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Clinical Pharmacology of High-dose Etoposide Associated with Cisplatin. Pharmacokinetic and Metabolic Studies

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Abstract—*Twelve patients were treated with high-dose etoposide given alone or in combination with cisplatin during a clinical trial. We had previously observed, in some subjects who received a combination of high-dose etoposide* (350 mg/m^2/*day* × 5 *days*) *and cisplatin* (40 mg/m^2/*day* × 5 *days*), *delayed hematological recovery after autologous bone marrow transplantation. Therefore we investigated the pharmacokinetics of etoposide and did not find any drug interaction with cisplatin. In four of these patients we also monitored etoposide salivary excretion and found saliva to plasma ratios in the range* 0.003–0.25. *The secretion of etoposide into saliva may be of concern if we consider that perioxidases in salivary glands are able to oxidize the drug leading to free radicals.* In vitro *experiments showed that sulfhydryl compounds are able to inhibit the formation of the etoposide radical. Furthermore, we were able to detect the presence of a new biotransformation product of etoposide in plasma samples of some patients. Fast atom bombardment liquid chromatography–mass spectrometry allowed us to identify this metabolite as the etoposide aglycone. The presence of this derivative in plasma* 48 h *after the last injection prompted us to delay autologous bone marrow transplantation to* 72 h *after the end of treatment, since the aglycone is cytotoxic and able to induce DNA strand breaks mediated by topoisomerase II.*

INTRODUCTION

CISPLATIN(CDDP) given with etoposide (VP16, **1**, Fig. 1) has shown synergistic therapeutic activity in both animal models [1] and clinical trials [2]. A more recent phase II study demonstrated the efficacy of the association of VP16 and CDDP with or without bleomycin in patients with testicular non-seminomas [3]. Although the prognosis of non-seminomatous germ cell tumors (NSGCT) thus clearly improved, refractory or relapsed patients still have poor survival rates.

Therefore the use of high-dose chemotherapy (HDC) followed by autologous bone marrow transplantation (ABMT) was evaluated in a group of poor prognosis young patients with NSGCT [4].

At conventional dosages (100–290 mg/m^2), myelosuppression is the dose-limiting toxicity of VP16. However, the doses of myelotoxic antineoplastic drugs can be escalated to more effective levels if followed by ABMT in order to prevent severe marrow suppression [5].

It has also been reported [6] that renal clearance

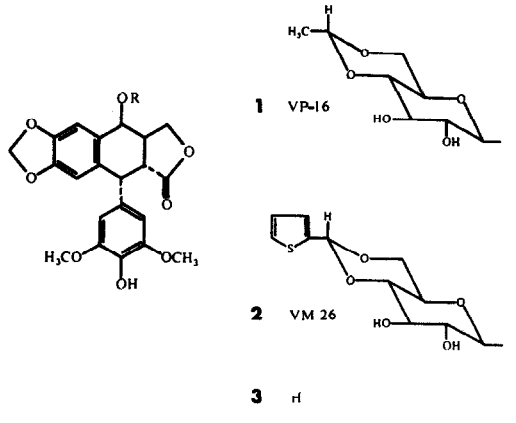

Fig. 1. Chemical structures of the epipodophyllotoxin derivatives: **1**, *etoposide or VP16;* **2**, *teniposide or VM26;* **3**, *aglycone.*

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of VP16 accounts for approx. 36% of the total plasma clearance. Since CDDP is nephrotoxic, co-administration of VP16 and CDDP may alter the pharmacokinetics of each compound, especially if renal clearance of VP16 is lowered because of renal insufficiency, thus prolonging the elimination half-life of this drug.

In fact, we observed during previous studies that some of our patients suffered from delayed hematological recovery. These results led us to plan pharmacokinetic studies in order to assess any potential drug interaction between VP16 and CDDP and to determine when ABMT should be done after the end of the treatment. We also used a liquid chromatographic–mass spectrometric interface in search of potential cytotoxic metabolites.

Finally, the dose-limiting toxicity observed following this high-dose VP16/CDDP/ABMT protocol is mucositis. Salivary VP16 excretion was also monitored in some patients and preliminary experiments were conducted to check if sulfhydryl compounds are able to prevent the formation of VP16 free radicals when the drug is incubated with peroxidases.

PATIENTS, MATERIALS AND METHODS

Patients

A total of 11 male (mean age: 28 years; range: 22–38) and one female patient (15 years old) took part in this study. These subjects were included for pharmacokinetic evaluation of VP16 either given alone (three patients) or in combination with CDDP (11 patients). Four of them were also selected for comparison of VP16 saliva levels with plasma concentrations. All subjects had poor prognosis NSGCT, 10 of testicular origin and two of extragonadal origin. They had previously been treated with different drugs including CDDP, VP16 at conventional dosages, cyclophosphamide, vincristine, vinblastine, actinomycin D or bleomycin. All of them had a normal renal function based upon creatinine blood levels (mean: 97 ± 21 μM; range: 62–138 μM). Prior to drug administration, bone marrow was harvested from the patient's anterior and posterior iliac crests and eventually from sternum, then cryopreserved using standard techniques [7].

Due to ethical considerations, only three patients were given only VP16, for five consecutive days, at 350 mg/m^2/day. Two of them were able to receive, 4–5 weeks later, the same chemotherapy course as the other subjects. The protocol included 350 mg VP16/m^2/day and 40 mg CDDP/m^2/day on days 1–5, 1.6 g cyclophosphamide/m^2/day (plus mercaptoethane sulfonate at 550 mg/m^2/day) on days 2–5 or bleomycin at 10 mg/m^2/day on days 1–5.

Blood samples were withdrawn and urine collections made at different time intervals for 8 days following the start of the treatment. Plasma samples were transfered to silanized tubes and frozen at −70°C. In one patient who had neurotoxicity symptoms, we obtained a sample of cerebrospinal fluid 4 h after the 4th injection of VP16. Saliva was collected at hourly intervals without any artefact to increase the salivary flow. These samples were received into silanized tubes.

Drugs

Pure VP16 and teniposide (VM26, **2**, Fig. 1) were generous gifts from Dr. J.-R. Kiechel (Laboratoires Sandoz, Rueil-Malmaison, France). Authentic samples of VP16 *cis*-picrolactone and hydroxy acid were kindly provided by Dr. M. Suffness (Natural Products Branch, NCI, Bethesda, U.S.A.). The authentic VP16 aglycone was prepared from VP16 according to the procedure described by Dow *et al.* [8] and purified by high performance liquid chromatography.

Pure CDDP was a gift from the late Dr. J.-P. Macquet and was used for our *in vitro* studies as well as for the calibration of the atomic absorption spectrophotometer.

Thirty minutes after i.v. infusion of VP16 (Vépéside, Sandoz®) over 30 min CDDP (Cisplatyl®, Laboratoires Roger Bellon) diluted in 3% hypertonic NaCl was administered i.v. in 1 h. In the same time, patients were under hyperdiuresis regime (3 l/m^2/day) and furosemide if necessary.

Drug analysis

Platinum concentrations were determined in biological samples with a Perkin–Elmer 560 atomic absorption spectrophotometer (pyrocoated tubes) according to the technique described by LeRoy *et al.* [9]. VP16 concentrations in all samples were measured by HPLC. Our procedure derives from previous publications [10–12] with minor modifications. The HPLC equipment (Waters Associates, Millipore) was used with a μBondapak® C18 column and connected to a Perkin–Elmer (Sigma 10) integrator. Concentrations were calculated from calibration curves using VM26 as an internal standard.

Liquid chromatography–mass spectrometry (LC–MS) [13]

A double-focusing mass spectrometer (VG 70–250 from VG Instruments, Le Chesnay, France) was equipped with a moving belt interface. The samples were loaded via a Rheodyne 7125 loop injector (50 μl) onto a reverse-phase column (Ultrasphere® Octyl, Altex, 5 μm, 2 mm i.d., 150 mm long) and eluted at 300 μl/min with a mixture of methanol and 0.025 M ammonium acetate buffer. The effluent emerged into a heated capillary that was used to spray the analytes onto the polyimide moving belt. The compounds were

ionized by fast atom bombardment (xenon) using a saddle field source (Ion Tech, U.K.) operated at 8 kV and 1 mA. Ions were accelerated at 6 kV. During the operation, the belt was continuously washed with a methanol:water (1:1) mixture containing up to 2.5% glycerol.

Electron paramagnetic resonance

EPR spectra were recorded on a Bruker instrument at room temperature (microwave frequency: 9.76 GHz, time constant: 1 s, field intensity: 3480 G, scan range: 100 G). VP16 was diluted in 0.1 M phosphate buffer (pH 7.0) at a concentration of 68 μM. Hydrogen peroxidase was added at a final concentration of 0.8 mM then 100 units of horseradish peroxidase were added. When cysteine was used, its final concentration was 0.412 mM.

In vitro *protein binding studies*

VP16 and CDDP were incubated with plasma samples, alone or in association for different times at several concentrations. Plasma ultrafiltrates were obtained by using cones (Amicon Corp.) with protein weight cut-offs at 50,000 or 25,000 daltons.

Pharmacokinetic analysis

Plasma concentration profiles were fitted to a biexponential decline. The main parameters wee calculated using the ADAPT program [14]. This software package allows parameter determination of models arising from pharmacokinetic applications and can accommodate linear as well as non-linear models with different inputs (no need to correct for infusion duration) and multiple outputs (plasma, urine, etc.) defined by differential equations.

RESULTS AND DISCUSSION

Protein-binding studies

By using the ultrafiltration technique, we were able to measure the extent of protein-binding of VP16 and platinum either separately or in association. At different concentrations up to 25 μg/ml, there is no significant change in the binding of VP16: an average of 98.0 ± 1.4% was bound to plasma proteins at 37°C. This result is higher than that reported by Allen and Creaven [15] who found 94%. For cisplatin, binding of platinum is time-dependent [16]. After 1 h and 24 h of incubation, 38.0 ± 4.1% and 92.4 ± 0.5% of the initial concentration of platinum were linked to proteins. When both drugs were associated, whatever the sequence of spiking plasma samples, at 24 h, the figures mentioned above were maintained: 98.2 ± 1.1% for VP16 and 91.8 ± 0.8% for CDDP. We could therefore conclude that there is no drug interaction between VP16 and CDDP as far as protein-binding is concerned.

Pharmacokinetic studies

During this clinical trial, we could only include three patients for the pharmacokinetic study of VP16 given as the only drug, for ethical reasons. Also, only two of the three were able to receive the VP16/CDDP combination after a wash-out period of 4–5 weeks. They were then treated in the same way as the other nine patients. When the main pharmacokinetic parameters were compared (Table 1), there was no significant change for VP16 with or without CDDP. It is highly probable that these two antitumor drugs do not interact *in vivo*.

In the total of 11 subjects, the mean elimination half-life is 6.8 h, the plasma and the renal clearances, 28.3 and 12.9 ml/min, respectively, for VP16. These parameters are in the same order of magnitude as previously published [6]. The apparent volume of distribution of the central compartment (5.8 l.) is in good agreement with the extensive protein-binding (Table 1). Renal excretion of platinum is also in agreement with those reported before [17].

In one patient who developed neurotoxicity symptoms, VP16 was analyzed in a cerebrospinal fluid sample and the concentration found was 1.52 μg/ml (platinum: 19.3 ng/ml). At the same time, plasma and saliva levels were 14.1 μg/ml and 2.29 μg/ml, 4 h after the 4th injection of VP16.

In four subjects, saliva was also collected at hourly interals. Out of 124 samples, the ratio of salivary to plasma concentrations ranged between 0.3 and 25%. Also, despite the fact that only a limited number of patients was followed, it appeared that the higher this ratio, the more severe the mucositis which is the dose-limiting toxicity when high-dose VP16 is administered with ABMT. We know that salivary glands contain peroxidases and that *in vitro* the hydrogen peroxide/peroxidase system is able to generate free radicals [18] and oxidize VP16 to an aromatic derivative [19]. In our laboratory, using EPR, we have shown (Fig. 2) that cysteine is capable of preventing VP16 free radical formation. This result has led us to investigate the potential role of thiol compounds, *N*-acetylcysteine for example, in preventing toxic side-effects of VP16 mediated by free radicals.

Metabolic studies

Since our previously mentioned results indicated that there is no drug interaction, we re-investigated plasma samples of patients who had a delayed hematological recovery after ABMT. We used a LC–MS system (moving belt interface) to look for potential cytotoxic (myelotoxic) metabolites of VP16. However, since VP16 and its major derivatives are not easily amenable to conventional mass spectrometry without derivatization, we used fast atom bombardment as the ionization technique. It

Table 1. Main pharmacokinetic parameters of VP16 when administered alone or in combination with cisplatin

Patients	VP16 alone $t_{1/2}\beta$ (h)*	Vc (l)†	Clp (ml/min)‡	Clr (ml/min)§	U (%)‖	VP16 + CDDP $t_{1/2}\beta$ (h)*	Vc (l)†	Clp (ml/min)‡	Clr (ml/min)§	U (%)‖	-Pt- U (%)
1	4.24	2.21	27.2	10.3	38.0						
2	3.47	4.57	31.7	7.7	24.2	3.99	4.84	31.6	5.3	16.7	26.3
3	3.53	2.72	35.9	13.4	37.3	4.14	3.94	31.4	12.8	40.6	27.5
4						6.12	3.96	26.8	8.6	31.9	34.3
5						3.62	2.07	28.6	11.9	41.7	26.4
6						4.64	7.47	44.8	24.6	54.8	31.5
7						3.34	2.96	35.3	14.9	42.1	33.5
8						6.85	5.30	23.3	12.3	53.0	32.3
9						12.6	5.83	23.2	—	—	—
10						11.6	9.19	21.8	—	—	—
11						4.34	7.79	20.7	—	—	—
12						13.5	9.95	23.8	—	—	—

*Elimination half-life.
†Apparent volume of distribution (central compartment).
‡Plasma clearance.
§Renal clearance.
‖Urinary excretion of unchanged drug (% of dose).

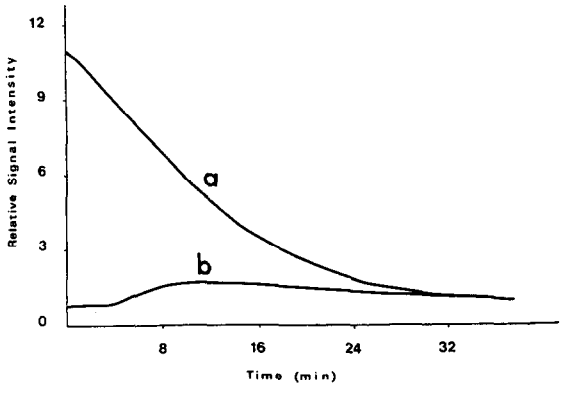

Fig. 2. EPR spectra of VP16 free radical obtained after treatment of VP16 by H_2O_2 and horseradish peroxidase: (a) without cysteine; (b) with cysteine.

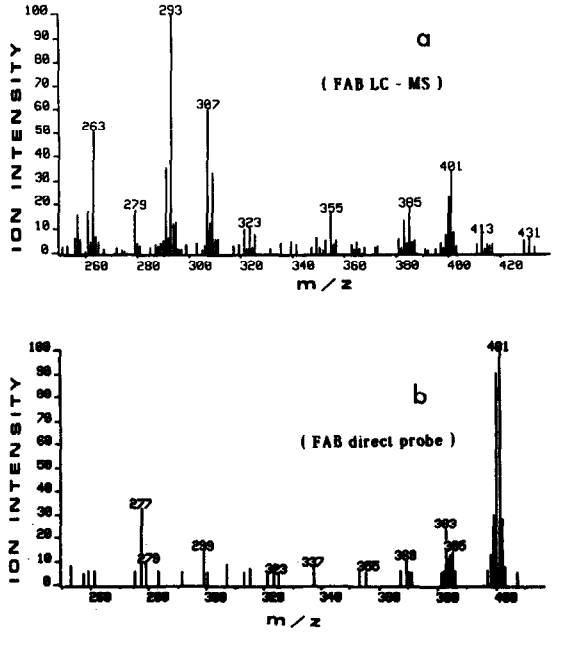

Fig. 3. Fast atom bombardment mass spectra: (a) human metabolite; (b) authentic aglycone.

is used for MS analysis of polar and/or thermolabile compounds and in this case the most intense ion is generally the quasi-molecular ion MH+ with minimal fragmentation. Thus, FAB LC–MS proved to be most useful in the analysis of plasma extracts. Besides the already identified metabolites of VP16, we detected a product with a molecular weight of 400 (m/z = 401 corresponding to the MH+ ion). The retention time and the mass spectrum (Fig. 3) are identical to those obtained with an authentic sample of the VP16 aglycone (**3**, Fig. 1). In a plasma sample collected 48 h after the 5th injection of VP16, the concentration of the aglycone was estimated to be between 5 and 7 ng/ml. Usually, when VP16 is administered at conventional doses, the aglycone is not found as such but as the glucuronide conjugate [20]. We also know that this product is cytotoxic [8] and is able to induce DNA strand breaks in the presence of topoisomerase II [21]. This important observation led us to observe a period of 72 h between the last injection of VP16 and ABMT, instead of the usual lag-time of 48 h based upon the elimination half-life of VP16. Our clinical results have been published elsewhere [4, 22, 23].

Note added in proof

Since this work was submitted for publication, a paper described the pharmacokinetics of high-dose VP16 in this Journal (Holthuis JJM, Postmus PE, Van Oort WJ *et al.* Pharmacokinetics of high-dose etoposide (VP16-213). *Eur J Cancer Clin Oncol.*

1986, **22**, 1149–1155). In that study, 12 patients were also included, salivary excretion was monitored and some CSF samples analyzed. So far, the results published by these authors and in our study are almost superimposable and this confirms that VP16 and CDDP do not interact. The only difference lies in the determination of the elimination half-life which depends mainly on the sensitivity of the assay.

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