The serum clearance (Cl) for tobramycin was calculated as the dose, expressed in mg,

divided by the AUC. The apparent volume of distribution (Vd_{area}) was calculated according to the following equation: $Vd_{area} = Cl/(BW*\beta)$, where BW is the bodyweight.

The time points for concentrations equal to 0.5 µg/mL were determined from serum concentration time curves estimated from determined pharmacokinetic parameters for the individual patients (Eksborg *et al.* 1985).

The influence of the infusion time on the maximum serum concentration (C_{max}) of tobramycin was predicted from the determined pharmacokinetic constants using the equations given by Eksborg *et al*. (Eksborg *et al.* 1985).

The chronopharmacokinetic effect was assessed by studying the correlation between systemic exposure expressed as AUC/mg/m^2 and administration time defined as hours after midnight.

3.6.2 Doxorubicin and doxorubicinol (Paper V, VI and preliminary results)

The pharmacokinetics of doxorubicin was evaluated using a limited sampling model (Eksborg 1990). Doxorubicin concentration data from the two studied patients (paper VI) were compared with previously published doxorubicin concentrations from 37 patients (median age: 5.48 years, range: 0.7 – 15.9 years; 20 boys). These patients were treated with a doxorubicin dose of 19.7 mg/m^2 (median value; range: 12.9 – 41.9 mg/m^2) administered as 4 h infusions (2 patients) and 24 h infusions (35 patients) (Eksborg, *et al.* 2000; Palm, *et al.* 2001).

Plasma concentrations of doxorubicin and doxorubicinol were measured 15 minutes and 1 hour prior to the end of infusion in patients treated with 4 h and 24 h infusion, respectively. Estimations of plasma clearance for doxorubicin were based on the observation that 69.5 and 86.5% of steady state (C_{ss}) were reached after 4 and 23 h constant rate infusions, respectively (Eksborg 1990). Plasma clearance for doxorubicin was calculated as the dose rate, expressed in mg/m^2/h, divided by the steady state concentration and based on the actual BSA value.

3.6.3 Etoposide (Paper VI)

Etoposide concentration data from the studied patient were compared with previously published etoposide pharmacokinetics from 16 patients (median age 8.3 years, range 0.3 – 22 years; 7 boys). The patients were treated with 130 mg/m^2 (median value, range: 32 – 210 mg/m^2 administered as 1 – 3 h infusions (Eksborg *et al.* 2000b). The plasma clearance for etoposide was calculated as the dose, expressed in mg/m^2, divided by the area under the plasma concentration time curve (AUC) and based on the actual BSA value.

3.7 STATISTICAL METHODS

Median values including their 95% confidence interval (95% CI) were calculated based on the Wilcoxon Signed-Ranks Test (paper III-V) (Daniel 1990). All statistical tests

were two-sided and p-values less than 0.05 were considered to be statistically significant.

3.7.1 Evaluation of sampling site differences and method comparisons

3.7.1.1 Correlation and regression analysis

Correlation analysis was performed by either the Spearman rank correlation test (paper I, III, V) or by the Pearson correlation coefficient (Paper IV, V).

Linear regression was used to evaluate the relationship between capillary and venous doxorubicin concentrations (Paper V). Deming's linear regression (orthogonal regression analysis) was used to evaluate the equations for estimation of the AUC using a limited sampling strategy since both the estimated and the determined AUC are subject to errors (Schellens *et al.* 1988). This type of regression was not initially used in paper I and V but was later on performed.

3.7.1.2 The Eksborg's plot

The Eksborg's plot (Eksborg 1981), a relative plot between two methods, was used to evaluate the methotrexate venous/capillary (Paper I) and the doxorubicin capillary/venous plasma (Paper V) concentration ratio. In paper II and III, the ratio plot was used for evaluation of the estimated C(0) based an over-estimation of the infusion time and the predicted C_{max}/C_{bol} for various infusion times. The equations used for estimation of the AUC using a limited sampling strategy were evaluated in paper IV.

3.7.1.3 Predictive performance

The bias and precision for the methotrexate concentrations in blood samples drawn from the subcutaneous access port (Paper I) and for the developed equations, in paper IV, were evaluated by calculation of the percentage of mean prediction error (ME%) and percentage of root mean square prediction error (RMSE%), respectively, according to Sheiner and Beal (Sheiner and Beal 1981).

3.7.2 Comparisons between observations or populations

The Wilcoxon matched-pairs signed-ranks test was used to analyze differences between paired observations while the Mann-Whitney *U*-test was used for comparison of values from two independent populations.

3.7.3 Randomization

In paper IV, the patients from paper III were randomized to either a model or a validation group (GraphPad StatMate version 1.01, GraphPad Software, Inc. La Jolla, USA).

3.7.4 Multiple regression

Stepwise multiple regression (Minitab 10.5Xtra, Minitab Ltd, Coventry, UK) was used to identify the most important sampling points for estimation of the AUC based on the tobramycin concentration data from the twelve patients in the model group.

Factors influencing the capillary/venous concentration ratio were evaluated by multiple regression with step-wise variable selection using Minitab version 10*Xtra* (Minitab Inc. State College, PA, USA). Age, gender, infusion time, body surface area, administered dose in mg/m^2 and drug concentrations in venous plasma were used as independent variables.

4 RESULTS

4.1 BLOOD SAMPLING FROM CENTRAL VENOUS ACCESSES (PAPER I)

The plasma concentration of methotrexate in the samples drawn from the central venous access (median value: 0.41 μmole/L; range: 0.08-21 μmole/L, n=71) did not differ from plasma concentrations in the capillary samples (median value: 0.43 μmole/L; range: 0.07-21 μmole/L, n=71), p=0.163. The concentrations of methotrexate in venous and capillary samples were closely correlated (Spearman rank correlation coefficient, r_s, was 0.98; p<0.0001; n=71), Figure 4A. The bias, ME%, was 2.39%, and the precision, RMSE%, was 22.3%. The slope from the Deming linear regression was not different from unity (1.162; 95% CI:0.9529 to 1.371).

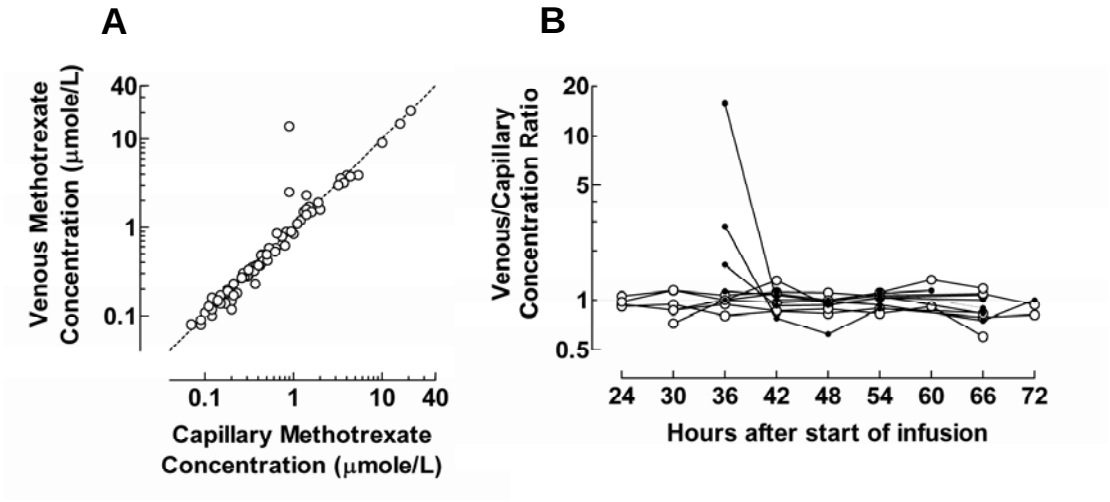

Figure 4. Comparison of venous and capillary concentrations of methotrexate (○, 4-hour infusion; ●, 24-hour infusion). **A**: Scatter plot of venous versus capillary plasma concentrations of methotrexate. The line of identity is given in the figure. The Spearman rank correlation coefficient, r_s, was 0.98 (p<0.0001; n=71). **B**: Eksborgs's plot of venous/capillary plasma concentration ratio of methotrexate versus sampling time. The dashed line represents a venous/capillary concentration ratio of unity.

The venous/capillary plasma concentration ratio was 1.00 (median value; interquartile range (IQR): 0.882-1.094); 85% of the data points were within the ratio 0.8-1.2, Figure 4B, independent of drug concentration (data not shown).

On three occasions considerably higher methotrexate concentrations were observed in blood samples drawn from the catheter, all in the first samples drawn after an infusion time of 24 hour, i.e. 36 hours after start of the methotrexate infusion. In one of the patients this could have resulted in an increased hydration, albeit without a change of the dose of calcium folinate. The methotrexate concentrations were 14.0 and 0.89 μmole/L in the blood sample drawn from the catheter and the capillary blood sample, respectively.

In one patient methotrexate concentrations were found to be falsely below 0.2 μmole/L (0.18 and 0.17 μmole/L) in the last two blood samples drawn from the central venous

access (66 h and 72 h after the start of a 4 hour infusion), compared to 0.23 and 0.21 μmole/L, respectively, in the capillary blood samples. This could have resulted in a too early cessation of the calcium folinate rescue. The present study indicate a 9.1% incidence for the need for changes in the calcium folinate rescue due to falsely enhanced methotrexate concentrations in samples from the central venous catheter (95% CI: 0.23 to 41.3%).

Blood sampling from the central venous access required an excessive blood volume (35-56 mL) per course of therapy compared to capillary blood sampling (2.5-4.0 mL), Figure 5, a highly significant difference (p=0.001).

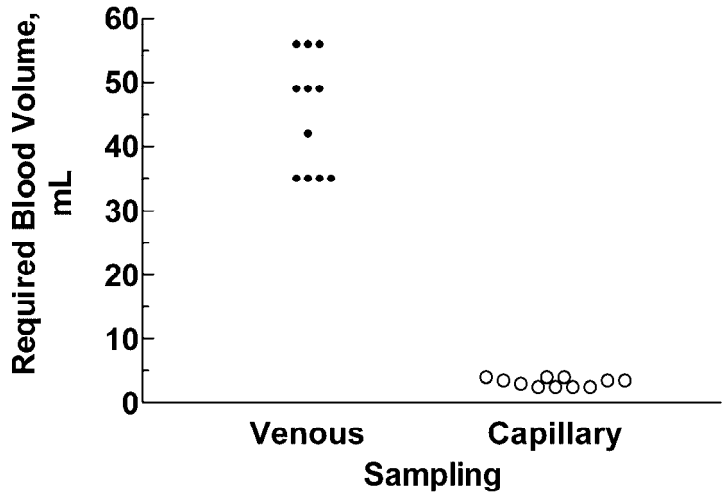

Figure 5. The total blood volume required for the individual patients during each treatment occasion. Data from venous blood sampling (●): 5 mL discarded volume and 2 mL sample volume. (○): 0.5 mL sample volumes.

4.2 STANDARDIZATION OF INTRAVENOUS DRUG INFUSIONS (PAPER II)

The solution withdrawn from the infusion sets (i.e. drip chamber and tubing), connected to the infusion bags filled with Giemsa solution (Figure 3A) initially consisted of pure saline. The absorbance in the withdrawn fractions increased with increasing withdrawn volume due to the appearance of Giemsa solution and reached 100% of the absorbance in the infusion bag after withdrawal of 38 mL from the IVAC Signature Edition™ infusion set, Figure 6.

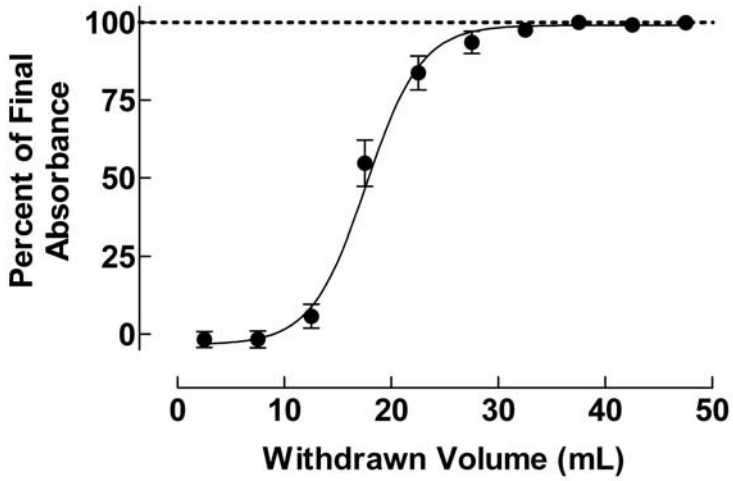

Figure 6. The mean with 95% confidence interval (n=6) for the absorbance in withdrawn solution *versus* withdrawn volume using the IVAC Signature Edition™ infusion set.

Over-estimation of the infusion time, due the concentration gradient within the subcutaneous access port and the tubing between the port and the distal stopcock, resulted in a decreasing accuracy of the estimated C(0) with decreasing infusion times and decreasing half-lives, Figure 7A and B.

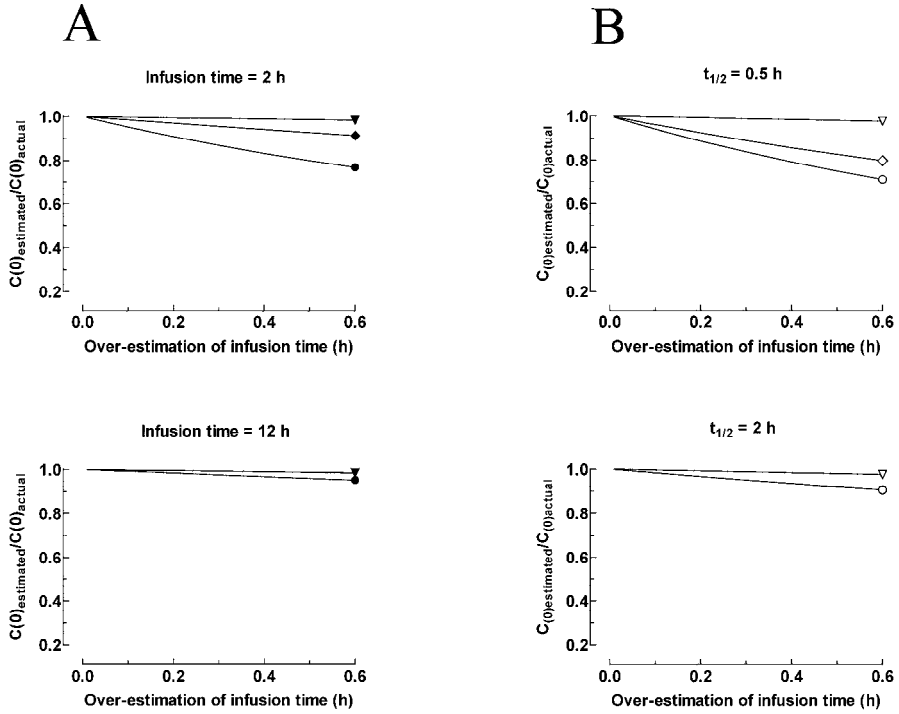

Figure 7. The impact of over-estimations of the infusion times on the accuracy of the estimated concentration. **A:** Eksborg's plot of $C(0)_{estimated}/C(0)_{actual}$ ratio for an infusion time of 2 and 12 hours *versus* over-estimations of the infusion times for half-lives of 0.1 h (●), 2 h (♦) and 12 h (▼). **B:** Eksborg's plot of $C(0)_{estimated}/C(0)_{actual}$ ratio for t1/2 of 0.5 and 2 hours *versus* over-estimations of the infusion times for infusion times of 0.5 h (○), 2h (◊) and 24 h (▽).

4.3 PHARMACOKINETICS OF TOBRAMYCIN (PAPER III)

A two-compartment model was used to describe the serum tobramycin concentration versus time curve after intravenous administration in all patients, exemplified in Figure 8.

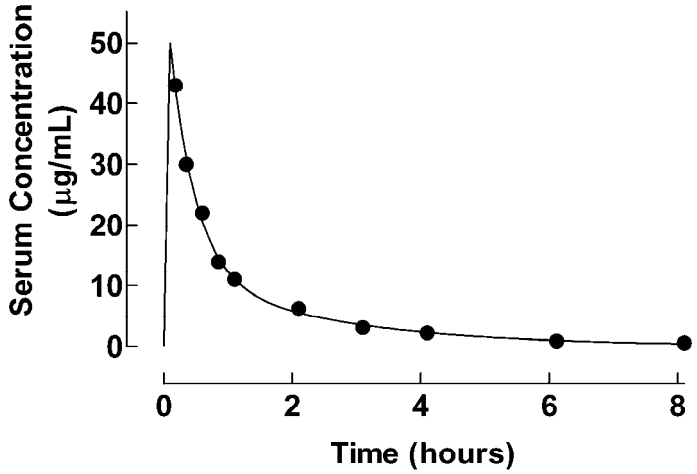

Figure 8. The serum concentration *versus* time curve for one female patient receiving tobramycin 8 mg/kg as an intravenous short time (5 minutes) infusion. The solid line is the predicted concentration curve obtained by pharmacokinetic modeling using a two-compartment model.

The systemic drug exposure (AUC), dose normalized by mg/m^2, was independent of age (p=0.1756), Figure 9a. In contrast, AUC normalized by the dose in mg/kg increased with increasing age of the patients (p=0.0034), Figure 9b. The AUC normalized for the dose in mg/m^2 was 0.24 µg*h/mL (median value; 95% CI: 0.22 to 0.27 µg*h/mL). The median AUC normalized for the dose in mg/kg was 6.8 µg*h/mL (95% CI: 5.9 to 7.8 µg*h/mL).

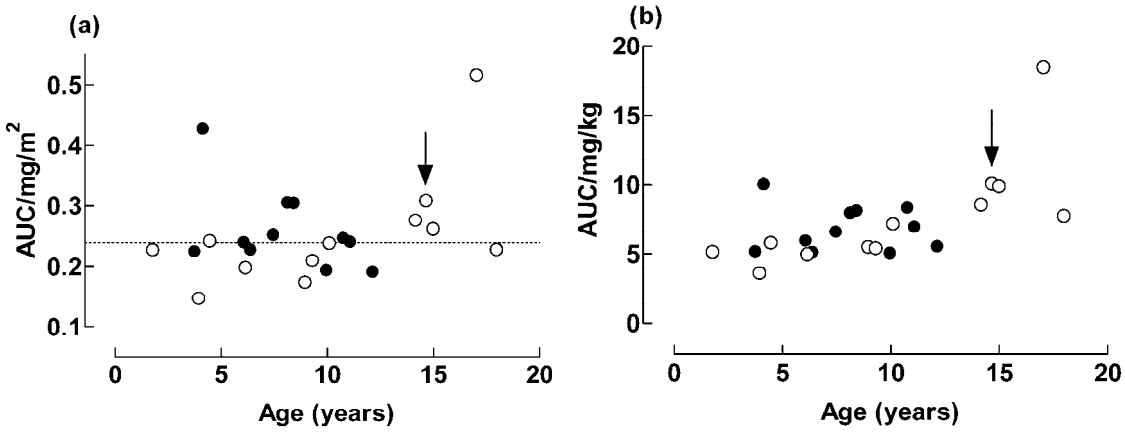

Figure 9. Dose-normalized areas under serum concentration *versus* time curve (AUC) of tobramycin *versus* age. AUC is expressed in µg*h/mL. **(a):** Dose-normalization based on body surface area. r_s=0.2925, p=0.1756. Median value (0.24 µg*h/mL) is given by the dotted line. **(b):** Dose-normalization based on body weight. r_s=0.5840, p=0.0034.

The median distribution and elimination half-lives ($t_{1/2\,\alpha}$ and $t_{1/2\,\beta}$) for tobramycin were 0.18 hours (95% CI: 0.13 to 0.24 h) and 1.5 hour (95% CI: 1.2 to 1.7 h), respectively. The elimination half-life showed no age dependence, Figure 10.

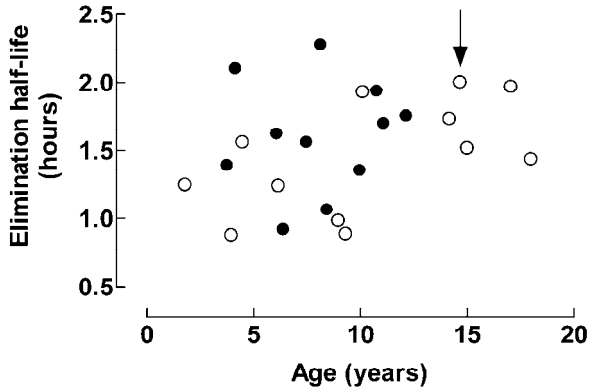

Figure 10. Closed symbols: females; open symbols: males. r_s=0.3538, *p*=0.0977.
The arrow shows the data from one patient with Down's and Klinefelter syndromes.

The estimated C_{max} normalized by mg/m^2 and mg/kg were 0.26 and 7.1 (median values; 95% CI: 0.23 to 0.30 and 6.1 to 8.4), respectively, and independent of age. Serum clearance (Cl) of tobramycin was 4.3 L/h (median value; 95% CI: 3.7 to 5.0 L/h). The volume of distribution (Vd_{area}) was 0.32 L/kg (median value; 95% CI: 0.29 to 0.36 L/kg).

Tobramycin concentrations were below the detection limit (0.5 µg/mL) 7.8 h (median value; 95% CI: 6.9 to 8.8 h) after start of the infusion. The time period within the dosing interval with serum concentrations below the detection limit was 16.2 hours (median value; 95% CI: 15.2 to 17.1 h).

The C_{max}, i.e. the maximum concentration at the end of infusion and the C_{max}/C_{bol} ratio (C_{bol}: estimated concentration for a bolus injection) decreased considerably with increasing infusion time (infusion time < 2 hours), Figure 11 and 12. A further increase in the infusion time to 4 hours had only minor influence on C_{max}/C_{bol}, Figure 12.

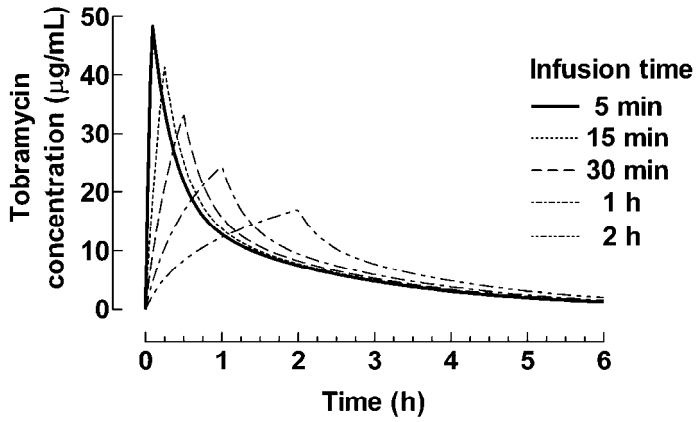

Figure 11. The predicted tobramycin concentration curves for various infusion times are based on the estimated pharmacokinetic parameters using an infusion time of 5 minutes. The solid line is the predicted concentration curve obtained by pharmacokinetic modeling using a two-compartment model.

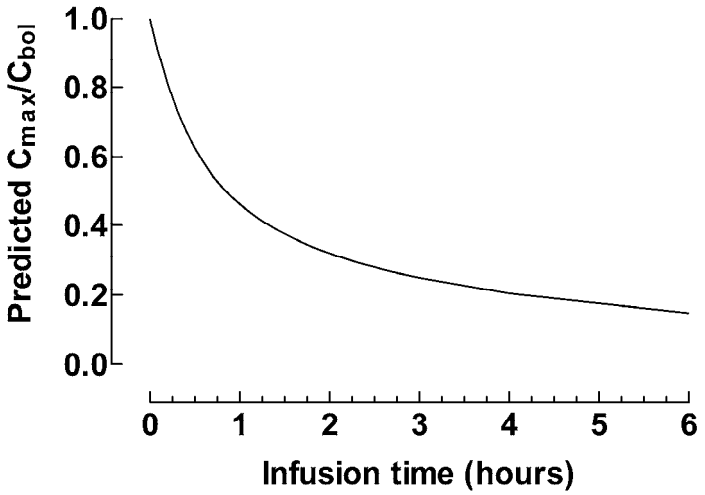

Figure 12. The influence of the infusion time on the C_{max}/C_{bol} ratio. The calculated curve is based on the estimated pharmacokinetic parameters. C_{max} is the predicted maximum concentration for various infusion times and C_{bol} is the maximum concentration after a bolus injection.

4.4 LIMITED SAMPLING STRATEGY FOR TOBRAMYCIN (PAPER IV)

Stepwise multiple regression, based on concentration data from the model group, showed that the most important sampling points for estimation of the AUC after a standardized short time (5.0 minutes) intravenous infusion of tobramycin were the 1, 6, 0.5 and 2 h , in decreasing order of importance, Table 1. Inclusion of additional sampling points did not result in a further improvement of the model for estimation of AUC.

Table 1. Equations for estimation of the area under the concentration time curve (AUC) after an intravenous infusion (5 minutes).

No. of sampling points	Time (h)	Equation AUC=	MPE (%)	RMSE (%)
1	1	$4.06*C_{1h}$	2.28	9.0
2	1, 6	$3.25*C_{1h}+ 8.38*C_{6h}$	-0.04	6.4
3	1, 6, 0.5	$2.26*C_{1h}+9.73*C_{6h}+0.528*C_{0.5h}$	0.33	4.3
4	1, 6, 0.5, 2	$0.905*C_{1h}+0.82*C_{6h}+0.684*C_{0.5h}+3.49*C_{2h}$	-0.83	2.9

The bias and the precision of the models are expressed by the percentage of the mean prediction error (MPE) and percentage of the root mean square prediction error (RMSE), respectively.
AUC is expressed in µg*h/mL. Concentration is expressed in µg/mL.

One sampling point (C_{1h}) after infusion will give an accurate estimate of the systemic drug exposure of tobramycin as indicated by the high correlation coefficient, Figure 13A. The slope from the Deming linear regression was not different from unity (0.915; 95% CI: 0.804 to 1.027), Figure 13A. The bias and precision expressed by the MPE and RMSE were 2.28% and 9.0%, respectively, Table 1. The median estimated/determined AUC ratio for the model group was 1.03 (median value; 95% CI: 0.97 to 1.11), Figure 14.

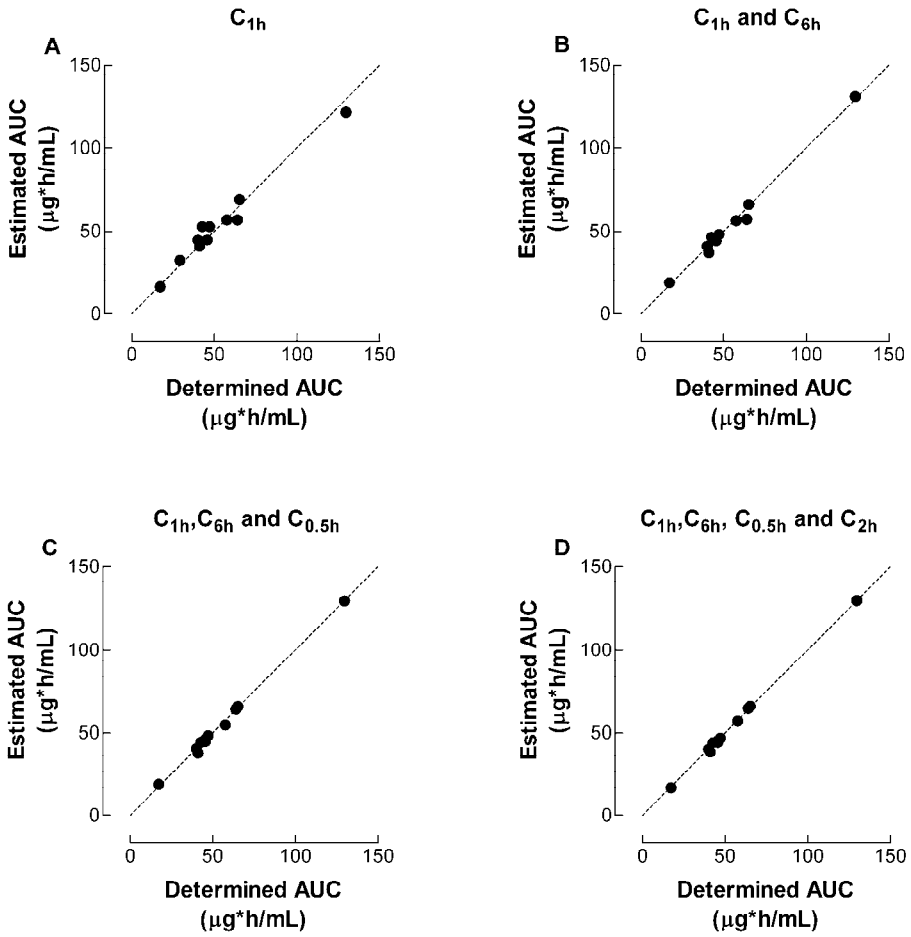

Figure 13. Scatter plot showing the relationship between estimated AUC and the determined AUC measured after intravenous short time infusion of tobramycin for the model group. Estimated AUC based on **A:** C_{1h}; **B:** C_{1h} and C_{6h}; **C:** C_{1h}, C_{6h} and $C_{0.5h}$; **D:** C_{1h}, C_{6h},$C_{0.5h}$ and C_{2h}. The dashed lined represents the line of identity. Deming linear regression, 95% CI confidence interval of the slope and the pearson correlation coefficient are: A: Y=5.245 + 0.915*X(0.804<slope<1.027); r=0.9855, B: Y=-0.426 + 1.005*X(0.926<slope<1.085); r=0.9945, C: Y=0.192+0.997*X(0.953<slope<1.041), r=0.9983, D: Y=-0.824 + 1.012*X(0.982<slope<1.024), r=0.9992.

The correlation coefficients were only increased to a minor extent when the number of sampling points for estimation of the AUC were increased, Figure 13B-D. The bias of the estimated AUC was below 2.28% in all models. The precision of the estimated AUC was improved by additional sampling points as shown by the decreasing RMSE (Table 1) and the decreased 95% CI, Figure 14.

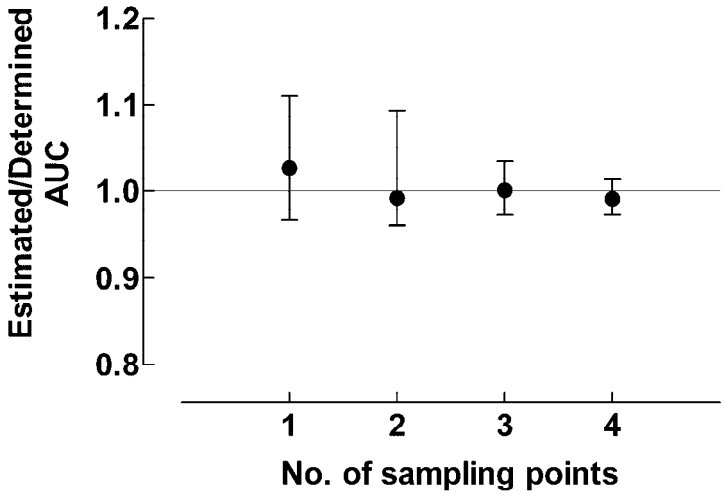

Figure 14. Eksborg's plot with the data representing the median value including the 95% CI showing the agreement of the estimated AUC using 1 to 4 sampling time points in relation to the determined AUC based on eleven sampling points after an intravenous short time infusion of tobramycin for the model group.

The derived equation using one sampling point (C_{1h}) for estimation of AUC (Table 1) was validated in another eleven patients (the validation group). The estimated and determined AUC was highly correlated (r=0.9579) with a slope of 1.002 (95% CI: 0.775 to 1.228) for the validation group, Figure 15A.

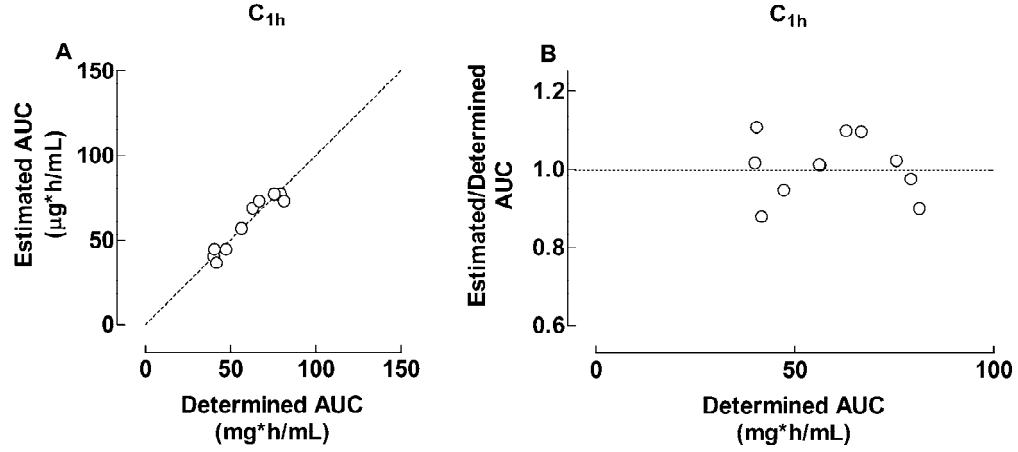

Figure 15. Scatter **(A)** and Eksborg's plot **(B)** showing the relationship and agreement, respectively, of the estimated AUC and determined AUC measured after intravenous short time infusion of tobramycin for the validation group. Estimated AUC based on the sampling point C_{1h}. **A:** Deming linear regression, 95% CI of the slope and the pearson correlation coefficient are: Y=0.168 + 1.002*X (0.775<slope<1.228); r=0.9579. The dashed line represents the line of identity. **B:** The dashed line represents the estimated/determined AUC ratio of unity.

AUC was estimated with a low bias (MPE: 0.04%) and a precision of 7.5% (RMSE) using the C_{1h} sampling point. The estimated/determined AUC ratio was close to unity (median value: 1.01; 95% CI: 0.95 to 1.06) for the validation group using solely one sampling point, Figure 15B. There was no significant difference ($p<0.8501$) between AUC estimated using C_{1h} and the determined AUC based on eleven concentration measurements.

4.4.1 The importance of an exact infusion time

A deviation in the infusion time within the range – 4 to + 5 minutes, resulted in a tobramycin concentration deviation of less than ±3% and ±5% for the sampling points C_{1h} and $C_{0.5h}$, respectively, Figure 16.

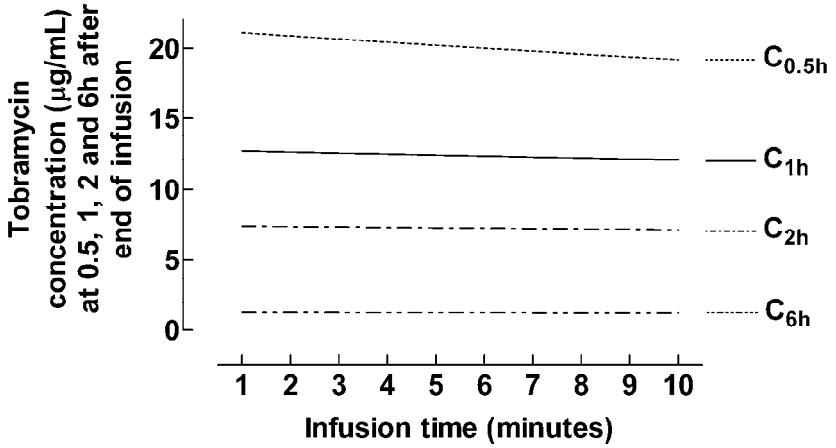

Figure 16. The influence of the infusion time on the predicted tobramycin concentration after a short time infusion (5 minutes) at the sampling time point 0.5, 1, 2 and 6 hours post infusion. Calculation based on the estimated serum concentration time curves from the pharmacokinetic parameters in paper III.

4.4.2 The importance of the actual sampling time

A deviation in the actual time for blood sampling influenced the tobramycin concentration considerably as illustrated in Figure 17. The earlier sampling time points ($C_{0.5h}$ and C_{1h}) were more sensitive to a deviation in actual time of blood sampling than later sampling time points, Figure 17. Blood sampling ten minutes earlier than the actual time point (C_{1h}) resulted in a 14% increase in tobramycin concentration while a ten minutes delayed sampling resulted in a 10% decreased concentration, Figure 17.

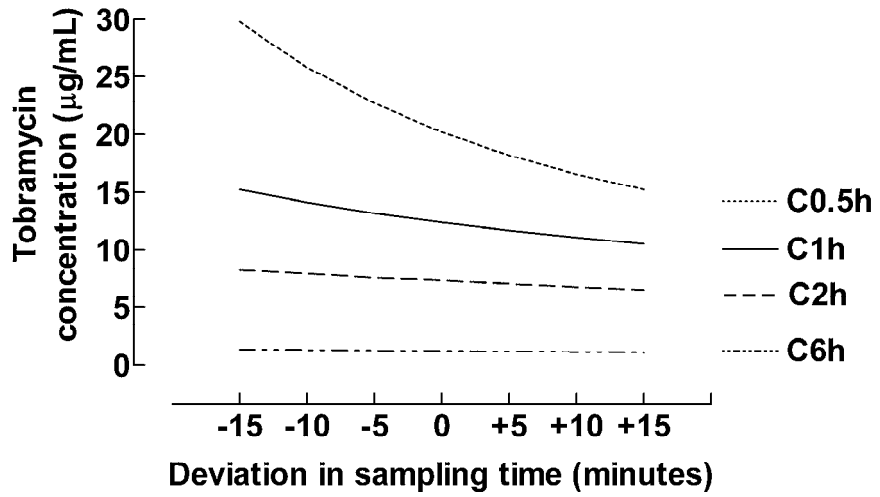

Figure 17. The influence of a deviation in the sampling time on the predicted tobramycin concentration after a short time infusion (5 minutes) at the sampling points 0.5, 1, 2 and 6 hours post infusion. Calculation based on the estimated serum concentration time curves from the pharmacokinetic parameters in paper III.

4.5 CAPILLARY BLOOD SAMPLING FOR DOXORUBICIN (PAPER V)

The median plasma concentration of doxorubicin found in capillary samples was 268 ng/mL (range 35.3 to 815 ng/mL) and in venous samples 248 ng/mL (ranges 34.8 to748 ng/mL). The concentrations of doxorubicin in capillary and venous samples were closely correlated (r=0.98; p<0.0001; n=16), Figure 18. The slope of the Deming linear regression was 1.173 (95% CI 1.043 to 1.303).

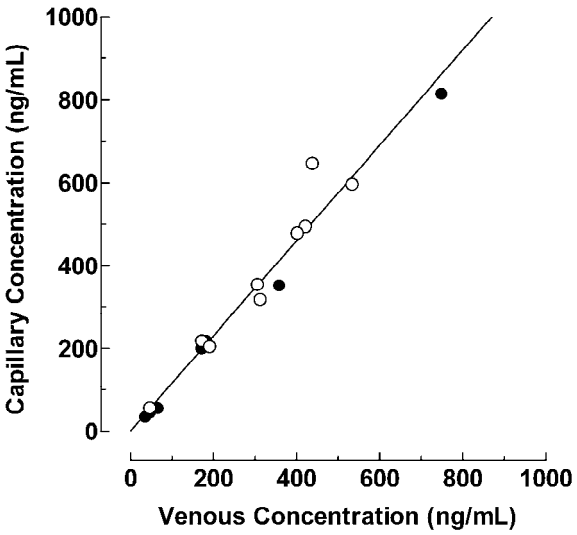

Figure 18. Capillary versus venous plasma concentration of doxorubicin: (●) females and (○) males. The line is obtained by linear regression analysis (r=0.98; p<0.0001).

The median capillary/venous plasma concentration ratio was 1.13 (95% CI: 1.06 to 1.20) and independent of drug concentration, Figure 19.

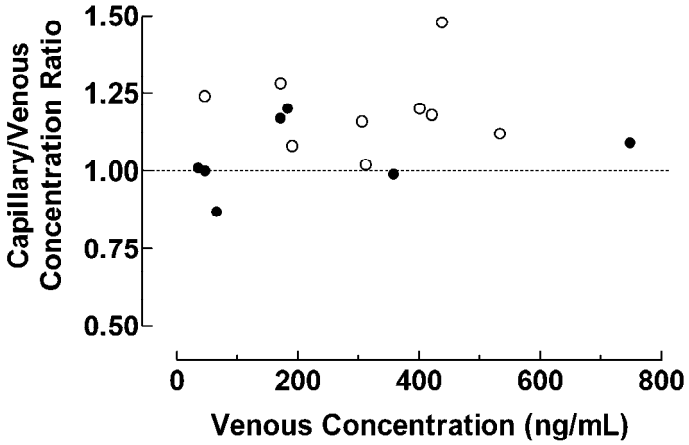

Figure 19. Capillary/venous plasma concentration ratio of doxorubicin versus age: (●) females and (○) males. The dashed line represents the capillary/venous ratio when capillary and venous concentrations are equal.

The plasma concentration ratio did not correlate with age or with body mass index (data not shown). The concentration ratio was significantly higher (p=0.03) in males (median value: 1.18; n=9) than in females (median value: 1.01; n=7). Multiple regression with step-wise variable selection revealed that gender was the only tested variable affecting the capillary/venous plasma concentration ratio.

The median plasma concentrations of the metabolite doxorubicinol found in capillary samples was 8.8 ng/mL (range 2.3 to 18.3 ng/mL) and in venous samples 8.3 ng/mL (range 2.5 to 25.1 ng/mL).

4.6 DOXORUBICIN AND ETOPOSIDE IN CLINICAL PRACTICE (PAPER VI, PRELIMINARY RESULTS)

4.6.1 Paper VI

In the studied obese male patient (Paper VI) the 4 hour plasma concentration of doxorubicin day 15 (first course), day 1 and 15 (second course), were 202, 181 and 162 ng/mL, respectively. The corresponding doxorubicinol concentrations were 11.8, 16.9 and 14.2 ng/mL. The plasma clearance of doxorubicin day 15 (first course), day 1 and 15 (second course), were 425, 476 and 532 mL/min/m^2, respectively, (median value: 476 mL/min/m^2), Figure 20, and did not differ from data in non-obese patients in whom the plasma clearance was 493 mL/min/m^2 (median value; range: 197 – 869 mL/min/m^2) (Eksborg *et al.* 2000a; Palm *et al.* 2001).

In the included obese female patient the 4 hour plasma concentration of doxorubicin and doxorubicinol were 175 and 14.7 ng/mL, respectively. The plasma clearance of 600 mL/min/m^2 did not differ from non-obese patients, Figure 20.

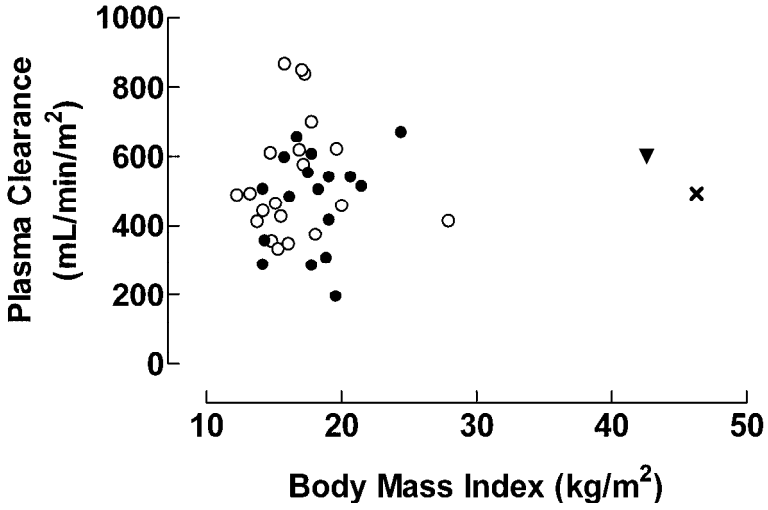

Figure 20. Plasma clearance (mL/min/m^2) of doxorubicin after intravenous infusions *versus* body mass index. (×) the median plasma clearance at the 3 studied occasions in the male obese patient. (▼) the plasma clearance at the first studied occasion the female obese patient. Closed and open symbols are doxorubicin data from females and males, respectively (Eksborg *et al.* 2000a; Palm *et al.* 2001)

Etoposide plasma clearance was 16.1 mL/min/m^2 in the studied obese male patient, which is in agreement with what has been observed in patients with normal BSA (14.9 mL/min/m^2; median value; range: 11.0 to 31.7 mL/min/m^2), Figure 21 (Eksborg *et al.* 2000b). The terminal half-life was 3.62 h as compared to 4.10 h (median value; range: 2.04 to 7.82 h) in non-obese patients (Eksborg *et al.* 2000b) (data not shown).

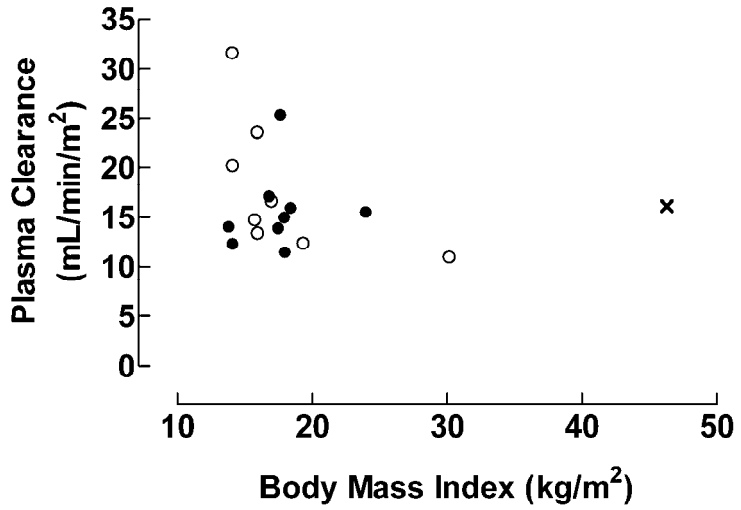

Figure 21. Plasma clearance (mL/min/m^2) of etoposide *versus* body mass index. (×) data from the morbidly obese male patient. Closed and open symbols are etoposide pharmacokinetic data from females and males, respectively (Eksborg *et al.* 2000b).

4.6.2 Preliminary results

The median dosnormalized doxorubicin concentration for standard risk patients were 1.6, 1.3 and 1.1 for day 1, 22 and 36, respectively. For intermediate risk patients the median dosnormalized doxorubicin concentrations were 1.6, 1.2 and 1.4 for day 1, 22, and 36, respectively. High risk patients had median dosnormalized doxorubicin concentrations of 1.1, 1.1, 1.0 and 1.2 at day 1, 8, 22 and 36, respectively.

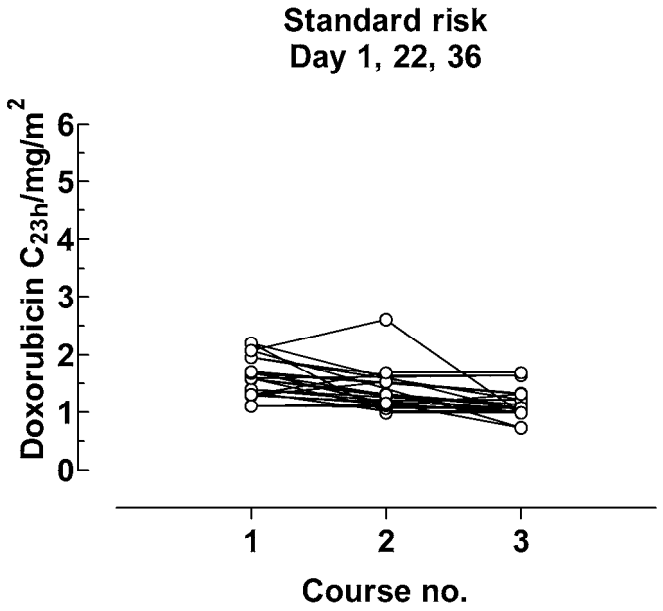

Figure 22. Dose-normalized plasma concentration of doxorubicin during repeated courses of treatment in twelve standard risk patients. Dose-normalization based on body surface area. Concentration expressed in ng/mL.

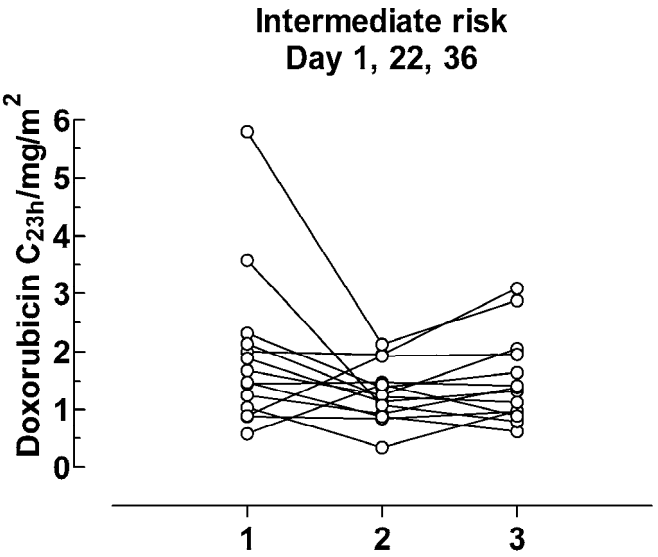

Figure 23. Dose-normalized plasma concentration of doxorubicin during repeated courses of treatment in fourteen intermediate risk patients. Dose-normalization based on body surface area. Concentration expressed in ng/mL.

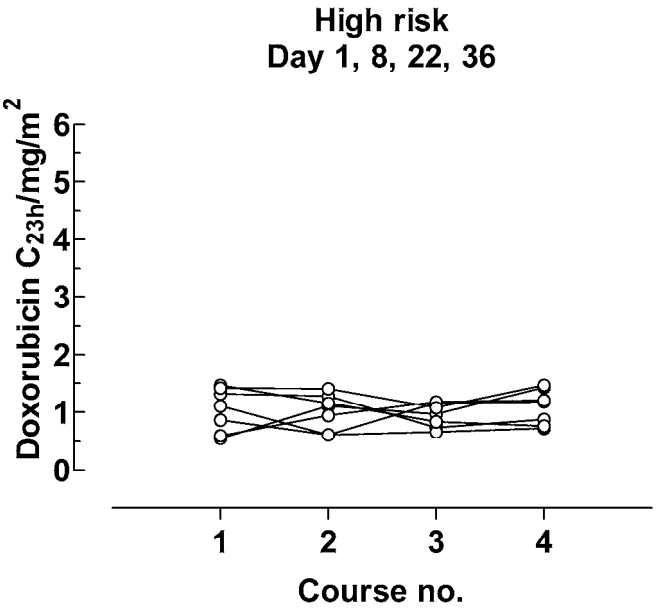

Figure 24. Dose-normalized plasma concentration of doxorubicin during repeated courses of treatment in seven high risk patients. Dose-normalization based on body surface area. Concentration expressed in ng/mL.

5 DISCUSSION

5.1 BLOOD SAMPLING FROM CENTRAL VENOUS ACCESSES (PAPER I)

Traditionally, capillary blood sampling has been used for therapeutic drug monitoring of methotrexate, but the ability to use the central venous access, e.g. a PORT-A-CATH®, for blood sampling would reduce the pain and anxiety, thereby improving the clinical care for pediatric cancer patients.

In the present study we did not find any statistically significant difference between the methotrexate plasma concentrations drawn from the subcutaneous access port and the capillary blood samples. These findings are in accord with those reported by Cash *et al.,* who compared methotrexate concentrations in peripheral venous blood samples and blood samples from central venous accesses during treatment with intermediate doses of methotrexate (Cash *et al.* 1999).

The high correlation between the concentrations of methotrexate in samples from central venous accesses and capillary samples might give the impression that the sampling sites can by used interchangeably, Figure 4A. However, the Eksborg's plot (Eksborg 1981) in Figure 4B, clearly demonstrates differences, emphasizing the need for a proper examination of the data before changing sampling sites in clinical practice. The most probable reason for the three cases of highly elevated concentrations in the samples drawn from the central venous catheter, Figure 4B, seems to be contamination of the blood samples with remaining drug solution in the catheter lumen, or in the stop-cock used for drug administration and/or in the tubing.

In 8 out of the first 11 paired samples very similar concentrations were obtained in the blood samples drawn from the two sampling sites, indicating that rinsing with 25 mL of saline after the methotrexate infusion, flushing with 10 mL of saline and discarding 5 mL of blood prior to blood sampling from the catheter is sufficient. Hence, rinsing might not have been performed according to the instructions in the three cases of highly enhanced concentrations in the blood samples from the catheter.

The need to rinse the catheter carefully prior to blood sampling, to ensure reliable drug concentrations, has been pointed out previously (McBeth *et al.* 2004; Mogayzel *et al.* 2008, Odum and Drenck 2002). The volume needed to rinse the catheter from drug solution is dependent on the drug and the type of catheter (McBeth *et al.* 2004). Some drugs, e.g. cyclosporine, aminoglycosides and thiotepa, have been suggest to absorb to the catheter during the drug infusion and subsequently be released during blood sampling (Busca *et al.* 1994; Carreras *et al.* 1988; de Jonge *et al.* 2003; Franson *et al.* 1987).

The risk of increased drug concentrations in samples drawn from a CVAD might be avoided by the use of double lumen catheters, using one lumen for drug administration and the other for blood sampling. However, blood sampling can only be performed by briefly interrupting the infusion or after the infusion (Huitema *et al.* 1999).

In a few cases the methotrexate concentrations in samples drawn from subcutaneous access port were remarkably lower than capillary methotrexate concentrations. In the present study a waste volume of 5 mL, often considered as a standard in clinical practice, was discarded prior to blood sampling from the central venous catheter (Frey 2003). Insufficient waste volumes prior to sampling from the catheter leads to a dilution of the sample with the saline used for flushing, resulting in falsely low methotrexate concentration (Odum and Drenck 2002; Shulman *et al.* 1998).

Capillary blood sampling was considered to be the reference method and used for guiding the rescue therapy in this study. One limitation with the present study is the lack of peripheral venous blood samples which would have been the optimal reference samples. There is a small possibility that the capillary blood sample is diluted with interstitial fluid, thereby lowering the methotrexate concentration (Kauffman and Kearns 1992, Palm *et al.* 2001). However, drawing additional peripheral venous blood samples was considered unethical, as capillary blood sampling was used clinically for methotrexate concentrations (Cole *et al.* 2006).

In the present study three considerably enhanced methotrexate concentrations were observed in the first blood samples drawn from the catheter after an infusion time of 24 hour, i.e. 36 hours after start of the infusion. At this time point, falsely high concentrations could have resulted in increased hydration since, according to the treatment protocol, the hydration should be increased, albeit without changing the dose of calcium folinate, if the methotrexate concentration exceeds 3 μmole/L.

ALL patients with plasma methotrexate concentrations >1 μmole/L at 42 h after the start of infusion are considered to be at high risk of developing toxicity and are given intensified calcium folinate treatment. Falsely elevated concentrations at that time point may lead to an increased dose of calcium folinate, which could compromise clinical efficacy (Bleyer 1978; Fotoohi *et al.* 2005). Falsely low concentrations might result in omitting to increase the calcium folinate dose at that crucial time point. Blood sampling from the central venous catheter at 42 h after start of methotrexate infusion would not have changed the calcium folinate rescue in any of the patients in the present study. Cash *et al*. reported that the clinical management of the leucovorin rescue would have been altered in 5 out of 33 patients (15.2%; 95% CI: 5.1 to 31.9%) if blood samples from central venous accesses were used for measuring methotrexate concentrations (Cash *et al.* 1999). In the present study including data from 11 treatment occasions, the potential risk of an incorrect change in the dose of calcium folinate could be quite high (up to 41.3%) using blood sampling from central venous accesses for dose adjustment.

A capillary blood sample taken prior to the end of the methotrexate infusion together with a plot of measured methotrexate concentration time data for the individual patient during the elimination phase, using a chart including a normal elimination curve, is mandatory for detecting deviating concentration data. Suspected erroneous methotrexate concentrations should be checked by an extra blood sample.

An excessive blood volume is required with blood sampling from the central venous access as compared to capillary blood sampling, Figure 5, a serious disadvantage

especially in the younger pediatric patients. Reinjection of the discarded volume would reduce the required blood volume (Adlard 2008), but precautions are needed to guarantee a high quality of the blood to be reinjected. The use of a push-pull technique (mixing technique) during blood sampling from central lines could be another way to minimize the discarded volume (Frey 2003). During cancer treatment hematological parameters are followed carefully due to the toxic effect of the chemotherapy. This will identify patients for whom excessive blood sampling for therapeutic drug monitoring is unsuitable. The feasibility of blood sampling from central venous accesses in specific pediatric patients should be taken into consideration by the physician.

5.2 STANDARDIZATION OF INTRAVENOUS DRUG INFUSIONS (PAPER II)

Starting an intravenous drug infusion using an infusion set pre-filled with saline results in a delayed drug delivery due to infusion of pure saline followed by a concentration gradient i.e. an initial non-constant drug delivery rate, Figure 6. This is appropriate in a therapeutic setting but unsuitable for pharmacokinetic studies since an exact time point for start of infusion is impossible to obtain. The delayed drug delivery and the concentration gradient can be avoided by the withdrawal, of at least 38 mL (i.e. twice the volume of the infusion set) of solution from the infusion set to obtain 100% of pure drug solution and an exact time point for start of the infusion, as indicated in the results presented in Figure 6.

The proposed standardized routine in Appendix 1 in paper II, using an excess of drug solution in the infusion bag allows a withdrawal of the required volume of solution from the infusion set prior to start of the infusion to avoid an initial concentration gradient. Together with a prompt disconnection of the infusion set once the predetermined volume has been infused, i.e. with flushing from the distal three-way stopcock toward the subcutaneous access port (Figure 3B), there will be almost exact time points for start and cessation of the infusion. The dispensing of an excess solution and infusion of a predetermined volume in our routines ensures that the prescribed drug dose is administered.

In the clinical routine complete drug delivery is assured by termination of the infusion with a flush drip but the exact time point for cessation of the infusion is impossible to record. An exact end point of the infusion is especially important for pharmacokinetic studies of drugs with short initial half-lives, e.g. doxorubicin ($t_{1/2\,\alpha}$ = 3 - 5 min) (Eksborg *et al.* 1985), vincristine ($t_{1/2\,\alpha}$ = 7.6 min) (Gidding *et al.* 1999) and tobramycin ($t_{1/2\,\alpha}$ = 11 min) (Paper III). A prompt disconnection of the infusion set without using our routines with excess of drug solution is unsuitable since the extent of drug remaining in the infusion set may be of such magnitude that the efficacy of the treatment might be jeopardized unless this is taken into consideration.

The dispensing of an extra 50 mL of drug solution is a potential risk for the patient as it could result in an erroneous administration of a too high drug dose. To maintain the safety, precautions e.g. proper labeling of the infusion bag and the infusion pump as

well as surveillance during drug administration is required. To avoid an erroneous drug administration the importance of labeling the infusion pump with the volume to be administered but also a note "Maximum infusion volume must not be exceeded" are emphasized. The withdrawal of drug solution prior to start of the infusion reduces the excess volume available in the infusion bag by approximately 20 mL. However, for low infusion volumes, i.e. 50 to 100 mL, the excess solution is still high, 58% and 29%, respectively, as compared to the prescribed infusion volume. In such cases the use of a syringe pump might be preferable since the required excess volume can be reduced. Less than 6% of excess drug solution is available for an infusion volume exceeding 500 mL and the consequence of an erroneous administration is obvious of considerably reduced.

The suggested (Appendix 1, Paper II) withdrawal of 40 mL from the infusion set using the distal stop-cock prior to start of an intravenous drug infusion will result in a concentration gradient solely within the subcutaneous access port and the tubing between the port and the distal stopcock (Figure 3B). The use of only one stopcock would be the most appropriate way for reducing the delay in drug administration (Roberts 1994). Two stopcocks connected in sequence are used according to clinical practice at Astrid Lindgren's Children's Hospital.

The estimated concentration for various compartments, C(0), is used for calculation of AUC, clearance and volume of distribution (Rowland and Tozer 1994). The accuracy in determining the actual time point for start of an infusion, dependent on a concentration gradient formed within the subcutaneous injection port as well as within the tubing between the injection port and the distal stop-cock, will influence the accuracy of C(0), and thus the subsequent estimated pharmacokinetic parameters. The importance of the actual time point for start of the infusion increases with decreasing half-lives and decreasing infusion times, Figure 7A and B. However, an over-estimation of the infusion time will only slightly influence the accuracy of C(0) and consequently the estimated pharmacokinetic parameters to a minor extent using our proposed routines.

The actual drug dose administered to the patient is a crucial point for pharmacokinetic studies as well as for therapeutic drug monitoring (Buclin *et al.* 2005; Parshuram *et al.* 2006). The proposed routines ensure administration of the prescribed drug dose but give also a possibility to collect samples from the infusion solution for concentration measurements which increase the accuracy in pharmacokinetic trials (Buclin *et al.* 2005). Unappreciated variability in the concentration of medication infusions may introduce significant bias in estimated pharmacokinetic parameters (Buclin *et al.* 2005; Parshuram *et al.* 2006; Parshuram *et al.* 2003). It has also been reported that some of the variability in drug concentrations might result from methodological problems in intravenous drug delivery (Buclin *et al.* 2005; Gould and Roberts 1979; Roberts 1981). The importance of drug delivery at a constant rate has to be highlighted since a change in the infusion rate affects the measured drug concentrations influencing the pharmacokinetic evaluation (Nahata 1988).

Protective equipments, i.e. gloves and an absorbent cover, together with proper handling during withdrawal of saline and disposal of the syringe are crucial to secure the personnel protection and to avoid contamination of surrounding areas (Connor and McDiarmid 2006). Priming the infusion set with drug solution can be used to ensure prompt drug delivery but has been recommended to be performed only inside a ventilated cabinet in the pharmacy and should be avoided bedside (Connor and McDiarmid 2006). Our proposed routines with disconnection of the infusion set, once the predetermined volume has been infused, without rinsing might expose the personnel for drug remaining in the tubing. Since the distal three-way stopcock including the 10 centimeter of tubing is rinsed from drug solution prior to disconnection the personnel drug exposure is minimized.

The removal of the distal stopcock after completion of drug administrations ensures that no drug remaining within the stopcock will be released during blood sampling influencing drug concentration measurements. Carefully standardized routines regarding rinsing, flushing as well as discarded waste volumes are crucial to avoid inaccurate drug concentrations when the same central venous access is used for both drug administration and blood sampling (Ritzmo *et al.* 2007).

5.3 PHARMACOKINETICS OF TOBRAMYCIN (PAPER III)

In this paper we investigated the pharmacokinetics of a once-daily dose of tobramycin in pediatric cancer patients since previously published pharmacokinetic data of both once and multiple daily dosing of tobramycin in pediatric cancer patients are very sparse (Dupuis *et al.* 2004; Hoecker *et al.* 1978). A standardized and accurate drug administration combined with a careful and extensive protocol for blood sampling was used to optimize the reliability of the concentration measurements and the subsequent pharmacokinetic evaluation. The very strict standardized drug administration and blood sampling procedures used in the present study probably explain the observed low inter-patient variability.

A two-compartment model, previously reported to be appropriate in adult patients (Aminimanizani *et al.* 2002), was used to describe the pharmacokinetics of tobramycin. We observed a short distribution half-life ($t_{\alpha 1/2}$ = 0.18 h) followed by a prolonged elimination half-life ($t_{\beta 1/2}$ = 1.5 h), both of which are shorter than those reported in adult patients with cystic fibrosis ($t_{\alpha 1/2}$ = 0.4 h, $t_{\beta 1/2}$ = 2.7 h) (Aminimanizani *et al.* 2002). The values of $t_{\beta 1/2}$ in the present study are in close agreement with earlier observations in pediatric patients with malignancies ($t_{\beta 1/2}$ = 1.6 h) (Hoecker *et al.* 1978), but shorter than those reported for children with cystic fibrosis ($t_{\beta 1/2}$ = 2.3 h) (Bragonier and Brown 1998). The distribution half-life in pediatric patients has not previously been described due to shortcomings in the pharmacokinetic studies, in which limited sampling procedures, e.g. only two to four samples with one-compartment modeling, or population pharmacokinetics have been used (Dupuis *et al.* 2004; Hoecker *et al.* 1978).

The AUC normalized by the dose in mg/m^2 showed no age dependence in the studied pediatric patient population suggesting that dosing should be based on BSA. In

contrast, AUC dose normalized by the body weight increased with increasing age of the patients giving an impression of age dependence. It therefore appears that dosing based on body weight is less appropriate in pediatric cancer patients. It has previously been shown that an increasing dose expressed in mg/kg with decreasing age is required to reach target serum concentrations of tobramycin in different age groups of neutropenic children undergoing stem cell transplantation (Dupuis *et al.* 2004). It has also been shown that dosing based on BSA results in a more uniform systemic drug exposure for gentamicin, an aminoglycoside similar to tobramycin, without the need for age adjustments of the drug dose (Siber *et al.* 1979). Dosing based on BSA for tobramycin seems reasonable since this drug is eliminated by the kidneys and the glomerular filtration rate (GFR) correlates well with BSA in children (> 2 years of age) with normal renal function (Bartelink *et al.* 2006). The inter-patient variability of AUC was also lower when normalized by BSA as compared to normalized by body weight in the present study. These findings are in accordance with our previous experiences of pediatric drug dosing (Eksborg *et al.* 2000a; Eksborg *et al.* 2000b).

Neither the optimal dose nor the optimal infusion time for a once-daily tobramycin administration has been established in pediatric cancer patients. The clinically used doses of tobramycin in children vary to a great extent (3 to 15 mg/kg/day) (Bragonier and Brown 1998; Dupuis *et al.* 2004; Hoecker *et al.* 1978; Knoderer *et al.* 2003; Turnidge 2003; Vic *et al.* 1998). The used infusion time varies considerable (from 5 min to 1 h) but generally an infusion time of 0.5 hours has been used (Bragonier and Brown 1998; Dupuis *et al.* 2004; Turnidge 2003; Vic *et al.* 1998) The strictly standardized infusion time of exactly 5 minutes used in the present study gives a possibility to predict the influence of the infusion time on the maximum peak concentration (i.e. at the end of infusion) with high accuracy. The peak concentration of tobramycin is strongly dependent on the infusion time, Figures 11 and 12. The importance of a sufficiently high peak concentration and peak concentration/MIC ratio for the therapeutic efficacy and prevention of resistance in gram-negative bacteria has been pointed out (Burgess 2005; Moore *et al.* 1987; Moore *et al.* 1984). Prolongation of the infusion time should be avoided in order to attain a high peak concentration. It has, however, to be kept in mind that the AUC is unaffected by the variation in infusion time.

The total systemic exposure expressed by AUC as well as AUC over MIC ratio also seem to be of importance for the therapeutic efficacy of aminoglycosides (Burgess 2005; Turnidge 2003; Zhanel and Craig 1994). Elevated trough concentrations of tobramycin are associated with nephrotoxicity (Burgess 2005; Knoderer *et al.* 2003) probably due to a delayed elimination resulting in drug accumulation (Begg *et al.* 1995). Therapeutic drug monitoring (TDM) with a concentration measurement immediately prior to the next administration ("trough concentrations") is often used to identify patients with delayed elimination. This type of TDM for once daily dosing has, however, been questioned since a dose modification will be belated (Begg *et al.* 1995). A sufficiently long drug free period is important to avoid drug accumulation (Begg *et al.* 1995; Kirkpatrick *et al.* 2002). The concentrations were below the detection limit (0.5 µg/mL) already at 7.8 hours (median value) after start of drug administration. The time period, within the dosing interval, with concentrations below 0.5 µg/mL is in

agreement with previously reported data (Dupuis *et al.* 2004) with an interval that indicates a safe margin to avoid drug accumulation.

There are inconsistent definitions of the wording peak concentration in published studies on tobramycin pharmacokinetics which make comparisons of reported concentration data difficult with risk of erroneous conclusions (Bragonier and Brown 1998; Dupuis *et al.* 2004; Hoecker *et al.* 1978; Moore *et al.* 1987; Vic *et al.* 1998). The maximum concentration (C_{max}) is defined from a pharmacokinetic point of view as the concentration obtained at the end of the infusion and used throughout the present study. The variability in infusion time as well as the time points for blood sampling for concentrations measurements also limits the possibility to estimate the actual C_{max} from previously published pharmacokinetic data (Aminimanizani *et al.* 2002; Bragonier and Brown 1998; Dupuis *et al.* 2004; Hoecker *et al.* 1978; Vic *et al.* 1998).

We found the volume of distribution to be lower compared with available information in children (Bragonier and Brown 1998; Dupuis *et al.* 2004; Hoecker *et al.* 1978; Vic *et al.* 1998). The reason for the somewhat divergent results in volume of distribution might be due the variability in the estimation of maximum serum concentration (Dupuis *et al.* 2004; Vic *et al.* 1998).

Our serum clearance for tobramycin was in agreement with previously published data in pediatric patients with malignancies but approximately 15% higher than in children with cystic fibrosis (Bragonier and Brown 1998; Dupuis *et al.* 2004; Hoecker *et al.* 1978).

The risk of nephrotoxicity has been reported to increase if an aminoglycoside is administered during the night (Prins *et al.* 1997; Turnidge 2003). In our limited number of patients we observed a slight tendency for a lower AUC, normalized by the dose in mg/m^2, when tobramycin was administered in the afternoon. The time of administration may thus influence both treatment efficacy and toxicity. Drug administration in the early afternoon and for the shortest period clinically feasible has therefore been recommended (Turnidge 2003).

The pharmacokinetic findings in the present study enables a possibility to adjust the dose to obtain predetermined values of AUC and AUC:MIC ratio. Optimal AUC values dependent on MIC for clinical use have been suggested by Drusano *et al*. (Drusano *et al.* 2007). Our pharmacokinetic data suggest that optimal dosage are 270 mg/m^2 for a target AUC of 65 µg*h/mL; MIC: 0.25 mg/L and 310 mg/m^2 for a target AUC of 75 µg*h/mL; MIC: 0.5 or 1 mg/L.

Even though optimal blood sampling should be performed using a sampling site separated from the site of drug administration this is not always feasible in pediatric patients. Both drug administration and blood sampling were performed using the only available central venous access of the patients, but standardized routines regarding rinsing, flushing as well as waste volumes were used to avoid inaccurate drug concentrations (Ritzmo *et al.* 2007).

5.4 LIMITED SAMPLING STRATEGY FOR TOBRAMYCIN (PAPER IV)

This study shows that the systemic drug exposure of tobramycin, i.e. the area under the serum concentration time curve (AUC), in pediatric cancer patients can be estimated using a limited sampling strategy. One blood sample at 1h (C_{1h}) after an intravenous short time (5 minutes) infusion gives an accurate estimate of the AUC as indicated by the high correlation coefficient (r=0.9855). Deming's linear regression with the 95% CI of the slope using solely the sampling point C_{1h} supports the conclusion of a suitable model for estimation of AUC since an ideal relationship between the investigated variables exists when the slope is not significantly different from unity (Schellens *et al.* 1988).

The suggested one sampling model, for estimation of AUC, with the precision expressed as RMSE of 9.0% and the bias expressed as MPE of 2.28% is therefore well suited as limited sampling model for estimation of tobramycin systemic exposure. Increasing the number of sampling points for estimation of the AUC resulted in a marginally improvement of the correlation coefficient in the present study. However, the precision increased as shown by the decreasing RMSE and the decreasing confidence interval of the estimated/determined AUC ratio, Table I and Figure 14. Previously published limited sampling strategies have also reported increasing precision with increasing number of samples (Liliemark *et al.* 1996; Ratain and Vogelzang 1987; Strömgren *et al.* 1993). A drawback with increasing the precision by additional sampling points is a more inconvenient model with decreasing applicability in clinical practice (van Warmerdam *et al.* 1994).

Limited sampling models have been developed for several drugs (Eksborg *et al.* 1994; Liliemark *et al.* 1996; Mahmood 2000; Ratain *et al.* 1991; Strömgren *et al.* 1993) and suggested for TDM but also for large-scale pharmacokinetic studies (van Warmerdam *et al.* 1994). Developed limited sampling models have generally been prospectively validated to ensure their applicability (Liliemark *et al.* 1996; Mahmood 2000; Ratain *et al.* 1991; Ratain and Vogelzang 1987; Strömgren *et al.* 1993). In the present study, the developed model for estimation of the AUC using one single sampling point (Table 1) was tested prospectively in separate set of patients. The high correlation coefficient (r=0.9579) and the slope equal to unity in combination with the Eksborg's plot (Figure 15A and B, respectively) confirm the usefulness of the suggested model.
The total systemic drug exposure expressed as AUC as well as AUC over MIC ratio seem to be of importance for the therapeutic efficacy of aminoglycosides (Drusano *et al.* 2007; Turnidge 2003). By estimation of the AUC after tobramycin administration an opportunity for optimizing the therapy might be based on a dose adjustment using the optimal values of AUC for various MIC values previously suggested (Drusano *et al.* 2007).

AUC has been suggested to be of significance for nephrotoxicity and the detection of an enhanced value at 1h after drug administration could enable an early dose adjustment already the following dose. An elevated AUC value estimated from C_{1h} may indicate a risk for toxicity but will not reveal a delayed elimination. We therefore recommend an additional serum concentration at 8 hours after drug administration to

identify patients with delayed elimination. The commonly used TDM with a tobramycin concentration measurement immediately prior to the next administration ("trough concentrations") has been questioned for once daily dosing since a dose modification will be unnecessary postponed in patients with delayed elimination (Begg *et al.* 1995).

The importance of a uniform duration and a constant rate of the infusion and an exact sampling time for the limited sampling models have previously been reported (van Warmerdam *et al.* 1994). In the present study, the models for estimation of the AUC were developed using a constant rate infusion during 5 minutes. However, a variation in infusion time between 1 to 10 minutes had only minor influence on the estimated of AUC using the tobramycin concentration measurement at the most important sampling point (C_{1h}) and were even less important for later sampling points (C_{2h}, C_{6h}) as shown in Figure 16. This is a great advantage in the routine clinical setting where a minor deviation in infusion time often occurs.

The importance of careful timing of the blood sampling to obtain a correct estimation of AUC has to be emphasized, Figure 17. The significance of the sampling time is most pronounced for earlier sampling time points since the distribution half-life of tobramycin is only 0.18 hour (Paper III). The use of a sampling point at 0.5 h is therefore less suitable for TDM.

An approach for dosing of aminoglycosides using an estimation of the AUC based on two concentration measurements after an infusion during 30 minutes has previously been presented (Begg *et al.* 1995). This method is, however, less appropriate in our pediatric population due to sampling during the distribution phase and the assumption of a one-compartment model. Furthermore, their method is less suitable in the clinical setting due to the extensive computations needed. We here suggest a more robust and simple method for estimation of AUC which is convenient for use in the clinical routine care.

The developed limited sampling technique is suitable for therapeutic drug monitoring but can also replace more extended sampling protocols for pharmacokinetic studies in large groups of pediatric patients.

5.5 CAPILLARY BLOOD SAMPLING FOR DOXORUBICIN (PAPER V)

The pharmacokinetics of the anthraquinone glycosides shows a very large inter-individual variability (Eksborg *et al.* 1985). Therapeutic drug monitoring has been suggested as a proper way to overcome the pharmacokinetic variability thereby improving therapeutic efficacy and/or reducing side effects (Desoize and Robert 1994). We have previously shown that the drug concentration in one single venous plasma sample drawn at the end of constant infusions gives highly accurate estimates of the systemic exposure of the anthraquinone glycoside doxorubicin and 4*'epi*-doxorubicin (Eksborg *et al.*1990). This approach may substitute a complete pharmacokinetic evaluation requiring at least 12 blood samples collected over a 24-h period (Eksborg *et al*. 1985). The previously established clinical applicability of this method (Eksborg *et*

al. 1992) is further improved by the use of capillary blood sampling, especially in pediatric patients, (Eksborg *et al.* 2000a).

Comparative studies of capillary and venous plasma concentration of anthraquinone glycosides have not previously been published. The very high correlation between concentrations of doxorubicin in capillary and venous plasma samples might falsely give the impression that capillary and venous sampling sites can be used interchangeable, Figure 18. In contrast, the ratio plots (Eksborg 1981) clearly demonstrate minor but significantly higher capillary plasma concentrations, Figure 19. The wide plasma concentration range of doxorubicin, obtained by inclusion of samples from patients treated with large variations in dose and infusion times, did not affect the relative concentrations of doxorubicin in capillary and venous samples.

The capillary/venous plasma concentration ratio of doxorubicin was significantly higher in males than in females. Multiple regression with step-wise variable selection revealed that gender was the only tested variable affecting the capillary/venous concentration ratio. Gender differences in plasma protein binding, body composition or blood flow might influence the amount of doxorubicin diffusing into the tissue, thereby affecting the capillary/venous concentration ratio. Gender specific sampling site differences of drug concentrations have to our knowledge not previously been reported.

The phenomenon and rationale of blood sampling site dependence on drug concentrations have been reviewed (Chiou 1989a; Chiou 1989b). Capillary blood is a mixture of arterial, venous blood, and interstitial fluid (Blumenfield *et al.* 1977). To minimize diluting of the blood with the interstitial fluid, the capillary blood sampling should, as done in the present study, be conducted with free flowing blood with minimum squeezing of the finger. A careful examination of drug concentration differences in capillary and venous blood samples is necessary prior to change of sampling site.

In fact, concentrations of a large numbers of drugs in capillary and venous blood samples have been compared (Bömelburg *et al.* 1987; Ericsson *et al.* 1993; Frazer *et al.* 1983; Gordi *et al.* 2000; Lewis *et al.* 1985; Profumo *et al.* 1995). However, the results are in general difficult to interpret due to unsuitable treatment of data, i.e. the use of scatter diagrams. A high correlation coefficient and/or a p-value < 0.05 are often considered sufficient for conclusions of interchangeable sampling sites. Hence it was concluded that methotrexate venous blood sampling can be substituted by capillary blood sampling, since a scatter plot of the concentration data showed correlation factor of 0.934 (Bomelburg *et al.* 1987). A closer examination of the data showed that the capillary/venous concentrations ratio ranged from 0.2 to 3.1, a fact that may have serious consequences when basing the leucovorin rescue on measured methotrexate concentrations.

5.6 DOXORUBICIN AND ETOPOSIDE IN CLINICAL PRACTICE (PAPER VI AND THE PRELIMINARY RESULTS)

5.6.1 Paper VI

Only limited but conflicting information on the disposition of anticancer drugs in obese patients is available (Cox *et al.* 1987; Eksborg *et al.* 1992; Fleming *et al.* 1991; Navarro 2003; Rogers *et al.* 2005; Rodvold *et al.* 1988; Thompson *et al.* 2009). Even though there does not appear to be enough evidence to support routine dose reduction in obese patients many protocols limit the upper BSA for dose calculations to 2.0 m^2 or suggest the use of the ideal rather than actual body weight to calculate BSA. However, such dose reductions might jeopardize the treatment efficacy (Griggs *et al.* 2005; Navarro *et al.* 2003).

The use of an adjusted BSA for dose calculations as performed in our two morbidly obese patients resulted in a dose reduction of doxorubicin and etoposide by approximately 25% as compared to dosing based on actual BSA. The reason for dose reductions was to avoid unnecessary toxicity, even though available information for proper dosing of chemotherapy in obese patients is non concordant. Therapeutic drug monitoring was performed in order to further evaluate the need for dose adjustment from a pharmacokinetic point of view.

It has been suggested that dosing of lipid-insoluble drugs, including doxorubicin, should be based on patients ideal body surface area or ideal body weight (Cox *et al.*1987). Clinical observations on the need for dose reduction of doxorubicin in obese patients are, however, conflicting. One case of cardiac death associated with doxorubicin treatment has been reported in an obese patient, despite an approximate dose reduction of 20% (Cox *et al.* 1987). The degree of obesity in breast cancer patients did not affect the pharmacokinetics of 4'*epi*-doxorubicin, an anthraquinone glycoside slightly more lipophilic than doxorubicin (Eksborg *et al.* 1992). The pharmacokinetics of doxorubicin and doxorubicinol has also been studied in obese adult patients with repeated sampling for 48 h (Rodvold *et al.* 1988). It was found that the maximum plasma concentration of doxorubicin at the end of 1 hour infusion and the terminal half life increased and plasma clearance decreased with increasing degree of obesity, albeit without increased toxicity. The influence of the degree of obesity on the doxorubicin pharmacokinetics was most pronounced in females, while only minor influences were observed in males.

For ethical reasons it was not feasible to keep our pediatric patients hospitalized for 48 h to perform a complete sampling for pharmacokinetic evaluation of doxorubicin and doxorubicinol. It has previously been shown that it is possible to get reliable estimates of the AUC and hence plasma clearance from measured maximum concentration of doxorubicin in one single blood sample drawn just before the end of infusion (Eksborg 1990). However, this has not been validated in obese patients. In the present study doxorubicin was administered as 4 hour infusions. From these concentration data it is possible to calculate the total plasma clearance, since the relation between concentrations of doxorubicin during constant rate infusions and the steady state level is known (Eksborg 1990). It has to be noticed that the use of blood sampling at 23 h

during 24 h infusions for therapeutic drug monitoring of doxorubicin is not completely validated, although this technique has been used in clinical practice, cf. (Eksborg *et al.* 2000a; Frost *et al.* 2002).

Pharmacokinetics of drugs given in various dose regimens can, under conditions with linear pharmacokinetics, be compared after dose-normalization. It has previously been shown that the pharmacokinetics of doxorubicin is linear within the dose range 20-60 mg/m^2 with infusion times varying between 3 min and 16 h (Eksborg *et al.* 1985). The pharmacokinetics of etoposide is linear within the dose range 32-210 mg/m^2 with infusion times between 1 and 3 h (Eksborg *et al.* 2000b). For this reason, dose normalized clearance of doxorubicin and etoposide, respectively, were calculated for comparison of the pharmacokinetic data in our morbidly obese pediatric patients with data from non-obese patients.

Data from previously published pharmacokinetic studies of doxorubicin in pediatric patients after intravenous infusions (Eksborg *et al.* 2000a; Palm *et al.* 2001) were used to estimate plasma clearance (cf. Eksborg 1990) for comparison with clearance data from our morbidly obese patient, Figure 20. Obviously, the plasma clearance of doxorubicin observed after 4 hour infusions in the patients (BMI 46.3 and 42.6kg/m^2) did neither deviate from our previous observations nor from other pharmacokinetic studies in non-obese pediatric patients (Eksborg *et al.* 2000a; Frost *et al.* 2002; Palle *et al.* 2006; Palm *et al.* 2001).

The concentration of the metabolite doxorubicinol just before the end of infusion of doxorubicin was within the range 5-10% of the intact drug in our patients. The pharmacologic activity of this metabolite is considered to be of the same order of magnitude as of intact doxorubicin (Bachur *et al.* 1976), but it accounts for only about 20% of the total AUC (Itoh *et al.* 2000). Due to lack of published pharmacokinetic data we were not able to compare doxorubicinol concentrations obtained in our patients with data from non-obese patients, but plasma concentration time curves of doxorubicinol were very similar in the three groups of patients with different degree of obesity described previously (Rodwold *et al*. 1988).

There is no information available in the literature supporting the need for dose adjustment of etoposide to obese patients. A potential risk of under dosing obese AML patients due to dose adjustments in the treatment with busulfan and etoposide has been reported (Navarro 2003). The plasma clearence of etoposide in the studied obese male patient did neither deviate from our previous observations in pediatric patients (aged 0.3 – 21.6 years; BMI : 13.8 - 30.1 kg/m^2) (Eksborg *et al.* 2000b) nor from other pharmacokinetic studies in non-obese pediatric patients (Boos *et al.* 1992; Boos *et al.* 1995; Palle *et al.* 2006). The plot of etoposide plasma clearance as a function of BMI, shown in Figure 21, suggest that there was no need for dose adjustment of etoposide in our obese male patient (Eksborg *et al.* 2000b). The terminal half-life, 3.62 h, is also in agreement with previous findings (Boos *et al.* 1992; Eksborg *et al.* 2000b).

More information on how to prescribe chemotherapy in obese patients is needed, especially in pediatric patients. Individual dosing based on TDM is a useful tool for optimizing treatment with antineoplastic drugs, i.e. to avoid the risk of under dosing

which could affect the efficacy and to avoid excessively high drug exposure leading to toxicity. From a pharmacokinetic point of view, the need for dose adjustments of doxorubicin and etoposide in our studied obese patients can be questioned.

5.6.2 Preliminary results

Capillary finger pricks can be very unpleasant due to pain and is therefore unsuitable for frequent sampling, e.g. 8 to 10 samples during a shorter time period such as an eight hour period for a pharmacokinetic study (Kauffman and Kearns 1992). However, capillary blood sampling is often recommended instead of venipuncture (Fradet *et al.* 1990).

Doxorubicin concentrations obtained prior to cessation of the drug infusion as mandatory using our limited sampling procedure makes a single lumen subcutaneous access port unsuitable for blood sampling. One capillary blood sample enables a feasible way to increase the pharmacokinetic information of doxorubicin during repeated courses of treatment, Figure 22-24.

A high inter-individual variability as well as a substantial intra-individual variability in peak plasma concentrations of doxorubicin in children has been published (Hempel *et al.* 2002). Blood sampling was performed when the infusion pump was temporarily stopped (Hempel *et al.* 2002). The relatively low inter-individual and intra-individual in our studied patients is probably due to the strict blood sampling during the constant rate infusion.

6 CONCLUSIONS

In this thesis, factors of importance for pharmacokinetic studies and therapeutic drug monitoring in pediatric cancer patients have been investigated.

Paper I:

- Blood sampling from the central venous access can be used under certain circumstances for therapeutic drug monitoring of methotrexate.

- Carefully evaluated standardized instructions regarding rinsing and flushing after drug administration is required if the central venous access is to be used for blood sampling for drug concentrations of the administered drug.

- It is important to minimize the total discarded blood volume, i.e. waste volume and sampling volume when using the central venous access.

Paper II:

- An exact time point for start and cessation of the intravenous infusion is possible using a standardized drug administration which is crucial when blood sampling is performed solely after the infusion.

Paper III:

- Dosing of tobramycin based on body surface area appears to be more consistent than dosing based on body weight. The pharmacokinetic findings enable a possibility to adjust the dose to obtain a predetermined target values of the systemic drug exposure expressed (AUC) and AUC:MIC ratio.

- The influence of the infusion time of the maximum serum concentration of tobramycin can be predicted from the determined pharmacokinetic data with the possibility to control the peak concentration without affecting AUC.

Paper IV:

- The development of a limited sampling strategy for estimation of the systemic drug exposure (AUC) of tobramycin was possible due to the standardized drug administration in paper III.
- The actual sampling time is of great importance while a minor deviation in the infusion time is of less significance for the estimation of AUC using the developed limited strategy.

Paper V, VI and preliminary results:

- Capillary blood sampling is an alternative to peripheral venipuncture for pharmacokinetic studies of doxorubicin based on limited sampling schedule using one blood sample per treatment occasion in pediatric patients.

7 FUTURE DIRECTIONS

This thesis emphasize the need for appropriate drug administration and blood sampling for pharmacokinetic studies but also for therapeutic drug monitoring in pediatric patients.

Most importantly, our developed techniques for standardized intravenous drug administration and blood sampling procedures enable us to perform pharmacokinetic studies with high quality in different patient populations, e.g. infants, patients with Down's syndrome and obese patients. However, this thesis raises many important working areas since several questions are still unanswered or requires further investigations.

The minimum blood volume needed to clear the subcutaneous access ports including tubing's remains to be studied. Furthermore, if the three-way stopcock contributes to the risk of enhanced concentrations in blood samples drawn from the subcutaneous access port this might be solved quit easily by replacing the stopcock after drug administration as recommended in paper II.

Reinjection of the withdrawn blood volume required to clear the catheter from contaminates when using blood sampling from the subcutaneous access ports would be interesting to further evaluate since it is a convenient way of minimizing the discarded blood volume.

The standardized drug administration in paper III enabled a great opportunity since it was possible to develop a limited sampling strategy. One retrospective investigation is currently ongoing with the goal to evaluate the appropriate dosing parameter, i.e. dosing based on body surface area as suggested in paper III, in a larger group of pediatric cancer patients using the limited sampling procedure. A further evaluation of the slight tendency of a chronopharmacokinetic effect on tobramycin is also ongoing. The limited sampling approach would also be of great interest for estimating the systemic drug exposure in infants and neonates.

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