THE MECHANISM OF THE ANTI-TUMOUR ACTION

OF FLAVONE ACETIC ACID

AND ITS XANTHENONE ACETIC ACID ANALOGUES

by

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ABSTRACT

The search for new compounds with anti-tumour activity is driven by the high mortality from disseminated visceral carcinomas, for which few effective treatments exist. The experimental agent flavone acetic acid (FAA) has stimulated interest because of its broad spectrum of activity against solid tumours in mice. This remarkable activity spectrum suggested an unusual mechanism of action. The aim of the thesis was to elucidate the mechanism of action of FAA and its analogues in mouse tumours, particularly the rapid induction of haemorrhagic necrosis.

Light and electron microscopic studies of Colon 38 tumours treated with 1.2 mmol/Kg FAA revealed a sequence of tumour cell separation and rounding, focal apoptosis, and finally confluent necrosis with blood vessel engorgement and rupture within 4 - 8 h. This was accompanied by a loss of energy metabolites. The combination of apoptotic and necrotic modes of cell death suggested that both ischaemic and non-ischaemic mechanisms were involved.

The role of blood flow failure was further investigated in Colon 38 and EMT6 tumours using a double label fluorescent vessel-labelling technique, which demonstrated a progressive and sustained fall in blood flow, beginning within 15 min of treatment. A mathematical model of ischaemic tumour cell killing using data from measurements of tumour perfusion, the rate of FAA-induced cell killing and the rate of cell death following global ischaemia indicated that mechanisms in addition ischaemia were involved. However, comparison of the resistance to FAA of avascular intraperitoneal tumour multicellular spheroids and vascularised tumours suggested that the major component of the anti-tumour effect was indeed blood vessel-dependent.

The vessel-*independent* effects of FAA were then studied *in vitro*, using EMT6 tumour multicellular spheroids which had been placed for six days in the peritoneal cavities of mice to become infiltrated by macrophages and lymphocytes. These *ex vivo* spheroids were more that twice as sensitive as spheroids grown entirely *in vitro*, when exposed to 2.5 mM FAA for 24 h. This provides evidence for immune cell mediation of the FAA anti-tumour effect.

To determine the relative importance of the vessel dependent and the immune mediated effects of FAA, a novel vascularised spheroid tumour system, which contained both avascular and vascular components, was developed and studied. FAA treatment caused necrosis only in the vascularised component, preceded by a loss in perfusion. The avascular tumour tissue also showed evidence of some cell loss by apoptosis. Thus, in tumour tissue not relying directly on blood vessels for metabolite exchange, only limited cell killing occurs, suggesting that treatment with FAA-like agents alone would have limited ability to cure.

The anti-tumour activities of a series of xanthenone acetic acid (XAA) analogues of FAA, were then compared with FAA by examination and measurement of morphological changes in subcutaneous tumours and spheroids, and by measurement of blood perfusion in tumours. Those XAA analogues which were active in inducing haemorrhagic necrosis also caused FAA-like morphological changes in tumours and spheroids, and inhibited blood flow, indicating a similar mode of action to FAA. The parent XAA and FAA were approximately equipotent, and 5-methyl XAA and 5,6-dimethyl XAA were about ten times as potent as FAA. 3-O-methyl XAA, 8-methyl XAA and xanthenone-4,5-diacetic acid showed no FAA-like activities.

Examination of non-tumour tissues after therapeutic doses of FAA and 5,6-dimethyl XAA showed cytolytic lesions, including necrosis, in the peripheral lymphoid tissues, the thymus and the uterus. Thus, although these agents lack the myelosuppressive effects of conventional chemotherapeutic agents, cell killing is not entirely specific for tumours. The sensitive tissues, including tumours, have in common a high content of macrophages or neutrophils, and regions of low vascular density.

The thesis studies have shown that FAA and its analogues differ from conventional cytotoxic (anti-proliferative) agents in their mechanism of action. Direct toxicity against tumour cells is of minor importance compared to macrophage mediated cytotoxicity and perfusion failure. If biological activity can be demonstrated in humans, FAA-like agents may show clinical utility in combination with radiotherapy, conventional or hypoxia selective cytotoxic agents.

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ABBREVIATIONS

3OMX	3-O-methyl xanthenone-4-acetic acid
5MX	5-methyl xanthenone-4-acetic acid
8MX	8-methyl xanthenone-4-acetic acid
ADP	Adenosine diphosphate
AMP	Adenosine monophosphate
ATP	Adenosine triphosphate
AVS	Avascular spheroid
BCG	Bacille Calmette Guerin
BDF_1	$(C52BL/6JxDBA/2J)F_1$ hybrid
CP	Creatine Phosphate
DMBA	7,12-dimethybenz[*a*]anthracene
DMSO	Dimethylsulfoxide
DMX	5,6-dimethyl xanthenone-4-acetic acid
Eq.	Equation
FAA	Flavone-8-acetic acid
FCS	Fetal calf serum
FITC	Flourescein isothiocyanate
GM	Growth medium
H&E	Haematoxylin and eosin
H33342	Hoechst 33342
HX	hypoxanthine
i.d.	Intradermal(ly)
i.m.	Intramuscular(ly)
i.v.	Intravenous(ly)
IFN	Interferon
IL	Interleukin
MTD	Maximum tolerated dose
NAO	10-nonyl acridine orange
NK	Natural killer
P	Probability
TNF	Tumour necrosis factor-α
PBS	Phosphate-buffered saline
s.c.	Subcutaneous(ly)
s.e.m.	Standard error of the mean
vol/vol	Volume/volume
VS	Vascularised spheroid
wt/vol	Weight/volume
X	Xanthene
XAA	Xanthenone-4-acetic acid
XDA	Xanthenone-4,5-diacetic acid

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