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Schedule-dependent topoisomerase II-inhibiting drugs

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Abstract. A number of topoisomerase II-acting drugs have been described, but few demonstrate schedule-dependent anti-tumour activity. The activity of the epipodophyllotoxins etoposide and teniposide and the acridine dye derivative amsacrine is clearly schedule-dependent, and this related not only to the observation that the activity of topoisomerase II varies throughout the cell cycle but also to the finding that these drugs are rapidly cleared from the cell following exposure, permitting DNA repair. Etoposide has been most clearly shown to be schedule dependent in clinical studies. The response rates of patients with small-cell lung cancer receiving a 24-h infusion was only 10% as compared with 89% when the same dose was given over 5 days. Pharmacokinetic studies performed in these patients demonstrated that although the total systemic exposure (area under the plasma concentration-time curve, AUC) was the same in both arms of the study, the duration of exposure to low levels of drug (>1 μg/ml) was doubled in the 5-day arm. Haematological toxicity was the same in both arms of the study, as was the duration of exposure to higher plasma levels (>5 μg/ml), suggesting that this toxicity may be associated with higher plasma concentrations, whereas anti-tumour activity is related to prolonged exposure to low levels of drug. This was confirmed in a subsequent study, where prolongation of treatment to 8 days compared to 5 days resulted in a similar exposure to low plasma concentrations and no difference in response rates or survival. Haematological toxicity in this study was worse in the 5-day arm, which also had an increased exposure to high levels of drug (>5 μg/ml). More recently, interest has focused on even more prolonged etoposide administration, typically involving small daily doses repeated for 14–21 days. Although this schedule shows high activity in relapsed small-cell lung cancer and lymphoma, it is associated with significant toxicity (around one-third of patients experience grade III/IV leukopenia or neutropenia), which may be related to the observation that the etoposide dose delivered per course in these studies is higher than that obtained with standard dosing over 3–5 days. Further randomised studies are required to determine the optimal dose and schedule of etoposide.

Key words: Topoisomerase II – Inhibition – Etoposide – Schedule

Introduction

Topoisomerase II is a nuclear enzyme that facilitates the passage of adjacent DNA strands during DNA synthesis, an action distinct from that of topoisomerase I, which principally catalyses DNA unwinding. A number of drugs have been described that exert a cytotoxic effect upon cells by inhibiting topoisomerase II, often involving different mechanisms of interaction but resulting in the failure of DNA strands to reseal, causing single- and double-strand breaks. Many of these inhibitors have secondary mechanisms of action additional to the formation of DNA breaks, and these may add to cytotoxicity.

Although there are a number of topoisomerase II-inhibiting drugs in clinical use, they fall largely into two classes: the anthracene compounds (anthracyclines and acridine dyes), which include doxorubicin, epirubicin, mitoxantrone and amsacrine, and the epipodophyllotoxins etoposide and teniposide. With some exceptions, within these two groups of compounds the schedule dependence is similar, with the anthracyclines exhibiting a lack of schedule dependence in pre-clinical, animal and clinical studies, whereas the epipodophyllotoxins are clearly schedule-dependent.

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Pre-clinical studies

Hill and colleagues [1] have investigated the activity of a number of drugs against murine neuroblastoma cells in vitro and demonstrated that these compounds generally fall into two groups. The first group, including etoposide and teniposide, induced little cell kill following a 1 h incubation, despite large increases in exposure concentration, and following a 24-h incubation showed dose-dependent increases in cytotoxicity with a clear plateau beyond which increases in concentration had no further effect. These were agents that acted at specific stages of the cell cycle, and short exposure periods resulted in few cells being affected. The second group included the anthracyclines, and these agents showed increasing cell kill with increasing drug concentrations, whether by 1-h or 24-h exposure, suggesting a lack of cell-stage-specific activity. More recent studies have shown that for both etoposide and teniposide to achieve the same degree of cell kill in a 1-h incubation period, concentrations 100 times higher than those used for continuous exposure were required [2].

Other reports have described that etoposide is rapidly removed from the cell when exposure to the agent is stopped and that short periods of exposure to high concentrations of drug may inhibit progression of cells through the cycle [3]. All of this in vitro evidence points to schedule-dependent activity of etoposide and teniposide.

A recent report compared the rate of DNA-strand rejoining observed following 2 h of incubation with a number of topoisomerase II-inhibiting drugs in L5178Y cells as shown in Table 1 [4]. The very rapid rate of strand resealing noted following exposure to amsacrine, menogaril and etoposide confirmed the importance of schedule in the use of these agents, whereas the other drugs tested showed only a very slow rate of repair (daunorubicin) or no repair within 8 h (mitoxantrone and doxorubicin). This prolonged damage resulting from anthracycline exposure is due to the drug concentrating in the nucleus and intercalating DNA, possibly causing an alteration in the conformation of DNA, which arrests the action of topoisomerase II. The avidity of this intracellular binding is reflected in the pharmacokinetics of the drugs, with etoposide having an elimination half-life of 6 h and a distribution volume of approximately 14 l [5], whereas the anthracyclines typically have half-lives of 30–50 h and distribution volumes of 25–30 l [6]. Amsacrine has been shown to bind to different sites on DNA compared to the epipodophyllotoxins and anthracyclines, which may explain the observed differences in activity relative to other topoisomerase II inhibitors [7]. Furthermore, amsacrine-induced strand breaks are greatest during the late S and G2 phases of the cell cycle, as are those caused by the epipodophyllotoxins, when topoisomerase II activity is maximal [8]. This agent, however, differs from etoposide and teniposide in that it shows very little, if any, activity against most of the solid tumours, and its use is largely restricted to the treatment of leukaemia, where it is typically used over several days.

Table 1. Rate of strand repair in L5178Y cells after a 2-h exposure to topoisomerase II-active drugs [4]

Fast (0.5 h)	Slow (3 h)	No repair within 8 h
Etoposide Amsacrine Menogaril	Daunorubicin	Mitoxantrone Adriamycin

Schedule dependence of anthracyclines

In keeping with the pre-clinical studies outlined above most anthracyclines show no schedule-dependence with regard to anti-tumour activity in clinical use. Because of this, they have typically been used as a single i.v. bolus or short infusion every 3 weeks. However, there is evidence to suggest that some of the toxic actions of doxorubicin may be schedule-dependent, particularly the cumulative cardiotoxicity. Several studies have reported decreased cardiotoxicity when the drug is given by continuous infusion rather than standard bolus dosing [9, 10], and the incidence of nausea and vomiting may be increased [10]. In contrast, mucositis has been reported to be more severe with prolonged exposure [10]. Other studies have reported that the area under the plasma concentration-time curve (AUC) for each of these schedules is the same, whereas peak plasma levels are obviously much higher with standard dosing, suggesting that anti-tumour activity is related to AUC, whereas cardiotoxicity is related to peak plasma levels. Free-radical formation, well documented for anthracyclines, has been implicated in this cardiotoxic mechanism, and circulating levels of these compounds are lower during continuous infusion than following standard dosing. A recent editorial by Workman [11] summarises this interesting pharmacology well.

Schedule dependence of podophyllotoxins

As previously described, the activity of both etoposide and teniposide has been shown to be schedule-dependent in preclinical systems. Animal studies carried out in the early 1970s demonstrated clear superiority of more extended schedules of etoposide administration against L1210 ascites tumours in mice and were perhaps the first indication that the activity of this agent is phase-specific [12]. More recent studies by Wolff et al. [13] have shown a clear relationship between the duration of exposure to etoposide and the drug's cytotoxicity against MOLT and 9812 tumour cell lines. The same degree of cell kill was achieved by exposure to concentrations of 10 μg/ml for 1 h and 0.5 μg/ml for 30 h.

Clinical studies

Although no single schedule used in the main phase I etoposide studies was superior [14], there was a general impression that weekly or twice-weekly doses were less effective than treatment given over 3–5 days [15–17].

Table 2. Schedule-dependent activity of single-agent etoposide in SCLC: 1-day vs 5-day arms [20]. All patients received 500 mg/m²

	24-h arm	5-day arm
Number of patients	20	19
Response rate (%)	10	89***
Nadir neutrophils (×10⁹/l)	2.6	2.6
Etoposide AUC/course (μg ml⁻¹ h)	483	472
Median time etoposide >10 μg/ml (h)	24	12*
Median time etoposide >5 μg/ml (h)	32	34
Median time etoposide >1 μg/ml (h)	49	97*

* $P < 0.05$, *** $P < 0.001$

Table 3. Schedule-dependent activity of single-agent etoposide in SCLC: 5-day vs 8-day arms [21]. All patients received 500 mg/m²

	5-day arm	8-day arm
Number of patients	48	47
Response rate (%)	81	87
Nadir neutrophils (×10⁹/l)	0.8	1.7 ($P = 0.05$)
Karnofsky performance score <70 (extensive-disease patients only)	20/31	9/28*
Etoposide AUC/course (μg ml⁻¹ h)	461	443
Median time etoposide >10 μ/ml (h)	11	3***
Median time etoposide >5 μg/ml (h)	32	24*
Median time etoposide >1 μg/ml (h)	93	109***

* $P < 0.05$, *** $P < 0.001$

The first study investigating the effect of schedule on etoposide activity was reported by Cavalli et al. in 1977 [18]. In this randomised study, 60 small-cell lung cancer (SCLC) patients (15 previously treated) received single-agent etoposide either as 250 mg/m² given i.v. weekly, 500 mg/m² given orally divided over 3 days weekly, or 850 mg/m² given orally divided over 5 days three-weekly. The results of this study are difficult to interpret due to the different doses and routes of administration used, but it was clear that the more prolonged schedules gave an improved response rate as compared with the weekly i.v. schedule (response rate: 20% in the weekly arm, 65% in the 3-day arm and 42% in the 5-day arm). Thereafter the majority of studies using etoposide involved administration over 3–5 days repeated three-weekly.

However, Mead et al. [19] failed to demonstrate an effect when etoposide in two different schedules was combined with doxorubicin and cyclophosphamide in 54 previously untreated SCLC patients. Both arms of this study had the same doses of the other two drugs but differed in that etoposide was given as a 500-mg/m² oral dose either as a single dose on day 1 or over 5 consecutive days. The response rates in the two arms were almost identical, either because etoposide adds little to this combination in SCLC or because the doses, which were similar to standard i.v. doses, were too small, given the 50% oral bioavailability of the drug.

Convincing evidence of the schedule dependence of etoposide came with two sequential studies of single-agent i.v. etoposide in previously untreated SCLC [20, 21]. In the first of these studies a total dose of 500 mg/m² was given either as a single 24-h infusion or as five daily 2-h infusions of 100 mg/m², each being repeated every 3 weeks [20]. The overall response rates were 10% in the 24-h arm and 89% in the 5-day arm (Table 2). Pharmacokinetic data from this study confirmed that the total systemic exposure (AUC) in each arm was identical but that the duration of exposure to low levels of drug (>1 μg/ml) was doubled in the 5-day arm, suggesting that prolonged maintenance of low levels of etoposide was an important determinant of anti-tumour activity. Additionally, haematological toxicity was the same in each arm, as was the duration of exposure to higher drug levels (>5 μg/ml).

Further confirmation of this hypothesis came in a subsequent study where patients again received the same total dose of drug (500 mg/m²) but either on the same 5-day schedule or on a more prolonged 8-day schedule [21]. There was no difference in anti-tumour activity between the two arms of this study (Table 3), despite the longer schedule in the 8-day arm, and the duration of exposure to low levels of drug (>1 μg/ml), although statistically signifantly different, were similar (93 h in the 5-day arm vs 109 h in the 8-day arm). Haematological toxicity was also more severe in the 5-day arm, as was the duration of exposure to etoposide concentrations of >5 and >10 μg/ml, again suggesting that myelosuppression may be related to higher concentrations of the drug. Performance status, however, was significantly lower in the 5-day arm of the study, which may have contributed to the increased myelotoxicity.

The importance of prolonged etoposide exposure, even when used in combination chemotherapy, has recently been described by Abratt et al. [22]. Poor-performance-status patients with limited-disease SCLC were randomised to receive 300 mg/m² etoposide either as a 300 mg/m² infusion given on day 1 or as a 60 mg/m² infusion given on day 1 and 120 mg/m² given orally on days 2–5, both with doxorubicin and vincristine. The 5-day arm had a significantly higher complete response rate (26% vs 53%), overall response rate (53% vs 75%) and survival duration (11 vs 14 months).

Prolonged etoposide schedules

Over the last 2–3 years a number of investigators have focused on even more prolonged schedules of etoposide using oral administration, typically for 14–21 days, with cycles being repeated every 3–4 weeks. These schedules have shown activity in patients previously unresponsive to conventional etoposide [23], and the impressive single-agent activity of 20% obtained in refractory lymphoma (with a reduction in tumour markers of >90% ocurring in a further 12%) [24] and of 46% obtained in relapsed or refractory SCLC [25], both with heavily pre-treated patients, confirms the activity of this schedule. A number of other studies have reported favourable response rates with this approach as first line treatment in SCLC [26, 27]. Typically, around one-third of patients in these prolonged oral administration studies experience grade III/IV neutropenia or leukopenia. This is not surprising as most use total doses/per course similar to that derived from the phase I study of Hainsworth et al. [23]. The data listed in Table 4 show the

Table 4. Total dose received per course in studies of prolonged oral and standard dose etoposide. Bioavailability, 50% at oral doses of 100 mg and greater and 60% at 50 mg

Reference	Route	Duration	Dose	Systemic dose
Slevin et al. [24]	i.v.	5–8 days	500 mg/m^2	500 mg/m^2
Carney et al. [29]	p.o.	5 days	800 mg/m^2	400 mg/m^2
Johnson et al. [25]	p.o.	21 days	50 mg/m^2	630 mg/m^2
Einhorn [24]	p.o.	21 days	50 mg/m^2	630 mg/m^2
Sessa et al. [27]	p.o.	21 days	100 mg	740 mg/m^2

commonly used doses and durations of treatment with prolonged oral dosing and compare the total dose received per course of treatment relative to that achieved with standard i.v. dosing and to that obtained with the well-tolerated but clearly highly active 5-day oral schedule reported by Smit et al. [28] and Carney et al. [29]. It is apparent that this is clearly not low-dose treatment and indeed involves doses much larger than those used in standard i.v. schedules. Although the impressive activity of prolonged oral dosing in refractory patients is provocative, its use both in this setting and as first line therapy requires closer examination in randomised trials.

A potential problem with oral dosing is the variability in bioavailability, even within patients studied on different days, as shown in Fig. 1 and recently reported by Sessa et al. [27]. Dose optimisation in individual patients on the basis of the AUC or a given plasma level is thus difficult to envisage with oral dosing, and this variability makes it difficult to correlate pharmacodynamic effects with a pharmacokinetic parameter. It is also difficult to quantitate the total systemic exposure per course of treatment. To overcome this problem, several investigators have used prolonged infusions of etoposide. Although this also exhibits marked variability in steady-state plasma concentrations, as has been reported by Thompson et al. [31] from a phase I study in which steady-state plasma levels in patients receiving 25 mg/m^2 ranged from 0.2 to 2.1 μg/ml (median, 0.7 μg/ml) in 13 patients from whom samples were collected, infusional administration offers the possibility of dose optimisation in individual patients by adaptive control. It is noteworthy that in this study, myelosuppression was mild or absent in most patients, with treatment interruption for 1–2 weeks and re-commencement of infusion at 75% of the original dose being carried out for grade III/IV leukopenia. Seven patients achieved a response to therapy, with five of ten patients with previously treated non-Hodgkin's lymphoma and two of three previously untreated SCLC patients responding. In some patients the treatment was remarkably well tolerated, with the duration of therapy ranging from 3 to 70 weeks (median, 17 weeks). One patient received a total dose of 8838 mg/m^2, which would be difficult to achieve with standard dosing. Data were not given as to whether the responding patients had a higher steady-state etoposide level. This is important, as in another report using infusional etoposide with cisplatin in previously untreated non-SCLC, Kunitoh and Watanabe [32] demonstrated responses in only 1 of 10 patients with a steady-state plasma concentration of <0.99 μg/ml as opposed to 5/11 patients with steady-state concentrations of >1.2 μg/ml. Furthermore, this mode of drug administration offers the possibility of adaptive control in individual patients to optimise dosing. It should also be noted that although long-term etoposide toxicity is less apparent in patients with solid malignancies, due largely to much lower rates of long-term survival, incorporation of these schedules into the curative treatment of malignancies may permit larger doses to be given at the expense of increased long-term toxicity, particularly for secondary neoplasms [33].

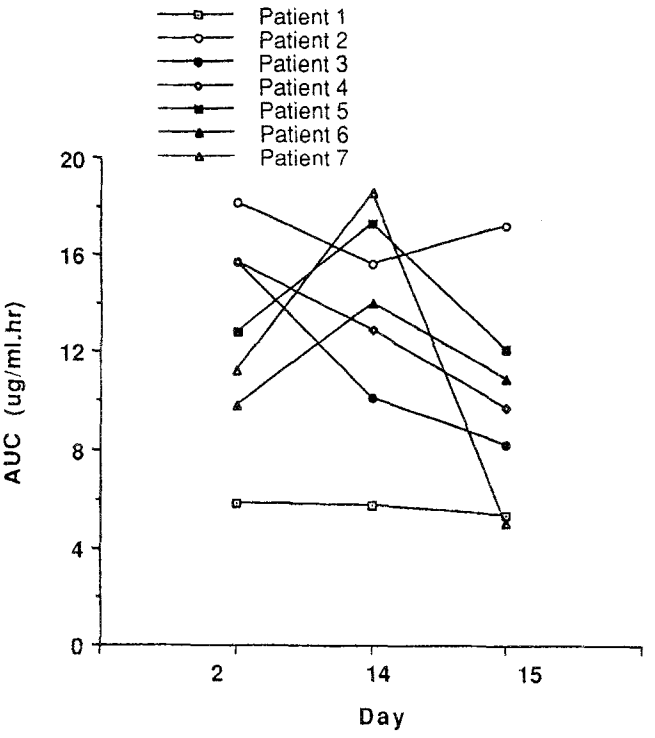

Fig. 1. Variability in AUC between days 2, 14 and 15 of a 15-day cycle in patients receiving 50 mg twice daily oral etoposide [30]. AUC shown is from 0–12 hours after a single 50 mg capsule

Although no clinical study has specifically addressed the effect of schedule on teniposide activity, pre-clinical studies suggest that this compound will have a schedule dependency similar to that of etoposide.

Conclusions

Of the topoisomerase II inhibitors in widespread clinical use, etoposide has most clearly demonstrated anti-tumour schedule dependency, although pre-clinical studies suggest that the activity of amsacrine and teniposide is also schedule-dependent. A number of clinical studies have confirmed that etoposide exhibits increased anti-tumour activity when given over several days rather than on a single day. Further studies have shown the activity of even more prolonged dosing using oral and, more recently, infusional etoposide, but the activity of these schedules has yet to be tested against standard dosing in clinical trials.

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