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Pharmacokinetics of 2-Methyl-9-hydroxyellipticinium Acetate (NSC-264137) in Cancer Patients (Phase I Study)

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Abstract—*In 12 cancer patients we studied the pharmacokinetics of a new antitumor compound, elliptinium, which is a quaternary ammonium derivative of ellipticine, after i.v. infusion. The main parameters were calculated by means of the ADAPT program based upon plasma concentrations and urinary elimination determined by a high performance liquid chromatographic assay and fluorescence detection. The mean apparent volume of distribution for the central compartment,* V_1, *is* 2.8 l, *the total plasma clearance is* 971 ml/min, *with a renal clearance of* 127 ml/min. *The mean elimination half-life,* $t_{\frac{1}{2}}$, z, *is* 23.7 hr, *but shows a large inter-individual variation. Urinary elimination is quite low* (16%), *with the main part* (12%) *excreted in the first* 24 hr. In vitro *plasma protein binding* (78%) *was found to be independent of the concentration* (0.1–5.0 μg/ml).

INTRODUCTION

ELLIPTINIUM, or 2-methyl-9-hydroxyellipticinium acetate (Fig. 1), is a quaternary derivative of the indole alkaloid ellipticine endowed with antitumor properties, as shown in animal experimental models [1] and in man [2–5].

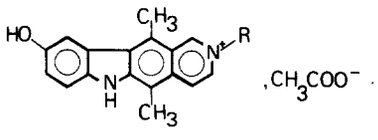

Fig. 1. Structural formulas of ellipticinium acetate (R = CH_3) *and of the internal standard* (R = n — C_4 H_9).

Following pharmacokinetic studies in mice and rats, including tissue distribution by means of autoradiography [6], we were interested in the fate of this new drug when administered by intravenous infusion to cancer patients during a phase I study.

In mice, after rapid intravenous administration this quaternary ammonium compound was quickly distributed into the kidneys, liver, lungs and salivary and thyroid glands [6]. The elimination half-life based upon drug concentration in total blood either by radioactivity measurements or by specific high-performance liquid chromatographic (HPLC) assay was 27 hr. The drug was mainly eliminated by the biliary route (70% of the total fraction of the dose eliminated) and to a lesser extent through the kidneys (30%). Within 64 hr 85 ± 8% of the dose was excreted unchanged: we could not detect under our experimental conditions any conjugation of the phenol or any reduction of the quaternary salt. However, we cannot rule out the possible formation of oxidation [7] or addition products [8].

This pharmacokinetic study is based upon plasma level measurements and urinary excretion as a function of time and dose. In six patients (one of these patients participated twice after a washout period of 4 weeks) the total dose was 104–160 mg [3]; two subjects were given high doses (300 mg), with mannitol-induced diuresis, and four of them received elliptinium on two or three consecutive days to study the possible accumulation of the compound in plasma.

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PATIENTS, MATERIALS AND METHODS

Patients (Table 1)

A total of 12 patients were included in this study (5 males and 7 females). Their age varied between 33 and 72 yr, with body surface areas between 1.45 and 1.90 m^2. All of them had proven malignancies with metastases and were refractory to all conventional therapy. Renal and hepatic functions were investigated in those patients prior to the start of the study.

Elliptinium

The drug was provided by the Laboratoires Labaz (SANOFI Group, Paris) as a freeze-dried powder in vials containing either 20 mg elliptinium and 40 mg urea (batch No. 878 040) or 50 mg elliptinium and 100 mg urea (batch No. 818 070). The appropriate dose was dissolved in sterile isotonic 5% glucose solution (to avoid the presence of chloride ions which drastically decrease the solubility of the compound). The amber vials were kept away from light. And, during the infusion, the solution and the catheter were covered with a black sleeve. The infusions were done by means of an infusion pump (Debitmatic, Midy) at doses reported in Table 1.

Blood sampling and urine collection

Blood samples during the first 12 hr were obtained through a Butterfly® catheter kept under diluted heparine, and thereafter by venipuncture. These samples (2.5–3.0 ml) were collected over lithium heparinate (Becton–Dickinson tubes) kept at 4°C, until centrifugation at 4°C (2500 rev/min for 10 min). The plasma was then transferred to silanized tubes to avoid any absorption on the glass surface. The plasma samples were obtained before the start of the infusion (blank) and 15, 30 and 45 min after the start of the administration and at the end of the infusion. After the infusion, samples were collected at 3, 5, 10, 15, 30, 45, 60 and 90 min, and 2, 3, 4, 5, 6, 8, 12, 24, 36, 48, 72 and 96 hr.

Urine was collected each time the patient urinated during the first 8 hr following the start of the infusion, then by 8-hr collections until the patient left the institution. The volume of each collection was carefully measured as well as the time of collection, and an aliquot (4 ml) was transferred into silanized tubes kept at 4°C until analysis within 24 hr. In some instances urine samples were frozen at −18°C until analysis (controls showed no degradation of the drug).

Drug assay

The assay method is a modification of that described by Muzard and Le Pecq [9]. The samples to be analyzed are spiked with the internal standard, 2-*n*-butyl-9-hydroxyellipticinium acetate (Fig. 1). Both the drug and the internal standard are extracted as ion-pairs (0.05 M sodium tetraphenyl borate) twice with ethyl acetate (2 × 3 ml). Extraction yield is 95%. The upper organic phases are combined in Reacti-vials® and evaporated to dryness under a gentle stream of nitrogen. The residue is then taken up into 100 μl anhydrous pyridine and the resulting solution is treated with 50 μl acetic anhydride. The esterification is complete within 10 min at room temperature. The solution is then evaporated to dryness and the residue redissolved in an adequate volume (100–1000 μl) of the solvent used for the liquid chromatography (methanol 74%, 0.05 M sodium heptane sulfonate aqueous solution 26%, with 1.8 ml acetic acid added per liter of eluent). The HPLC apparatus (Waters S.A) is equipped with a reversed phase μ-Bondapak® C-18 column

Table 1. Patients' characteristics and dosage

Patient	Sex	Age, yr	Body surface area, m^2*	Diagnosis	Dose, mg/m^2	Infusion duration, min
1	M	70	1.77	Kidney cancer	90.4	67
1	,,	,,	,,	,,	,,	52
2	F	68	1.58	Epithelioma	94.9	100
3	F	69	1.63	Melanoma	98.2	58
4	F	48	1.45	Breast cancer	103.4	53
5	M	46	1.75	Rhabdomyosarcoma	80.0	60
6	F	51	1.56	Breast cancer	89.7	44
7	F	68	1.66	Fibrosarcoma	180.7†	60
8	M	53	1.74	Kidney cancer	172.4†	62
9	M	54	1.73	Gastric schwannoma	57.7 × 3 (Days 1, 2, 3)	60
10	F	65	1.60	Ovarian adenocarcinoma	187.5 × 2 (Days 1, 2)	360
11	F	72	1.45	Breast cancer	137.9 × 3 (Days 1, 2, 3)	60
12	M	33	1.90	Acute myeloblastic leukemia	105.3 × 3 (Days 1, 2, 3)	60

*BSA (m^2) = height $(cm)^{0.725}$ × body weight $(kg)^{0.425}$ × 7.184×10^{-3}.
†With mannitol-induced diuresis.

and with a spectofluorimetric detector (Schoeffel 970). The excitation wavelength is 305 nm and the emission wavelength 470 nm. The areas under the peaks corresponding to the elliptinium and the internal standard are computed by an integrator (Sigma 10, Perkin-Elmer). Drug concentrations are calculated from linear calibration curves, from 10 ng/ml to 5 μg/ml in plasma and from 0.5 to 20 μg/ml in urine. The spectrofluorimetric detection is specific, the limit of sensitivity of the assay being 10 ng/ml in plasma with a precision of 10%. At higher concentrations the variation coefficient is about 5–6%. Two typical chromatograms are depicted (Figs. 2 and 3).

Protein-binding studies

The protein binding of elliptinium was studied *in vitro* according to the ultracentrifugation technique described by Scholtan [10] with tubes containing 4 ml of pooled human plasma, centrifuged at 40,000 rev/min for 16.5 hr (SW 51-1 rotor, Kontron). The assay of the drug was based on radioactivity measurements using [1-^{14}C]-elliptinium [11]. The protein binding has been measured at 0.1, 0.5, 1.0 and 5.0 μg/ml, with 5 tubes for each concentration.

Pharmacokinetic calculations

The pharmacokinetic parameters were computed based upon a 3-compartment body model [12] using the ADAPT program [13] on a VAX/VMS (1.60 version) computer (Digital Equipment) that allowed the calculation of the translocation 'microscopic' rate constants between compartments, the elimination (biliary and urinary) rate constants and the apparent volume of distribution V_1. Starting from the 'microscopic' rate constants k_{ij} from compartment i to compartment j, one can compute the 'macroscopic' rate constants λ_1, λ_2, and λ_z, which characterize the triexponential plasma pattern with respect to time.

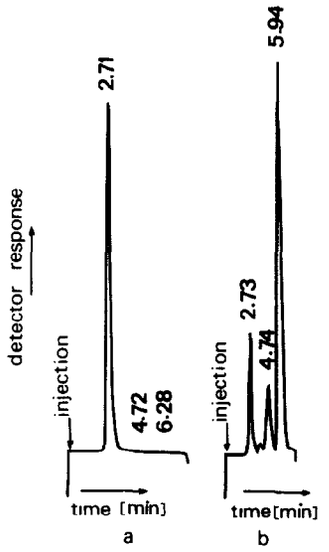

Fig. 2. HPL chromatograms of plasma samples: (a) blank; (b) 20 ng/ml *elliptinium acetate (retention time:* 4.74 min*) and* 200 ng/ml *internal standard (RT:* 5.94 min*).*

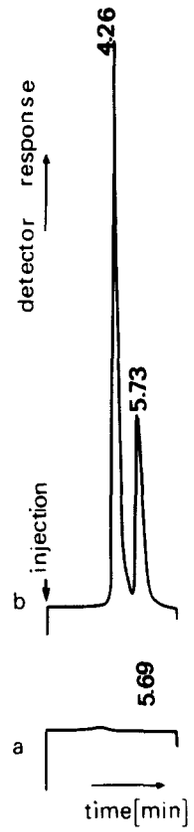

Fig. 3. HPL chromatograms of urine samples: (a) blank; (b) 14 μg/ml *elliptinium acetate (RT:* 4.26 min*) and* 5μg/ml *internal standard (RT:* 5.73 min*).*

If we write:

$$\lambda_1 . \lambda_2 . \lambda_Z = k_{10} . k_{21} . k_{31} = A,$$

where $k_{10} = k_{1U} + k_{1B}$ (k_{1U} and k_{1B} are the urinary and biliary elimination rate constants respectively),

$$\lambda_1 . \lambda_2 + \lambda_1 . \lambda_Z + \lambda_2 . \lambda_Z =$$
$$k_{21} (k_{10} + k_{13} + k_{31}) + k_{31} (k_{10} + k_{12}) = B$$

and

$$\lambda_1 . \lambda_2 + \lambda_Z = k_{10} + k_{12} + k_{13} + k_{31} = C.$$

Then λ_1, λ_2 and λ_Z are the roots of the following equation:

$$x^3 - C.x^2 + B.x - A = 0$$

with $\lambda_1 > \lambda_2 > \lambda_Z$ and the corresponding half-lives

$$t_{\frac{1}{2}}(\lambda_1, \lambda_2, \lambda_Z) = \ln 2/\lambda_1 \text{ (or } \lambda_2 \text{ or } \lambda_Z).$$

The total plasma clearance CL is equal to $CL = k_{10} . V_1$ and can be used to calculate the area under the curve AUC = D/CL (where D = dose) that can be compared to the experimental AUC

computed according to the linear and logarithmic trapezoidal rule [14].

RESULTS

Plasma concentrations

Typical plasma–time curves are drawn in Fig. 4 (single infusion) and Fig. 5 (three infusions on three consecutive days) and peak plasma concentrations are given in Table 2. By the end of the infusion, plasma concentration decreased very sharply, which is probably due to rapid distribution into tissues as seen in animals [6].

In the patients who received two or three consecutive doses, we did not see any accumulation in plasma, except for patient No. 11, for whom peak concentration was 1053 ng/ml on day 1 and 1536 ng/ml on day 3 (Fig. 5). Here the elimination half-life was 36.3 hr.

Pharmacokinetic parameters

In Table 3 we have summarized the individual pharmacokinetic parameters based upon plasma levels and urinary excretion. The mean volume of distribution is 2.8 l, the mean total plasma clearance is 971 ml/min and the mean renal clearance 127 ml/min. The elimination half-life is quite variable in our patients, from 8.1 to 51.2 hr, with a mean of 23.9 hr.

Urinary excretion is quite low, the average percentage of the dose excreted through the kidneys being 16%, the main part, i.e. 12% of the dose, being excreted in the first 24 hr.

The experimental areas under the plasma–time curves are in good agreement with the theoretical values obtained from the ratio of the dose to the total plasma clearance.

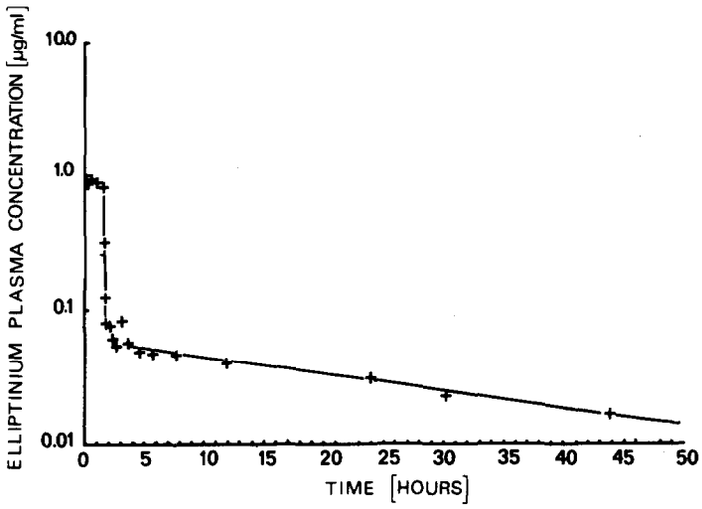

Fig. 4. Semilogarithmic plot of the plasma concentration of elliptinium acetate vs time in patient No. 2 after a single i.v. infusion of 150 mg *over* 100 min (+: *experimental data*; ——— : *computer-adjusted curve*).

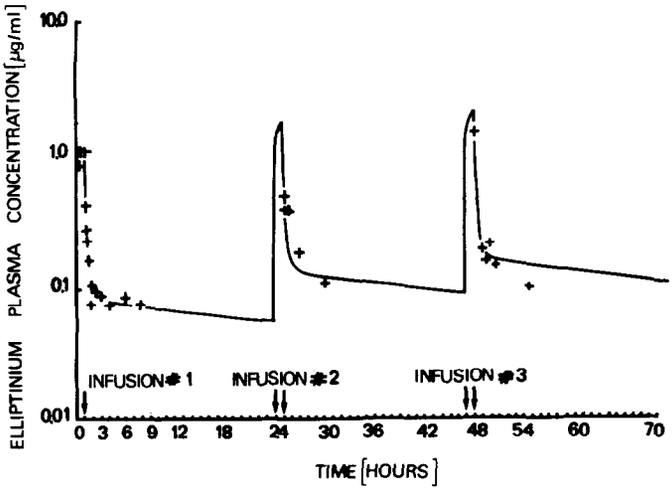

Fig. 5. Semilogarithmic plot of the plasma concentration of elliptinium acetate vs time in patient No. 11 after i.v. infusions of 200 mg *over* 60 min *on three consecutive days* (+: *experimental data*; ——— : *computer-adjusted curve*).

Table 2. Plasma concentrations (ng/ml) *by the end of the i.v. infusion of elliptinium and* 24 hr *after the start of the administration*

Patient	Peak concentration			Concentration 24 hr after the beginning of the infusion	
1		1213		13	
1		2004		ND*	
2		936		31	
3		1633		15	
4		2352		26	
5		824		16	
6		2310		24	
7		3790		11	
8		2531		69	
9	462 (Day 1)	—	468 (Day 3)	24 (Day 1)	14 (Day 2)
10	478 (Day 1)	487 (Day 2)		35 (Day 1)	—
11	1053 (Day 1)	—	1536 (Day 3)	26 (Day 1)	—
12	1165 (Day 1)	1233 (Day 2)	1062 (Day 3)	12 (Day 1)	11 (Day 3)

*ND: not detectable (<10 ng/ml).

Protein binding

In Table 3 we have summarized the percentage of elliptinium acetate bound to plasma proteins as a function of the concentration. There is no statistical difference between concentrations, hence we can assume that there is no dose-dependence in the 'therapeutic' range as far as protein binding is concerned. The mean percentage bound to plasma proteins is 78%, with a standard deviation of 7%.

DISCUSSION

Along with a phase I study, we have investigated the pharmacokinetic parameters of elliptinium at different dosages. The main parameters were calculated according to a three-compartment model since the statistical analysis of the residuals [16] indicated that a bi-compartmental fitting of the experimental data was not satisfactory.

During the administration plasma concentration rose very quickly and a steady state was almost reached by the end of the 1-hr infusions. In patient No. 10, who received a total dose of 300 mg over 6 hr, plasma concentration plateaued at about 460 ng/ml and remained stable for the last 4 hr of the infusion (i.e. 440 ng/ml at 30 min, 430 at 1 hr, 478 at 2 hr, 468 at 3 hr, 462 at 4 hr and 457 at the end of the infusion).

Upon cessation of the infusion the concentration decreased very steeply, as seen with adriamycin. The half-life of the first phase $t_{\frac{1}{2}}(\lambda_1)$ could only be detected by the computer and cannot account for any physiological process. The second phase, with $t_{\frac{1}{2}}(\lambda_2)$ = 14.5 min (range 1.2–73.4 min), more probably reflects tissue distribution. In animals we have seen by means of autoradiographic studies [6] that, 5 min after i.v. bolus injection, the drug had already been distributed into the liver, the lungs and some other organs (salivary glands).

The elimination half-life $t_{\frac{1}{2}}z$, with a mean of 23.9 hr, shows a great inter-individual variation (range 8.1–51.2 hr). But there is no indication that the elimination half-life depends on the administration schedule. Since we have observed that long infusions (more than 2 hr) are not well tolerated due to vein toxicity and that high doses may, in some patients, induce (reversible) tubulopathy [3, 17], we did not include more subjects on long-infusion or high-dose protocols.

However, in two patients (Nos 7 and 8) we infused a total dose of 300 mg under mannitol-induced diuresis without noticing any modification of the starting biological parameters used to monitor the renal function. In those two patients the renal clearance was 112 and 114 ml/min respectively. The main effect of the induced diuresis was the dilution of the urine samples.

The apparent volume of distribution (central compartment), 2.8 l., is in the same order of magnitude as that of plasma. This value is in good agreement with a 78% plasma protein binding. The total clearance based upon total (free and bound) drug in plasma is quite high, with a mean of 971 ml/min and a range of 573–1405 ml/min. This suggests that the hepatic extraction ratio is high, since the renal clearance is about 127 ml/min, with a range from 39 to 225 ml/min.

If we now consider the renal and hepatic functions of our patients before the start of the study, we may make a few remarks.

(a) The renal excretion may involve the three different processes: glomerular filtration, tubular secretion and reabsorption: in some cases the renal clearance of elliptinium is less than the

Table 3. Individual pharmacokinetic parameters

Patient	V_1, l.*	CL, ml/min	CL_R, ml/min	Urinary elimination (% of the dose) Total	Urinary elimination (% of the dose) At 24 hr	AUC, μg × hr/ml Experimental	AUC, μg × hr/ml Calculated	Half-lives $t_{1/2}(\lambda_1)$, min	Half-lives $t_{1/2}(\lambda_1)$, min	Half-lives $t_{1/2}z$, hr	Renal functions†	Hepatic functions†
1	3.06	1,314	—	—	‡	2.18	2.03	0.82	9.4	13.9	N	N
1	2.92	1,241	138	11.1	8.05	1.92	2.15	—	—	7.5	N	N
2	2.78	726	41	5.6	4.2	3.33	3.44	1.04	11.2	23.9	N	N
3	2.11	1,037	118	11.4	11.4	2.50	2.57	0.75	4.9	14.3	N	P
4	3.48	573	88	15.4	10.9	4.56	4.36	1.52	13.2	11.0	N	P
5	2.18	928	215	23.2	14.5	2.36	2.51	0.40	10.2	51.2	N	N
6	3.90	728	149	20.4	11.5	2.65	3.20	1.46	12.1	33.3	N	N
7	1.44	1,141	112	9.8	7.9	3.87	4.38	0.66	4.5	13.3	N	N
8	2.58	786	114	14.5	12.4	5.90	6.36	0.35	1.2	21.7	P	N
9	2.62	1,405	225	16.0	§	1.33	1.19	—	—	14.1	N	P
10	2.52	889	39	4.4	4.2	5.28	5.62	0.64	5.0	45.8	P	N
11	4.36	590	96	16.3	12.9	5.85	5.65	0.56	14.2	36.3	N	P
12	2.74	1,271	188	14.8	13.5	2.12	2.26	0.58	73.4	8.1	N	N

*Apparent volume of distribution of the central compartment; other symbols are those proposed by Rowland and Tucker [15].

†N: normal, P: perturbed.

‡5.5% recovery in the first 9 hr, and a non-quantitated loss of urine at night.

§24, 18 and 5.9% were eliminated within 24 hr after the first, second and third infusions respectively.

Table 4. In vitro *protein binding of elliptinium*

Plasma concentration, μg/ml	0.1	0.5	1.0	2.0	5.0
Fraction bound*	0.75	0.83	0.72	0.74	0.84
Standard deviation	0.01	0.006	0.02	0.05	0.06

*Mean of 5 determinations except at 0.5 μg/ml (3 tubes only).

glomerular filtration rate, suggesting that tubular reabsorption is taking place. And, in some other patients, the renal clearance is higher than the glomerular filtration rate, indicating a possible tubular secretion process.

(b) As most of the drug is excreted by extra-renal (mainly biliary) routes, the dosage must not be modified in case of slight to moderate renal insufficiency. In the case of patients with severe renal insufficiency, pharmacokinetic parameters should be determined, as the drug may cause tubular toxicity.

(c) In the case of hepatic dysfunction, we could not find any rule to modify the drug dosage. For example, in a patient (No. 9) with hepatic metastases, the total clearance, hence hepatic clearance, was not modified. In fact, this patient was given a reduced dosage (100 mg each day for 3 days), as proposed for adriamycin [18]. However, in two other patients (Nos 4 and 11) total clearance was 573 and 590 ml/min. In the first patient (No. 4), the elimination half-life was not prolonged, contrary to what we observed in the second subject (No. 11). Nevertheless, care must be taken in patients with hepatic insufficiency and drug monitoring is advisable (phase II studies).

Based upon these preliminary results, and on the fact that there is no side-effect on white blood cells and platelets, this drug can be administered as a 1-hr infusion on a weekly schedule and at a dose of 100 mg/m^2. However, this does not rule out other administration schedules such as divided dose infusions on five consecutive days every three weeks, with follow-up of the renal function.

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