Flavone Acetic Acid (NSC 347512)-induced DNA Damage in Glasgow Osteogenic Sarcoma in Vivo

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ABSTRACT

Flavone acetic acid (FAA) is a new antitumor agent with broad activity against transplantable solid tumors of mice but with only scant or no activity against leukemias and lymphomas. The technique of alkaline elution was used to study DNA lesions in s.c. implanted Glasgow osteogenic sarcoma in C57BL/6 x DBA/2 F, mice treated i.v. with FAA. At efficacious dosages (235 and 200 mg/kg), FAA produced extensive single strand breakage. Formation of single strand breaks was dependent on time of assay after exposure to FAA with only minimal damage occurring prior to 5 h posttreatment. Apparently Glasgow osteogenic sarcoma had no capacity to repair single strand breaks for at least 45 h after drug administration. Thus, FAA differs in its mechanism from other scission agents (e.g., VP-16). Neither interstrand cross-links nor DNA-protein cross-links were detected. DNA single strand breaks did not occur in the bone marrow cells or in the unresponsive P388 leukemia cells at dosages causing extensive DNA damage in solid tumor cells.

INTRODUCTION

FAA* [2-phenyl-8-(carboxymethyl)-benzopyran-4-one, LM-975, NSC 347512] is a new antitumor agent (1–5) synthesized by Lyonnaise Industrielle Pharmaceutique (LIPHA, Lyon, France) (Fig. 1). This agent is presently undergoing Phase I clinical trials at six institutions in the United States and Europe. FAA has been shown to be active against 11 transplantable solid tumors of mice, including five colon adenocarcinomas (07, 10, 26, 38, 51), two pancreatic adenocarcinomas (02 and 03), and an Adriamycin-resistant mammary adenocarcinoma (16/C/Adr) (1–3). Of particular interest is the fact that we have been unable to find a transplantable solid tumor of mice that is unresponsive to FAA. This is totally unlike the behavior of any other chemotherapeutic agent for which only a small percentage of solid tumors tested are usually responsive (6–9). Furthermore, it is essentially inactive in vivo against a variety of hematological tumors, including P388 leukemia (4, 5). Considering the breadth of activity of FAA against solid tumors, knowledge of its mechanism of action is of obvious importance.

Many members of the flavone and flavonoid series are known to possess a wide range of biological and biochemical activities including inhibition of many enzymes, binding to macromolecules, inhibition of ATP synthesis, catalyzing electron transport, scavenging of free radicals, and binding to metals which themselves catalyze the production of free radicals (10–12).

Both the broad spectrum of antitumor activity of FAA and the variety of biochemical activities of flavonoids led us to examine for damage directly at the DNA molecule. Using the alkaline elution technique, the time-response and dose-response relationships for FAA-induced SSB as well as the absence of DNA-protein and DNA-DNA cross-links were examined in vivo in a responsive tumor (Glasgow osteogenic sarcoma).

MATERIALS AND METHODS

Mice. Male C57BL/6 x DBA/2 F, (hereafter called B6D2F,), C57BL/6, or DBA/2 mice (8–14 weeks old) were bred in-house from strains obtained from The Jackson Laboratory, Bar Harbor, ME. Food and water were supplied ad libitum.

Tumors. Glasgow's 125Pb-induced osteogenic sarcoma (13) was chosen for this study since it is responsive to FAA (3) and monodispersed cell suspensions, necessary for alkaline elution studies, can be prepared easily. The tumor was maintained in the mouse strain of origin (C57BL/6) and transplanted into the appropriate F, hybrid (B6D2F,) for therapy trials. The experimental animals were implanted bilaterally s.c. with 30–60-mg tumor fragments by a 12-gauge trocar on day 0. P388 leukemia was maintained in the mouse strain of origin (DBA/2) by i.p. injection. The experimental animals were implanted i.p. with 3 x 10' cells on day 0. All mice weighed more than 20 g at the start of therapy.

Drug. FAA was obtained from the Drug Evaluation Branch, National Cancer Institute. FAA was dissolved in a solution of sodium bicarbonate 24 h before injection and stored at room temperature in the dark. The final concentration of bicarbonate was 5%; the volume of injection was 0.5 ml/mouse.

Efficacy Trial. The technique of chemotherapy and data analysis have been described elsewhere (8, 14). Tumors were allowed to grow to 300–600 mg before the mice were given i.v. injections of a single dose of FAA. Tumors were measured by caliper three times weekly and tumor weights were calculated as

\[
\text{Tumor wt (mg)} = \frac{a \times b^2}{2}
\]

where a and b are the tumor length and width (mm), respectively.

The following end points were used to assess antitumor activity: (a) tumor growth inhibition (T/C in percentage) where T and C are the median tumor burden for the treatment group and the control group, respectively. For these experiments, day 13 was used. A T/C equal to or less than 42% is considered significant antitumor activity by the Developmental Therapeutics Program, Division of Cancer Treatment, National Cancer Institute (15); (b) tumor growth delay (T – C in days) where T and C are the median times required for the treatment group and the control group, respectively, to reach a predetermined size (1000 mg); (c) response criteria, in which complete response is regression below the limit of palpation and partial response is regression greater than 50% in tumor weight; (d) the log,0 cell kill/dose calculated as

\[
\log_{10} \text{cell kill/dose} = \frac{T - C}{3.32 (T_0)} \text{ (no. of doses)}
\]

where T is the tumor doubling time (in days) and was estimated from the best-fit straight line from a log-linear growth plot of the control group tumors in the 100- to 1000-mg range. The log,0 cell kill (gross) was the log,0 cell kill/dose multiplied by the number of doses (14).

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The abbreviations used are: FAA, flavone acetic acid; AUC, area under the curve; GOS, Glasgow osteogenic sarcoma; PK, proteinase K; PSA, Puck's solution A; SSB, single strand breaks; T/C, treated/control; SSF, strand scission factor.
Treatment and Preparation of Cell Suspension for Alkaline Elution. Chemotherapy for alkaline elution experiments was started when GOS tumors reached 300–600 mg. The mice were sacrificed at various times after a single i.v. injection of FAA and the excised tissues were processed immediately.

Tumors were minced into small fragments and further disrupted in 5 ml of ice-cold PSA using a Stomacher-80 for 20 s. The material was then poured through an 80 mesh sieve; residual material was forced through by finger using a sterile glove. The sieve was rinsed twice with cold PSA. The suspension was then centrifuged (110 × g) for 8 min at 4°C and the cells were resuspended in PSA. The cell suspension was forced slowly through a 20-gauge needle, 1 inch long, to disrupt clumps. The cells were counted with a hemocytometer.

Bone marrow cell suspensions were obtained by flushing the cells from the femurs with PSA (1 ml/femur) using a 23-gauge needle. The cells were counted with a hemocytometer, with an average yield of 1.6 × 10^7 cells/femur.

Mice bearing i.p. P388 leukemia were treated on day 3 with FAA (i.p.) and assayed 6, 8, and 24 h later. The cells were removed from the peritoneal cavity and adjusted to a concentration of 10^7 cells/ml.

Alkaline Elution. The procedure described by Kohn (16) was used, with a fluorometric determination of the DNA (17, 18). Except where noted, 5 × 10^6 cells were placed onto a 47-mm-diameter, 0.8-μm polycarbonate filter (Nucleopore Corp., Pleasanton, CA). The cells on the filters were washed with 10 ml of cold PSA-EDTA, lysed with 10 ml of a pH 10.0 solution (2 M NaCl-0.04 M tetrasodium EDTA-0.2% Sarkosyl) either with or without PK at a concentration of 0.5 mg/ml. The filters were washed with 10 ml of 0.02 M tetrasodium EDTA, pH 10.3. The DNA was eluted with 0.1 M tetrapropylammonium hydroxide containing 0.02 M EDTA (free acid), pH 12.14, at a constant flow rate of 0.04 ml/min. Fractions were collected every 90 min for 15 h. The entire procedure was conducted with minimal vibration, constant temperature, and aluminum foil light shields. Assays for both SSB and intrastrand cross-links were performed under deproteinization conditions; PK was added to the lysis solution and the lysate on the filter was retained 45 min at room temperature. This treatment greatly reduced the influence of any DNA-protein-linked molecules in the elution profile (19, 20). For interstrand cross-linking assay, the cells received 500 rads at 0°C from a Gammacell 137Cs irradiator (Mark I, Model 68; J. L. Shepherd and Associate, Glendale, CA) prior to elution. A positive control for DNA-protein cross-links (data not presented here) was carried out with L1210 cells in vivo using VP-16 as the cross-linking agent. Results identical to those obtained in other laboratories were observed (21). Similarly, a positive control for DNA-DNA cross-links (data not presented here) was carried out with L1210 cells in vivo using cyclophosphamide as the cross-linking agent.

Filters were removed with forceps, minced into small pieces, and placed into scintillation vials containing 5 ml of washing solution. The DNA retained on the filter fragments was removed by heating to 60°C for 30 min and vortexed for 5 min. DNA remaining in the filter holder or barrel was recovered by flushing with a 5-ml EDTA wash solution. For each experiment, a blank column with filter but with no cells was run to correct for background fluorescence.

DNA Assay. The relative DNA concentration in each fraction was determined using the fluorescent Hoechst dye 33258 (22). A 1-ml aliquot was withdrawn from each fraction, including the filter and wash solutions, and transferred to a 13- x 100-mm borosilicate glass culture tube. Each sample was neutralized with 0.4 ml of 0.2 M KH₂PO₄ and the volume was adjusted to 2 ml with 0.6 ml of distilled water. Finally, 1 ml of Hoechst dye (1.5 × 10⁻⁶ M in standard saline citrate) was added and the tubes were vortexed. The fluorescence intensity was determined using a Perkin-Elmer 650 fluorescence detector with the excitation wavelength set at 350 nm and the emission at 460 nm. The sample fluorescence, blank fluorescence, and volumes were analyzed by a computer program (23).

Quantitation of Strand Scission. A dose-response calibration curve was generated to express the degree of DNA strand scission in relation to the dose of radiation. GOS cells were prepared from tumors grown in vivo and were then irradiated at 4°C in vitro prior to analysis by alkaline elution. Elution curves of the DNA from GOS cells exposed to different doses of radiation are shown in Fig. 2. The calibration curve was constructed by plotting the SSF (18) versus dose of radiation. The SSF was calculated as

\[
\text{SSF} = \frac{\log_{10} \% \text{ of DNA retained in the irradiated sample after 9 h of elution (18 ml eluted)}}{\% \text{ of DNA retained in the control sample after 9 h of elution (18 ml eluted)}}
\]

This curve was then used to convert the strand scission factor of drug-treated samples to rad equivalents.

FAA High-Performance Liquid Chromatography Assay in Plasma, Tumors, and Bone Marrow Cells. Blood samples were obtained by cardiac puncture immediately after the sacrifice of the animals using heparinized syringes. The blood was centrifuged at 13,000 × g for 5 min and the plasma was collected and stored at −20°C until assay. The tumors were excised, washed free of blood, carefully weighed, and frozen until analysis. The bone marrow samples were obtained by flushing the femurs with ice-cold PSA (1 ml/femur) using a 23-gauge syringe. The bone marrow cells were counted using a hemocytometer and frozen until assay. Both tumor and bone marrow samples were homogenized using a Polytron for 30 s (type PT10/35; Brinkman Instruments, Westbury, NY). After protein precipitation using cold 5% trichloroacetic acid (40 μl), plasma and homogenate samples (200 μl), or dilutions thereof, were extracted with 1 ml methanol. The samples were then centrifuged (13,000 × g, 5 min) and 25 μl of the supernatant (100 μl for the bone marrow homogenates) were injected onto a C₁₈ reverse-phase column (μBondapak; Waters Associates) protected by a
precolumn. FAA was eluted with a mobile phase composed of acetonitrile, water, and acetic acid (40:60:2) at a flow rate of 1 ml/min (Waters pump M-45). Under these conditions, FAA eluted at 8 min and was detected at 300 nm (Spectroflow 773, Kratos). If needed, samples were diluted to fall within the concentrations used for the calibration curves. The calibration curves were linear from 0 to 10 μg/ml with a coefficient of correlation near unity. Peak heights were used for quantification.

Histology. Portions of the s.c. tumors were removed and immediately immersed in 4% buffered formalin solution. The tissues were processed through graded alcohols and embedded in paraffin. Sections (6 μm) were prepared and stained with hematoxylin and eosin. The status of the tumor tissue was evaluated for areas of necrosis and cellular integrity.

RESULTS

Efficacy Trial. GOS was chosen for these studies because FAA has been shown to be active against early stage GOS at a dosage of 200 mg/kg (T/C = 3%) (3). GOS is a fast growing tumor (doubling time, 1.5 days). It is highly sensitive to alkylating agents (e.g., cyclophosphamide or L-phenylalanine mustard), moderately to highly responsive to selective antimetabolites (tiazofurin, 6-thioguanine), and unresponsive to DNA binders and mitotic inhibitors (e.g., Adriamycin and vincristine).

The effects of i.v. administered FAA were evaluated on advanced s.c. GOS (300—600 mg). There were no drug death at dosages less than or equal to 235 mg/kg, but frank toxicity was reached with a 380-mg/kg dosage (80% lethal dose) (Table 1). FAA was moderately active with the single dose schedule used here, with a T/C of 18.5% at the highest nontoxic dosage (235 mg/kg) and a T/C of 35.6% at a 200-mg/kg dosage. The two lower dosages (150 and 95 mg/kg) were inactive with T/C greater than 45% (14).

Time Response of the DNA Damage. GOS-bearing mice were treated with a single dose (200 mg/kg) of FAA and tumors were removed and assessed for DNA damage at times thereafter. FAA caused DNA SSB as shown by the greater rate of DNA elution from the treated cells compared to the untreated control cells (Fig. 3). The rate of DNA elution increased with time following the FAA administration. These experiments were run with proteinase K in order to eliminate any possible DNA-protein cross-links. The extent of DNA damage was converted to rad equivalents, using the calibration curve as defined in “Materials and Methods.” Values of 45, 61, and 280 rad equivalents for 0.5, 3, and 5 h FAA exposures, respectively, were obtained. Exposures to FAA greater than 8 h and as long as 45 h produced DNA damage equivalent to or greater than a 500-rad exposure.

Since undiminished strand breakage was seen up to 45 h after drug injection, the damage produced by FAA did not appear to be repairable. The drug concentration in plasma at 45 h was 1 μg/ml (data not shown), which is less than 1% of an effective level.

Dose Response of the DNA Damage. The doses used ranged from inactive to active, matching the efficacy trial (Table 1). The time chosen between the FAA treatment and the harvest of tumor cells was 8 h inasmuch as significant DNA strand breakage was observable at this time. DNA elution profiles from GOS cells after treatment are shown in Fig. 4. Increasing the dose of FAA resulted in a faster rate of elution of tumor DNA relative to that of the untreated control indicating that the extent of DNA strand breakage was dose dependent.

The activity or inactivity of the drug correlated with the presence or absence of DNA strand breakage (Fig. 4; Table 1). Extensive strand breakage (equal or greater than 500 rad equivalents) was observed in GOS cells after FAA treatment of both 235- and 200-mg/kg dosages, which gave T/C values in mice of 18.5 and 35.6%, respectively. Dosages of either 150 or 95 mg/kg yielded little, if any, strand breakage and were inactive based on their T/C values (Table 1).

Assessment of DNA-Protein and DNA-Interstrand Cross-Links. For DNA-protein cross-link evaluation, mice bearing 300—600-mg GOS tumors were treated with a single dose of FAA (200 mg/kg). The tumors were removed 3 and 5 h after treatment and tumor cells were lysed in the presence or absence of PK. If DNA-protein cross-links were present, treatment with PK would increase the rate of DNA elution. The DNA alkaline elution profiles indicated little if any DNA-protein linkages (data not shown). The differential between the elution profiles with and without PK averaged 4.5 rads 3 hours after treatment and 48 rads 5 h after treatment. In a similar experiment done with 235 mg/kg of FAA, we failed to demonstrate any DNA-protein cross-links 5 h after treatment.

To determine the presence or absence of DNA-interstrand cross-links, we treated mice with FAA (200 mg/kg) and examined the DNA of the GOS cells at 0.5, 3, 5, and 8 h after treatment, with or without exposure to 500 rads. If DNA-interstrand cross-links were present, the rate of elution for the cells exposed to both FAA and radiation would be less than that for radiation alone. The DNA elution of the 500-rads-irradiated FAA-treated cells was found to closely follow that of the untreated irradiated (500 rads) control cells (data not shown). Thus, it is clear that FAA did not produce DNA-interstrand cross-linking.

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Table 1 Response of advanced stage Glasgow osteogenic sarcoma to FAA treatment in vivo

<table>
<thead>
<tr>
<th>Dosage* (mg/kg/dose)</th>
<th>Drug death (days of death)</th>
<th>Median tumor burden/mouse on day 13 in mg (range)</th>
<th>T/C (%) on day 13</th>
<th>Partial response*</th>
<th>Time for median tumor burden to reach 1000 mg (days)</th>
<th>Tumor growth delay (days) T—C</th>
<th>Log$_a$ cell kill</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>4/5 (11, 11, 11, 11)</td>
<td>1350 (720—1670)</td>
<td>35</td>
<td>2/5</td>
<td>18</td>
<td>6.0</td>
<td>1.2</td>
</tr>
<tr>
<td>380</td>
<td>0/5</td>
<td>250 (180—740)</td>
<td>18</td>
<td>0/5</td>
<td>18</td>
<td>14.5</td>
<td>2.5</td>
</tr>
<tr>
<td>235</td>
<td>0/5</td>
<td>480 (240—1160)</td>
<td>35</td>
<td>0/5</td>
<td>13.5</td>
<td>13.5</td>
<td>1.5</td>
</tr>
<tr>
<td>200</td>
<td>0/5</td>
<td>720 (600—1200)</td>
<td>53</td>
<td>0/5</td>
<td>13</td>
<td>13</td>
<td>1.0</td>
</tr>
<tr>
<td>150</td>
<td>0/5</td>
<td>810 (810—1510)</td>
<td>81</td>
<td>0/5</td>
<td>13</td>
<td>81</td>
<td>0.2</td>
</tr>
<tr>
<td>95</td>
<td>0/5</td>
<td>1100 (810—1510)</td>
<td>81</td>
<td>0/5</td>
<td>13</td>
<td>81</td>
<td>0.2</td>
</tr>
</tbody>
</table>

* FAA was prepared in 5% NaHCO$_3$ in distilled water and injected i.v. (0.5 ml/mouse); mice = 26 g average. Drug treatment began on day 10 at 4 dosage levels (1.6-log increment); an extra dosage (200 mg/kg) was added due to the narrow window of efficacy of this drug.

* Regression greater than 50% in tumor weight.
Effect of FAA on Bone Marrow Cells. The DNA damage produced in a normal cell population of the host, specifically bone marrow cells, was studied. Cells examined 5 h after treatment of GOS-bearing mice with 200 mg/kg of FAA revealed no DNA damage in bone marrow cells while there was a 280-rad equivalent damage to the DNA in the tumor cells (Fig. 5). A comparison of the FAA concentrations in the plasma, tumors, and bone marrows is listed in Table 2. The overall AUC for the tumors at 200 mg was 29% higher than the AUC in the bone marrow for the same dose. Since the absence of DNA damage at the bone marrow level could have been due to a lower AUC in this tissue compared to tumors, GOS-bearing mice were then treated with a higher single dose of FAA (235 mg/kg, the maximum tolerated dose) in an attempt to obtain a higher AUC in the bone marrow. The AUC for the bone marrow at a 235-mg/kg dosage was higher than the AUC in the tumor at a dosage of 200 mg/kg (Table 2). At this higher dose, there was still no detectable effect on the DNA of the marrow cells, 1, 3, 5, 12, and 24 h post-FAA administration (data not shown). This contrasted with the extensive strand breakage seen in the tumor at the 200-mg/kg dosage (Fig. 4). In a separate experiment DNA damage was found in the tumor cells at 6, 14, and 24 h after a 235-mg/kg FAA treatment (the damage was equal to or greater than 500 rad equivalents, data not shown).

Correlation between DNA Lesions, Histology, and Efficacy. It was of interest to note that at efficacious dosages, FAA caused hemorrhage and necrosis within the tumor 24 h posttreatment at 200-mg/kg dosage or greater. Hyperemia (without necrosis) was seen as early as 6 h after injection of a 235-mg/kg dosage.
Table 2  FAA concentrations in plasma, tumors, and bone marrows, at different times after an i.v. bolus administration in B6D2F, mice bearing advanced stage Glasgow osteogenic sarcoma

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Dosage (mg/kg)</th>
<th>FAA concentrations ($\mu$g/ml)</th>
<th>0.5 h</th>
<th>3 h</th>
<th>5 h</th>
<th>8 h</th>
<th>12 h</th>
<th>24 h</th>
<th>AUC*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma*</td>
<td>200</td>
<td></td>
<td>155.6 ± 36.3</td>
<td>23.5 ± 8.2</td>
<td>16.0 ± 4.1</td>
<td>17.5 ± 5.8</td>
<td>8.0 ± 5.7</td>
<td>0.84 ± 0.25</td>
<td>505 ± 122</td>
</tr>
<tr>
<td></td>
<td>235</td>
<td></td>
<td>363.0 ± 37.3</td>
<td>118.6 ± 28.2</td>
<td>85.0 ± 12.8</td>
<td>47.7 ± 8.8</td>
<td>4.9 ± 2.1</td>
<td>4.7 ± 3.9</td>
<td>1256 ± 74</td>
</tr>
<tr>
<td>Tumor</td>
<td>200</td>
<td></td>
<td>121.4 ± 66.0</td>
<td>26.4 ± 6.6</td>
<td>13.2 ± 2.3</td>
<td>13.3 ± 3.7</td>
<td>7.1 ± 4.1</td>
<td>4.0 ± 3.2</td>
<td>402 ± 68</td>
</tr>
<tr>
<td></td>
<td>235</td>
<td></td>
<td>150.4 ± 40.7</td>
<td>79.5 ± 48.4</td>
<td>43.2 ± 6.4</td>
<td>30.3 ± 3.3</td>
<td>28.3 ± 20.1</td>
<td>5.4 ± 0.8</td>
<td>902 ± 275</td>
</tr>
<tr>
<td>Bone marrow</td>
<td>200</td>
<td></td>
<td>59.8 ± 36.6</td>
<td>9.9 ± 4.2</td>
<td>8.7 ± 0.5</td>
<td>10.3 ± 3.3</td>
<td>11.7 ± 1.0</td>
<td>7.9 ± 0.3</td>
<td>311 ± 82</td>
</tr>
<tr>
<td></td>
<td>235</td>
<td></td>
<td>121.7 ± 50.5</td>
<td>38.9 ± 17.7</td>
<td>26.6 ± 3.8</td>
<td>18.7 ± 0.6</td>
<td>17.3 ± 15.3</td>
<td>10.6 ± 7.9</td>
<td>532 ± 173</td>
</tr>
</tbody>
</table>

* FAA concentrations were determined by high performance liquid chromatography as described in “Materials and Methods.”

$\beta$ half-lives were 4.0 and 4.3 h for the 200- and 235-mg/kg doses, respectively.

Effect of FAA on P388 Leukemia. FAA has been shown to be essentially inactive against P388 in vivo (2–5). Therefore, as a negative control, we examined the effect of FAA on the DNA of these cells. Mice bearing approximately $5 \times 10^8$ P388 leukemia cells (i.p.) were treated i.p. with 235 mg/kg of FAA. The alkaline elution profiles indicated little if any damage to the DNA of these leukemic cells at 6, 8, and 24 h posttreatment.

DISCUSSION

The technique of alkaline elution was used to study FAA-induced DNA lesions in s.c. implanted GOS tumors in BD2F,
mice. Based on the data presented, it is clear that FAA produces nonrepairable DNA SSB in a time- and dose-dependent manner. The time needed to develop significant strand breakage was relatively long, with minimal or no SSB occurring until 5 h after treatment. It is of significance that the presence of strand breaks correlated with the efficacy of FAA, with inactive dosage failing to produce SSB and active dosages producing extensive SSB.

The interpretation of any mechanism of strand breakage from alkaline elution data is difficult. The DNA damage can be either a direct effect of FAA or an indirect effect consequent upon another effect. Although the specific mechanism by which FAA causes SSB is presently unknown, two possible models appear to be most reasonable. First, FAA itself or a metabolite could be clastogenic for DNA. However, upon examination of the structure of FAA, there is no obvious highly reactive center which could act directly on the DNA to produce the breaks. The benzo-γ-pyrones show little evidence of aromatic character. It is stabilized by π overlap of the orbitals of the double bonds, the intracyclic heteroatom (oxygen) and the carbonyl (24). Therefore it is likely that FAA needs activation in order to cause strand breakage. The metabolism of flavonoid compounds in mammals was recently reviewed by Hackett (25) and includes the following biotransformations: heterocyclic ring fission (mediated by intestinal microorganisms); methylation and conjugation with glucuronic acid or sulfate; and oxidation by introduction of one or more hydroxyl groups (this reaction would be catalyzed by microsomal monooxygenases in mammalian tissues). Hydroxylation may be important in the case of FAA, since reactive species (semiquinone radicals, anions) are unlikely to occur without this step. Metabolites more polar than FAA have been detected in the mouse and dog (26). It should be noted that FAA was selectively cytotoxic in vivo for solid tumors over leukemias in a soft agar colony formation assay, suggesting that the agent did not need to be metabolized to active species outside the tumor cell (3). A second model is that FAA or a metabolite inhibits ATP synthesis either as a sole mechanism or in combination with a DNA clastogenic effect. This would explain the lack of repair of SSB noted following FAA exposure. Indeed, ATP is not detected within GOS cells by 4 h following treatment with therapeutically effective doses of FAA and remains undetectable for at least 48 h, as demonstrated by 31P surface coil nuclear magnetic resonance (27).

Furthermore, FAA might inhibit mitochondrial succinocoxidase and NADH oxidase activities thereby not only inhibiting ATP synthesis but also leading to the production of superoxide and hydrogen peroxide (12) which may be responsible for the clastogenic effect noted on DNA. However, we cannot exclude the possibility that FAA-induced DNA damage could be a consequence of cell death secondary to other cytotoxic events.

Clearly, FAA does not behave as any of the other antitumor agents known to produce strand breakage. For example, Adriamycin-like DNA binders and VP-16 cause SSB but also produce DNA-protein cross-links (21, 28). FAA does not produce these cross-links. Thus FAA may represent a unique class of strand breaker.

Contrary to most antineoplastic agents, FAA in bolus dosages produces only minimal or no toxicity to the bone marrow of mice or dogs (4). As expected, there was minimal or no SSB in the bone marrow cells at dosages that produced extensive breaks in the tumor. The AUC for bone marrow cells of mice treated i.v. with 235 mg/kg of FAA was higher than the AUC in the tumors of mice treated i.v. with 200 mg/kg of FAA. Thus, drug exposure was not the cause of the bone marrow insensitivity. In addition to the lack of leukopenia (4), the very weak responsiveness of leukemias to FAA and the absence of SSB in DNA of P388 cells are consistent with the innate insensitivity of hematopoietic cells and tumors derived from these cells. Possibly FAA is metabolized in the responsive tumors to an active form, whereas bone marrow and leukemias are unable to efficiently accomplish this activation step.

In conclusion, FAA represents a novel class of anticancer agents, selectively active for solid tumors and capable of producing nonrepairable DNA strand breaks. Thus, the agent (and selected analogues) may be a promising candidate for tumor therapy either alone or in combination with other chemotherapeutic agents or with radiation.

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FLAVONE ACETIC ACID-INDUCED DNA DAMAGE IN VIVO


Flavone Acetic Acid (NSC 347512)-induced DNA Damage in Glasgow Osteogenic Sarcoma in Vivo

Marie-Christine Bissery, Frederick A. Valeriote, Guy G. Chabot, et al.


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