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Letter to the Editor

Flavone Acetic Acid (NSC 347512) Induces Haemorrhagic Necrosis of Mouse Colon 26 and 38 Tumours

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FLAVONE-8-ACETIC ACID (NSC 347512; FAA) is a synthetic flavonoid recently found to have unexpectedly high cytotoxic activity against subcutaneously implanted (s.c.) Colon 38 tumours in mice [1, 2]. Its spectrum of activity embraces a variety of murine solid tumours [3], but it is much less active in leukaemia models. FAA and its diethylaminoethyl ester (NSC 293015) are at present undergoing Phase I clinical trials [4] and the mode of their cytotoxic action is of great interest. Naturally occurring flavonoids can affect cells at numerous biochemical sites, for instance by inhibition of cell membrane ATPase pumps, inhibition of prostaglandin cyclooxygenase [5] and induction of gene expression [6]. We show here that the action of FAA and NSC 293015 against Colon 26 and 38 tumours in mice involves the rapid induction of haemorrhagic tumour necrosis.

Colon 26 and 38 tumours were provided by Mason Research Institute, Worcester, Massachussetts, U.S.A., and passaged in BALB/cJ and C57BL/6J mice, respectively, as s.c. fragments by standard procedures. Mice were bred in the laboratory from stock provided by the Jackson Laboratory (Bar Harbor, Maine, U.S.A.) under constant temperature and humidity with sterile food and bedding. One mm^3 fragments of a 1 cm diameter tumour were implanted s.c. in male 18–22 g mice and tumours were allowed to grow to a diameter of 5–10 mm before treatment. Groups of six mice were injected intraperitoneally (i.p.) with FAA (330 mg/kg single dose, dissolved in 5% $NaHCO_3$; drug kindly provided by Dr. K. Paull, Developmental Therapeutics Programme, National Cancer Institute, U.S.A.), or with NSC 293015 (150 mg/kg, every 4 days × 3, dissolved in water; drug kindly provided by Dr E. Szarvasi, Lyonnaise Industrielle Pharmaceutique, Lyon, France). All tumours were subsequently measured in two dimensions using digital calipers three times weekly, and tumour volumes were calculated as $0.52a^2b$ where a and b are the minor and major tumour diameters. FAA and NSC 293015 induced average delay times in the Colon 38 tumour of 11.3 days and 13 days, respectively, in agreement with previous results for mouse solid tumours [2, 3]. FAA (330 mg/kg) also caused delay of 7 days in the growth of Colon 26 tumour.

A histological study of the effect of FAA was carried out. Groups of animals bearing advanced Colon 38 tumours were treated with a single i.p. injection of FAA (330 mg/kg) from 2 to 24 hr prior to excision. Animals were killed by cervical dislocation and tumours were excised, fixed in 10% formalin, embedded in paraffin wax, sectioned and stained with haematoxylin and eosin by standard methods. Portions of liver and small bowel from all mice were also prepared for histological examination. Untreated tumours grew as a solid, lightly encapsulated non-invasive tumour, with a rich vascular supply. A small degree of central necrosis was present in small tumours and the degree increased with tumour size. Small areas

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of peripheral haemorrhage were sometimes present in larger tumours. Tumours removed from mice treated 2 hr previously with FAA were similar to untreated tumours. However, from 4 to 24 hr after treatment, tumours became grossly haemorrhagic throughout, with tumour softening but an intact capsule. Histologically there was full thickness haemorrhage and necrosis. Examination of other murine organ systems 24 hr after treatment failed to reveal any obvious abnormalities. Similar effects were observed in Colon 38 tumours following NSC 293015 (330 mg/kg), and in Colon 26 tumours following FAA (330 mg/kg).

Tumours were also examined by flow cytofluorimetry 24 hr following treatment with FAA (330 mg/kg). Subcutaneous tumours were removed and minced with crossed scalpels. Tumour mince (300 mg) was placed into a glass centrifuge tube and incubated with stirring at 37° C for 45 min with pronase (1 mg/ml, 1 ml per 60 mg of tumour mince) dissolved in alpha-modified minimal essential growth medium containing 10% foetal calf serum. The suspension was then washed three times to remove pronase, stained with diamidinophenylindole (10 μg/ml) in 0.4% Triton X [7], and the cells analysed for DNA content using an Ortho Instruments ICP22A cytometer and Model 2103 multichannel analyser. There was no significant increase in the proportion of S or G2 phase cells in cell suspensions from treated tumours. FAA thus differs in its action from the majority of clinical cytotoxic agents, which generally arrest cells in G2 phase [8].

Previous studies on the effect of FAA on experimental solid tumours have utilized s.c. inocula [1, 2]. In order to determine whether FAA acted on tumours in other locations, hepatic metastases of the Colon 26 and 38 tumours were induced by intrasplenic inoculation [9] of male BALB/cJ and C57BL/J mice (19.0–23.0 g), respectively. Single cell suspensions were prepared from s.c. tumours using the above pronase procedure (cell yield 1–3 × 10^7 cells/g of tumour). Recipient animals were anaesthetized with i.p. pentobarbital (90 mg/kg, dissolved in 0.2 ml saline) and a small (0.5 cm) tranverse left subcostal incision made. The spleen was gently mobilized on to the external abdominal wall, and 10^6 cells (0.1 ml) were injected slowly into the upper pole of the spleen via a 30-gauge needle, raising a pale weal in the splenic pulp which vanished over a period of 1–2 min. Neither laceration of splenic parenchyma nor leakage of the cell suspension occurred provided the needle was positioned carefully under the splenic capsule. Two minutes after inoculation, the splenic ligaments and vessels were firmly tied using fine silk, and the spleen resected to avoid residual tumour growing in the spleen itelf. The peritoneum and skin were closed in one layer with a small Michel wound clip. The proportion of animals developing tumours was 100% and approx. 20% for Colon 26 and 38, respectively. Groups of mice were treated with a single dose of FAA (330 mg/kg) and after 24 hr livers from treated and untreated animals were removed and sectioned. Extensive necrosis of the treated tumours was observed, but haemorrhage was not as conspicuous as with the s.c. tumours.

In summary, we have shown that as early as 4 hr after treatment with FAA, treated s.c. colon tumours show evidence of extensive haemorrhagic necrosis. After 24 hr, there are few tumour cells remaining in either s.c. or liver tumours which are recognizably viable. The histological observations obtained here bear remarkable similarities to those reported for the action of tumour necrosis factor (TNF) on Colon 26 and several other transplantable mouse tumours [10]. Preliminary experiments in this laboratory have shown that recombinant human TNF induces haemorrhagic necrosis in the Colon 26 tumour, although the Colon 38 tumour is considerably less sensitive. Further work is in progress to determine whether TNF is involved in the action of FAA, and whether FAA is acting in other ways as a biological response modifier rather than a direct cytotoxic agent.

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