Cancer Chemother Pharmacol (1988) 22: 47–50

Cancer Chemotherapy and Pharmacology


# Dose-dependent pharmacokinetics of flavone acetic acid in mice

**Giovanna Damia, Maria Luisa Zanette, Cosmo Rossi, Rosella Mandelli, Aurora Ferrari, and Maurizio D'Incalci**

Istituto di Ricerche Farmacologiche "Mario Negri", via Eritrea 62, Milan, Italy

**Summary.** The pharmacokinetics of the novel anticancer agent, flavone acetic acid (FAA) were investigated in Balb-c mice treated with i.v. doses of 100 mg/kg or 300 mg/kg, using an HPLC assay. The kinetics of disappearance from plasma was monoexponential and dose-dependent. After 100 mg/kg, the plasma peak level was 250 ± 11 μg/ml, t½ was 0.5 h, and AUC was 309 μg/ml per h. After 300 mg/kg, the plasma peak level was 710 ± 57 μg/ml, t½ was 2.1 h, and the area under the curve (AUC) 1771 μg/ml h. Mouse plasma protein binding of FAA was about 70%. As is the case with plasma, in all tissues analyzed, the FAA-AUC values were disproportinately greater after 300 mg/kg than after 100 mg/kg. The highest drug concentrations were found in the liver and small intestine; concentrations were intermediate in lung, heart, and spleen, and lowest in brain. Less than 5% of the FAA dose was eliminated as unchanged drug in the stool. Total excretion of FAA as unchanged drug in the urine collected up to 96 h after drug treatment corresponded to 75% and 60% of the i.v. doses of 100 and 300 mg/kg, respectively. A minor fraction of FAA dose, corresponding to 1% and 6% of the two doses, was eliminated in the urine as a FAA glucuronide or sulfate.

## Introduction

Flavone acetic acid (FAA) is a novel anticancer agent that has recently entered phase II clinical trials. In preclinical screening it has shown a peculiar spectrum of activity against murine tumors, being much more active on slow-growing tumors than on fast-growing ones [3, 4, 6, 7]. For example, it is curvative for mouse colon 38 and pancreatic adenocarcinoma 03, but has only marginal activity against L1210 or P388 leukemias.

The dosage schedule appears crucial for its antitumor activity in mice. Initial pharmacokinetic studies indicated that effective plasma levels are very close to the toxic ones; in addition, nonlinear pharmacokinetics of FAA in mice has been suggested [9].

No information is available on the metabolism of this drug. A better understanding of the metabolism and pharmacokinetics of FAA is needed as a basis for correct studies on its mode of action and also for rational clinical investigation of this drug. In the present study we investigated the pharmacokinetic properties of FAA in Balb-c mice.

*Offprint requests to:* G. Damia

## Materials and methods

*Animals and treatments.* Male and female Balb-c mice (24 ± 2 g) obtained from Charles River (Italy) were used. FAA was kindly provided by Dr P. Briet, Lipha, Lyons, France. The drug was dissolved in 5% $NaHCO_3$ and administered as a bolus injection over 30 s at doses of 100 and 300 mg/kg.

*Sample collection.* Animals were killed before and 5, 15, 30 min and 1, 2, 4, 8, 16, and 24 h after treatment. At each time plasma and tissues (liver, kidney, small intestine, spleen, brain, heart, lung, and epididymal adipose tissue) were collected separately from four animals. Urine and stools were collected from mice in metabolic cages at different intervals up to 96 h. Urine was collected in tubes containing 200 μl 4 *N* HCl. The urine and stools of six mice were pooled at each collection time for drug measurement. All samples were immediately frozen at −20° C until analysis.

*Protein-binding studies.* Plasma protein binding was determined by equilibrium dialysis at 37° C. Undiluted plasma (250 μl) taken from animals 2 h after treatment with 100 and 300 mg/kg i.v. was dialyzed for 15 h against isotonic Krebs buffer (pH 7.4) in Teflon containers separated by a cellulose membrane with an average pore radius of 24 Å (Dianorm System). Dialysis was also carried out with plasma against isotonic Krebs buffer containing different concentrations of FAA. After 15 h the drug was assayed in the liquids on both sides of the membrane. Each protein-binding fraction value was the mean of four determinations.

*Metabolic incubation.* Mice liver microsomes were homogenized in 0.25 *M* sucrose to give a 20% homogenate. The 9000 *g* supernatant was prepared by differential centrifugation. Microsomes were obtained after addition of $CaCl_2$ [8] and resuspended in phosphate buffer. Samples of 5, 10, 100, and 200 μg/ml were incubated with microsomes equivalent to 0.22 g liver/ml of incubation medium (phosphate buffer). The incubation medium was fortified with 3 m*M* $MgCl_2$ and cofactors. The reaction was started by adding the drug and carried out at 37° C under shaking and under air or nitrogen flow. At the end of incubation period, the mixtures were processed as for biological fluids.

*Analytic assay.* FAA was quantified by a modification of the Kerr et al. HPLC method [5]. Briefly, 50 or 100 μl plas-

ma or urine was added to 0.5 ml $H_2O$, 200 µl 5% TCA, and 50 or 100 µl 3-methylflavone-8 carboxyl acid as internal standard (100 µg/ml), kindly provided by Dr D. Nardi, Recordati, Milan, Italy. FAA was extracted with chloroform:isopropanol (1:1) and then mixed at room temperature for 1 h. The precipitate and the aqueous phase were sedimented by centrifugation for 15 min at 3000 rpm. The organic layer was then removed and dried under vacuum. The samples were resuspended in 200–600 µl methanol. Tissues were homogenized (in water 1:5 or 1:10) and 0.5 or 1 ml of the homogenate was processed as described for biological fluids. Extracts were injected into a Waters Model 6000 A HPLC equipped with a UV detector set at 254 nm. Separation was performed using an isocratic solvent system of 0.001 *M* phosphoric acid:acetonitrile: ethanol (60:30:10) at a flow rate of 1 ml/min with a 25-cm-long $C_{18}$µBondapak column (Water Assoc., New York, NY).

Recovery was about 95% and sensitivity was 100 ng/ml and 200 ng/g for plasma and tissue samples, respectively. The curve was linear in the range 0.01–40 µg/ml; CV less than 10%.

The presence of conjugates with glucuronic acid or sulfate was determined by collecting urines in tubes containing 200 µl 4 *N* HCl. Urine was injected directly in the HPLC, the peak attributable to glucuronic or sulfate conjugate was collected and incubated overnight at 37° C with 0.5 ml 0.1 *M* acetate buffer (pH 4.6) and with or without adding 10 µl β-glucuronidase-arylsulfatase (from *E. coli* K12, Boehringer Biochemical Robin, Milan, Italy). After the incubation period, the samples were injected in the HPLC and FAA derived from FAA conjugate quantified. During the incubation, in the sample controls without β-glucuronidase-arylsulfatase, a 10% spontaneous degradation of the conjugate was observed.

*Pharmacokinetic analysis.* The results were processed using a one-compartment open model after i.v. administration described by the equation $C = A\,e^{-\alpha t}$, where C is the concentration at time t, A is the intercept on the ordinate at time zero, and α the slope of the exponential segment. All pharmacokinetic parameters were processed using a nonlinear fitting procedure using the weighted least-squares criterion and a microcomputer program. The half-life and total clearance were computed as follows:

$$t_{1/2} = 0.693/\alpha \qquad Cl = Dose/AUC$$

## Results

Figure 1 shows the FAA disappearance curve from plasma of Balb-c male and female mice after i.v. doses of 100 or 300 mg/kg. At both doses and in both sexes, the rate of disappearance appeared to be monoexponential, the elimination rate being faster after 100 mg/kg than after 300 mg/kg. As can be seen in Table 1, higher $t_{1/2}$ and lower clearance values were found after the dose of 300 mg/kg. The peak plasma levels after 300 mg/kg were approximately 3 times those after 100 mg/kg, while the AUC was about six times greater.

After repeated daily doses, FAA concentrations in plasma and liver at 15 min and 2 h were not statistically different from those after the first dose, suggesting that after multiple doses the drug does not accumulate or induce changes in drug elimination (see Table 2). The concentrations of FAA in plasma and blood were essentially the same (data not shown).

As can be seen in Table 3, plasma protein binding of FAA was between 60%–80%; values were similar for plasma from FAA-treated mice or after addition of FAA to mouse plasma. The fraction of FAA bound to plasma proteins also did not vary over a range of concentrations between 5 and 300 µg/ml.

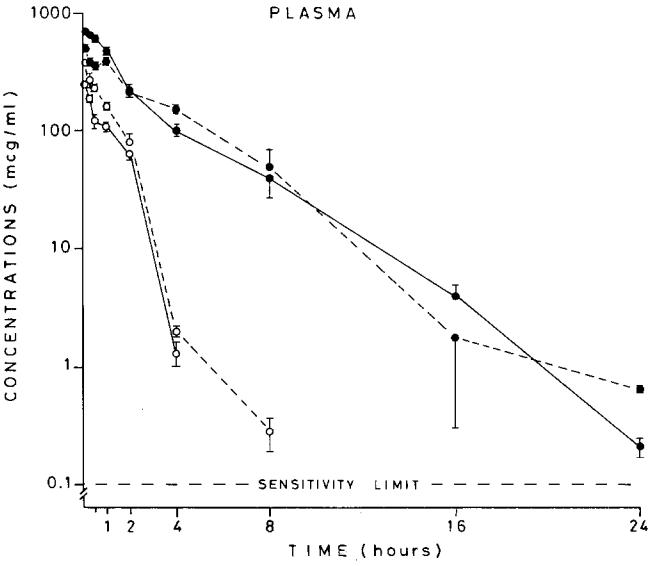


**Fig. 1.** FAA plasma disappearance curves in female (– – – –) and male (———) Balb-c mice treated with 100 (○) and 300 (●) mg/kg i.v.

**Table 1.** Plasma pharmacokinetic parameters of animals treated with 100 and 300 mg/kg FAA (flavone acetic acid) i.v.

| Dose (mg/kg) | 100 | 300 |
|---|---|---|
| Peak (µg/ml) | 250±11 | 710±57 |
| $t_{1/2}$ (h) | 0.5 | 2.1 |
| Total body Cl (ml/h per kg) | 320 | 169 |
| Renal Cl (ml/h per kg) | 240 | 101 |
| $AUC_{0\rightarrow\infty}$ (µg/ml per h) | 309 | 1771 |

**Table 2.** Levels of FAA in plasma and liver of mice after single or repeated doses

| | Plasma | | Liver | |
|---|---|---|---|---|
| | 15 min | 120 min | 15 min | 120 min |
| Group A | 123±10 | 3.2±0.8 | 161±15 | 15±2 |
| Group B | 142± 9 | 6.7±3.3 | 185± 6 | 13±2 |
| Group C | – | 4.0±0.8 | – | 13±1 |

Balb-c male mice were each given a single i.p. dose of 100 mg/kg (group A), or pretreated for 14 days with 50 mg/kg i.p. and then on day 15 given a last i.p. dose of 100 mg/kg (group B), or pretreated for 14 days with 100 mg/kg per day i.p. (split into two injections of 50 mg/kg); on day 15 they received a last i.p. dose of 100 mg/kg (group C). In groups B and C, FAA concentrations were determined only after the last dose. Each group consisted of 5 mice. No statistical differences were found between the groups (Dunnett's test)

**Table 3.** In vitro and in vivo plasma protein binding of FAA

| | | | |
|---|---|---|---|
| In vitro | FAA concentration μg/ml | | % Binding[a] |
| | 5 | | 69 |
| | 50 | | 80 |
| | 100 | | 73 |
| | 200 | | 66 |
| | 300 | | 66 |
| In vivo | FAA i.v. dose mg/kg | Time | % Binding[b] |
| | 100 | 5 min | 73±8 |
| | | 120 min | 76±4 |
| | 300 | 5 min | 70±7 |
| | | 120 min | 64±6 |

[a] Protein binding was evaluated in two samples for each concentration and the mean values are reported

[b] Values represent the mean + SE of four Balb-c male mice

Figure 2 illustrates FAA concentrations in mouse tissues at different intervals after drug injection and Table 4 summarizes the tissue pharmacokinetic parameters. The drug disappeared from tissues much more slowly after 300 mg/kg than after 100 mg/kg. FAA AUC values in all tissues were disproportionately greater at the higher dose.

The pattern of distribution was similar with both doses. The highest drug concentrations were found in liver, kidney, and small intestine; intermediate concentrations were found in lung, heart; and spleen and the lowest drug levels were in brain (Fig. 2).

The data concerning urinary excretion of FAA are shown in Table 5. Urine was collected with 200 μl of 4 *N* HCl because of the presence of the conjugate metabolite, which undergoes degradation at alkaline or neutral pH (G. G. Chabot, personal communication). The total excretion of FAA (unchanged drug plus FAA glucuronide or sulfate) after 96 h, accounted for approximately 75% and 60% of the respective i.v. doses of 100 and 300 mg/kg. At the dose of 100 mg/kg, most urinary drug elimination occurred within 16 h. However, after 300 mg/kg, a significant proportion of drug was also excreted in the intervals between 16–24 h and 24–48 h. FAA conjugate accounted for 0.86% and 6.7% of the total doses of 100 and 300 mg/kg, respectively.

Fecal excretion of FAA as unchanged drug in the 48 h after i.v. administration accounted for 4.8% and 3.8% of the doses of 100 and 300 mg/kg, respectively.

No HPLC peaks attributable to FAA metabolites were seen in either plasma or tissue extracts.

## Discussion

The pharmacokinetics of FAA appear to be dose-dependent in mice. Drug clearance was twice as high follow-

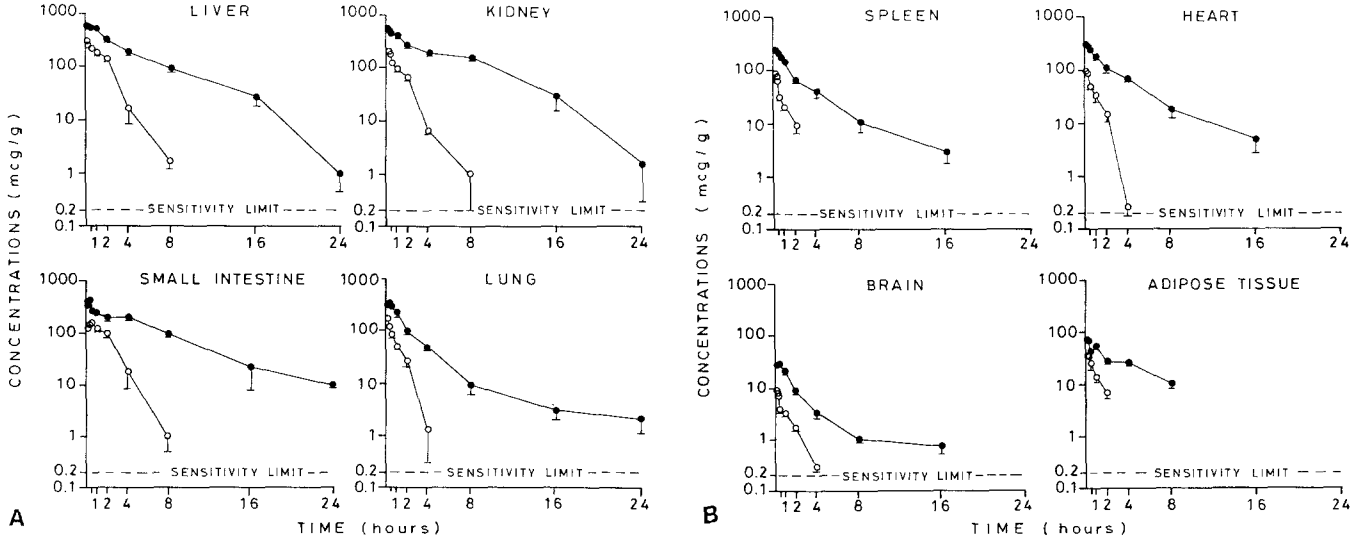


**Fig. 2.** FAA tissue disappearance curves in male Balb-c mice treated with 100 (○) and 300 (●) mg/kg i.v. [Panel A shows FAA concentrations in liver, kidney, small intestine, and lung; panel B shows FAA concentrations in spleen, heart, brain, and adipose tissue]

**Table 4.** Tissue pharmacokinetic parameters of animals treated with 100 and 300 mg/kg i.v. FAA

| Dose (μg/kg) | 100 | | | 300 | | |
|---|---|---|---|---|---|---|
| | Peak (μg/g) | $t_{1/2}$ (h) | $AUC_{0\rightarrow\infty}$ (μg/g · h) | Peak (μg/g) | $t_{1/2}$ (h) | $AUC_{0\rightarrow\infty}$ (μg/g · h) |
| Liver | 321±15 | 1.0 | 611 | 596±14 | 2.6 | 2716 |
| Kidney | 177± 8 | 0.9 | 303 | 557±56 | 3.0 | 2766 |
| Small intestine | 183±31 | 1.1 | 419 | 459±57 | 4.4 | 2210 |
| Lungs | 188±33 | 0.6 | 167 | 359±28 | 1.6 | 796 |
| Heart | 99± 6 | 0.5 | 105 | 340±23 | 2.2 | 886 |
| Spleen | 96± 6 | 0.5 | 73 | 255±13 | 2.2 | 563 |
| Adipose tissue | 35± 3 | 0.8 | 45 | 74± 8 | 3.1 | 269 |
| Brain | 10± 2 | 0.9 | 11 | 28± 2 | 1.6 | 69 |

**Table 5.** Urinary excretion of FAA (as unchanged drugs plus conjugate metabolite – % of the total dose) in animals treated with 100 and 300 mg/kg

| Interval (h) | Dose (mg/kg) | |
|---|---|---|
| | 100 | 300 |
| 0– 8 | 55 | 34 |
| 8–16 | 17 | 10 |
| 16–24 | 1.3 | 9.6 |
| 24–48 | 0.6 | 5.2 |
| 48–72 | 0.6 | 0.4 |
| 72–96 | 0.2 | 0.4 |
| 0–96 | 74.7 | 59.6 |

ing an i.v. dose of 100 mg/kg as after an i.v. dose of 300 mg/kg.

Even though few data on the kinetic properties of FAA are available so far, there are other indications of nonlinear pharmacokinetics of FAA in mice [2, 9]. The phenomenon appears to occur regardless of sex (see Fig. 1), the strain of mice, or the presence of a tumor, since Bissery et al. suggested dose-dependent kinetics of FAA in C57Bl mice bearing advanced colon 38 [1]. The dose-dependent kinetics of FAA may explain why a small increase in the dose may result in disproportionately greater toxicity [9].

As in plasma, FAA concentrations were disproportionately higher in tissues after 300 than after 100 mg/kg. Therefore a saturation of tissue distribution can be excluded as a possible mechanism at the basis of the nonlinear pharmacokinetics of FAA in mice. The highest concentrations were found in organs that may well be associated with drug elimination, such as kidney, liver, and small intestine. The high concentrations seen in liver and small intestine, together with the observation that less than 5% of the FAA is eliminated as unchanged drug in the feces, suggest there may be some enterohepatic circulation of FAA.

It was of interest to investigate whether FAA was present in the brain and if so in what amount, because the drug may be of potential use in tumors or metastases of the CNS and also because neurological side-effects appear to be its dose-limiting toxicity.

The concentrations found in brain were much lower than those found in other tissues, indicating that the drug does not cross the blood-brain barrier efficiently. However, the concentrations achieved after 300 mg/kg, which gave a peak level of about 30 μg/g, appeared high enough to exert toxicity, which was manifested in these animals as lethargy lasting a few minutes after drug injection. Alternatively, some unidentified metabolite able to cross the blood-brain barrier could be responsible for these neurological side-effects. This possibility appears unlikely, and in fact we could not detect any metabolite of FAA in plasma or tissues of mice treated with the drug. In addition, no metabolism was detected in vitro, incubating FAA with fortified mouse liver microsomes or with the supernatant of 9000 *g* mouse liver fraction in the presence or absence of oxygen (data not shown). Therefore, the dose-dependent kinetics of FAA are apparently not due to saturation of its metabolism. In the urine, however, we found a conjugate of FAA that was converted into the parent drug upon incubation with β-glucuronidase arylsulfatase. As already shown by Chabot et al. [2], this metabolite is unstable at neutral or alkaline pH. Since a much greater relative amount of this metabolite was eliminated after the higher dose than after the lower dose (6% vs 1%), it can be excluded that the saturation of conjugation may play a role in the FAA dose-dependent kinetics. The relatively greater conjugation of FAA after 300 mg/kg than after 100 mg/kg may be related to the much higher and longer lasting levels of FAA in the liver in mice receiving the higher dose.

In mice, plasma protein binding of FAA corresponded to about 70% of the total drug concentration at both doses investigated. Binding of FAA to human plasma protein is higher, reaching about 90% (J. Collins, personal communication; and data from this laboratory, not shown).

Renal excretion appears to be a major route of elimination of FAA in mice. Within 96 h of injection, the amount of FAA recovered in the urine corresponded to 75% and 60% of the doses of 100 and 300 mg/kg respectively. The lower proportion of FAA excreted after 300 mg/kg, particularly in the first 16 h, may help to explain the lower clearance found with this dose. As drug protein binding is similar at both doses, presumably similar glomerular filtration of FAA occurs. Saturation of tubular secretion therefore appears to be the most likely mechanism of the dose-dependent kinetics of FAA.

*Acknowledgements.* This work was supported in part by a research grant from the Consiglio Nazionale delle Ricerche, Rome (Contract No. 86.02608.44) and by the Italian Association for Cancer Research.
Giovanna Damia is the recipient of a fellowship from the Italian Association for Cancer Research.

## References

1. Bissery MC, Chabot GG, Corbett TH, Rutkowski K (1986) Flavone acetic acid (FAA, NSC-347512): Non linearity of area under the concentration x time curves with changing dosage. Proc Am Assoc Cancer Res 27: 282
2. Chabot GG, Bissery MC, Corbett TH, Rutkowski K (1987) Causes of the nonlinear pharmacokinetics of flavone acetic acid (FAA NSC-347512) in mice. Proc Am Assoc Cancer Res 28: 436
3. Corbett TH, Bissery M-C, Wozniak A, Plowman J, Polin L, Tapazoglou E, Dieckman J, Valeriote F (1986) Activity of flavone acetic acid (NSC-347512) against solid tumors of mice. Invest New Drugs 4: 207
4. Flavone acetic acid. A clinical brochure (1985) NSC 347512. National Cancer Institute, Bethesda, MD
5. Kerr DJ, Kaye SB, Cassidy J, Dutta S, Setanoians A, Forrest G, Cunningham D, Soukop M, Vezin WR (1985) A clinical pharmacokinetic study of LM985 and LM975. Br J Cancer 52: 467
6. O'Dwyer PJ, Shoemaker D, Zaharko DS, Grieshaber C, Plowman J, Corbett T, Valeriote F, King SA, Cradock J, Hoth DF, Leyland-Jones B (1987) Flavone acetic acid (LM975, NSC347512). A novel antitumor agent. Cancer Chemother Pharmacol 19: 6
7. Plowman J, Narayanan VL, Dykes D, Szarvasi E, Briet P, Yoder OC, Paull KD (1986) Flavone acetic acid: A novel agent with preclinical antitumor activity against colon adenocarcinoma 38 in mice. Cancer Treat Rep 70: 631
8. Schenkman JB, Cinti DL (1972) Hepatic mixed function oxidase activity in rapidly prepared microsomes. Life Sci 11: 247
9. Zaharko DS, Grieshaber CK, Plowman J, Cradock JC (1986) Therapeutic and pharmacokinetic relationships of flavone acetic acid: An agent with activity against solid tumors. Cancer Treat Rep 70: 1415

Received August 14, 1987/Accepted January 20, 1988