Chapter 10

SUMMARY AND CONCLUSIONS

The nuclear enzyme DNA topoisomerase II (topo II) is a target of many anticancer agents, including several effective chemotherapeutic drugs which are used in the clinic, e.g. the anthracyclines and etoposide. In **Chapter 1** an overview is given of the function of topo II, the classic topo II drugs, and their interaction. Central in this interaction is the formation of a cleavable complex comprising DNA and topo II, which is stabilized by the drug. Stabilization of the complex results in persistent DNA strand-breaks and this will initiate a process that leads to cell-death. Therefore these drugs have been designated topo II poisons. Their activity is directly related to the amount of cleavable complex that is formed and thus to the topo II level and activity. Recently a new class of topo II directed drugs has been identified. These agents are direct inhibitors of topo II catalytic activity and do not stabilize the cleavable complex. In contrast to the topo II poisons, topo II catalytic inhibitors are most effective when the topo II levels are low. Therefore, these new drugs may be of value when topo II specific tumor drug-resistance (atMDR = altered topo II multidrug resistance) occurs.

The clinical studies discussed in this thesis had the purpose to explore some possibilities of innovative use of the classic topo II drug etoposide, to investigate the oral administration of the etoposide prodrug etoposide phosphate and to study the toxicity and pharmacokinetics of the topo II catalytic inhibitor fostriecin.

Etoposide is a topo II poison with proven efficacy in several malignant diseases, especially when it is used in combination with other chemotherapeutic agents. The combination of etoposide with cisplatin shows synergy in vitro and is one of the most effective anticancer drug combinations for the treatment of germ cell tumors and small cell lung cancer. **Chapter 2** concerns the application of this drug combination in advanced ovarian cancer. This clinical phase II study was designed to investigate drug-efficacy in a special group of patients: those in whom a small tumor residue (< 2 cm) remains after induction chemotherapy and second-look laparotomy. Cisplatin was administered intraperitoneally because this offers the highest drug level at the peritoneal tumor sites, and etoposide was given intravenously. Of the 36 patients, 64% had a clinical response and in seven patients a pathological complete response was found at surgical

evaluation. The median progression-free survival of all patients was 11 months. This indicates that, despite the high response rate, most patients developed a relapse within a relative short interval, including a considerable number intraperitoneal relapses. The latter indicates an insufficient local effect of this treatment. Results might be improved by the use of higher cisplatin doses and more restricted selection of patients (e.g. microscopic lesions only).

In **Chapter 3** the published studies on prolonged low-dose etoposide monotherapy are reviewed. In vitro studies demonstrated the rapid reversibility of etoposide induced DNA-lesions once the drug is removed and therefore provide a rationale for prolonged dosing. Furthermore, the effect of etoposide is cell-cycle dependent. In randomized clinical studies it was demonstrated that multiple-days dosing is superior to single-dose schedules. Subsequently, the efficacy of prolonged ($\geq$10 days) low-dose etoposide treatment was investigated in a large number of phase II studies. This is facilitated by the possibility of oral administration. The results of these trials show that this is an effective treatment in small-cell lung cancer and malignant lymphoma. The activity is promising in patients with breast or ovarian cancer and either disease refractory to standard treatment or a relapse shortly thereafter. However, important questions remain to be answered. There is a lack of comparative trials to conventional etoposide dose-schedules and it was not definitely proven that the toxicity-profile of prolonged low-dose etoposide compares favorably.

In **Chapter 4** the activity of prolonged low-dose oral etoposide was investigated in 17 patients with advanced epithelial ovarian cancer and progressive disease during, or relapsing after, prior chemotherapy. Because reliable tumor measurements are difficult in such patients, the CA-125 serum level was used as disease parameter. The course of this tumor marker during oral etoposide treatment was analyzed and compared to its course during the preceding time interval. We found a marked overall decrease of the rate of CA-125 progression during etoposide treatment: the mean CA-125 doubling-time increased more than ten-fold. Because disease stabilization is a valid goal in such patients and because of the advantages of oral administration, oral etoposide can be considered a valuable treatment option.

The erratic bioavailability is an important drawback of oral etoposide. Etoposide phosphate is a water-soluble prodrug of etoposide and equivalent to etoposide

after intravenous administration. In **Chapter 5** we compared the plasma-pharmacokinetics of etoposide after oral etoposide and oral etoposide phosphate in 15 patients with solid tumors. The median AUC_{inf} (area under the concentration versus time curve from zero to infinity) of etoposide was 77.7 mg/l.h after etoposide phosphate (95% CI 61.3-100.5) and 62.0 mg/l.h after oral etoposide (95% CI 52.2-76.9). There was a small but statistically significant difference in favor of etoposide phosphate: median 9.9 mg/l.h (95% CI 0.1-32.8). However, this difference represents only 10% increase of the bioavailability and there remained a large inter-patient variability in the plasma levels of etoposide (coefficient of variation 42.3%). Thus, oral etoposide phosphate does not offer a pharmacologically relevant benefit over oral etoposide.

To investigate the gastrointestinal handling of the prodrug etoposide phosphate, we performed the study described in **Chapter 6.** In this study it was analyzed in vitro whether prodrug conversion to etoposide occurs in human gastric juice or in bile. Prodrug conversion to etoposide in gastric juice was negligible. The percentage of prodrug converted to etoposide in bile at pH 8 after 1 h was 78 ± 18% (mean ± SD) for a 0.1 mg/ml prodrug solution and 36 ± 26% for 0.5 mg/ml. At pH 7 conversion was less, but after 1 h 22% of prodrug was converted when incubated with bile at 0.1 mg/ml and 10% at 0.5 mg/ml. It is probable that conversion of prodrug to etoposide was caused by alkaline phosphatase present in the bile. This study shows that variable conversion to etoposide can be expected within the gastrointestinal lumen. This may affect the supposed pharmacological advantage of the prodrug etoposide phosphate after oral administration.

In **Chapter 7** the preclinical literature on the topo II catalytic inhibitor fostriecin is reviewed. It was found that several tumor cell lines with decreased topo II levels and drug-resistance to classic topo II poisons retained their sensitivity to fostriecin. In GLC_4 human small cell lung cancer cell lines an inverse relation was found between topo II levels and fostriecin cytotoxicity. Recently possible other mechanisms of action of fostriecin were reported in addition to the effects on topo II, in particular inhibition of the nuclear protein phosphatases PP1 and PP2A and histone phosphatases. Further investigation is needed to determine the relative contribution of the different mechanisms of action to the antitumor activity of fostriecin.

In **Chapters 8 and 9** a clinical phase I study of fostriecin is described. Fostriecin was administered as 1-hour intravenous infusions for 5 days in 20 solid tumor patients. The dose was escalated to 20 mg/m²/day. The major toxicities of fostriecin were elevated blood liver transaminases and a decrease of renal function. Dose-limiting toxicity, of the liver, was encountered in one of two patients at 20 mg/m²/day. However, the maximum tolerated dose was not reached, because the fostriecin supply was stopped. The latter was due to the fact that the supplier, the US National Cancer Institute, detected an unacceptable percentage impurities (8%) in the clinical batches and was unable to solve this problem.

The liver and renal toxicities peaked after the first 1 to 2 out of 5 doses per course and did not increase with continued administration. There was only a limited increase of the liver toxicity with increasing fostriecin doses and no relation between the decrease in renal function and the dose-level at doses ≥4 mg/m²/day. These observations might be explained by saturation or depletion of the reduced-folate carrier mechanism, which is responsible for the cellular uptake of fostriecin. However, this explanation requires demonstration of the presence of this carrier in normal human liver and kidney cells. Another question raised by the observed course of the toxicities is whether the possible antitumor effects of fostriecin are similarly limited at the present dose-schedule.

The renal toxicity of fostriecin was further analyzed with accurate studies on renal hemodynamics in eight patients. A median decrease of 36% in GFR was observed during fostriecin administration. A 150-fold increase in urinary β_2-microglobulin concentration during fostriecin administration indicates that fostriecin caused a renal tubular abnormality. The observed renal hemodynamic changes were compatible with renal tubular damage. Because the renal toxicity recovered immediately after drug administration and within 1-2 weeks, it is likely that fostriecin induces primarily functional alterations and only limited morphological damage. The exact mechanism of the renal toxicity needs further investigation.

The pharmacokinetic studies revealed that fostriecin has a short plasma half-life: the median terminal half-life in the two-compartment model was 1.5 h. About 15% of the drug is renally excreted unaltered. A metabolite was detected which was most probably dephosphorylated fostriecin. This metabolite is of particular interest, because the presence of the phosphate group is, at least in vitro, essential for the antitumor activity of fostriecin.

We also investigated whether the drug plasma levels achieved in vivo could

be related to a significant in vitro antitumor effect. Therefore cells of a teniposide (VM-26) resistant cell line, GLC_4/VM_{20x}, were incubated with patient plasma samples obtained during fostriecin infusion. GLC_4/VM_{20x} was selected because the teniposide resistance was due to a reduced topo II level. This cell line can be considered an in vitro model of the perceived target population for the clinical use of fostriecin. The results suggested that further fostriecin dose-escalation with at least 50% to 100% is required before a clinical antitumor effect can be expected. The present data on the toxicities of fostriecin do not preclude this approach. However, several observations in this study indicate that the fostriecin dose-schedule should be reconsidered. Because of the short plasma half-life, longer infusion might be preferred with regard to efficacy. On the other hand, the relation between the dose-schedule and the toxicities and the possible role of the reduced folate carrier also need to be elucidated. In view of the present clinical data further investigation of fostriecin is justified and no final conclusions about the clinical potential of this drug are possible at the present.

The studies described in this thesis explored some of the possibilities for improvement of anticancer drug therapy aimed at topo II. Other options are discussed in chapter 1. In the nearby future, results of clinical studies with topo II catalytic inhibitors will define the possible clinical role of these agents. Further clinical development of rational drug combinations including topo II directed drugs, in particular combinations with topo I inhibitors, is warranted. The relation between topo II level and/or activity of tumors in patients and the sensitivity to particular drugs is an important field for clinical research. Other strategies foreseen in the future may include manipulation of the drug target at the topo II-gene level.