Polyamine analogues as anticancer drugs

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Abstract
Just over 30 years ago, the late Diane Russell published the first in a series of papers linking polyamines and cancer. These early studies led to a flurry of research activity in the polyamine field that continues to this day attempting to identify a role for the polyamines in cancer development, treatment and/or prevention. The recognition that polyamines are critical for the growth of cancer cells, and consequently the identification of their metabolic pathways as a target for therapeutic intervention, led to the development of a number of useful inhibitors of polyamine biosynthesis. Arguably the most significant addition to the polyamine field in the last 30 years was the synthesis of α-difluoromethylornithine (DFMO), which is being tested currently as a cancer chemopreventative agent in man and is used also as a highly effective trypanocidal agent. Although an extremely useful tool experimentally, DFMO has been disappointing in clinical trials with little therapeutic efficacy. Despite this setback, the polyamine pathway is still considered a viable target for chemotherapeutic intervention. This has led to the development of the polyamine analogues as multifunctional inhibitors that will produce inhibition of tumour cell growth, polyamine depletion and optimum therapeutic efficacy.

Polyamines and cancer: initial studies
The initial studies [1–3] showed increased amounts of one or more of the polyamines in the serum or urine of patients with leukaemias, melanomas, adenocarcinomas and lymphomas (reviewed in [4]). Polyamines were, therefore, proposed as biochemical markers of neoplasia, with elevated concentrations of polyamines in physiological fluids being diagnostic of malignant disease. Disappointingly, raised amounts of polyamines were not restricted to malignant conditions, and high concentrations were also found in body fluids of patients with cystic fibrosis [5], in psoriasis [6] and during pregnancy [3].

The focus then switched from measuring polyamines as a diagnostic tool to using polyamine content as a means of monitoring therapeutic efficacy. It had been shown in a number of cases that patients in remission had urinary polyamine outputs similar to those found in normal individuals and that this remained within the normal range while the patient remained in remission [3,7]. More importantly, urinary polyamine output was found to increase when patients suffered a tumour recurrence. Although this finding still holds true, disappointingly, little use is made of this fact in clinical practice. There is, however, a clear link between increased polyamine content and cancer, and thus it is reasonable to suggest that strategies aimed at depletion of polyamine content will have anti-proliferative activity.

Single enzyme inhibitors
Enzyme inhibitors have been developed for virtually all the enzymes involved in polyamine metabolism (Figure 1). The best known of these is the ornithine decarboxylase (ODC) inhibitor, α-difluoromethylornithine (DFMO; also known as Elformithine). DFMO was one of a series of ornithine and putrescine analogues that were synthesized by Merrell Dow in Strasbourg in the late 1970s and early 1980s [8]. As an inhibitor, it has survived the test of time in that it is still being evaluated today as a potential agent in the cancer armoury. Currently, DFMO is being tested as a chemopreventative (agents designed to prevent cancer development) as opposed to a chemotherapeutic drug [9,10]. As a suicide inhibitor of ODC, DFMO was hailed as a breakthrough in cancer therapy having been designed rationally to inhibit ODC selectively [8].

In vitro, DFMO prevented cell growth through depletion of both putrescine and spermidine. The intracellular spermine content in DFMO-treated cells, however, remained unchanged [11]. This would argue that spermine is not a growth-supporting polyamine since, despite the maintenance of its intracellular content, cell growth was decreased.

Despite the success in vitro, DFMO as a monotherapy proved to be disappointing, with cytostatic rather than cytotoxic effects being observed in vivo. For example, in the Roser T-cell leukaemia model in rats, DFMO decreased the number of circulating blast cells by more than 90% and increased survival by more than 3 days. However, when
organ infiltration was investigated, there was almost no effect of the drug [12]. Further analysis revealed that the rat chow contained more than 300 nmol of polyamine/g of food (G. McLachlan and H.M. Wallace, unpublished work). The polyamines in the chow were taken up by individual organs and tissues in polyamine-depleted rats to a greater extent than in the untreated animals, with the net result being no change in tissue content. Similar responses to DFMO treatment were observed in humans, and so the use of DFMO as a single anticancer drug has been all but discontinued. DFMO does, however, show promise as a chemopreventative agent. It is a relatively non-toxic drug, and is currently being evaluated in conjunction with non-steroidal anti-inflammatory drugs (or NSAIDs) as a chemopreventative agent in humans.

A number of other single enzyme inhibitors directed against the polyamine pathway were synthesized and evaluated, including compounds such as methylglyoxal bis(guanylhydrazone) (MGBG), S-(5′-deoxy-5′-adenosyl)-methylthioethyl-hydroxylamine (AMA), 5′-{[(Z)-4-amino-2-butenyl]methylamino}-5′-deoxyadenosine (AbeAdo), [2,2-bipyridine]-6,6′-dicarboximidamide (CGP-39937) and 4-amidinoindan-1-one 2′-amidinohydrazone (CGP-48664). Unfortunately, all these compounds suffered from similar problems in that either they did not deplete all three polyamines to a sufficient extent and/or were highly toxic in humans (for reviews, see [13–16]).

Although disappointing, the results with the single enzyme inhibitors did provide proof of the concept that inhibition of the polyamine pathway was a viable option for the production of anti-proliferative drugs. This conclusion led to the development and synthesis of a range of polyamine analogues that were designed to compete or inhibit more than one reaction in polyamine metabolism. The strategy behind the analogues is outlined in Table 1. These will deplete polyamine content by a combination of preventing uptake, negative-feedback inhibition of biosynthesis and increased metabolism and export. The analogues will not, however, be able to substitute for the natural polyamines in terms of function. In practice, the analogues can be divided into categories; polyamine mimetics and polyamine anti-metabolites [17]. The mimetics are taken up by the polyamine transporter system and displace the polyamines from intracellular binding sites, thus preventing function. However, the mimetics do not necessarily cause significant polyamine...
depletion. The anti-metabolites, on the other hand, also enter the cell via the polyamine transporter and deplete polyamine content by the feedback inhibition of ODC and S-adenosylmethionine decarboxylase (ADOMETDC), and up-regulation of spermidine/spermine N\(^1\)-acetyltransferase (SSAT) and polyamine export.

### Polyamine analogues

Development of polyamine analogues with potential to be antitumour agents began in the mid-1980s [15]. The early analogues, such as 1-amino-oxy-3-aminopropane (‘APA’), an analogue of putrescine, and 1-aminoxy-3-N-[3-aminopropyl]aminopropane (‘AP-APA’) and N-[2-aminoxyethyl]-1,4-diaminobutane (AOE-PU), analogues of spermidine, were found to be effective at inactivating and inhibiting ODC and spermidine synthase and ODC and ADOMETDC respectively [18,19]. However, these analogues were found to be highly reactive, and therefore were not developed further. These analogues did, however, provide the initial evidence that analogues of the polyamines might be successful anti-proliferative drugs.

### Symmetrically substituted analogues

The first generation of these polyamine analogues were the N,N'-bis(ethyl)polyamines. These were symmetrical, terminally alkylated analogues of either spermidine or spermine. Bis(ethyl) norspermine (BENSpm), bis(ethyl)spermine (BESpm) and bis(ethyl) homospermine (BEHSpm) have proved the most successful analogues in depleting intracellular polyamine content and exhibiting a range of therapeutic activities against cancer cells with a selective cell type cytotoxicity. One of the unpredicted effects of these analogues was the induction of SSAT. This effect, in fact, turned out to be a bonus in that induction of SSAT leads to increased export of acetylpolyamines, and therefore facilitates further polyamine depletion [20]. Initial studies using, primarily, non-small-cell lung carcinoma and melanoma cells also suggested that the ability of an analogue to cause cell death was related to its ability to induce SSAT activity: the greater the induction of SSAT, the greater the toxicity [21]. The term ‘superinducer’ of SSAT was coined to refer to large increases in enzyme activity, greater than 1000-fold, produced in response to treatment with some of these analogues. The direct relationship between SSAT induction and toxicity did not, however, hold true for all analogues, or indeed all cell types. For example, 1,12-dimethyl spermine (‘DMSpm’) is a potent inducer of SSAT, but shows little or no toxicity in human large-cell lung carcinoma cells [22]. Interestingly, similar cells respond to BESpm with a superinduction of SSAT [23]. A study using the anti-tumour agent, etoposide, showed significant cell death in human cancer cells, but only a limited ability to induce SSAT [24]. These data suggested that induction of SSAT and cell death are linked, but not in a direct or causal manner in all cases.

The bis(ethyl) analogues are taken up by the polyamine transport systems [25] and deplete all three intracellular polyamines by inhibiting the activity of ODC and ADOMETDC via negative-feedback mechanisms. Unfortunately, in Phase II clinical trials, BENSpm was found to have little activity. In addition, toxicity was a problem and eventually, with the merger of the various drug companies involved, the compound was shelved.

### Unsymmetrically substituted analogues

Pat Woster at Wayne State University in Detroit, MI, U.S.A., developed the next group of analogues. These were the unsymmetrically substituted alkylpolyamines, and were almost exclusively spermine analogues [26]. The first compounds synthesized were N\(^1\)-propargyl-N\(^{11}\)-ethyl-norspermine (PENSpm) and N\(^1\)-cyclopropyl-methyl-N\(^{11}\)-ethyl-norspermine (CPENSpm). Both of these compounds were found to exhibit greater cytotoxicity and SSAT induction than BESpm [27], again suggesting that, in some cell types, analogue toxicity correlated positively with the ability to induce SSAT [28]. These analogues were shown to down-regulate ODC and ADOMETDC, but were not able to substitute for natural polyamines in terms of function [29].

The third compound in this series, N\(^1\)-cyclohexylmethyl-N\(^{11}\)-ethyl-norspermine (CHENSpm), was found to be a potent cytotoxic drug, but had little effect on polyamine depletion and SSAT activity, indicating that toxicity was not occurring by the proposed analogue theory (Table 1), but by an alternative mechanism [15,24]. Further analogues belonging to the same family were synthesized, and again no correlation between cytotoxicity and ability to induce SSAT activity was demonstrated, reinforcing the conclusion that there was more than one mechanism responsible for the antitumour effects induced by these analogues.

Although less well characterized than the bis(ethyl) analogues, these analogues have been found to have significant anti-proliferative activity in vitro. Both CHENSpm and CPENSpm [(S)-N\(^1\)-(2-methyl-1-butyl)-N\(^{11}\)-ethyl-4,8-diazaadecane] showed cytotoxicity in human leukaemic (HL-60) cells [17,30]. CPENSpm, on the other hand, was not cytotoxic under these conditions, but did show some anti-proliferative activity. This was an interesting observation in view of the controversy over the role of SSAT induction in cytotoxicity, as CPENSpm was the most potent inducer of SSAT, but the least effective in terms of growth inhibition. These analogues do, however, produce apoptosis [17,30,31], although the mechanism of induction of cell death is not yet clear.

### Other analogues

The latest generation of analogues has been synthesized by the SLIL Biomedical Corporation [32]. These analogues can be divided roughly into three groups: the oligoamines, amines with inserted double bonds and those with internal cyclic structures. These compounds have also shown a range of therapeutic activities, including some anti-proliferative effects against human tumour cells (A.V. Fraser and H.M. Wallace, unpublished work), and additionally offer promise in the management of diseases other than cancer.
In the late 1980s, Merrell Dow synthesized a series of bis(benzyl)polyamine analogues, which were developed for their potential anti-malarial activity [33]. Other analogues with a 4-4-4 backbone were designed on the basis of computer modelling of polyamine-DNA interactions. These compounds essentially had a quaternary amine group and were effective cytotoxic agents. Addition of another ethyl group increased the cytotoxicity further, making these highly toxic agents [34].

Summary
Polyamine analogues provide a novel and potentially effective class of compound for the inhibition of tumour cell growth as a result of interference with polyamine metabolism and/or function. The analogues are taken up by the polyamine-transport system and, as this transport system is more active in tumour cells, this may provide a starting point for the selectivity required by successful anticancer drugs. The analogues can be either polyamine anti-metabolites that deplete intracellular polyamine concentrations, or polyamine mimetics that act by displacing the natural polyamines from binding sites, but do not substitute in terms of function. The efficacy of each type of analogue in cancer therapy and prevention remains to be characterized fully.

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References

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