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THE MECHANISM OF THE ANTI-TUMOUR ACTION

OF FLAVONE ACETIC ACID

AND ITS XANTHENONE ACETIC ACID ANALOGUES

by

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A thesis submitted in accordance with the requirements

for the degree of

Doctor of Philosophy

in the field of Pathology

University of Auckland, May 1992
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 than 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.
# TABLE OF CONTENTS

ABSTRACT 2

TABLE OF CONTENTS 4

LIST OF FIGURES AND TABLES 10

ABBREVIATIONS 12

ACKNOWLEDGEMENTS 13

PUBLICATIONS ARISING FROM THE THESIS 15

CHAPTER 1 - CANCER THERAPY AND FLAVONE ACETIC ACID 16

1.1. Cancer chemotherapy perspective 16

1.1.1. Thesis overview 16

1.1.2. The development of chemotherapy 16

1.1.3. Resistance to treatment 16

1.2. Finding a new basis for selective toxicity 22

1.2.1. New tumour cell targets 22

1.2.1a. Tissue specificity 22

1.2.1b. Growth factors 22

1.2.1c. Immunotherapy 22

1.2.1d. Other cell-directed agents 22

1.2.2. Therapies directed at hypoxic cells 22

1.2.3. Therapies aimed at the tumour vasculature 22

1.3. Tumour vessels and blood flow 25

1.3.1. Tumour neovascularisation 25

1.3.2. Structure of the tumour vasculature 25

1.3.3. Physiological control of tumour vessels 25

1.3.4. The endothelial layer 25

1.4. The low exchange environment 30

1.4.1. Distance of tumour cells from the vasculature 30

1.4.2. High intra-tumour pressures 30

1.4.3. Fluctuating blood flow 30

1.4.4. The necrotic zone 30

1.4.5. Implications of microenvironmental heterogeneity 30
1.5. Immune cell cytotoxicity in solid tumours

1.5.1. Immune cells in human and experimental tumours
1.5.2. Specific and non-specific anti-tumour effects
1.5.3. Macrophage tumouricidal activity
1.5.4. Tumour necrosis factor-α
1.5.5. Direct tumour cell toxicity of TNF
1.5.6. Indirect anti-tumour effects of TNF
1.5.7. The role of specific immunity in TNF activity
1.5.8. Relationship of TNF to FAA

1.6. Flavone acetic acid

1.6.1. Early pharmacokinetic and mechanistic studies
1.6.2. Hypotheses to explain FAA activity
1.6.2a. Direct cytotoxicity after in vivo activation
1.6.2b. Stimulation of immune-mediated cytotoxicity
1.6.2c. Inhibition of tumour blood flow
1.6.2d. Specificity for tumour microenvironment

1.6.3. AIMS OF THE THESIS

CHAPTER 2 - MECHANISMS OF FAA-INDUCED INJURY IN TUMOUR TISSUE:
A MORPHOLOGICAL AND METABOLIC STUDY

2.1. Introduction

2. M. Methods

2. M.1. Mice and tumours
2. M.2. Flavone acetic acid
2. M.3. Histological assessment
2. M.4. Electron microscopy
2. M.5. Tumour ATP and related metabolites

2. R. Results

2. R.1. Histology of untreated Colon 38 tumours
2. R.2. Histological effects of FAA treatment
2. R.3. Ultrastructure of untreated Colon 38 tumours
2. R.4. Ultrastructural effects of FAA treatment
2. R.5. Histology of globally ischaemic Colon 38 tumours
2. R.6. Effects of FAA on ATP and related metabolites

2. D. Discussion

2. D.1. Spontaneous necrosis in Colon 38 tumours
2. D.3. Non-ischaemic mechanisms of tumour cell killing
2. D.4. Ischaemic mechanisms of tumour cell killing
2. D.5. The distribution of necrosis
2. D. 7. Conclusions

CHAPTER 3 - THE ROLE OF ISCHAEMIA IN THE ANTI-TUMOUR ACTION OF FAA

3. I. Introduction

3. M. Methods

3. M. 1. Mice and tumours
3. M. 2. FAA and fluorescent perfusion markers
3. M. 3. Assessment of tumour perfusion: single label studies
3. M. 4. Assessment of tumour perfusion: double label studies
3. M. 5. Clonogenic assays on i.m. EMT6 tumours
3. M. 6. Effects of FAA on EMT6 i.p. spheroids and i.m. tumours

3. R. Results

3. R. 1. Single label blood flow study: Colon 38 tumours
3. R. 2. Double label studies: fluorescent dyes in control tumours
3. R. 3. Staining patterns in control EMT6 tumours
3. R. 5. Fluctuating perfusion in tumours
3. R. 8. Modelling FAA-induced cell killing as an ischaemic process

3. D. Discussion

3. D. 1. Assessment of tumour perfusion
3. D. 2. Spontaneous changes in tumour perfusion
3. D. 3. The evidence for an ischaemic FAA mechanism
3. D. 4. The evidence for non-ischaemic FAA mechanisms
3. D. 5. The evidence against in vivo activation
3. D. 7. The mechanism of perfusion failure

CHAPTER 4 - THE ROLE OF MICROENVIRONMENTAL FACTORS AND IMMUNE EFFECTOR CELLS IN FAA-INDUCED TUMOUR CELL INJURY

4. I. Introduction

4. I. 1. The spheroid as a model of the tumour microenvironment
4. I. 2. Infiltration of spheroids by immune cells in the peritoneum

4. M. Methods

4. M. 1. EMT6/Ak spheroids
4. M. 2. Oxic and anoxic exposure of EMT6 cells to FAA
4. M. 3. Clonogenic assays
4. M. 4. Eosin exclusion fraction
4. M. 5. Ex vivo (peritoneal) spheroids
4. M. 8. Effects of oxygen concentration on spheroid response to FAA
4. M. 9. Light and electron microscopy of spheroids
4. M. 10. Statistical analysis

4. R. Results
4. R. 1. Effects of hypoxia on the direct toxicity of FAA
4. R. 2. Composition of host cells infiltrating ex vivo spheroids
4. R. 3. FAA cytotoxicity in intact in vitro and ex vivo spheroids
4. R. 4. Light microscopy of spheroids
4. R. 5. Scoring histological features of spheroids
4. R. 7. Effects of FAA concentration
4. R. 8. Effects of FAA exposure time
4. R. 10. Effects of phorbol myristate acetate (PMA)
4. R. 11. Effects of oxygen concentration on ex vivo spheroids

4. D. Discussion
4. D. 1. Direct cytotoxicity of FAA
4. D. 2. Immune cell infiltration of EMT6 spheroids
4. D. 3. Host mediated cytotoxicity of FAA
4. D. 4. Which infiltrating cells mediate the FAA-induced effects?
4. D. 6. Relevance to the anti-tumour action of FAA
4. D. 7. Utility of the ex vivo spheroid tumour model
4. D. 8. Conclusions

CHAPTER 5 - COMPARISON OF FAA EFFECTS IN AVASCULAR AND VASCULAR EMT6 SPHEROID TUMOUR TISSUE

5. I. Introduction

5. M. Methods
5. M. 1. Histological studies
5. M. 2. Fluorescent vascular marker studies

5. R. Results
5. R. 1. Histology of untreated spheroids
5. R. 2. Fluorescent markers in untreated VS
5. R. 3. Histology of spheroids after FAA treatment
5. R. 4. Histology of infiltrative tumour deposits
5. R. 5. Effects of FAA on fluorescent marker distribution
CHAPTER 6 - THE ANTI-TUMOUR ACTIVITY OF XANTHENONE ACETIC ACIDS: COMPARISON WITH FAA

6.1. Introduction

6. M. Methods

6. M. 1. Drugs
6. M. 2. Maximum tolerated dose (MTD) and necrotising activity
6. M. 3. Perfusion studies
6. M. 4. Spheroid experiments

6. R. Results

6. R. 1. Necrotising activity in Colon 38 tumours
6. R. 2. Perfusion studies
6. R. 3. Effects of XAAAs on ex vivo EMT6 spheroids

6. D. Discussion

6. D. 1. FAA-like activities of XAA analogues
6. D. 2. Potencies of the XAAs
6. D. 3. Effects of hydralazine and vincristine on tumour perfusion
6. D. 4. The double label perfusion technique
6. D. 5. Conclusions

CHAPTER 7 - TOXIC EFFECTS OF FAA AND 5,6-DIMETHYL XANTHENONE ACETIC ACID: CELL KILLING IN NON-TUMOUR TISSUES

7.1 Introduction

7. M. Methods

7. M. 1. Histological studies
7. M. 2. Fluorescent vascular marker studies

7. R. Results

7. R. 1. Histological effects of FAA and DMX
7. R. 2. Fluorescent marking of non-tumour tissues
7. R. 3. Effects of FAA treatment on perfusion
7. D. Discussion

7. D. 1. Cytolytic and vascular effects in non-tumour tissues
7. D. 2. The lymphotoxic effects of FAA and DMX
7. D. 3. The basis of the tissue selectivity of FAA and DMX
7. D. 4. Relationship of cytolytic lesions to systemic toxicity
7. D. 5. Comparison of FAA and DMX
7. D. 6. Conclusions

CHAPTER 8 - GENERAL DISCUSSION

8. 1. The mechanisms of the anti-tumour action of FAA

8. 1. 1. The role of perfusion failure in the anti-tumour effect
8. 1. 2. The mechanism of perfusion failure
8. 1. 2a. The coagulation system
8. 1. 2b. Arterial hypotension
8. 1. 2c. Haemorrhage
8. 1. 2d. Approaches to further investigation
8. 1. 3. Non-ischaemic anti-tumour mechanisms
8. 1. 3a. The role of macrophages
8. 1. 3b. The relevance of immune mediated cytotoxicity
8. 1. 3c. The relationship of immune effects and perfusion failure
8. 1. 3d. The role of specific immunity
8. 1. 3e. The relationship of FAA and TNF
8. 1. 4. The role of the tumour microenvironment
8. 1. 5. Activation of FAA in vivo
8. 1. 6. Mechanisms of the xanthenone acetic acid (XAA) analogues
8. 1. 7. Toxic effects of FAA and 5,6-dimethyl XAA (DMX)

8. 2. Clinical implications of FAA mechanisms

8. 2. 1. Perfusion failure in anti-cancer treatment
8. 2. 2. Combinations with other treatments
8. 2. 2a. Hypoxia selective cytotoxic agents
8. 2. 2b. Conventional anti-proliferative agents
8. 2. 2c. Radiation
8. 2. 2d. Immunotherapy
8. 2. 2e. Other vessel-directed agents
8. 2. 3. Reasons for the failure of FAA in the clinic

8. 3. Concluding remarks

APPENDIX: The double label fluorescent marker technique: additional experiments and final discussion

MATERIALS AND EQUIPMENT

REFERENCES
LIST OF FIGURES AND TABLES

TABLES

Table 1.1 - Activity of FAA in mice
Table 4.1 - Percentage of host cells in spheroids
Table 6.1 - Drug-induced necrosis in Colon 38 tumours
Table 7.1 - Distribution of cytolytic lesions

FIGURES

Figure 1.1 - Flavone acetic acid and xanthenone acetic acid
Figure 1.2 - Growth patterns of solid and dispersed tumours
Figure 1.3 - Tumour vascularisation and growth
Figure 1.4 - Colon 38 tumour treated with TNF
Figure 2.1 - Histology of untreated Colon 38 tumours
Figure 2.2 - Histology of FAA-treated tumours
Figure 2.3 - Ultrastructure of untreated Colon 38 tumours
Figure 2.4 - Ultrastructure of FAA-treated Colon 38 tumours
Figure 2.5 - Histology of globally ischaemic Colon 38 tumours
Figure 2.6 - Effect of FAA on energy metabolites
Figure 3.1 - Perfusion of Colon 38 tumours after FAA - single label
Figure 3.2 - Fluorescent staining of Colon 38 tumours
Figure 3.3 - Perfusion of tumours after FAA - double label
Figure 3.4 - Cell death in EMT6 tumours after FAA
Figure 3.5 - Cell death in globally ischaemic EMT6 tumours
Figure 3.6 - FAA-induced cell killing as an ischaemic process
Figure 3.7 - Cell death in spheroids and tumours after FAA
Figure 4.1 - Lack of FAA cytotoxicity against dispersed EMT6 spheroid cells
Figure 4.2 - Flow cytometric assessment of ex vivo spheroid cell surface markers
Figure 4.3 - FAA cytotoxicity in EMT6 spheroids
Figure 4.4 - Histological effects of FAA on spheroids
Figure 4.5 - Scoring of morphological features
Figure 4.6 - Ultrastructure of EMT6 spheroids
Figure 4.7 - Effects of FAA concentration on ex vivo spheroids
Figure 4.8 - Histological effects of concentration and exposure time
Figure 4.9 - Effects of dexamethasone
Figure 4.10 - Comparison of PMA and FAA
Figure 4.11 - Histological effects of PMA on ex vivo spheroids
Figure 4.12 - Effect of oxygen concentration

Figure 5.1 - Histology of EMT6 spheroids
Figure 5.2 - Fluorescent staining of untreated VS
Figure 5.3 - Histological changes in VS after FAA treatment
Figure 5.4 - Effects of FAA on infiltrating tumour deposits
Figure 5.5 - Perfusion loss in VS after FAA treatment
Figure 5.6 - Vascularisation and FAA treatment of spheroids

Figure 6.1 - Structures of FAA and XAA
Figure 6.2 - DMX-treated Colon 38 tumour
Figure 6.3 - Perfusion changes in Colon 38 tumours
Figure 6.4 - Effect of DMX dose on tumour perfusion
Figure 6.5 - Scoring of morphological changes in EMT6 spheroids
Figure 6.6 - Histological effects of XAAs on spheroids

Figure 7.1 - Histology of non-tumour tissues
Figure 7.2 - Distribution of fluorescent markers in untreated non-tumour tissues

Figure A.1 - Comparison of methods for assessing fluorescence in tissue sections
### ABBREVIATIONS

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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</thead>
<tbody>
<tr>
<td>3OMX</td>
<td>3-O-methyl xanthenone-4-acetic acid</td>
</tr>
<tr>
<td>5MX</td>
<td>5-methyl xanthenone-4-acetic acid</td>
</tr>
<tr>
<td>8MX</td>
<td>8-methyl xanthenone-4-acetic acid</td>
</tr>
<tr>
<td>ADP</td>
<td>Adenosine diphosphate</td>
</tr>
<tr>
<td>AMP</td>
<td>Adenosine monophosphate</td>
</tr>
<tr>
<td>ATP</td>
<td>Adenosine triphosphate</td>
</tr>
<tr>
<td>AVS</td>
<td>Avascular spheroid</td>
</tr>
<tr>
<td>BCG</td>
<td>Bacille Calmette Guerin</td>
</tr>
<tr>
<td>BDF&lt;sub&gt;1&lt;/sub&gt;</td>
<td>(C52BL/6JxDBA/2J)&lt;sub&gt;F&lt;sub&gt;1&lt;/sub&gt;&lt;/sub&gt; hybrid</td>
</tr>
<tr>
<td>CP</td>
<td>Creatine Phosphate</td>
</tr>
<tr>
<td>DMBA</td>
<td>7,12-dimethylbenz[a]anthracene</td>
</tr>
<tr>
<td>DMSO</td>
<td>Dimethylsulfoxide</td>
</tr>
<tr>
<td>DMX</td>
<td>5,6-dimethyl xanthenone-4-acetic acid</td>
</tr>
<tr>
<td>Eq.</td>
<td>Equation</td>
</tr>
<tr>
<td>FAA</td>
<td>Flavone-8-acetic acid</td>
</tr>
<tr>
<td>FCS</td>
<td>Fetal calf serum</td>
</tr>
<tr>
<td>FITC</td>
<td>Flourescein isothiocyanate</td>
</tr>
<tr>
<td>GM</td>
<td>Growth medium</td>
</tr>
<tr>
<td>H&amp;E</td>
<td>Haematoxylin and eosin</td>
</tr>
<tr>
<td>H33342</td>
<td>Hoechst 33342</td>
</tr>
<tr>
<td>HX</td>
<td>hypoxanthine</td>
</tr>
<tr>
<td>i.d.</td>
<td>Intradermal(ly)</td>
</tr>
<tr>
<td>i.m.</td>
<td>Intramuscular(ly)</td>
</tr>
<tr>
<td>i.v.</td>
<td>Intravenous(ly)</td>
</tr>
<tr>
<td>IFN</td>
<td>Interferon</td>
</tr>
<tr>
<td>IL</td>
<td>Interleukin</td>
</tr>
<tr>
<td>MTD</td>
<td>Maximum tolerated dose</td>
</tr>
<tr>
<td>NAO</td>
<td>10-nonyl acridine orange</td>
</tr>
<tr>
<td>NK</td>
<td>Natural killer</td>
</tr>
<tr>
<td>P</td>
<td>Probability</td>
</tr>
<tr>
<td>TNF</td>
<td>Tumour necrosis factor-α</td>
</tr>
<tr>
<td>PBS</td>
<td>Phosphate-buffered saline</td>
</tr>
<tr>
<td>s.c.</td>
<td>Subcutaneous(ly)</td>
</tr>
<tr>
<td>s.e.m.</td>
<td>Standard error of the mean</td>
</tr>
<tr>
<td>vol/vol</td>
<td>Volume/volume</td>
</tr>
<tr>
<td>VS</td>
<td>Vascularised spheroid</td>
</tr>
<tr>
<td>wt/vol</td>
<td>Weight/volume</td>
</tr>
<tr>
<td>X</td>
<td>Xanthene</td>
</tr>
<tr>
<td>XAA</td>
<td>Xanthenone-4-acetic acid</td>
</tr>
<tr>
<td>XDA</td>
<td>Xanthenone-4,5-diacetic acid</td>
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ACKNOWLEDGEMENTS

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PUBLICATIONS ARISING FROM THE THESIS


ORAL PRESENTATIONS OF THESIS MATERIAL

Tumour Blood Flow.

The role of blood flow in the anti-tumour action of flavone acetic acid.

The role of the vasculature in the anti-tumour action of flavone acetic acid.

FAA: mechanisms and clinical implications.
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