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Review paper

The clinical pharmacology of alkylating agents in high-dose chemotherapy

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Alkylating agents are widely used in high-dose chemotherapy regimens in combination with hematological support. Knowledge about the pharmacokinetics and pharmacodynamics of these agents administered in high doses is critical for the safe and efficient use of these regimens. The aim of this review is to summarize the clinical pharmacology of the alkylating agents (including the platinum compounds) in high-dose chemotherapy. Differences between conventional and high doses will be discussed. [© 2000 Lippincott Williams & Wilkins.]

Key words: Alkylating agents, clinical pharmacology, high-dose chemotherapy.

Introduction

High-dose chemotherapy with autologous or allogeneic bone marrow transplantation is a well-established treatment option in hematological malignancies. The development of the highly effective serotonine antagonist anti-emetics, hematopoietic growth factors and peripheral blood progenitor cell transplantation has contributed greatly to the feasibility and safety of high-dose chemotherapy. These advances have made hematological toxicity manageable and allow substantial dose escalation. Over the last decade, high-dose chemotherapy has widely been investigated for the treatment of solid tumors such as breast, ovarian and germ cell cancer.[1] Prerequisits for a useful application of anticancer agents in high-dose regimens are a steep dose–response curve, antitumor activity at standard doses and dose-limiting hematological toxicity at standard doses allowing substantial dose escalation if bone marrow protection is applied. Most alkylating agents share these properties and therefore form the cornerstone of current high-dose chemotherapy regimens. Since alkylating agents generally lack cross-resistance, combinations of alkylating agents have been employed extensively. The use of these combinations may reduce the likelihood of resistance and increase therapeutic benefit.[1] Alkylating agents can be divided in several classes based on their chemical structures. The main classes are the nitrogen mustard derivatives (e.g. cyclophosphamide, ifosfamide and melphalan), ethylenimines (e.g. thioTEPA), alkyl sulfonates (e.g. busulfan), nitrosoureas (e.g. carmustine) and the triazenes (e.g. dacarbazine and temozolomide). Alkylating agents cause cytotoxicity by the formation of DNA alkyl adducts which leads to apoptosis. Most alkylating agents are bifunctional and form interstrand DNA cross-links. The platinum compounds cisplatin and carboplatin strictly do not belong to the alkylating agents. The common denominator, however, is that these drugs are DNA–adduct-forming agents that kill cells by inducing apoptosis. The therapeutic use of platinum compounds in high-dose chemotherapy is in principal similar to the true alkylating agents and therefore these agents are also considered in this respect. The chemical structures of the compounds most frequently used in high-dose chemotherapy are shown in Figure 1.

Up to 20 times the standard dose of these agents has been administered in high-dose regimens (Table 1).[1] Profound knowledge of the clinical pharmacology of these high-doses is considered crucial for the safe use

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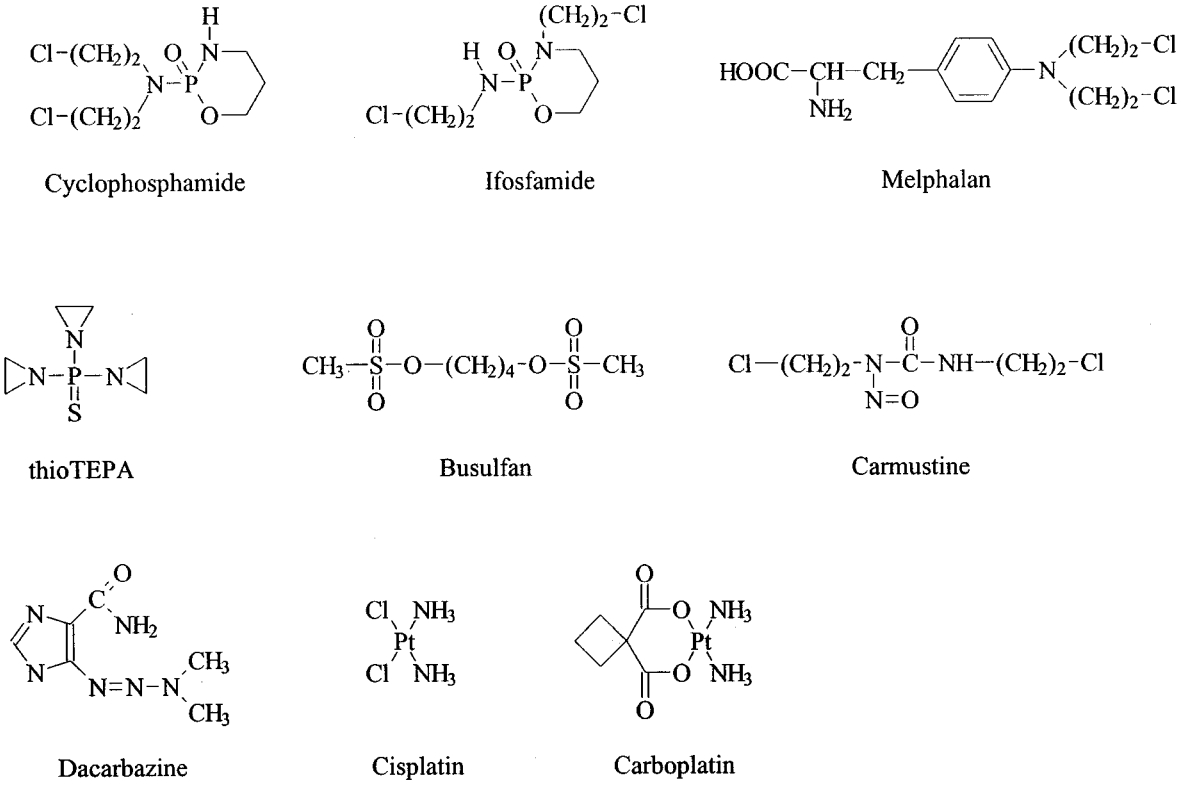

Figure 1. Chemical structures of alkylating agents commonly used in high-dose chemotherapy.

Table 1. Alkylating agents commonly used in high-dose chemotherapy

Drug	Doses in conventional dose regimens	Doses in high-dose regimens	Major toxicity in high-dose regimens	Main elimination pathway	Other
Cyclophosphamide	0.5–1.5 g/m^2	4–8 g/m^2	bladder, pancarditis	CYP	prodrug, auto-induction, saturable elimination?
Ifosfamide	5–9 g/m^2	10–16 g/m^2	renal, bladder, CNS	CYP	prodrug, auto-induction, saturable elimination?
Melphalan	30 mg/m^2	140–220 mg/m^2	GI	degradation, renal	
ThioTEPA	65 mg/m^2	320–1000 mg/m^2	GI, hepatic, CNS	CYP, GSH	saturable elimination, active metabolites
Busulfan	2–10 mg/day	16 mg/kg	hepatic, CNS	metabolic, GSH	increased elimination in children
Carmustine	200 mg/m^2	300–1200 mg/m^2	pulmonary, hepatic	degradation, CYP	
Dacarbazine	0.75–1 g/m^2	4–10.5 g/m^2	hypotension, GI	CYP	prodrug, saturable elimination
Carboplatin	250–400 mg/m^2	800–2400 mg/m^2	Neuro-, oto-, renal toxicity	renal	
Cisplatin	75–100 mg/m^2	150–200 mg/m^2	neuro-, oto-, renal toxicity	renal	

GI = gastrointestinal; CNS = central nervous system, GSH = glutathione conjugation; CYP = cytochrome P450-mediated metabolism.

of these drugs. For instance, saturation of elimination pathways may occur, resulting in non-linear and unpredictable pharmacokinetics. Moreover, most alkylating agents are extensively metabolized both to active and inactive metabolites, and administration of high-doses and combinations may result in shifts in metabolic pathways. The aim of this review is to summarize the current knowledge of the clinical pharmacology of the alkylating agents in high-dose chemotherapy including differences between the use at conventional and high-doses.

The doses, toxicity and elimination pathways of alkylating agents commonly used in high-dose chemotherapy are summarized in Table 1. Table 2 shows the main pharmacokinetic parameters of these agents both in conventional and high-dose regimens. The clinical pharmacology of the different classes of alkylating agents is discussed in detail below.

Nitrogen mustard derivatives

The class of nitrogen mustard derivatives includes the oxazaphosphorines (cyclophosphamide and ifosfamide), melphalan, mechlorethamine and chlorambucil. Due to their chemical instability and/or relative slow cytotoxic effect, mechlorethamine and chlorambucil have rarely been included in high-dose chemotherapy regimens and are therefore not included in this review.

Oxazaphosphorines

Cyclophosphamide and ifosfamide belong to the group of the oxazaphosphorines. Other, rarely used, oxazaphosphorines are trofosfamide and mafosfamide. The potential of cyclophosphamide as a chemotherapeutic agent was first described in 1958.[68] Oxazaphosphorines are stable derivatives of nitrogen mustard. Metabolism of the oxazaphosphorines plays a crucial role in the clinical pharmacology of these drugs. Both ifosfamide and cyclophosphamide are prodrugs undergoing a variety of metabolic pathways consisting of both activation and inactivation reactions.[69] The metabolic scheme of cyclophosphamide is depicted in Figure 2. The first step in the activation is the 4-hydroxylation mediated by the cytochrome P450 (CYP). The resulting intermediate (4-hydroxycyclophosphamide) is in equilibrium with its ring-opened form aldophosphamide. CYP 2A6, 2B6, 2C9, 2C18, 2C19, 3A4 and 3A5 have been related with the 4-hydroxylation of cyclophosphamide, of which CYP2B6 has the highest activity.[70–73] Non-enzymatic β-elimination of acrolein results in the formation of the final cytotoxic metabolite phosphoramide mustard. Due to its polar nature, phosphoramide mustard is not capable of penetrating cell membranes and therefore only intracellularly formed phosphoramide mustard is considered to be the cytotoxic fraction. Cytotoxicity of phosphoramide mustard is mediated by the formation of interstrand DNA cross-links.[69] In this process, chloroethylaziridine, formed from phosphoramide mustard, may be involved.[74] Glutathione conjugation mediated by glutathione S-transferase may play an important role in the cellular resistance to the oxazaphosphorines.[75] Side-chain oxidation by CYP3A4 of cyclophosphamide results in 2-dechloroethylcyclophosphamide and an equimolar amount of chloroacetaldehyde.[76] Two major inactivation routes of 4-

Table 2. Summary of the pharmacokinetics of alkylating agents in conventional and high-dose regimens

Drug	Conventional dose regimens (without hematological support)				High-dose regimens (with hematological support)			
	Cl (1/h)[a]	V_{SS} (1)[a]	$t_{1/2,\beta}$ (h)	Reference	Cl (1/h)[a]	V_{SS} (1)[a]	$t_{1/2,\beta}$ (h)	Reference
Cyclophosphamide[c]	4.7–5.6	34–63	4.2–8.2	2–7	2.5–5.8	21–35	4.6–8.7	2,3,8–14
Ifosfamide[c]	3.1–7.2	23–88	3.8–15	15–17	11.6[d]	NR	NR	18
Melphalan	14–67	17–70	0.7–1.4	19–21	28–55	25–70	0.5–1.4	22–29
ThioTEPA	19–81	30–125	1.2–2.4	30–33	14–35	40–110	1.3–3.7	34–41
Busulfan[b]	14–16	NR	2.5	42	11–14	46–63	2.3–4	43–48
Carmustine	235	228	0.37	7,49	113–326	357	0.37–4.3	50–52
Dacarbazine	12–65	39–104	0.7–2.9	53–55	18	65	3	56
Carboplatin[e]	4.2–16	17–35	1.7–6.3	57	5.1–11	21–74	1.8–6.3	18, 58–62
Cisplatin[e]	33–63	19–23	0.6–2.0	63–66	NR	26–33	NR	67

NR, not reported.
[a]Cl and V_{SS}, when necessary normalized for BSA = 1.73 m^2 and body weight = 70 kg.
[b]Pharmacokinetic parameters after oral administration in adults (CL/F and V/F) are reported.
[c]Cl and $t_{1/2}$ of day 1 are given.
[d]Mean clearance during 96 h infusion.
[e]Pharmacokinetics of ultrafilterable platinum.

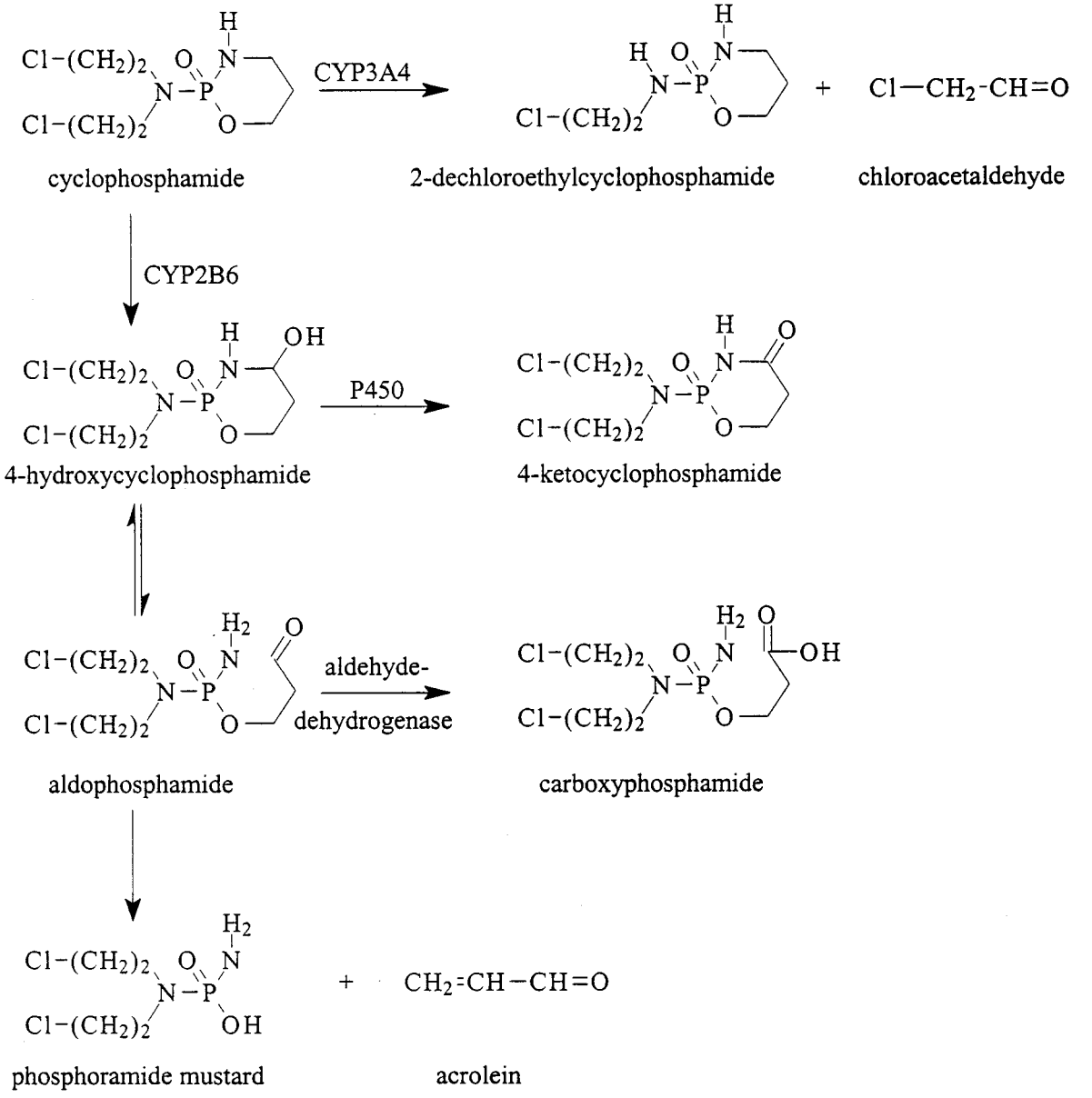

Figure 2. Metabolic scheme of cyclophosphamide.

hydroxycyclophosphamide exist. Oxidation of 4-hydroxycyclophosphamide by CYP results in 4-ketocyclophosphamide, while oxidation of aldophosphamide by aldehyde dehydrogenase results in the formation of carboxyphosphamide.[69] Ifosfamide is an isomer of cyclophosphamide with a chloroethyl moiety on both the exocyclic and endocyclic N-atom (Figure 1). Ifosfamide shows a similar metabolic scheme. Side chain oxidation of ifosfamide, however, results in the formation of two dechloroethylated metabolites (2- and 3-dechloroethylifosfamide). Both activation and inactivation routes of ifosfamide are mediated by CYP3A4.[71,73]

The inactivation routes of cyclophosphamide and ifosfamide result in metabolites which may induce several side effects. Chloroacetaldehyde is held responsible for the neurotoxicity associated with ifosfamide therapy and acrolein may cause severe bladder toxicity (hemorraghic cystitis). To prevent acrolein associated side effects, 2-mercaptoethanesulfonate (MESNA) is co-administered in both ifosfamide and cyclophosphamide therapy. These toxic metabolites

are not formed from glufosfamide, a recently developed prodrug of ifosforamide mustard.[77]

Cyclophosphamide is one of the most frequently used alkylating agents in high-dose chemotherapy. Doses of cyclophosphamide in conventional dose chemotherapy are in the range of 500–1500 mg/m,^{2} while in high-dose combination chemotherapy doses of 6 g/m^2 are commonly used. Dose-limiting toxicity of these high-doses is cardiotoxicity. In doses over 1.55 g/m^2/day, cardiotoxicity occurred in 25% of the patients.[78] Hemorraghic cystitis has been observed in 12–35% of the patients receiving high-dose cyclophosphamide.[79]

Several studies have described the pharmacokinetics of parent cyclophosphamide and metabolites in high-doses[2,3,8–13,80–86] (Table 2). Urinary excretion of cyclophosphamide accounted for 12–30% of the dose.[2,11,80,87] The fraction of the dose converted to 2-dechloroethylcyclophosphamide is 4–7%.[2,8] Busse *et al.* compared the pharmacokinetics of conventional (500 mg/m^2) with high-dose (100 mg/kg) cyclophosphamide and demonstrated that total body clearance was equal at both doses, although the clearance of the bioactivation route was significantly reduced in the high-dose scheme (66 versus 48.5% of the total clearance). Furthermore, renal clearance and formation of 2-dechloroethylcyclophosphamide were increased after high-dose cyclophosphamide compared to the conventional dose regimen.[3] This indicates that, although cyclophosphamide shows linear pharmacokinetics, the balance between the different metabolic pathways may shift in favor of the inactivation routes. In several studies, auto-induction of cyclophosphamide metabolism has been shown. Auto-induction resulted in an increased clearance and thus a decreased AUC of cyclophosphamide,[2,8–11,14,82,85,86,88] an increased exposure to 4-hydroxycyclophosphamide,[2,8,10,14] phosphoramide mustard[9,85] and 2-dechloroethylcyclophosphamide[2] during treatment. This suggests that both the activation pathway (4-hydroxycyclophosphamide and phosphoramide mustard) and the inactivation pathway (2-dechloroethylcyclophosphamide) are induced, which is reflected in an increased urinary excretion of the different metabolites.[87] The induced metabolic elimination resulted in a reduced renal elimination of the parent drug during treatment,[2,11] although this finding was not demonstrated in the study of Joqueviel *et al.*[87] In general, auto-induction is detectable within 24 h after the start of the treatment and may result in a 2-fold increase in clearance and subsequent 2-fold decrease in elimination half-life of cyclophosphamide.[2,3,8,10,11] Ren *et al.* showed that the increased exposure to 4-hydroxycyclophosphamide cannot solely be explained by an increased formation by auto-induction and thus a decreased elimination of this metabolites may also play a role.[8]

Hassan *et al.* developed a mechanism-based enzyme model for the auto-induction of the bioactivation route.[86] The model comprised a hypothetical amount of enzyme of which the formation rate was increased by the cyclophosphamide concentration resulting in an increased clearance during treatment. Consequently, a direct relationship between plasma concentration and rate of auto-induction was used. Chen *et al.* and Anderson *et al.* found a convex downward shaped concentration time curve after a 90 min infusion of 4 g/m^2, which was explained by saturation of elimination. K_m values of 247 and 575 μM were found for this process.[80,83,84] Saturable elimination has not been described in other studies. As demonstrated in the study of Hassan *et al.*,[86] the rate of auto-induction may be directly related to the cyclophosphamide plasma concentration. In the studies of Chen *et al.* a high-dose was administered in a short infusion resulting in very high plasma concentrations.[80,83] The observed convex-shaped curves can, therefore, also be explained by a very rapid development of auto-induction.

Anderson *et al.* and Chen *et al.* found an increased AUC of cyclophosphamide and decreased AUC of 4-hydroxycyclophosphamide in combination with thioTEPA compared to cyclophosphamide as single agent.[80,84] In a later study, a strong schedule dependency of 4-hydroxycyclophosphamide pharmacokinetics in combination with thioTEPA has been demonstrated.[89] These findings have been explained by CYP inhibition by thioTEPA.[84,89] Furthermore, CYP-based interactions with cyclophosphamide have been described for the CYP inducers phenytoin,[13] pehenobarbital[90] and ondansetron[67,91] (resulting in reduced exposure to cyclophosphamide and increased bioactivation), and the inhibitor fluconazole.[92] A positive correlation between elimination half-life of cyclophosphamide and body weight after conventional dose has been described with an approximately 2-fold increase in half-life in obese patients.[6] Juma *et al.* found a significant longer half-life of cyclophosphamide and phosphoramide mustard in patients with renal insufficiency,[93] although in two case reports the administration of high-dose cyclophosphamide (120 mg/kg divided over 2 days) proved feasible in patients requiring hemodialysis.[94,95] Ayash *et al.* and Petros *et al.* demonstrated that both occurrence of heart failure and efficacy (tumor response and survival) were inversely related to cyclophosphamide AUC in high-dose combination therapy.[81,96] This finding was explained by an

inverse relation between the AUC of the parent compound and activated metabolites which has been demonstrated by others.[13]

Ifosfamide is less frequently used in high-dose chemotherapy than cyclophosphamide, mainly because non-hematological toxicity becomes dose limiting just above the doses used in conventional dose chemotherapy.[1] For ifosfamide, renal failure, hemorraghic cystitis and neurotoxicity have frequently been encountered in high-dose regimens. In conventional dose regimens, doses up to 9 g/m^2 have been used, while in high-dose combinations, doses of 10–16 g/m^2 have been applied.[1] The clinical pharmacology of ifosfamide in high-doses is, therefore, not essentially different from conventional dose regimens as reviewed by others.[15–17] In contrast to cyclophosphamide, dechloroethylation of ifosfamide and concomitant formation of chloroacetaldehyde play a major role in the elimination of ifosfamide. Chloroacetaldehyde is held responsible for the neurotoxicity and renal failure caused by ifosfamide, possibly mediated by depletion of intracellular glutathione.[97] Moreover, a cytotoxic effect for chloroacetaldehyde comparable to 4-hydroxyifosfamide has been described, which may explain the lack of complete cross-resistance between ifosfamide and cyclophosphamide.[97,98] Chloroacetaldehyde neurotoxicity may be prevented by the co-administration of methylene blue by interference with the mitochondrial terminal respiration chain. This may allow further dose escalation.[99] As for cyclophosphamide, ifosfamide shows auto-induction with similar metabolic consequences.[15,16] A mechanism-based pharmacokinetic model for the auto-induction of ifosfamide with the same principles as used by Hassan *et al.* for cyclophosphamide[86] has been developed recently.[100] Cerny *et al.* described saturation of activation and inactivation routes of ifosfamide although the AUC of the parent compound increased linear with increasing dose (12–18 g/m^2 as monotherapy).[101] In this study, however, interpatient variability was wide and the number of patients per dose level was relatively small. In a study of Wright *et al.*, ifosfamide (16 g/m^2) was administered in combination with etoposide and carboplatin during a 96 h infusion with bone marrow transplantation. Renal insufficiency precluded further dose escalation of ifosfamide. A correlation between the ifosfamide levels at 16–22 h and renal toxicity was found in this study suggesting a role for pharmacokinetic-guided dosing.[18]

Melphalan

Melphalan was first synthesized in 1953. It is the phenylalanine derivative of nitrogen mustard.[102] The cellular transport mechanism of melphalan is unique since it is mainly transported into the cell by a selective L-amino acid transporter. Other substrates of this transport system are the amino acids glutamine and leucine.[103] As for other alkylating agents, sensitivity and resistance to melphalan may be mediated by the intracellular formation of glutathione conjugates.[104,105] Melphalan has frequently been used for a variety of tumors in both adults and children, and is incorporated in a well-established high-dose regimen in combination with carmustine, etoposide and cytarabine (BEAM) for hematological malignancies. Dose escalation of melphalan is limited by the occurrence of severe mucositis. In a study of Moreau *et al.*, 13 out of 16 patients experienced grade 4 mucositis after 220 mg/m^2 followed by stem cell tranplantation.[24]

Melphalan is hydrolyzed in aqueous solutions and whole blood/plasma to the inactive mono- and dihydroxy metabolites.[27,106–108] The protein binding of melphalan is 80–90% of which 30% is covalently bound.[19,106] Between 6 and 30% of the dose is excreted unchanged in urine.[19,109] Penetration of melphalan in cerebrospinal fluid (CSF) is limited and possibly clinically insignificant.[27,110] A CSF/plasma ratio of approximately 0.1 in adults has been demonstrated.[110] Linear pharmacokinetics of melphalan have been reported.[20,24,26] Melphalan is almost completely eliminated within 24 h after administration, allowing rapid reinfusion of stem cells.[22,27,29] Variability in clearance is up to 10-fold in patients treated with similar doses.[22–24] Clearance is weakly related to creatinine clearance[22,25] and high-dose melphalan has safely been administered to patients with renal failure without dose adjustments.[23] In a study of Peters *et al.*, however, two patients with renal failure showed a prolonged half-life and experienced severe toxicity.[26] Studies in children did not reveal age dependency of the pharmacokinetics.[27,29,109] The wide interpatient variability encountered justifies a pharmacokinetically guided dosing strategy. Tranchand *et al.* studied the feasibility of the use of a test dose to quantitate exposure. A low test dose administered within 24 h of the high dose accurately predicted exposure to the high dose administered subsequently.[20] In a subsequent study of the same group in conventional dose therapy, the dose was individualized after a standard dose and the deviation of the target AUC was found to be less than 15% in all patients. Moreover, significant relationships between AUC and hematological toxicity were observed in this study.[111]

Besides i.v. administration, melphalan has been administered orally in combination with stem cell transplantation.[112,113] Melphalan absorption involves

an energy-dependent system which can be inhibited by amino acids.[114,115] Both intrapatient and interpatient variability in bioavailability were extremely high—a range of 25–100% was reported, with some evidence for saturable absorption.[112,113,116,117] Variability may partly be due to variations in the gastric pH, which may also be an explanation for the reduced bioavailability in combination with cimetidine.[118] Therefore, oral administration cannot be recommended for high-dose melphalan therapy.

Ethylenimines

ThioTEPA is the major representative of group of the aziridine or ethelenimine alkylating agents. The first compound synthesized in this class was triethylenemelamine which was the synthetic precursor of *N*,*N*′,*N*″-triethylenephosphoramide (TEPA). TEPA showed a profound cytotoxic effect, but was chemically too unstable to be used in clinical practice.[119] ThioTEPA (*N*,*N*′,*N*″-triethylenethiophosphoramide) is a more stable sulfur analog of TEPA and also possesses strong alkylating activity.[120] *In vitro* studies showed that thioTEPA exerts cytotoxicity by the production of single-strand DNA breaks, while TEPA causes alkali-labile DNA lesions.[121] ThioTEPA is registered as antineoplastic agent for the treatment of bladder, breast and ovarian cancer. Van Maanen and Beijnen recently reviewed the chemistry and pharmacology of thioTEPA.[122]

In dose-escalating studies in the high-dose setting, central nervous system toxicity, gastrointestinal toxicity (mucositis) and hepatic toxicity became dose limiting.[34,38] Less than 2% of the administered dose of thioTEPA is eliminated unchanged in urine.[123–125] Therefore, metabolism is considered to be the major elimination route of thioTEPA. The metabolic scheme of thioTEPA is shown in Figure 3. The first metabolite of thioTEPA identified was TEPA,[126,127] which is formed by an oxidative desulfuration reaction mediated by the CYP 2B and 2C enzymes.[128,129] Recently, two other metabolites of thioTEPA were identified. The conversion of an aziridinyl function of TEPA into a β-choroethylphosphoramide moiety results in the formation of monochloroTEPA and conjugation of thioTEPA to glutathione with subsequent loss of two amino acids results in the formation of thioTEPA-mercapturate.[130] Both metabolites have only been detected in urine of patients treated with high-dose thioTEPA. The role of these metabolites needs to be investigated in future studies. Renal

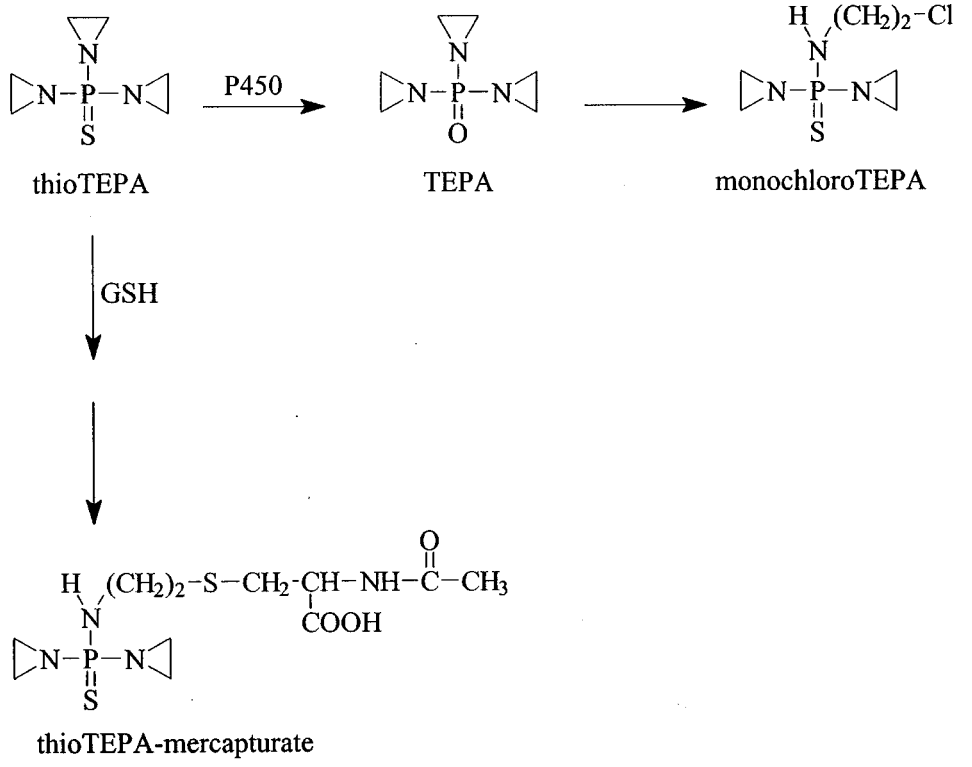

Figure 3. Metabolic scheme of thioTEPA.

elimination of thioTEPA, TEPA, monochloroTEPA and thioTEPA-mercapturate accounted for approximately 0.5, 11, 0.5 and 12% of the administered dose, respectively, indicating that other metabolic routes must exist.[130] All metabolites show considerable alkylating activity. The alkylating activity of TEPA is comparable to that of thioTEPA.[130,131]

ThioTEPA and TEPA both exhibit excellent penetration into CSF.[31] Protein binding of thioTEPA is about 10%.[132] Correlation between the volume of distribution and body weight and between clearance and liver function have been demonstrated.[40,41] Nevertheless, the influences of these co-variables are possibly clinically irrelevant. Ackland *et al.* found that two patients with moderate liver dysfunction had a significantly lower thioTEPA clearance than a patient with normal liver function receiving the same dose.[37] Both in conventional dose and in high-dose regimens, saturable elimination of thioTEPA has been described,[30,31,35,38] while others did not report a decreased clearance at higher doses.[32–34,37,40] Lazarus *et al.* and Ackland *et al.* found a linear increase of the AUC when doses were increased from 45 to 405 mg/m^2/day or 1.8 to 7.0 mg/kg/day in short infusions, respectively.[34,37] Hussein *et al.* showed a substantially lower clearance at doses of 750–900 mg/m^2/day compared to 300–450 mg/m^2/day in a short infusion,[38] which is considerably higher than the doses used by Lazarus *et al.* and Ackland *et al.* Henner *et al.* found an inverse relation between dose and clearance with doses between 180 and 900 mg/m^2 in a 96 h infusion.[35] These results indicate that at higher plasma concentrations saturation of the metabolic enzymes may occur, and the combination of the applied infusion duration and the dose may explain the different findings with respect to non-linearity.

TEPA is detectable within few minutes after the start of the thioTEPA infusion and has a considerably longer half-life than the parent compound. The half-life ranged between 3.0 and 4.6 h, although half-lives up to 21 h have also been reported.[30,31,39] The AUC of TEPA generally exceeds the AUC of thioTEPA. Przepiorka *et al.* found a ratio of the TEPA and thioTEPA AUC of 1.3–1.5 in a high-dose regimen with doses of 150–250 mg/m^2.[40] Heideman *et al.* and O'Dwyer *et al.* demonstrated saturable formation of TEPA at a conventional dose, which may explain the non-linear elimination of thioTEPA.[30,31] Interpatient variability in clearance of thioTEPA has been reported to be between 28 and 90%.[34,35,37–39] Variability in TEPA AUC in high-dose thioTEPA was 15–50%.[39,40] At conventional doses, variability in TEPA pharmacokinetics exceeded the variability in thioTEPA pharmacokinetics.[30,31]

Hussein *et al.* found a correlation between thioTEPA AUC and development of severe mucositis in a dose-escalating study of thioTEPA in combination with cyclophosphamide and cisplatin.[38] Antman *et al.* have reported that the occurrence of regimen-related toxicity of high-dose thioTEPA correlated to thioTEPA AUC, but TEPA pharmacokinetics were not evaluated in this study.[133] Significant correlations between non-hematopoietic toxicity and the sum of thioTEPA and TEPA AUC and TEPA peak concentrations above 1.75 μg/ml were demonstrated in the study of Przepiorka.[40]

Alkyl sulfonates

Busulfan is the only representative of the alkyl sulfonates widely used in high-dose chemotherapy. Busulfan is a bifunctional methane sulfonate capable of spontaneous alkylation of DNA. High-dose busulfan is frequently used in combination with hematopoietic transplantation for a variety of hematological malignancies in children and adults. Due to its low solubility in water, busulfan is usually administered orally. In 1999 a busulfan formulation for i.v. administration (Busulfex®) has been approved by the FDA. Therapy usually consists of 4 mg/kg/day orally administered in four divided doses during 4 days of treatment. Hepatic veno-occlusive disease (VOD) and neurotoxicity (convulsions) are the main regimen-related toxicities with VOD occurring in 20–40% of the patients.[134] For the prevention of neurotoxicity often prophylactic anticonvulsant therapy has been used. Bioavailability has been reported to be approximately 70%.[135,136] Busulfan is mainly eliminated by metabolic routes, while approximately 1–5% of the dose is excreted unchanged in urine.[42,43,137] Glutathione conjugation, predominantly catalyzed by gluthatione S-transferase A1-1, results in the formation of the tetrahydrothiophenium ion,[43,138] while in urine sulfolane and 3-hydroxysulpholane have been identified as metabolites.[43] Busulfan was detected in CSF with a mean CSF:plasma ratio of 0.95–1.39 with some evidence for non-linear CSF distribution.[43,139,140] Protein binding of busulfan has been reported to be low (7%).[43] In adults, the mean AUC after the standard 16 mg/kg regimen was 1300–2000 μM·min[46,48,141] with an interpatient variability of up to 9-fold, which can partly be explained by the wide interpatient variability in bioavailability (varying between 22 and 100%).[135,136] Oral clearance (CL/F) was increased and the elimination half-life and AUC were decreased in young children (0.5–3 years) compared to older children (7–19 years) and adults.[48,140,142,143] Therefore, body

surface area-based dosing has been proposed for young children and resulted in comparable exposures in these children compared to adults.[140,144–146] A circadian rhythm has been observed in the pharmacokinetics of which the clinical relevance is unclear.[45,48,137,141] Moreover, disposition may be influenced by type of disease and body mass.[44,147] In children, the occurrence of neurotoxicity was dose-dependent and possibly related to prolonged brain exposure to busulfan and/or its metabolites.[140] Some studies have described decreased plasma levels during treatment, possibly caused by auto-induction[43,48] although it has been shown that phenytoin, in these studies used as prophylactic anti-convulsant, induces busulfan metabolism,[47] which may also explain this phenomenon. The disposition of busulfan has been associated with both treatment outcome and (hepatic) toxicity. Treatment related mortality and occurrence of VOD has been correlated to busulfan AUC.[46,141,148,149] Moreover, in a study of Slattery *et al.* busulfan steady-state levels below the median value (917 ng/ml) were associated with an increased risk of relapse.[134] Grochow *et al.* identified a toxic AUC of 1500 μM·min as a relatively sharp cut off level above which there is very high risk of VOD. Subsequently, the possibility of AUC guided dose adjustments was studied.[45] Patients with an AUC value above the toxic level after the first dose received a dose reduction for the fifth and subsequent doses (total 16 doses). Dose adjustment reduced the incidence of VOD from 75 to 18%.[45] The relative risk for occurrence of VOD in patients with an AUC$>$1500 μM·min has been reported to be 11 ($p<0.01$).[141] Dix *et al.* performed a similar study but no reduction in occurrence of VOD was observed in the group with the reduced dose, which may be explained by the late dose reduction achieved in this study (after the 10th dose).[141] To avoid this problem, the exposure can be determined after a test dose followed by AUC guided dosing of the subsequent doses. In a two studies, it was demonstrated that this approach lead to accurate and precise predictions of the exposure.[150,151] For rapid assessment of exposure limited sampling models (two or three samples) have been developed.[150,152]

Nitrosoureas

Carmustine (BCNU) is the most frequently used member of the nitrosourea-class of alkylating agents. Other, less frequently used, members of this class are lomustine, semustine and streptozocin. Cytotoxicity of the nitrosoureas is mediated by the formation of DNA interstrand crosslinks in a two-step reaction. The first step is the alkylation of guanine in the O^6 position, followed by interstrand crosslinking with cytosine.[153] The repair enzyme O^6-alkylguanine alkyltransferase (AGT) is involved in cellular resistance to nitrosoureas.[154,155] Significant relationships between AGT levels and outcome in patients treated with carmustine have been described.[156,157] Due to the high lipophilicity, these drugs readily cross the blood–brain barrier and have therefore frequently been used for the treatment of brain tumors.[158] In conventional dose regimens, the nitrosoureas show an unusually delayed myelosuppression. High-dose carmustine is complicated by the occurrence of pulmonary toxicity (lung fibrosis) and hepatic toxicity (VOD).[51,52,159-162] Pulmonary toxicity has been encountered even up to 17 years after chemotherapy.[163] At doses above 1500 mg/m^2, dose-related neurotoxicity and cardiotoxicity were encountered.[160]

Several putative metabolic routes have been identified. Hydrolytic decomposition of carmustine at physiological pH has been described resulting in both active and inactive products.[164] Furthermore, exposure to carmustine was decreased after pre-treatment with phenobarbital, suggesting a role for microsomal metabolism.[165] Both NADPH-dependent microsomal denitrosation and glutathione-dependent denitrosation have been described in preclinical studies.[166–169] The importance of these metabolic routes for the activity of carmustine has been reviewed by Lemoine *et al.*[170] Plasma protein binding of carmustine has been reported to be 77%.[50]

The AUC of carmustine varied over a 15-fold range in patients receiving the same dose.[51,159] Due to this high-interpatient variability, no difference in AUC in patients receiving 450 or 600 mg/m^2 could be demonstrated.[159] Pharmacokinetic parameters were similar to those reported for conventional dose suggesting linear pharmacokinetics.[50] In a study of Mbidde *et al.*, four cases of irreversible late marrow failure were found and these cases were associated with the reinfusion of bone marrow within 20 h after treatment since carmustine levels were detectable 24 h after treatment.[52] In a study of Jones *et al.*, 53% of the patients experienced acute pulmonary toxicity after 600 mg/m^2 carmustine in combination with cyclophosphamide and cisplatin. In this study, an association between the AUC of carmustine and the occurrence of lung toxicity was demonstrated. A threshold AUC of 600 μg·min/ml, above which the risk for toxicity was significantly increased, was identified.[51] In rats, both the AUC and the variability of the AUC of carmustine were increased after pretreatment with cyclophosphamide and cisplatin.[171] This may be in agreement with the increased

risk for pulmonary toxicity encountered in the combination of carmustine with cyclophosphamide and etoposide.[162]

Triazene derivatives

Dacarbazine (DTIC) and temozolomide belong to the group of the triazene derivatives. A simplified metabolic scheme of these agents is depicted in Figure 4. DNA methylation by dacarbazine involves oxidative *N*-demethylation mediated by CYP 1A1, 1A2 and 2E1 resulting in the formation of 5-[3-methyl-triazen-1-yl]-imidazole-4-carboxamide (MTIC), which rapidly liberates aminoimidazol carboxamide (AIC) and the methylating product methane diazohydroxide.[172] Temozolomide decomposes to MTIC at pH > 7 and therefore temozolomide yields the same activated metabolites but without the involvement of microsomal enzymes.[173,174]

Temozolomide is a relatively new cytotoxic agent, which is currently used for a variety of tumors.[175–178] Although myelosuppression is the major toxicity of temozolomide, this drug has not been included in high-dose regimens in combination with hematological support, thus far. Dacarbazine is included in the widely used ABVD regimen in combination with adriamycin, bleomycin and vincristine. Doses of dacarbazine in combination with hematological support up to 10.5 g/m^2 have been used with hypotension being dose limiting.[56,179,180] The occurrence of mild to severe stomatitis and diarrhea proved dose dependent.[180]

Adkins *et al.*, studied the pharmacokinetics of dacarbazine in three patients receiving 4394 mg/m^2 in combination with autologous bone marrow transplantation.[56] Clearance found in this study was substantially lower (±50%) than previous studies in conventional dose regimens.[54] Moreover, in the conventional dose study of Buesa and Urréchaga, dacarbazine elimination and the formation of the AIC metabolite seemed to be limited after higher dacarbazine doses.[54] Together, these results suggest non-linear pharmacokinetics of dacarbazine and its main metabolite. Urinary excretion of unchanged dacarbazine and AIC accounted for 11–63 and 1.2–25% of the dose, respectively.[53–55] An elimination half-life of 0.75–3.25 h for AIC has been reported.[53,54]

Platinum compounds

Cisplatin and carboplatin are platinum(II) coordination complexes, able to form both intrastrand and interstrand DNA cross-links. The largely comparable pharmacology and clinical activity of the platinum compounds has been reviewed recently.[181] Cisplatin contains a platinum(II) atom with two amino and two chlorine moieties, while in carboplatin the two chlorine groups are replaced by a cyclobutanedicarboxylate group. Both compounds are activated by hydrolysis, partially determined by the chloride concentration, to monoaquo and diaquo species. The conversion rate of carboplatin is lower than for cisplatin, which may explain the higher dose of carboplatin necessary to obtain a similar cytotoxic

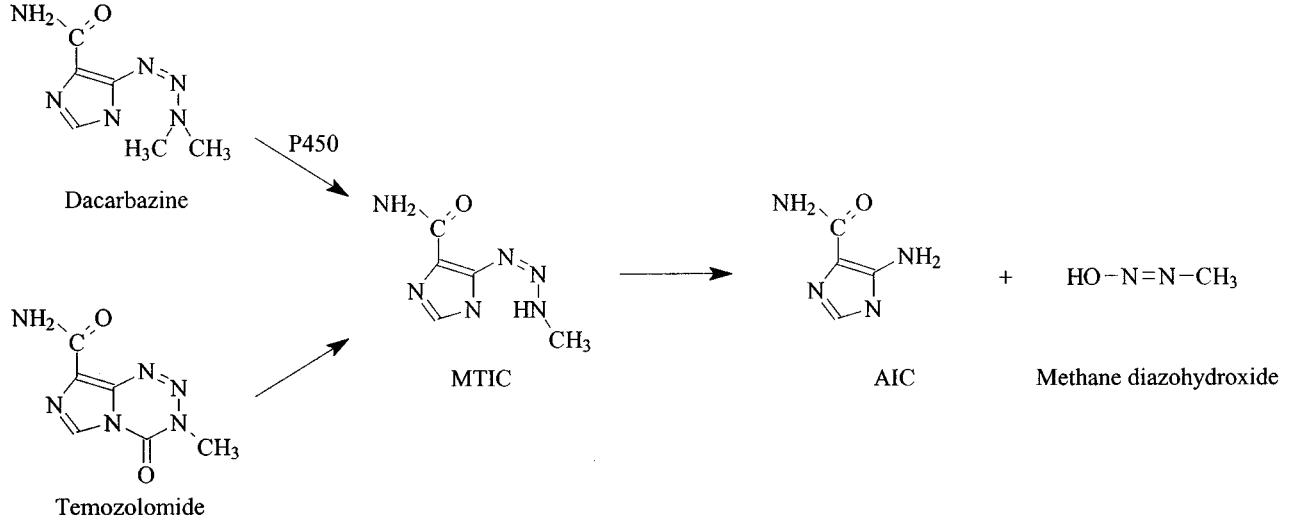

Figure 4. Simplified metabolic scheme of the activation of dacarbazine and temozolomide (MTIC=5-[3-methyl-triazen-1-yl]-imidazole-4-carboxamide; AIC=aminoimidazol carboxamide).

effect.[182] Both compounds bind irreversibly to plasma proteins while the unbound fraction is considered pharmacologically active. Dose-limiting toxicity of carboplatin in conventional doses is myelosuppression, while dose escalation of cisplatin is limited by ototoxicity. Therefore, cisplatin doses used in high-dose chemotherapy are not substantially higher than doses applied in conventional dose therapy. At the maximum tolerated dose, carboplatin is less emetogenic, nephrotoxic, neurotoxic and ototoxic than cisplatin.

For carboplatin, the dose range in conventional dose therapy is 250–400 mg/m^2, while in high-dose therapy, doses up to 2400 mg/m^2 have been used with oto-, cardio- and renal toxicity being dose limiting.[1] Pharmacokinetic parameters of carboplatin were not different in various schedules, combinations or doses.[57] After administration, carboplatin can be detected in CSF, ascites and pleural effusions.[61,183] Clearance is dominated by renal elimination with 40–80% of the dose excreted in urine within 24 h after therapy.[57] Close correlations between clearance and renal function have been observed.[184,185] Sørensen *et al.* compared [^{51}Cr]EDTA clearance as measure of the glomerular filtration rate (GFR) and carboplatin clearance, and concluded that renal elimination of carboplatin occurred by glomerular filtration followed by tubular reabsorption.[186] Non-renal elimination of carboplatin accounted for approximately 30% of total clearance and was not related to body surface area.[185,186] De Lemos recently reviewed the relationships between AUC and toxicity/effectivity.[187] In a study with conventional doses of carboplatin, the AUC of free platinum was positively related to peripheral blood platinum–DNA adduct levels, a surrogate marker for cytotoxicity.[188] Calvert *et al* developed a simple formula for AUC guided dosing of carboplatin: dose=target AUC·(GFR+25), in which the GFR was estimated using determination of the [^{51}Cr]-EDTA clearance.[185] This method is, however, not always available and, therefore, creatinine clearance (Cl_{cr}) has been used instead of GFR. Cl_{cr} can be measured with 24 h urine collection or calculated from serum creatinine with the Cockcroft–Gault formula.[189] Recently, Wright *et al.* developed a formula for the estimation of the GFR from serum creatinine.[190]. Chatelut *et al* developed a formula for the prediction of carboplatin clearance from serum creatinine using a population pharmacokinetic analysis,[191] which was later refined for the use in obese patients.[192] These formulas have been validated in several regimens.[193–196] In two studies, the performance of the Chatelut formula was better than the modified Calvert formula using the Cockcroft–Gault formula,[193,195] while in the study of Okamoto *et al.*, the Cockcroft--Gault formula and 24-h creatinine clearance proved superior.[194] Differences may be explained by differences in the study population and method for the determination of serum creatinine. Depending on the method used for the determination of serum creatinine, adjustment of the proposed methods has been suggested.[197] Other methods for carboplatin dose individualization are based on the administration of a standard dose of carboplatin followed by estimation of the exposure using sparse individual data and subsequent individualization. Bayesian approaches[60,62,196,198,199] and limited sampling procedures[200–203] have been developed for this purpose. The single-sample procedure of Sørensen *et al.*[201] provided an accurate and precise estimation of the AUC in a high-dose combination with thioTEPA and cyclophosphamide,[196,204] while the Ghazal–Aswad procedure,[200] using a 24-h plasma sample for total platinum analysis, did not predict exposure in the same regimen.[205] Disadvantages of these methods are the necessity for exact timing of infusion and sampling. Bayesian dosing strategies are in general more flexible and have been developed in high-dose combinations with paclitaxel and cyclophosphamide[60] and thioTEPA and cyclophosphamide.[197] In general, methods for the estimation of the exposure after a standard dose perform better than the *a priori* dosing formulas.[197]

Few studies have investigated the pharmacokinetics and possible relationships with pharmacodynamics in high-dose chemotherapy.[18,58–61,85] A relationship between cumulative AUC and ototoxicity was described in a high-dose combination with cyclophosphamide and thioTEPA.[59] Wright *et al.* showed that the free platinum concentration determined early during a 96 h infusion in combination with ifosfamide and etoposide was predictive for the development of renal toxicity.[18]

The use of cisplatin in high-dose chemotherapy is complicated by its prominent non-hematological toxicity. For cisplatin, a relation between plasma exposure, DNA adduct formation and tumor response in conventional dose has been described.[206] The pharmacokinetics of cisplatin in doses up to 200 mg/m^2 have been reviewed by Holleran and DeGregorio.[207] No indications for non-linear pharmacokinetics exist. In a high-dose combination of cisplatin with paclitaxel and cyclophosphamide, no correlations between non-hematological toxicity and cisplatin pharmacokinetics were observed.[208] Cagnoni *et al.* found a possible reduction in cisplatin AUC when combined with ondansetron, a widely used antiemetic in high-dose chemotherapy.[67]

Table 3. Summary of relationships between pharmacokinetics and pharmacodynamics of alkylating agents in high-dose chemotherapy

Drug	Pharmacodynamic parameter	Relationship	Reference
Cyclophosphamide	cardiotoxicity/treatment outcome	inversely related to cyclophosphamide AUC	81,96
Ifosfamide	renal toxicity	positively related to drug levels early during 96-h infusion	18
ThioTEPA	mucositis	positively related to thioTEPA AUC	38
ThioTEPA	severe regimen related toxicity	positively related to thioTEPA AUC	133
ThioTEPA	severe regimen related toxicity	positively related to thioTEPA + TEPA AUC and to TEPA $C_{SS} > 1.75$ μg/ml	40
Busulfan	risk of relapse	increased risk in patients with $C_{SS} < 917$ ng/ml	134
Busulfan	VOD	increased risk in patients with ACU > 1500 μM·min	45,141
Carmustine	pulmonary toxicity	increased risk in patients with ACU > 600 μg·min/ml	171
Carboplatin	ototoxicity	positively correlated to cumulative AUC	59

Discussion

The alkylating agents have been used in cancer chemotherapy for over four decades. Over the last decade, these agents have been increasingly used for high-dose chemotherapy. The clinical pharmacology of these agents in high-dose chemotherapy is, however, not well established. This may partly be explained by the extensive metabolism of several alkylating agents resulting in active or inactive metabolites which are often very unstable. Moreover, clinical pharmacology in high-dose combinations may be very complicated, e.g. by the occurrence of drug–drug interactions, auto-induction and non-linear pharmacokinetics.

Several pharmacokinetic interactions between alkylating agents and frequently co-administered drug have been identified as examplified by the interactions between ondansetron with cyclophosphamide and phenytoin with busulfan. For cyclophosphamide, ifosfamide, thioTEPA and dacarbazine, possible saturable elimination has been described, while ifosfamide, cyclophosphamide and possibly busulfan show auto-induction of metabolic pathways. These phenomena may become prominent in high-dose regimens and may therefore be involved in the wide interpatient variability encountered in the pharmacokinetics of several alkylating agents in high-dose chemotherapy.

Although hematological toxicity (e.g. leukocyte nadir) cannot be used as pharmacodynamic endpoint for relationships between pharmacokinetics and pharmacodynamics in high-dose chemotherapy, a variety of relationships between exposure and toxicity or even efficacy have been identified. These relationships are summarized in Table 3. Moreover, a wide interpatient variability in exposure has been found for various agents in high-dose chemotherapy. These findings may form the basis of pharmacokinetic-guided dosing in high-dose chemotherapy. For carboplatin, clear relationships between patient characteristics (renal function) and exposure have been described, allowing dose individualization based on these characteristics. For other alkylating agents such relationships have not been identified up to now. For these agents, dose adaptation based on pharmacokinetic measurements after standard doses has been used. For busulfan, dose adaptation early during treatment has dramatically reduced the risk for development of VOD. For other alkylating agents (e.g. the oxazaphosphorines, thio-TEPA and the triazene derivatives), pharmacokinetic-guided dosing is more complicated due to the formation of active and/or unstable metabolites.

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