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Flavone acetic acid: a nonlinear pharmacokinetic model

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Summary. Flavone acetic acid pharmacokinetics were studied in 31 patients in a phase I clinical trial. The drug was given by i.v. infusions over 1, 1.5, 3, and 6 h at doses ranging from 0.5 to 6.4 g/m^2. The pharmacokinetic parameters were determined according to a nonlinear model including Michaelis-Menten-type kinetics. The mean elimination half-life is 4.8 h and the mean volume of distribution of the central compartment, 7.6 l. Our model predicted a maximal tolerated dose (MTD) of 11.1 g/m^2 on the basis of the "therapeutic window" concept, very close to the clinically observed MTD of 10 g/m^2. This model is also operational when different protocols of inoculation are considered, such as a divided-dose schedule vs a unique infusion, and indicates that, at the MTD, injections should be made every 72 h to avoid drug accumulation.

Introduction

Flavone acetic acid (FAA, LM975, NSC-347512, Fig. 1) has a broad spectrum of antitumor activity in relatively refractory, slow-growing animal models [3, 13] and was assessed clinically in a phase I trial following the phase I assessment of flavone acetic acid ester (LM985), which has proven to be a prodrug for FAA [10].

The preclinical pharmacology of FAA has recently been described by Zaharko et al. [17], who proposed the existence of a "therapeutic window" for tumor-bearing mice with boundary plasma concentrations of 100 and 600 mg/l (357 and 2143 μM). Toxicity was related to a brief drug exposure above 600 mg/l or prolonged exposure within the therapeutic range. Therefore, the therapeutic window exists in a two-dimensional sense, governed by plasma concentration and time. In addition, it has been noted that FAA pharmacokinetics are nonlinear in mice [1], dogs [17], and cancer patients [11].

Nonlinear pharmacokinetics have been described for diverse drugs including propanolol [12], amobarbital [5], and fluorouracil [16]. Despite the fact that saturation kinetics are applicable to FAA, the pharmacokinetic parameter estimation thus far carried out has been based on linear models [11, 17], and these parameters are therefore subject to bias and inappropriate for use in simulating plasma concentration-time profiles.

In this study, we used the ADAPT program [4] for both parameter determination and simulations where Michaelis-Menten kinetics were taken into account. This program has numerous applications [7–9] since it can accommodate (1) repetitive dosing when changing doses are given, (2) different infusion times, (3) dosage interval variations, (4) nonlinear models, (5) models with various outputs (i.e., plasma, urine), and (6) different weighing procedures; it can be used for simulation purposes when the model is defined either in an integrated form or by differential equations.

We report the results of the nonlinear pharmacokinetic modelling of FAA in phase I cancer patients and its potential applications in clinical practice.

Materials and methods

Patients [11]. All patients had histologically proven metastatic cancer refractory to any conventional therapy. Eligibility criteria included (a) adequate performance status (WHO grade 0–2), (b) adequate pretreatment bone marrow (WBC $>3\ 10^9$; platelets $>100\ 10^9$), and (c) normal hepatic (bilirubin $<20\ \mu M$) and renal (serum creatinine $<120\ \mu M$) functions. This clinical trial for FAA pharmacokinetic analysis involved 31 patients.

Drug. FAA was provided by Lipha (Lyon, France) as a freeze-dried powder (1 g/vial) to be dissolved in sterile water (10 ml). The drug was then diluted in 0.5–1.0 l 0.9% saline and given by i.v. infusion over 1, 1.5, 3, or 6 h.

Drug analysis. A high-pressure liquid chromatographic (HPLC) assay was used with paradimethylaminobenzaldehyde (BDH Chemicals, UK) as an internal standard. The mobile phase was composed of 12.5% methanol, 12.5% isopropanol, and 0.005 *M* phosphoric acid as previously described [11].

Pharmacokinetic analysis. The ADAPT program package was made available by Prof. D. Z. D'Argenio (Biomedical Simulations Resource, Los Angeles) and implemented on a VAX 11–780 computer (Digital Equipment Co) at the Gustave-Roussy Institute. Different models were tested for goodness of fit, all including saturable kinetics. Usually

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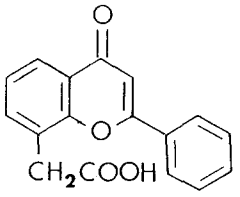


Fig. 1. Flavone acetic acid

those kinetics are described by the Michaelis-Menten equation:

$$-\frac{dC}{dt}=\frac{V_{max}\cdot C}{K_m+C}, \quad (1)$$

where $-\frac{dc}{dt}$ is the rate of decline of the concentration at time t, V_{max} is the theoritical maximal rate of the process, and K_m is the Michaelis constant equal to the concentration at which the process rate is equal to one-half its theoretical V_{max}.

When K_m is much larger than C, the kinetics are first-order and are said to be linear. However, when the drug concentration is greater than K_m, the rate of decline is independent of the drug level (zero-order kinetics).

The model we have used (Fig. 2) is defined by the following differential equations:

$$\dot{X}_1=-\left(\frac{V_{max}\cdot V_C}{V_C\cdot K_m+X_1}+k_{10}+k_{12}\right)\cdot X_1+k_{21}X_2+r, \quad (2)$$

$$\dot{X}_2=k_{12}X_1-k_{21}X_2, \quad (3)$$

$$\text{and } X_1(0)=0 \quad (4)$$

$$C=X_1/V_C. \quad (5)$$

$\dot{X}_1$, and $\dot{X}_2$ are the instantaneous changes in the amount of drug in compartments 1 (central) and 2 (peripheral), V_c is the apparent volume of distribution of the central compartment, k_{12} and k_{21} are the rates of the transfer processes between compartments, k_{10} is the rate constant of a possible first-order elimination mechanism, and r is the infusion rate of the drug. Data were weighted according to $w_i = 1/C_i^2$. The areas under the curves (AUC) were calculated by the linear and/or logarithmic trapezoidal rule [6].

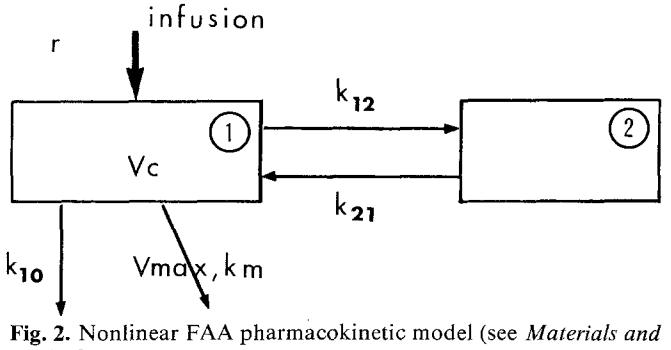


Fig. 2. Nonlinear FAA pharmacokinetic model (see *Materials and methods*)

Results

Pharmacokinetic parameters (Table 1)

The use of a nonlinear model (Fig. 2) allows FAA plasma level fitting with high coefficients of correlation. The use of such a nonlinear model is statistically better (Fig. 3) than that of a linear approach (Fig. 4). In fact, many of the plasma profiles observed in our patients proved to be curvilinear instead of linear when represented as semilogarithmic plots. The intrinsic plasma clearance was calculated when the drug concentration, C, approached near-zero values; that is,

$$Cl = V_C\cdot\left(k_{10}+\frac{V_{max}}{K_m}\right), \quad (6)$$

since the conventional relationship between clearance, dose, and AUC,

$$Cl=\frac{dose}{AUC}, \quad (7)$$

does not hold when we are dealing with nonlinear or saturable processes. The elimination half-life was computed when concentrations were well below K_m values because if the half-life is calculated when the kinetics are not first-order, it appears that the half-life is overevaluated. It should be noted that mean elimination half-life in our patients was 4.8 h. Additional, in our model it does not appear to be dependent on the dose. The mean volume of distribution is around 7.6 l. As far as total plasma clearance is concerned, it seems that our patient population can be divided into two groups, one with a low FAA clearance (below 125 ml/min 1.7 m^2) and one with a higher clearance (up to 372 ml/min 1.7 m^2).

Areas under the curves and time above therapeutic threshold

The total AUCs were computed by the log/linear trapezoidal rule [6] and could be compared to those situated above the minimal therapeutic concentration (100 mg/l or 357 μM) or above the toxic level (600 mg/l or 2143 μM), both previously defined by Zaharko et al. [17] in preclinical studies. The results are reported in Table 1. We also computed the time during which plasma levels remained above these values (Table 1), since this parameter may also be a prognosis factor for toxicity.

Simulations

Our model was used to simulate concentration vs time profiles according to different protocols of inoculation. First, we considered increasing doses of 1, 3.6, 6.4, and 10 g/m^2, infused over 6 h. The simulations were made from the individually determined parameters, not from pooled data. The maximal concentration at the end of the infusion is not linearly correlated to the dose but is a power function of the dose (Fig. 5). We made a similar observation with AUCs. If we relate to Fig. 5, it appears that the minimal effective dose to be infused in 6 h is 2.5 g/m^2 and the maximal tolerated dose would be 11.1 g/m^2. With this model it is also possible to compare the FAA plasma profiles when doses are increased (Fig. 6). Thus, it appears clear that, if plasma concentrations are not monitored for long periods of time (less than 24 h), the apparent elimination half-life

Table 1. Pharmacokinetic parameters of FAA

Patients	Dose (g/m^2)	Infusion time (h)	C_{max} (μM)	$T_{1/2}$ (β) (h)	V_c ($1/m^2$)	K_m (μM)	V_{max} (μM/h)
1	0.5	1.0	125	1.53	7.09	215	381
2	0.5	1.0	200	6.15	10.57	39	25
3	0.5	1.0	239	3.18	6.22	201	298
4	1.0	1.0	200	7.36	11.91	67	69
5	1.5	1.0	496	6.51	10.14	426	356
6	1.5	1.0	379	2.72	9.33	404	507
7	1.5	1.0	464	2.66	7.32	561	377
8	2.0	1.0	604	4.13	8.05	993	103
9	2.0	1.0	464	5.56	10.00	210	214
10	2.0	1.5	736	2.48	7.63	390	188
11	2.7	1.0	739	4.79	10.62	1760	379
12	2.7	1.0	789	4.82	8.83	1285	133
13	2.7	1.0	686	6.48	13.37	570	311
14	2.7	1.0	829	1.77	6.67	103	157
15	3.6	1.0	1311	5.39	10.27	119	114
16	3.6	1.0	1850	4.76	8.87	250	62
17	3.6	1.5	779	3.45	13.48	927	228
18	4.8	1.0	5339	4.50	2.24	104	116
19	4.8	1.0	1621	3.54	4.24	307	75
20	4.8	1.5	3050	12.04	3.48	580	45
21	4.8	6.0	918	2.99	–	–	–
22	4.8	6.0	1079	2.85	3.26	2301	760
23	4.8	6.0	761	5.99	8.69	280	234
24	6.4	1.0	6914	3.79	2.00	117	673
25	6.4	1.0	1832	3.04	10.86	2912	95
26	6.4 (●)	3.0	2157	6.88	5.83	822	141
27	6.4 (●)	3.0	3721	6.31	2.88	1390	1390
28	6.4 (●)	3.0	4118	2.54	2.00	160	957
29	6.4	6.0	989	7.08	–	–	–
30	6.4	6.0	1143	3.69	–	–	–
31	6.4	6.0	714	8.80	–	–	–
Mean	(●) 0–1 h : 3.6 g/m_2			4.77	7.63		
SD	1–3 h : 2.8 g/m_2			2.27	3.46		

C_{max}, plasma concentration at end of infusion; $T_{1/2}$ (β), elimination half-life; V_c, apparent volume of distribution of the central compartment; AUC, total area under the plasma concentration-time curve; AUC_{357}, area above "therapeutic" threshold (100 mg/l or 357 μM); AUC_{2143}, area above "toxic" level (600 mg/l or 2143 μM); Δt_{357}, time during which concentration stays above 357 μM); Δt_{2143}, time during which concentration remains above 2143 μM); Cl, intrinsic plasma clearance

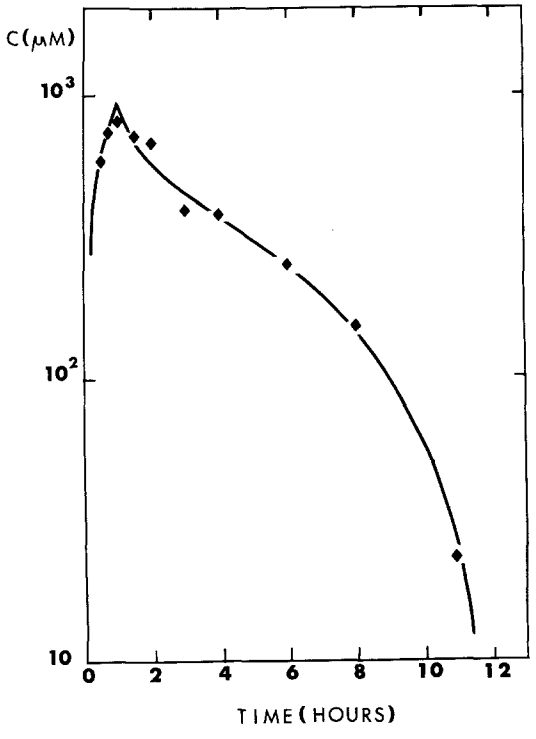


Fig. 3. Nonlinear fitting of FAA kinetics (patient 14)

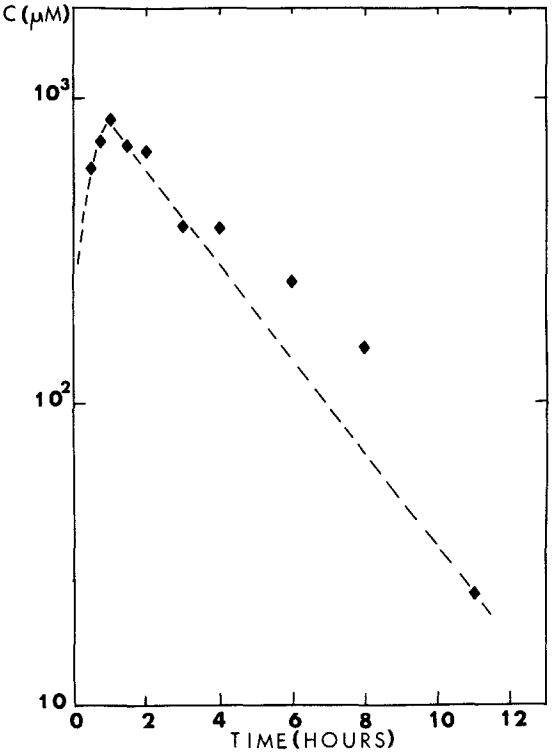
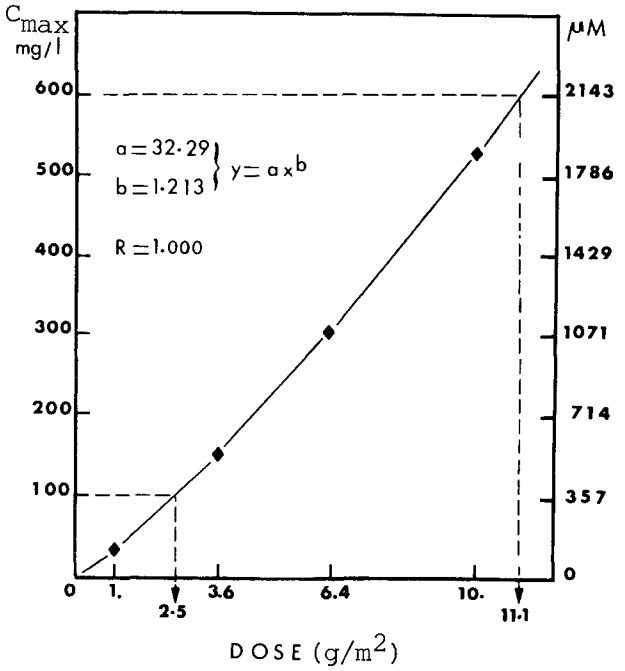


Fig. 4. Linear modelling of FAA plasma profile (same data as Fig. 3)



Fig, 5. Correlation between doses (6-h infusions) and the maximum achieved concentrations

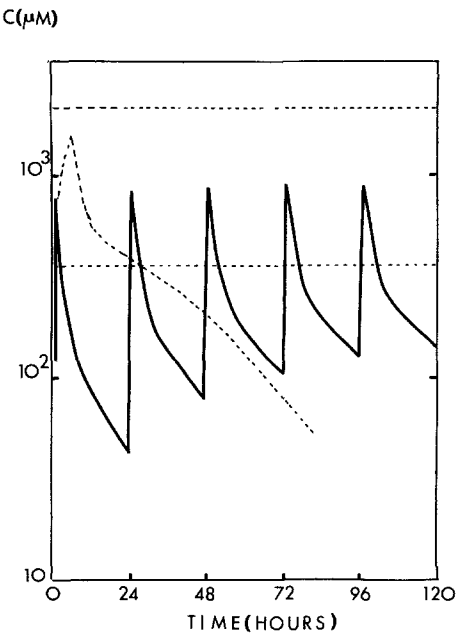


Fig. 7. Simulation of FAA plasma levels after divided-dose inoculation (2.7 g/m^2 X 5) (———) and a single infusion (10 g/m^2) (– – – –). The therapeutic window is located between the two horizontal dashed lines

increases with the dose. On the contrary, if the drug is measured down to the limit of the HPLC assay, the intrinsic elimination half-life is, in fact, unchanged for every dose.

Finally, we compared the effect of a divided-dose schedule to a unique infusion, taking into account both the AUC and time above the therapeutic threshold. When a dose of 2.7 g/m^2 is injected i.v. over 1 h for 5 consecutive days, the AUC and time above the threshold are less than when FAA is infused over 6 h at a dose of 10 g/m^2 (Fig. 7). Therefore, it may be wiser not to use a divided-dose protocol but rather to give FAA in single doses repeated every 72 h, thus avoiding drug accumulation above the 600 mg/l or 2143 μ*M* level.

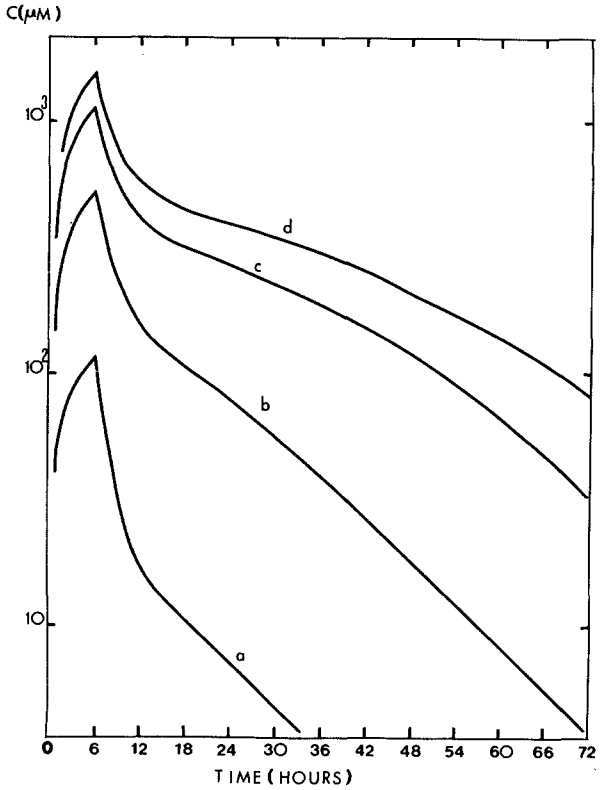


Fig. 6. Simulated plasma profiles at different doses: *a*, 1 g/m^2; *b*, 3.6 g/m^2; *c* 7.5 g/m^2; *d*, 10 g/m^2

Discussion

FAA, a flavonoid derivative whose mechanism of cytotoxicity is still unknown, does not induce strand breaks in DNA (M. D'Incalci, private communication), nor does it intercalate between DNA base pairs. However, some plant-secreted flavones can induce the expression of nodulation genes in *Rhizobium* [15]; those natural substances are mainly hydroxylated flavones. Such hydroxylated derivatives of FAA could be generated in vivo by oxidases and be responsible, in part, for cytotoxicity. If this still remains hypothetical, we know that the biotransformation of drugs is generally enzyme-mediated and therefore prone to saturation. Some other disposition processes (i.e., tubular secretion and/or reabsorption, transport) may also follow nonlinear kinetics [14]. It has previously been reported that FAA undergoes such nonlinear pharmacokinetics [1, 17]. However, the relevant parameters were obtained using linear models, although some authors have claimed that the kinetics were not first-order [17]. The half-lives and clearances should then be revised accordingly, as might those of doxorubicin [2].

In this paper we propose a nonlinear bicompartmental model defined by differential equations including Michaelis-Menten-type kinetics. This model appears to be most relevant when we compare both nonlinear (Fig. 3) and linear (Fig. 4, this paper; Fig. 2 A from [17]) fittings. When ex-

Table 1. (continued)

Patients	AUC ($\mu M \cdot h$)	AUC_{357} ($\mu M \cdot h$)	Δt_{357} (h)	AUC_{2143} ($\mu M \cdot h$)	Δt_{2143} (h)	Cl plasma ml/min per 1.7 m^2
1	173	0	0	0	0	372
2	497	0	0	0	0	203
3	286	0	0	0	0	276
4	502	0	0	0	0	367
5	874	1.3	0.14	0	0	256
6	652	2.5	0.19	0	0	340
7	1050	80.4	1.0	0	0	190
8	1145	139	1.2	0	0	171
9	1318	125	1.3	0	0	369
10	2190	405	2.5	0	0	128
11	2745	519	2.8	0	0	113
12	1940	378	1.8	0	0	163
13	1382	131	1.3	0	0	253
14	3414	766	4.0	0	0	326
15	4791	1316	4.4	0	0	281
16	5003	1704	4.5	0	0	92
17	5597	1354	6.0	0	0	94
18	17834	12602	9.1	4374	2.7	72
19	8311	4094	7.5	0	0	44
20	9278	5773	5.1	699	1.3	35
21	1805	600	7.5	0	0	–
22	8669	3631	9.7	0	0	62
23	4823	1154	6.0	0	0	213
24	17964	11823	11.3	4081	2.0	327
25	8431	4007	7.4	0	0	79
26	9942	5761	7.0	1.1	0.08	81
27	13415	1942	8.9	212	1.0	49
28	15879	3196	8.1	729	1.9	340
29	7397	708	8.0	0	0	–
30	10370	4554	10.5	0	0	–
31	9242	1538	8.5	0	0	–

C_{max}, plasma concentration at end of infusion; $T_{1/2}$ (β), elimination half-life; V_c, apparent volume of distribution of the central compartment; AUC, total area under the plasma concentration-time curve; AUC_{357}, area above "therapeutic" threshold (100 mg/l or 357 μM); AUC_{2143}, area above "toxic" level (600 mg/l or 2143 μM); Δt_{357}, time during which concentration stays above 357 μM); Δt_{2143}, time during which concentration remains above 2143 μM); Cl, intrinsic plasma clearance

perimental data are plotted on a semilogarithmic scale, the profiles are curvilinear, indicating that there is an inflexion point (second derivative equal to zero) on the plasma concentration-time curve. It must be noted that for linear models there is not such an inflexion point. We could then determine apparent K_m and V_{max} parameters. Some patients have quite low K_m and display obvious nonlinear kinetics (Fig. 3). Others have higher K_m and curvilinear plots are less evident: the concentration is much less than K_m and the model becomes linear. However, those K_m and V_{max} values are model-dependent and may not reflect the K_m and V_{max} parameters of an enzyme-mediated process. In the literature we have found some information on 5-fluorouracil, with $K_m = 10.9\ \mu M$ and $V_{max} = 8400\ \mu M$/h [16], and on propranolol, with $K_m = 116\ \mu M$ and $V_{max} = 386\ \mu M$/h [12]. According to this model, intrinsic elimination half-lifes and plasma clearances were calculated from the derived transfer rate constants and from the apparent volume of distribution of the central compartment when concentrations became negligible compared with K_m. Thus, the FAA elimination half-life, 4.8 h, appears to be dose-independent.

Our model was also used for simulation purposes and, starting from the individual pharmacokinetic parameters, allowed us to demonstrate the nonlinear increase of peak plasma concentrations with the dose. If we consider the therapeutic window concept developed by Zaharko et al. [17] to be applicable to patients (Fig. 5), it is then possible to predict the maximal tolerated dose: 11.1 g/m^2 as a 6-h infusion. In fact, the actual maximal tolerated dose, clinically, is 10 g/m^2 in a 6-h i.v. infusion (D. Kerr, personal communication). Another phase I clinical trial is in progress (J. P. Armand, A. Gouyette) and is based on our findings: FAA is given by 6-h infusions every 72 h without any hydration or urine alkalinization. The first three patients were treated at 2.7 g/m^2; the peak plasma levels of FAA were 97.6, 156.8, and 38.6 mg/l (mean, 97.7 mg/l) for a predicted value of 98.1 mg/l (Fig. 5). At 3.6 g/m^2, the first and second patients had a C_{max} of 144.7 and 60.9 mg/l (predicted, 153.4 mg/l).

Furthermore, when the model was used to compare a divided-dose schedule with a unique infusion, we could understand why the divided-dose protocol is less effective in animals [17], since both the AUC and duration above the therapeutic threshold are lower (3833 μMh vs 8098 μMh) and shorter (19.3 h vs 27.2 h) when the drug is given at 2.7 g/m^2 for 5 consecutive days (total dose, 13.5 g/m^2) vs 10 g/m^2 by a single infusion. This is the

consequence of the dose-dependent pharmacokinetics as, with high doses, biotransformation and/or elimination processes are saturated, hence the clearance lowered and apparent elimination half-life increased.

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