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# A phase I and pharmacokinetic study of 12-h infusion of flavone acetic acid

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**Summary.** This phase I study investigated flavone acetic acid (FAA) given as a 12-h intravenous infusion every 3 weeks in the absence of urinary alkalinisation. Cohorts of three patients were treated at doses of 7, 10 and 13 g/m$^2$. One subject had colon cancer; 5, renal cancer; and 3, lung cancer. The Eastern Cooperative Oncology Group (ECOG) performance status was 0 in four patients, 1 in two subjects and 2 in three cases. The maximum tolerated dose was 13 g/m$^2$. The dose-limiting toxicities were WHO grade 3 hypotension and grade 3 diarrhoea. Other toxicities included lethargy and dizziness, nausea, temperature fluctuation, myalgia and dry mouth, but no significant myelosuppression was encountered. One patient receiving 10 g/m$^2$ for renal cancer showed a partial response that lasted for 3 months and included the resolution of pulmonary and cutaneous metastases. The pharmacokinetics showed large interpatient variability. At 12–16 h post-infusion, the plasma elimination profile entered a plateau phase, with frequent increases in concentration suggesting enterohepatic recycling. Neither peak FAA levels nor AUC values were dose-dependent at the doses studied. Peak plasma levels were 101–402 μg/ml and AUC (0–48 h) values were 75–470 mg ml$^{-1}$ min. Plasma protein binding varied with total concentration. Two metabolites were detected in the plasma, and both also underwent apparent enterohepatic recycling. Repeat dosing resulted in decreases of up to 48% in peak levels and AUC values for FAA in three of six patients. Of the total FAA dose, 39%–77% was excreted in the urine as FAA or metabolites within 2 days. The dose recommended for further phase II studies is 10 g/m$^2$.

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## Introduction

Flavone acetic acid (FAA) is a synthetic flavonoid selected for clinical development because of its preclinical activity in a range of murine solid tumours, particularly colon adenocarcinoma 38, despite its poor activity in P388 and L1210 murine leukaemias [9, 21]. Unfortunately, poor clinical activity has been reported from phase I studies [18, 24]. In phase II studies conducted by the Early Clinical Trials Group of the European Organization for Research and Treatment of Cancer (EORTC) no activity was found in breast, colon, lung or head and neck cancers or in melanoma, and the Cancer Research Campaign found no activity in melanoma or colorectal cancer [17, 19].

There have been several suggestions as to the mechanism of action of FAA. In vitro studies indicated that high drug concentrations or long exposure periods were necessary for direct drug cytotoxicity but these were not achieved in mice in vivo, suggesting an indirect mechanism for anti-tumour effects against subcutaneous murine tumours [2, 5]. In vivo, irreparable single-strand DNA breaks were demonstrated in the Glasgow osteogenic sarcoma but may not have been a primary cause of tumour cell death [3]. One component of the indirect activity may involve the immune modulatory activity of FAA. In patients receiving the drug, natural killer-cell activity is increased and interferons are induced [8, 16, 23]. Another mechanism of action may be vascular shutdown and reduced tumour blood flow following FAA administration [13, 25]. All of these factors make it difficult to ascertain the optimal scheduling of the drug. Certainly, total drug exposure has been calculated as being greater for 6-h infusions as compared with 1-h infusions in phase I studies [18].

Although clinical trials of FAA have been carried out in the absence of urinary alkalinisation [1], most have used prior alkalinisation of the urine by sodium bicarbonate to prevent potential nephrotoxicity caused by the crystallisation of FAA at acidic pH in the renal tubules [18, 19, 24]. Murine studies, however, have suggested that alkalinisation inhibits the immunomodulatory and immunother-

apeutic effects of FAA, and it may induce similar effects in human clinical trials [14]. We report the results of a phase I and pharmacokinetics study of FAA infused over 12 h in the absence of prior urinary alkalinisation.

## Patients and methods

*Patient selection.* Adult patients presenting with histologically confirmed solid tumours refractory to conventional therapy were entered into this study after their written informed consent had been obtained. The protocol was reviewed and approved by the Institutional Ethics and Research Committees. Patients were required to have recovered from prior chemotherapy and radiotherapy, with an interval of $\geq 4$ weeks having elapsed since the last treatment; those who had undergone irradiation of >50% of their bone marrow were excluded from the study. An Eastern Cooperative Oncology Group (ECOG) performance status of 0–2 and a life expectancy of $\geq 8$ weeks were also required. Subjects exhibiting a serious concomitant illness, particularly cardiovascular, neurological, respiratory or bleeding disorders, those with cerebral metastases, and pregnant or lactating women were excluded. Eligibility criteria included a pretreatment WBC of $\geq 4 \times 10^9$/l, a neutrophil count of $\geq 2 \times 10^9$/l, a platelet count of $\geq 100 \times 10^9$/l and a haemoglobin value of $\geq 10$ g/dl. Liver- and renal-function test results were required to be within 1.5 times the upper limit of the normal range.

Pretreatment evaluation included a complete history and examination, a full blood examination, determinations of bleeding time, a biochemistry profile, urinalysis and electrocardiography. Clinical examinations, X-rays, nuclear and computed tomography (CT) scans were performed pretreatment and then as required to evaluate the tumour size. Blood pressure was measured every 30 min during the infusion and then hourly for 24 h. Patients were examined and subjected to full blood tests weekly. Standard World Health Organization (WHO) toxicity criteria were used in patient assessment [20].

*Drug administration.* FAA was provided by the Division of Cancer Treatment, National Cancer Institute (Bethesda, Md., USA). It was supplied in sterile vials as a lyophilised powder (1 g/vial) and was reconstituted with sterile water so as to obtain a solution of 100 mg FAA/ml, with sodium hydroxide being added to adjust the pH to 7–9. The drug was diluted in 500 ml 0.9% NaCl and infused intravenously over 12 h in the absence of prior urinary alkalinisation. The solution was protected from light during the infusion [22]. The dose was repeated every 3 weeks if the patient had recovered from the previous toxicities. Cohorts of three subjects were entered at each dose tested (7, 10 and 13 g/m$^2$). In no case was dose escalation implemented.

*Sampling.* A pharmacokinetics study was performed for each course on each patient. Blood samples were obtained before treatment, at 1, 3, 6, 9 and 12 h during the infusion, and then at 1, 2, 3, 4, 6, 9, 12, 18, 24, 28, 32 and 36 h post-infusion. Blood was collected into lithium heparin-coated tubes and immediately centrifuged at 1000 *g* for 10 min, following which the plasma supernatant was removed and stored at –70° C. Urine was collected and the volume recorded pretreatment, during the infusion and post-infusion. A 10-ml aliquot of each specimen was stored at –70° C.

*Drug analysis.* Plasma and urine were assayed for FAA and metabolites using a modification of a published reverse-phase high-performance liquid chromatography (HPLC) method [10]. Briefly, plasma proteins were precipitated with acetonitrile (1 : 2, v/v) containing the internal standard hesperidin (Aldrich). Protein binding was estimated in selected samples by ultrafiltration using a Centrifree micropartition system equipped with a YMT membrane with a molecular-weight cut-off of 30,000 Da (Amicon). Ultrafiltrates were diluted 1 : 1 with acetonitrile containing hesperidin. Urine specimens were diluted 1 : 9 with water, then mixed with acetonitrile (1 : 2, v/v) containing 4-dimethylaminobenzaldehyde (BDH) as the internal standard. This internal standard was used because hesperidin co-eluted with a urinary metabolite. All samples were vortexed and centrifuged, and the supernatant (10 µl) was injected onto a C-18 Novapak column equipped with a µBondapak C-18 Guard -Pak and eluted with a mobile phase of 20% isopropanol and 80% 17.5 mM ammonium phosphate (pH 5.5) at 2.0 ml/min. Approximate retention times for plasma in minutes were: 2.3 (M-1), 2.8 (hesperidin), 3.2 (M-2) and 4.5 (FAA); extra peaks in urine were observed at 2.5 (M-3), 2.7 (M-4) and 8.9 (internal standard) min. Ultraviolet detection was carried out at 303 nm. Because metabolite standards were unavailable, metabolites were measured as FAA equivalents.

**Table 1.** Characteristics of patients

| Characteristics | | |
|---|---|---|
| Patients (*n*) | | 9 |
| Median age (years) | | 62 (range, 36–68) |
| Sex (M/F) | | 6/3 |
| Performance status: | | |
| | 0 | 4 |
| | 1 | 2 |
| | 2 | 3 |
| Prior treatment: | | |
| | None | 3 |
| | RT only | 3 |
| | CT only | 1 |
| | RT and CT | 2 |
| Primary tumour type: | | |
| | Kidney | 5 |
| | NSCLC | 2 |
| | SCLC | 1 |
| | Rectum | 1 |
| Median number of courses | | 2 (range, 1–5) |

RT, Radiotherapy; CT, chemotherapy; NSCLC, non-small-cell lung cancer; SCLC, small-cell lung cancer

*Pharmacokinetics.* The area under the plasma concentration versus time curve from 0 to 48 h ($AUC_{0-48\,h}$) was calculated by the trapezoidal rule. The terminal elimination half-life was not measured due to apparent enterohepatic recycling. The approximate total clearance was determined by dividing the dose by the $AUC_{0-48\,h}$ value.

*Metabolite identification.* Urine samples (1 ml) obtained from patient E were incubated in a shaking water bath at 37° C with β-glucuronidase (Type VII-A, Sigma) in a final volume of 10 ml containing 1,000 IU enzyme in phosphate buffer (pH 6.8). As controls, identical urine samples were incubated with 9 ml sodium phosphate buffer (pH 6.8) in the absence of enzyme. Samples of 200 µl were removed for HPLC assay at 0, 2, 7, 23, 47 and 78 h.

## Results

In all, 9 patients received 22 courses of FAA and all were evaluable for toxicity (Table 1).

### *Toxic effects of FAA*

At the maximum tolerated dose of 13 g/m$^2$, the dose-limiting toxicities were grade 3 diarrhoea in all three patients and hypotension, with one subject showing a decrease of 80 mmHg in systolic blood pressure and another, a 30-mmHg fall (Table 2). Grade 2 diarrhoea lasting several days was seen at doses of 7 and 10 g/m$^2$. During the infusion, particularly towards the end of the 12-h period,

**Table 2.** Numbers of patients exhibiting grade 2 or 3 toxicities[a]

| Toxicity | FAA dose level[b] | | |
|---|---|---|---|
| | 7 g/m² | 10 g/m² | 13 g/m² |
| Diarrhoea: | | | |
| Grade 2 | 1 | 1 | – |
| Grade 3 | – | – | 3 |
| Hypotension (fall, ≥30 mmHg) | 1 | 2 | 2 |
| Nausea and vomiting: | | | |
| Grade 2 | 2 | 2 | 2 |
| Lethargy: | | | |
| Grade 2 | – | 1 | – |
| Dizziness | 2 | – | – |
| Myalgia: | | | |
| Grade 2 | – | 1 | 1 |
| Other: | | | |
| Grade 2 | 1[c] | 1[d] | 2[e] |

[a] NCI toxicity grades: 0, none; 1, mild; 2, moderate; 3, severe; 4, very severe
[b] 3 patients were treated at each dose
[c] Headache
[d] Renal
[e] Indigestion

falls of >30 mmHg in blood pressure were recorded at all dose levels; two subjects reported dizziness on the completion of the infusion. Grade 2 nausea and vomiting was observed at all dose levels. Grade 2 myalgia was experienced by two patients, and two other subjects showed a transient deterioration in renal function. Headache and indigestion were reported by single patients. Mild lethargy was common but was recorded as grade 2 in only one case. Five patients displayed mild elevations in temperature or "flushing" during the infusion, and this occurred at all dose levels. No significant haematological toxicity was recorded.

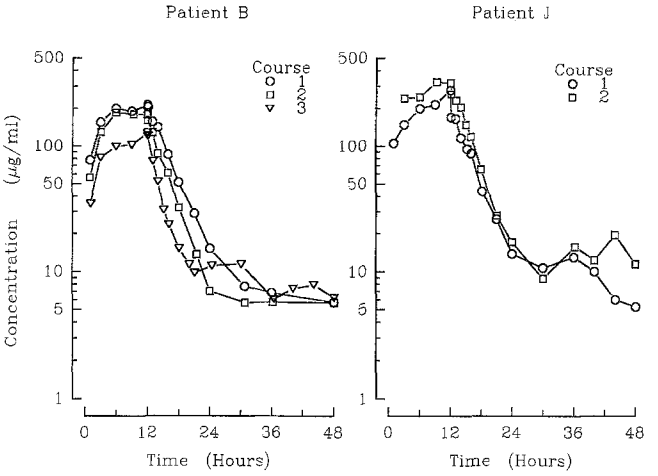


**Fig. 1.** Semi-logarithmic plasma concentration-time plots of total FAA for two representative patients receiving multiple courses of 7 (*Patient B, left*) or 13 (*Patient J, right*) g/m² FAA as a 12-h infusion

### *Therapeutic response*

One patient receiving 10 g/m² (patient E) for metastatic renal cancer achieved a partial response that lasted for 3 months. After two cycles of FAA had been completed, a metastasis on his nephrectomy scar resolved and pulmonary metastases cleared as judged from his CT scan. Irregularities in the renal bed remained, and a needle biopsy of the area confirmed persistent disease. The patient received a total of five cycles of FAA.

### *Pharmacokinetics*

Pharmacokinetic analysis was done for all courses of FAA, and the pharmacokinetic parameters for the first course of treatment are summarised in Table 3. The peak plasma concentration and AUC values for FAA were highly variable between patients and were independent of dose. Peak levels (calculated as FAA equivalents) of the first metabolite (M-1) were 6%–20% of FAA levels, whereas the

**Table 3.** Summary of the pharmacokinetics of FAA and metabolites as calculated from plasma-concentration data for the first course of FAA for each patient

| Patient | Dose delivered over 12 h (g/m²) | FAA | | | M-1[a] | | M-2[a] | |
|---|---|---|---|---|---|---|---|---|
| | | Peak (μg/ml) | $AUC_{(0-48\,h)}$ (mg ml$^{-1}$ min) | Clearance (ml min$^{-1}$ m$^{-2}$) | Peak (μg/ml) | $AUC_{(0-48\,h)}$ (mg ml$^{-1}$ min) | Peak (μg/ml) | $AUC_{(0-48\,h)}$ (mg ml$^{-1}$ min) |
| A | 7 | 321 | 272 | 26 | 18 | 23 | 49 | 49 |
| B | 7 | 212 | 187 | 37 | 27 | 31 | 78 | 68 |
| C | 7 | 160 | 120 | 58 | 21 | 18 | 68 | 53 |
| D | 10 | 101 | 75 | 133 | 19 | 17 | 65 | 52 |
| E | 10 | 402 | 411 | 24 | 31 | 37 | 97 | 95 |
| F | 10 | 240 | 175 | 57 | 16 | 17 | 73 | 56 |
| G | 13 | 381 | 470 | 27 | 52 | 97 | 216 | 349 |
| H | 13 | 270 | 217 | 59 | 46 | 62 | 85 | 94 |
| J | 13 | 278 | 205 | 62 | 55 | 57 | 196 | 170 |

[a] Since pure metabolite standards were unavailable, results are presented as FAA equivalents

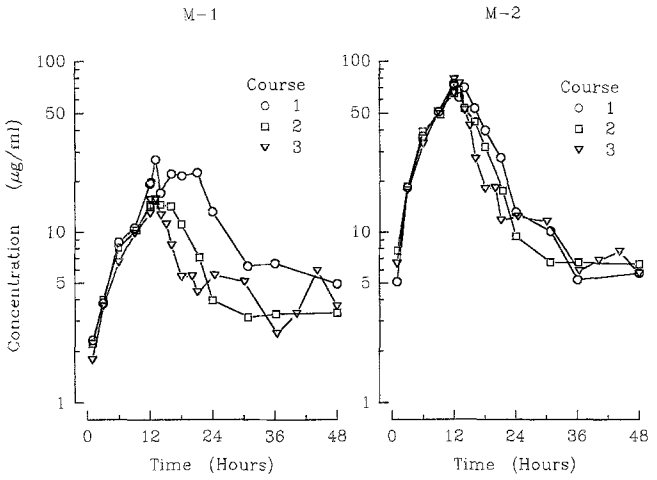


**Fig. 2.** Semi-logarithmic concentration-time plots of two FAA metabolites (*M-1, left; M-2, right*) in plasma from a representative patient (patient B) receiving three courses of FAA at 7 g/m$^2$ as a 12-h infusion

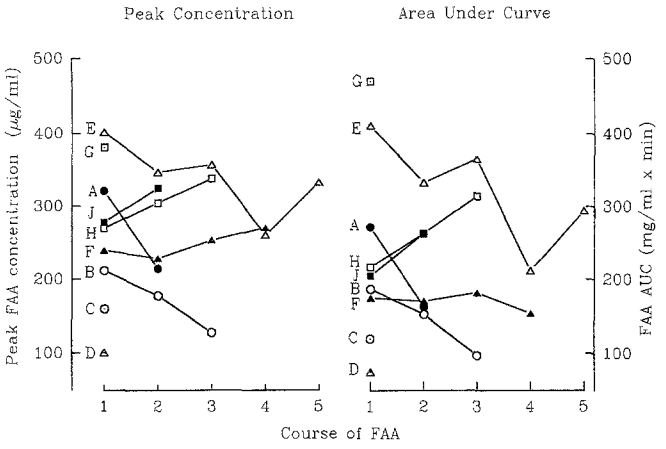


**Fig. 3.** Peak FAA plasma concentration (*left*) and $AUC_{(0-48\,h)}$ (*right*) for each course of FAA. Doses of 7 (*Patients A – C*), 10 (*Patients D – F*) or 13 (*Patients G, H, J*) g/m$^2$ were given as a 12-h infusion

second metabolite (M-2) was present at 15%–71% of FAA concentrations. The plasma concentration versus time curves for FAA in two patients who received multiple courses are shown in Fig. 1. Increases or plateaus in the plasma concentration of FAA following the completion of the 12-h infusion were seen in 18 of 22 courses. Thus, the accurate calculation of a terminal elimination half-life was not possible. The half-life immediately after the infusion was 2–4 h. Only 3 of the 22 courses could be described as following Michaelis-Menten kinetics. Typical concentration-time profiles for M-1 and M-2 are shown in Fig. 2. In general, M-1 but not M-2 continued to accumulate post-infusion, but thereafter the apparent elimination profiles for both of these metabolites were similar to that of FAA, with frequent increases in plasma concentration being found after 12 h post-infusion.

Six of the nine patients received more than one course of FAA at the same dose level. Figure 3 shows the peak plasma concentrations and the AUC values for total FAA for each subject. As compared with the first course, subsequent courses indicated striking decreases in the AUC value (40%–48%) and peak concentrations (33%–40%) determined for three patients. One subject showed no change, and two others displayed increases of up to 45% in peak concentrations and AUC values. This intrapatient variability in the pharmacokinetics of FAA could not be correlated with changes in renal or liver function, toxicity, or disease state. However, the two patients exhibiting consistent increases in peak concentration and AUC received the top dose of FAA. It is also interesting that the patient who responded did not receive the highest dose yet showed the highest initial peak concentration and AUC values, although the concentrations decreased with subsequent courses.

Free FAA and metabolites were quantitated in some patients at selected time points. Table 4 indicates the percentage of free drug or metabolite measured at the end of the infusion (when the peak plasma FAA concentration was reached) and at 12 h thereafter (when FAA concentrations were less than 25% of the peak). In general, more

**Table 4.** FAA protein binding: percentage of free drug measured at the end of the 12-h FAA infusion and at 12 h thereafter

| Patient and course | Total FAA (μg/ml) | | % Free drug | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 12 h | 24 h | FAA | | M-1 | | M-2 | |
| | | | 12 h | 24 h | 12 h | 24 h | 12 h | 24 h |
| B-1 | 212 | 15 | 25 | 2 | ND | ND | ND | ND |
| B-2 | 178 | 7 | 24 | 6 | ND | ND | ND | ND |
| D-1 | 101 | 7 | 27 | 4 | 48 | 11 | 42 | 10 |
| E-1 | 402 | 117 | 27 | 8 | 65 | 9 | 53 | 26 |
| E-4 | 251 | 22 | 29 | 8 | 78 | 25 | 51 | 7 |
| G-1 | 381 | 98 | 47 | 57 | 72 | 42 | 51 | 55 |
| H-2 | 305 | 37 | 47 | 77 | 74 | 17 | 62 | 42 |
| H-3 | 338 | 44 | 49 | 61 | 76 | 26 | 60 | 58 |
| J-1 | 278 | 14 | 47 | 18 | 48 | 5 | 40 | 10 |
| J-2 | 320 | 17 | 55 | 58 | 70 | 8 | 54 | 24 |

ND, Not determined

**Table 5.** Renal excretion of FAA and metabolites[a]

| Patient and course | % Dose excreted as: | | | Total[c] |
|---|---|---|---|---|
| | FAA | M-2[b] | M-4 | |
| A | 19 | 17 | 38 | 77 |
| B | 6 | 17 | 37 | 63 |
| C | 10 | 14 | 25 | 52 |
| D | 1 | 7 | 46 | 56 |
| E | 10 | 11 | 29 | 52 |
| F | 7 | 14 | 45 | 68 |
| G | 6 | 13 | 18 | 39 |
| H | 5 | 16 | 25 | 51 |
| J-1 | 17 | 21 | 7 | 53 |
| J-2 | 4 | 17 | 19 | 45 |

[a] Values are shown for the first course of treatment except for patient J. In other patients on multiple courses, renal excretion did not vary significantly

[b] M-1 values were <5%, and M-3 values were usually 0

[c] Sum of the renal excretion of FAA plus that of all four metabolites measured as FAA equivalents

drug was free at high total concentrations, indicating that protein binding is saturable. The percentage of unbound drug was highly variable among patients, at different plasma FAA concentrations, and even within the same patient when the plasma concentration reached during the infusion was again attained post-infusion. For example, when the total concentration of FAA in patient A reached 109 μg/ml during the infusion, 4% occurred as free drug, whereas at 2 h after the infusion had been completed, 14% of the total concentration of 116 μg/ml represented free drug. Similarly, in patient B, 2% of the 97 μg/ml total FAA measured at 1 h into the infusion occurred as free drug vs 18% of the 91 μg/ml measured at 4 h post-infusion.

Table 5 summarizes the renal excretion of FAA and four metabolites over 48 h. The percentage of the total dose that was excreted as M-1 amounted to <5%, and M-3 was only rarely detected, although it may have been obscured by the large M-4 peak. FAA and M-2 excretion were comparable, whereas M-4 was generally present in quantities 2- to 4-fold those of FAA. Of the total FAA dose, 39%–77% was excreted in the urine as FAA or metabolites within 2 days.

After a 2-h incubation of urine with β-glucuronidase, no M-4 remained (Table 6). Immediately after addition of the enzyme, FAA concentrations had trebled, whereas M-4 levels were reduced by nearly one-third (cf. the zero time points for buffer and enzyme). M-1 and M-2 were far less sensitive to the actions of β-glucuronidase. M-1 concentrations increased and then decreased, suggesting interconversion from FAA or another metabolite. Incubation in buffer alone indicated that M-4 decomposes, and when this finding is taken together with the increasing concentrations of M-1, M-2 and FAA, it becomes apparent that interconversion occurs amongst all of these species.

UV absorption scans obtained using the HPLC UV detector (Waters model 490) during a run gave chromophores that were nearly identical for FAA and the metabolites (determinations for M-3 were not done), resulting in the following lambda maxima: FAA, 260 and 303 nm; M-1, 257 and 303 nm; M-2, 259 and 306 nm; and M-4, 257 and 303 nm. The exact concentration yielding each scan was not determinable, but qualitative analysis indicated that the compounds are all very similar.

## Discussion

In the present study, one patient presenting with renal cancer achieved a partial response to FAA. This is unique

**Table 6.** Incubation of urine from patient E with β-glucuronidase or buffer

| Incubation time (h) | Concentration (μg/ml or FAA equivalents) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Sodium phosphate buffer, pH 6.8 | | | | β-glucuronidase, pH 6.8 | | | |
| | FAA | M-1 | M-2 | M-4 | FAA | M-1 | M-2 | M-4 |
| Course 1: | | | | | | | | |
| 0 | 32 | 45 | 182 | 381 | 119 | 39 | 154 | 151 |
| 2 | 41 | 52 | 259 | 199 | 202 | 41 | 148 | 0 |
| 7 | 51 | 74 | 313 | 71 | 202 | 47 | 147 | 0 |
| 23 | 81 | 144 | 308 | 54 | 228 | 70 | 144 | 0 |
| 47 | 126 | 142 | 249 | 0 | 231 | 68 | 122 | 0 |
| 78 | 191 | 126 | 212 | 0 | 269 | 67 | 118 | 0 |
| Course 3: | | | | | | | | |
| 0 | 58 | 44 | 192 | 315 | 175 | 48 | 203 | 119 |
| 2 | 67 | 56 | 267 | 167 | 245 | 54 | 197 | 0 |
| 7 | 78 | 75 | 300 | 57 | 237 | 63 | 181 | 0 |
| 23 | 110 | 139 | 289 | 11 | 264 | 85 | 165 | 0 |
| 47 | 156 | 148 | 251 | 10 | 274 | 73 | 137 | 0 |
| 78 | 212 | 122 | 212 | 0 | 305 | 62 | 122 | 0 |

Two urine samples obtained from patient E following two different courses of FAA that contained similar concentrations of FAA and metabolites (see 0-h sample in buffer) were selected, and 1 ml was incubated with β-glucuronidase (final volume, 10 ml; 1000 IU, in buffer, pH 6.8) or buffer (as control) at 37° C. Samples (200 μl) were removed at selected times for HPLC analysis of FAA and metabolites (as FAA equivalents)

in that despite the encouraging preclinical activity of FAA, responses have rarely been seen in clinical trials. These disappointing results may have been attributable in part to the inhibition of activity in studies that used concomitant alkalinisation, or perhaps the optimal scheduling had simply not been identified [14]. Prolongation of exposure may be more important than bolus dosing.

The toxicities reported herein paralleled those previously found in other phase I trials [18, 24]. It is interesting that side effects such as hypotension, diarrhoea, nausea, temperature changes, myalgia and slight, if any, myelosuppression are more usually associated with biological agents rather than conventional chemotherapeutic substances.

The most noticeable feature of the pharmacokinetics in this study was its variability both between and within patients. Whereas the dose level fluctuated by a factor of 1.9, the peak plasma FAA concentration, AUC, and estimated total body clearance varied by factors of 4.0, 6.3, and 5.5, respectively. This variation did not correlate with the dose and indicates large interpatient variability in FAA elimination.

Previous studies [18, 24] found dose-dependent pharmacokinetics at doses of <6 g/m$^2$, whereby larger than proportionate increases in peak FAA levels and AUC were noted, and clearance values were greater at low doses. At higher doses or during prolonged infusions, clearance was not dose-dependent. Saturable metabolism and protein binding were suggested as possible explanations for the nonlinear pharmacokinetics. Gouyette et al. [15] developed a nonlinear pharmacokinetic model for FAA and used it to predict peak plasma levels of FAA for a 6-h infusion. Not all patients in that study displayed obvious nonlinear kinetics, since individual patients exhibited different $K_m$ values (the Michaelis constant; in this case, the concentration of drug at which the rate of metabolism is half-maximal) and because the model becomes linear when the plasma concentrations are much lower than the $K_m$ values.

In the present study, the plasma concentration versus time curves after the infusion were curvilinear on a semilogarithmic scale for 3 of 22 courses. The plots were further complicated by increases in plasma FAA concentrations at >12–16 h post-infusion. Enterohepatic recirculation of FAA has been demonstrated in mice [7, 12], but there is no indication in the literature that this also occurs in humans. However, Weiss and colleagues [24] stated that 24-h levels were higher than those predicted from their earlier data, and enterohepatic recycling might explain this finding. The reconversion of a metabolite to FAA could also account for some of the increases in plasma concentrations.

Two inactive metabolites have been identified in human plasma [11]. Both were probably glucuronides and were converted to the parent FAA under physiological conditions [7]. These were the only plasma metabolites found by these investigators, and no others have been reported in the literature. The UV spectra of the two major plasma metabolites M-1 and M-2 are similar to those reported by Cummings and co-workers [11], and the retention times are similar as determined using comparable HPLC assays. It is therefore likely that the previously identified metabolites are the same as the major plasma metabolites (M-1 and M-2) that were detected in our study.

Protein binding of FAA has been investigated [4, 6, 7] and proved to be saturable within the therapeutic dose range. Consistent with this observation, the present study also found more free FAA, M-1, and M-2 at higher total FAA concentrations. There was considerable variability among patients, and even when the same total plasma FAA concentration was reached before and after the infusion, the percentage of free FAA was greater after the infusion. This suggests possible competition for protein binding sites by metabolites, which would occur at higher concentrations post-infusion.

The urinary excretion of FAA and up to two metabolites has previously been reported [11, 24]. Unlike the patients in those trials, our subjects did not receive sodium bicarbonate infusions, and this may explain the discrepancy between the patterns of urinary metabolites. The generally lower amounts of FAA together with the significant presence of M-4 (up to 46% of the dose) suggests a possible conversion of FAA to this metabolite in the urine. M-4 disappeared rapidly following the incubation of urine with β-glucuronidase, and FAA concentrations simultaneously increased, further supporting this possibility. Nevertheless, the total percentage of the FAA dose that was excreted as FAA or metabolites (39%–77%) was comparable to previously reported values (61%–82% [11], 63% [24]).

The present study demonstrates that a 12-h infusion of high-dose FAA in the absence of urinary alkalinisation results in large interpatient variability in pharmacokinetics. Peak levels and AUC values after infusions of 7, 10 and 13 g/m$^2$ were independent of dose and also varied widely in individual patients receiving multiple courses. The patient who responded displayed the highest plasma levels of FAA. A 12-h infusion of 13 g/m$^2$ represents the maximum tolerated dose, and 10 g/m$^2$ is the dose recommended for further studies. The use of a more regular dosing schedule without urinary alkalinisation would be worth examining.

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