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# Flavone acetic acid distribution in human malignant tumors

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**Summary.** The pharmacokinetics of flavone acetic acid (FAA) after a dose of 4.8 mg/m$^2$ given i.v. over 1 h was investigated in 13 patients with different solid tumors. The mean volume of distribution and clearance were $52 \pm 4$ l/m$^2$ and $2.6 \pm 0.2$ l/h $\times$ m$^2$, respectively. A tumor or metastasis biopsy was obtained from six patients 2 h after the end of infusion. Tumor FAA levels ranged from 39.6 to 148.8 μg/g and were similar to those obtained after a therapeutic i.v. dose of 200 mg/kg FAA in animals bearing Pan/03 tumor, which is very sensitive to the drug. Although FAA tumor concentration could be detected only during one interval and we therefore cannot draw a definitive conclusion, differences in the agent's antitumor activity in mice and patients (i.e. very active in the former and inactive in the latter) are apparently not due to discrepancies in drug distribution and pharmacokinetics.

## Introduction

Flavone acetic acid (FAA) is a recently discovered anticancer agent currently under evaluation in phase II clinical trials. The preclinical data held out considerable promise for the following reasons: (a) its structure and pharmacological data suggest that its mechanism of action is different from that of other antitumor agents [18]; (b) it is one of the most effective drugs against several mouse tumors as well as some human tumors transplanted in immunodeprived mice [6, 9, 14]; and (c) it causes no bone marrow or gastrointestinal toxicities, which are the two most common side effects produced by available anticancer agents [13].

Although the clinical investigations are not yet complete, the results are thus far disappointing, since none of the cancer patients in phase II trials have responded to FAA. Why FAA is so effective in mice but not in humans remains to be elucidated. This study was undertaken to determine the tumor distribution and plasma kinetics of FAA in humans to determine whether differences in its kinetic behaviour in the two species could account for the discrepancies in its efficacy.

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## Materials and methods

*Patients.* A total of 13 adult patients (10 men and 3 women) ranging in age between 26 and 66 years (mean, 60 years) entered this study: 4 were suffering from lung carcinoma, 4 had colon adenocarcinoma, 3 had melanoma, 1 suffered from carcinoma of the tongue and 1 had laryngeal carcinoma. Their performance status, according to the WHO scale, was 0–2. Only 4 of 13 patients had previously been treated with chemo-immunotherapy or radiotherapy. All patients had normal hepatic and renal functions.

*Animals and tumors.* C57B1 female mice ($18 \pm 2$) (Charles River, Calco, Italy) were used. The animals underwent subcutaneous transplantation of a fragment (2–3 mm) of a murine adenocarcinoma of the pancreas (Pan/O3). The pharmacokinetic study was done on day 21 after transplant. Each value represents the mean of data obtained from three animals.

*Drug treatment.* FAA for clinical use was obtained through the Early Clinical Trials Group (ECTG) of EORTC. The drug was given at a dose of 4.8 g/m$^2$ in 500 ml saline as a 1-h infusion once a week for at least 3 weeks. To prevent crystallization in the renal tubules, 500 ml 1.26% sodium bicarbonate was given before and after FAA. Pharmacokinetic studies were done after the first drug cycle. FAA for animal experiments was obtained from Lypha (Lyon, France) and was given i.v. over 30 s at a dose of 200 mg/kg in 5% $NaHCO_3$.

*Sample collection.* Blood samples were drawn through an indwelling cannula from the arm that did not receive the drug, put into heparinized tubes and spun down at 400 *g*. Samples were taken at the following times: 0 (before treatment) and 0.25, 0.5, 0.75, 1, 2, 4, 8, 12, 16, 24, 36, 48 and 72 h after the end of infusion. When possible, tumors or metastatic lymph nodes were biopsied 2 h after the end of infusion (e.g. in patients 1 and 2, a metastatic lymph node; in cases 3 and 4, subcutaneous metastases; in patients 5 and 6, melanoma). Fragments of the same biopsies were analysed to verify their pathological features. The weight of biopsies of tumors and metastases ranged from 0.050 to 0.875 g. Murine plasma and tumors were obtained 2 h after FAA treatment.

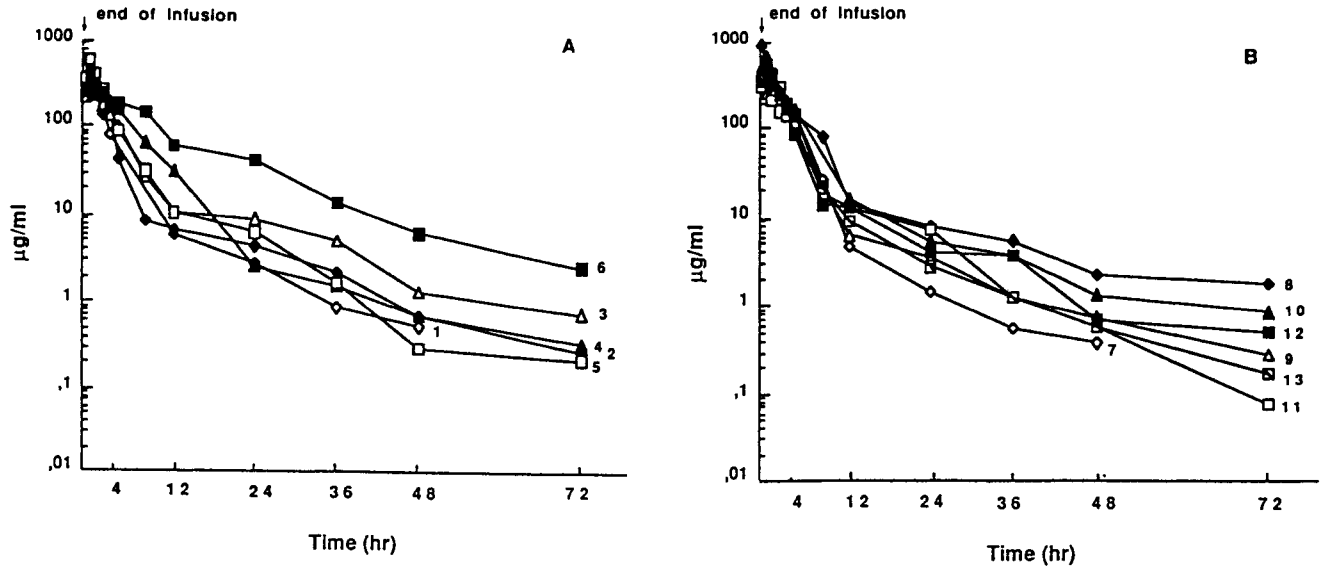


Fig. 1. FAA plasma disappearance curves in 13 patients receiving 4.8 g/m² of the drug over 1 h. A: patients from whom it was possible to obtain a tumor or lymphnode biopsy. B: patients from whom it was not possible to obtain biopsies

Table 1. FAA levels in plasma and tumors

| | Patients[a] | | | | | | Mice[b] |
|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | |
| Plasma | 157 | 138 | 188 | 208 | 256 | 245 | 174 ± 40 |
| Tumor | 39.6 | 110.5 | 44.3 | 148.8 | 118.5 | 73.7 | 100 ± 8 |
| T/P | 0.25 | 0.80 | 0.23 | 0.71 | 0.46 | 0.30 | 0.57 |

[a] Biopsies of tumor or metastasis were obtained 2 h after the end of infusion

[b] Animals bearing Pan03 tumors were treated i. v. with 200 mg/kg FAA on day 21 after implantation. Plasma and tumors were obtained 2 h after treatment. Each value represents the mean (± SE) of data from three animals

T/P Tumor-to-plasma FAA concentration ratios

*Drug assay.* FAA was quantified by a modification of the HPLC method of Kerr et al. [12]. Briefly, 50 or 100 µl plasma was added to 0.5 ml $H_2O$ and 200 µl 5% trichloroacetic acid (TCA), with 50 or 100 µl 3-methylflavone-8-carboxyl acid as an internal standard (100 µg/ml), kindly provided by Dr. D. Nardi (Recordati, Milan, Italy). FAA was extracted with chloroform: isopropanol (1 : 1, v/v) and then mixed at room temperature for 1 h. The precipitate and aqueous phase were sedimented by centrifugation for 15 min at 3,000 rpm. The organic layer was then removed and dried under vacuum. The samples were resuspended in 200–600 µl methanol. Tissues were homogenized (in 1 : 5 or 1 : 10 parts $H_2O$), and 0.5 or 1 ml 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 done using an isocratic solvent system of 0.001 *M* phosphoric acid : acetonitrile : ethanol (60 : 30 : 10, by vol.) at a flow rate of 1 ml/min with a 25-cm-long $C_{18}$ µ-Bondapak column (Water Associates, New York, NY, USA). The recovery of the extraction from plasma or tissues was evaluated by comparing a calibration curve of external standards with an internal calibration curve. For the calibration curves, at least three drug concentrations were added to either human plasma or a murine tumor homogenate. 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 of 0.01–40 µg/ml, and the CV was <10%.

*Pharmacokinetic calculations.* Pharmacokinetic parameters were determined as follows: the AUC was calculated using the trapezoidal rule from time 0 to the last measured time point and then extrapolated from the last point to infinity ($AUC_{0\to\infty}$); the plasma clearance ($Cl_p$) = dose/$AUC_{0\to\infty}$; the volume of distribution ($V_d\beta$) = drug clearance divided by the elimination rate constant (β); the elimination half life = 0.693/β.

## Results

The data on the clinical activity and toxicity of FAA will be reported separately in a paper summarizing the EORTC phase II clinical trial. However, none of the 13 patients investigated responded to the drug, and only minor toxicity was encountered (all but 2 patients experienced a feeling of warmth all over the body during the infusion, and 5 of 13 developed grade 1 gastrointestinal toxicity according to the WHO scale). Plasma FAA levels of 13 patients who received 4.8 g/m² as a 1-h infusion reachd a peak at the end of infusion, then disappeared following a biphasic decay pattern (data not shown), with a terminal half-life ranging from 7.6 to 23.9 h (mean ± SE, 14.7 ± 1.3 h). Mean AUC, $V_d\beta$ and Cl values were 2,021 ± 166 µg/ml × h, 51.8 ± 3.9 l/m² and 2.58 ± 0.17 l/h × m², respectively (Fig. 1).

In six patients, we assayed FAA in biopsies of primary tumor or metastases taken approximately 2 h after the end of infusion. Table 1 shows FAA levels in patients' plasma and neoplastic tissues in comparison with those in plasma and tumor of the mouse tumor Pan/03. The pancreatic Pan/03 tumor was selected for these studies because of its high sensitivity to FAA [6]; a dose of 200 mg/kg (600 mg/m² FAA) is active against this tumor, causing a 65% reduction in tumor weight and a 44% increase in survival (data from this laboratory, not shown). FAA

tumor levels and the ratios of FAA tumor-to-plasma concentrations appeared to be similar in humans and mice.

## Discussion

Phase II clinical studies on FAA have thus far been unsuccessful, with no objective responses. These disappointing results clearly contrast with the striking antitumor activity seen against mouse tumors. We hypothesized that the difference might be related to drug pharmacokinetics. Therefore, we investigated the pharmacokinetics of FAA in patients under phase II study and compared their plasma and tumor drug concentrations with those found in mice bearing tumors against which FAA has proved to be effective.

The plasma pharmacokinetic parameters obtained for FAA in the present study agree with those previously reported for patients receiving similar doses during the phase I clinical trials, except that the $t^1/_2\beta$ ranged from 7.6 to 23.9 h in our study as opposed to 3.54 and 4.5 h in the phase I studies [10, 13]. The higher $t^1/_2\beta$ observed by us might reflect the different sampling times of at least 48 h in our study and up to 24 h in the others. According to the published reports [4, 7, 8, 18], clearance of FAA in the mouse ranges between 0.5 and 3.9 $l/h \times m^2$, depending on the dose, which was 117–900 $mg/m^2$. Therefore, it seems unlikely that the efficacy of FAA in mice is due to slower drug elimination than in humans. Although the tumor drug concentrations could be investigated in only a few patients and during only one interval (i.e. 2 h), they did not appear to be lower than in the sensitive mouse tumor (Pan/03) (Table 1). The drug concentrations in biopsies of human melanomas or neoplastic lymph nodes were certainly much higher than either those reported by Pratesi et al. [15] in a chemically induced mouse colon tumor or those obtained by Bibby et al. [1] in the murine MAC26 tumor, although both of the latter are sensitive to FAA.

Although the mechanism of action of FAA has not yet been fully elucidated, most data suggest that its antitumor activity is mediated by the host. FAA is only weakly cytotoxic against some cancer cell lines exposed to very high concentrations [3] but is very active *in vivo* against several mouse tumors as well as human tumors transplanted in nude mice [6, 9, 14]. No active metabolites have thus far been identified in mice or humans given FAA; thus, it would appear that FAA activity *in vivo* is not mediated by the formation of active metabolites.

Recent studies have demonstrated that FAA can activate NK cells in both mice and humans [5, 11, 16] and potentiate interleukin-2 (IL-2) stimulation of LAK cells [17]. It is therefore possible that it has shown no clinical activity because the disease of patients who entered clinical investigations was too advanced for them to benefit from immunological activation, which potentially could help eradicate only a very small residual tumor. In contrast to this hypothesis, in mice the drug has shown similar activity against small and large tumors, sometimes even stronger activity against large ones [6]. Another hypothesis recently put foward [2] on the mode of action of FAA is that it may induce tumor necrosis by reducing the blood supply. Findings in MAC tumors support this view; however, to our knowledge, no comparative studies of the effects of FAA on blood flow in human and mouse tumors have yet been undertaken.

In conclusion, it would appear that the activity of FAA in mice but not in patients is not a question of differences in drug distribution and pharmacokinetics; it is more likely related to interspecies pharmacodynamic differences. Biochemical and immunological factors involved in the antitumor effect of FAA should be identified, because they may help in planning rational use of FAA in treating selected populations of patients who present the features necessary for the drug to be active.

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