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Review

Flavone 8-acetic acid: our current understanding of its mechanism of action in solid tumours

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Summary. Flavone 8-acetic acid (FAA) represents a novel chemical structure undergoing clinical trials as an anticancer drug. Its unusual properties tend to distinguish it from a conventional cytotoxic compound, particularly in the response of solid murine tumours; as a consequence, novel mechanisms of action are currently under investigation. In this review we summarised these mechanisms into one of the three categories (a) direct cytotoxicity, (b) biologic response modifier and (c) pharmacologic effector and considered the evidence for and against each. FAA is cytotoxic to tumour cells in vitro, but only at high concentrations and after long exposures. In vivo it is considerably more cytotoxic to the same cells, and it is unlikely that direct cytotoxicity alone can account for this difference. FAA stimulates NK cell activity, induces interferon α and synergises with interleukin 2 in the treatment of murine renal cancer. However, a definite link between immunomodulation and antitumour activity has still to be confirmed. Perhaps FAA's most unusual property is its ability to reduce tumour blood flow dramatically, which may provide the appropriate conditions for reactive chemistry to occur. Finally, a combination of the above mechanisms probably work together in producing the drug's unique spectrum of antitumour activity.

Introduction

Flavone 8-acetic acid [2-phenyl-8-(carboxy methyl)-benzopyran-4-one; LM975, NSC 347512, FAA] (Fig. 1), along with its sister compound flavone 8-acetic acid dimethylaminoethyl ester [LM985, NSC 293015], are the first two synthetic flavonoid compounds to enter clinical trials for the treatment of cancer. They therefore represent novel structures whose mechanism of action is of considerable interest because of the possibility of a large number of second-generation analogues. It is the aim of this review to categorise the many novel mechanisms of action currently under investigation and consider the evidence for and against each. As LM985 has now been shown to be a prodrug for FAA [17], this review will concentrate solely on FAA.

Flavonoids are a large class (500+) of natural lipophilic products found ubiquitously throughout the plant kingdom in the thylakoid membrane, where they act as catalysts in electron transport reactions and regulators of ion channel fluxes [16]. They exhibit many properties deemed desirable in an anticancer drug: they are relatively nontoxic to man, induce interferons, inhibit tyrosine kinase activity of oncogene products, intercalate DNA yet at the same time can mop up toxic oxygen free radicals and induce aryl hydroxylase and epoxide hydrolase, two key enzymes involved in the detoxification of reactive chemical species [27]. Clinical trials with other flavonoids under non-cancerous conditions have shown that their high biologic activity is not normally translated into high therapeutic efficacy; in this respect, the flavonoids resemble other natural products such as interferons and streptomycin [16].

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Pharmacology

To date FAA has been given to over 100 patients in phase I and II clinical trials carried out in Europe as well as many more patients in the United States [12]. Peak plasma drug levels well in excess of those required in mice to produce spectacular antitumour activity can easily be reached without encountering undue toxicity. Nevertheless, no tumour responses have been reported [18, 19]. Dose-limiting toxicities included hypotension and myalgia [18]. A possible explanation for the lack of activity in man may be species differences in drug disposition and metabolism rather than a failure in the FAA molecule to produce the critical cytotoxic event in human tumours or at other sites of action. Contrary to the behaviour of most drugs, FAA is cleared by mice 10–50 times slower than by man. The high rate of clearance in man is due to extensive metabolism to inactive glucuronides, which are rapidly excreted in the urine [8]. These metabolic pathways are absent or operate at a low level in mice. Slower clearance in mice is coupled to high drug uptake into solid tumours [8]. Thus, although equivalent peak plasma concentrations are achieved in man and mouse, it is possible that insufficient drug is delivered to the sites of action in patients due to competition from metabolic pathways.

Activity profile in tumour-bearing mice

Originally FAA was screened in mice against colon 38, a tumour type with a low response rate to new compounds, where it demonstrated high activity that prompted its development towards clinical trials [21]. It is now clear that almost all transplantable solid murine tumours will re-

spond to FAA, albeit to a greater or lesser degree [7, 20]. In contrast, P388 and L1210 ascites respond poorly, although the drug is given i.p. [25]. These results have led to the conclusion that FAA is inactive only against rapidly growing haematologic tumours [3]. However, if drug-sensitive solid tumour cells such as MAC15A or Lewis lung carcinoma are grown i.p. as ascites or even as small nodules in the lung, they also lose their ability to respond to FAA [10, 14]. Thus, the critical feature for activity in vivo is the location of the tumour.

Solid tumours respond to FAA in a dramatic manner that has been likened to the effects of tumour necrosis factor α (TNFα), where massive haemorrhagic necrosis occurs, commencing as early as 2–4 h after drug administration [11, 24]. In vitro, high drug concentrations and long drug exposures are required for only modest cytotoxicity [1]. In fact, FAA has been determined to be much more cytotoxic to the same cells (Lewis lung) when grown s.c. as a solid tumour than in a monolayer culture [14]. Although not all solid murine tumours respond to the same extent, patterns in activity have emerged and these have provided clues as to how the drug may work. Slowly growing, well-differentiated tumours such as MAC26 respond better than poorly differentiated, faster growing tumours such as MAC13, and early tumours respond less well than later tumours [1]. All of these factors indicate that a well-established solid tumour is probably the key to the activity of FAA.

Mechanisms of action

The mechanisms of action that have been proposed to date fall into one of three broad categories:

1. FAA or a metabolite is directly cytotoxic to solid tumours and this alone accounts for its activity (direct cytotoxicity).
2. FAA or a metabolite acts indirectly as an immunomodulator through the effects of one or more cytokine(s) (biologic response modifier).
3. FAA or a metabolite directly alters the biology of solid tumours in a way that is highly cytotoxic (pharmacologic effector).

In the remainder of this review we consider the evidence for and against each of these.

Direct cytotoxicity

Arguing in favour of direct cytotoxicity, Corbett and coworkers [3] have demonstrated in vivo that FAA induces unique, irreparable single-strand DNA breaks in the Glasgow osteogenic sarcoma (GOS) after tumours have been disaggregated and cells have been harvested and subjected to the alkaline elution assay [3]. The additional observation of these authors by phosphorous nuclear magnetic resonance (NMR) that FAA depletes GOS of ATP was cited to explain the inability of the tumour cells to repair the damage [13]. There are two main problems with this mechanism. Firstly, the above authors themselves found it difficult to explain their own findings, especially when they could see no obvious reactive centre within the FAA molecule that could produce selective single-strand breaks (and only in tumour cells, without affecting bone marrow cells isolated from the same animals), nor could they detect the presence of any metabolite that could be responsible for the damage. Secondly, DNA damage was recorded only 4–5 h after drug administration, by which time substantial areas of necrosis would normally have appeared. Depletion of ATP is a well-known characteristic of cell death. When FAA was incubated with tumour cells for only 2 h, no damage was detected in DNA but both DNA and RNA synthesis were inhibited [4]. Although it is always difficult to distinguish between cause and effect in vivo, it would appear that Corbett et al. were looking at the consequences of tumour cell death rather than the primary cause.

In the presence of 9,000 g supernatants of mouse liver homogenates (S9), cytotoxicity of FAA to human colon tumour cells is enhanced [5]. The implication is that the drug is biotransformed to more active metabolites, although actual conversion to metabolites was not demonstrated nor were metabolite species identified. Conjugation with glucuronic acid to yield inactive products represents the major pathway of biotransformation of FAA [8]. After the administration of a therapeutic dose of drug to either BALB-c or NMRI mice bearing sensitive tumours, only trace levels of a single biotransformed product exhibiting the characteristics of a conjugate were detected in plasma [8, 9]. In addition this metabolite is not taken up into the tumour. Flavonoids in nature act as catalysts in electron transport reactions, stimulating the flow of reducing equivalents to molecular oxygen. This property of FAA may explain its enhanced activity in the presence of S9.

It has recently been postulated that a resonance form of FAA may be the active moiety directly cytotoxic to the tumour. This structure envisages a stabilised oxonium/carboxyl ion pair and a reductive site at the oxygen atom on position C-4 [22] (Fig. 1) and can only occur when the acetic acid grouping is attached to C-8 of the flavone ring system. In favour of this hypothesis is the fact that flavonoids without an acetic acid substituent are inactive antitumour agents and positional isomers flavone 3, 5 and 7 acetic acid also show no activity. Nevertheless, this mechanism remains only a theoretical possibility at this stage. Formation of the ion pair is favoured only at alkaline pH, whereas tissues tend to be slightly more acidic than plasma. However, such a mechanism cannot be completely ruled out and may be important if the correct conditions are present in tumours.

Biologic response modifier

Two groups have independently reported that FAA can stimulate natural killer cell activity in non-lymphoid tissue such as the liver and kidney as well as the spleen [6, 28, 29]. Furthermore, FAA synergises with interleukin 2 (IL2) in the treatment of murine renal cancer in BALB/c mice. Depletion of asialo-GM_1 positive leucocytes significantly impairs the efficacy of the combination. Several thousand

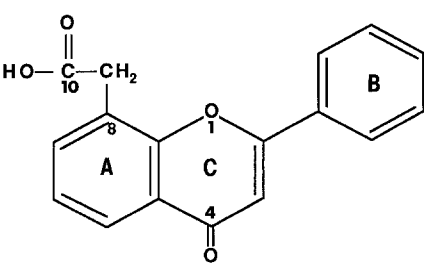

Fig. 1. The molecular structure of flavone 8-acetic acid

units of interferon/ml plasma are induced after treatment with the drug, and exogenous interferon α can partially replace FAA in the IL2 combination. Together, these data suggest that the drug may be acting indirectly through one or more cytokines. In patients receiving the drug, therapeutically peripheral blood NK cell activity is boosted and interferons are induced [26, 28].

Against the biologic response modifier mechanism is the fact that no positive evidence exists for a direct involvement of NK cells or any other cytotoxic leucocyte effector cell in the antitumour action of FAA. Indeed, in a murine model in which stimulation of NK cell activity in the spleen occurs and the drug induces haemorrhagic necrosis in the tumour, the injection of anti-asialo GM_1 antibody has prevented only the former without altering the latter [6], thus indicating that both effects can work via independent mechanisms that can operate side by side without necessarily overlapping. Although peripheral blood NK cell activity is boosted and interferons are induced in patients, no anticancer activity has been reported. FAA cannot stimulate NK cell activity or induce interferons in vitro, nor has a metabolite been identified that can do so [29]. Other investigators have failed to detect NK cell stimulation in tumour-bearing animals and patients, and in one report an inhibition was detected in patients when drug plasma concentrations exceeded 500 μg/ml [15, 23].

Pharmacologic effector

One of the earliest events associated with the administration of FAA is a dramatic reduction in tumour blood flow, ranging from 60%–80%. This observation has been made in two different tumours by two different techniques: Evans blue dye perfusion in MAC 26 [11] and H-2 NMR with deuterated water in GOS [13]. Most of the unusual properties of the drug fit in with this mechanism: high activity against well-differentiated solid tumours; poor activity against early established solid tumours, small avascular tumours, nodules and ascites tumours. In MAC15A growing s.c. there is a relationship between vascularity and response: the greater the vascular composition of the tumour, the greater the antitumour activity [2].

Postulating this mechanism also involves problems: it is difficult to envisage how reducing tumour blood supply alone is likely to induce rapid and massive necrosis, since it is well known that solid tumour cells are adapted to survive in low oxygen tension environments. Nevertheless, it may act as a precursor, providing the ideal conditions of O_2 tension and pH (among others) for other mechanisms to come into play.

Conclusions

In conclusion, although FAA is cytotoxic to tumour cells in vitro, it is unlikely that direct cytotoxicity alone can account for the dramatic necrosis observed in vivo in solid tumours. There is little doubt that FAA is an immunomodulator, but whether a direct relationship to antitumour activity exists still remains unclear. The drug profoundly reduces tumour blood flow, which may precipitate a series of as yet unidentified events, resulting in high tumour cell kill. Finally, a combination of these mechanisms probably work in concert, with some being more important than others in different tumour models.

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