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Polyamine analogues – an update

Minireview Article

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Summary. The polyamines are growth factors in both normal and cancer cells. As the intracellular polyamine content correlates positively with the growth potential of that cell, the idea that depletion of polyamine content will result in inhibition of cell growth and, particularly tumour cell growth, has been developed over the last 15 years. The polyamine pathway is therefore a target for development of rationally designed, antiproliferative agents. Following the lessons from the single enzyme inhibitors (α-difluoromethylornithine DFMO), three generations of polyamine analogues have been synthesised and tested *in vitro* and *in vivo*. The analogues are multi-site inhibitors affecting multiple reactions in the pathway and thus prevent the up-regulation of compensatory reactions that have been the downfall of DFMO in anticancer chemotherapy. Although the initial concept was that the analogues may provide novel anticancer drugs, it now seems likely that the analogues will have wider applications in diseases involving hyperplasia.

Keywords: Cancer – Spermidine – Spermine – Polyamine analogues – DFMO – Disease

Abbreviations: AOE-PU, N-[2-aminooxyethyl]-1,4-diaminobutane; APA, 1-aminooxy-3-aminopropane; AP-APA, 1-aminooxy-3-N-[3-aminopropyl]-aminopropane; BCNU, 1,3-bis(2-chloroethyl)-1-nitrosourea; BEHSpm/DEHSpm/BE-4-4-4, N^1,N^{14}-bis(ethyl)-homospermine; BENSpm/DENSpm/BE-3-3-3, N^1,N^{11}-bis(ethyl)-norspermine; BES, N^1,N^8-bis(ethyl)spermidine; BESpm/DESpm/BE-3-4-3, N^1,N^{12}-bis(ethyl)spermine; CHENSpm, N^1-ethyl-N^{11}-((cycloheptyl)methyl)-4,8-diazaundecane; CPENSpm, N^1-ethyl-N^{11}-((cyclopropyl)methyl)-4,8-diazaundecane; DFMO, α-difluoromethylornithine; IPENSpm, N^1-ethyl-N^{11}-((isopropyl)-methyl)-4,8-diazaundecane; MGBG, methylglyoxal bis(guanylhydrazone); ODC, ornithine decarboxylase; SAMDC, S-adenosylmethionine decarboxylase; SSAT, spermidine/spermine N^1-acetyltransferase

Introduction

It is now well accepted that polyamine concentrations in mammalian cells correlate with the rate of cell growth: high concentrations are found in rapidly proliferating cells and low concentrations are present in slow growing or quiescent cells (Jänne et al., 1978; Tabor and Tabor, 1984; Wallace et al., 2003). Early studies using inhibitors of the biosynthetic enzymes such as methylglyoxal bis(guanylhydrazone) (MGBG) showed that interference with polyamine production could negatively influence the rate of cell growth in L1210 leukaemia cells (French et al., 1960). This, together with the fact that almost all cancer cells contain increased concentrations of polyamines (reviewed by Wallace et al., 2003), has lead to the polyamine biosynthetic pathway being highlighted as a target for the development of new anticancer drugs.

DFMO was one of the first rationally designed antitumour agents being an irreversible, suicide inhibitor of ornithine decarboxylase (ODC), the first enzyme in the polyamine biosynthetic pathway (Metcalf et al., 1978). DFMO has significant growth inhibitory effects in cancer cells *in vitro* (reviewed by Meyskens and Gerner, 1999). However, as an anticancer drug, it has not lived up to its original promise with perhaps the exception of the work of Victor Levin's group in gliomas (Levin et al., 1992). In general, the effects of DFMO as a monotherapy were found to be cytostatic rather than cytotoxic (Mamont et al., 1984). There are two major reasons for this, firstly DFMO does not usually deplete spermine to any great extent in mammalian cells and secondly, inhibition of biosynthesis results in up regulation of polyamine transport from the extracellular milieu, thus negating any depletion resulting from treatment with the inhibitor (Alhonen-Hongisto et al., 1980). In an attempt to overcome this setback, DFMO was combined with a number of other cytotoxic agents. However, the results were mixed with both attenuation and potentiation of the growth inhibitory

- **Cytotoxics**
  - **MGBG, BCNU**
    - ***Enhanced cytotoxic effects***
      - *Seidenfeld et al., 1987*
- **Immunosuppressives**
  - **Cyclosporin A**
    - ***Synergistic inhibition of growth***
      - *Smart et al., 1989a, b*
- **Antibiotics, PAO inhibitor and polyamine free diet**
  - ***Greatly enhanced antitumour effects***
    - *Quemener et al., 1994*

Scheme 1. Theory of the polyamine analogues

⇨ Polyamine analogues will deplete polyamine content by multiple mechanisms

- Polyamine analogues will be sufficiently similar to the polyamines that they
  - Will regulate the pathway
  - **Will not** substitute for function
- Inhibit biosynthesis
  - Feedback inhibition of the decarboxylases
- Compete for uptake
  - With the natural polyamines
- Induce the catabolic enzymes and export
  - SSAT and PAO

Scheme 2. DFMO in combination with other drugs

effects being observed (Scheme 1). In our own studies combining DFMO with cyclosporin A in a T cell leukaemia model in rats we observed significant decreases in circulating blast cells but little effect was seen on organ infiltration or survival (Smart et al., 1989a, b). In the wake of DFMO, a number of inhibitors of individual enzyme reactions were synthesized (reviewed by Wallace and Fraser, 2004) but to date no single enzyme inhibitor directed against the polyamine pathway is currently used alone in cancer chemotherapy.

The studies with DFMO did, however, provide "proof of concept" that preventing polyamine biosynthesis can inhibit the growth of tumour cells. The results also highlighted several other important issues that need to be considered when attempting to regulate cell growth by polyamine depletion. The first point is that polyamine depletion needs to include loss of all three polyamines. DFMO only produced loss of putrescine and spermidine (reviewed by Meyskens and Gerner, 1999) and MGBG resulted in loss of only spermidine and spermine (Porter et al., 1980). The second feature is that account must be taken of the compensatory mechanisms that result from blockage of polyamine biosynthesis. In the case of DFMO, the increase in uptake of polyamines from the diet or the circulation which negates the effects of blocking biosynthesis must be considered.

Bearing in mind these provisos the polyamine pathway, with its pivotal role in cell growth, is still a worthy target for the development of new antiproliferative drugs.

Polyamine analogues

In response to the limitations of the single enzyme inhibitors Porter and Bergeron in the 1980s proposed that analogues of the polyamines might be an alternative way in which the pathway of polyamine metabolism could be blocked. The theory of the analogues is that they will interfere at multiple sites in the pathway thus preventing or, at least, minimising the compensatory changes seen with the single enzyme inhibitors (Scheme 2). Essentially, the analogues were designed to regulate polyamine metabolism through the mechanisms used by the natural polyamines themselves (reviewed by Wallace and Fraser, 2003). However, as the analogues could not substitute for the polyamines in terms of function, intracellular polyamine pools should decrease leading to growth inhibition and cell death. An early observation with the analogues was that they superinduce spermidine/spermine N^1-acetyltransferase (SSAT) (Casero et al., 1989) which forms part of the retroconversion pathway recycling spermine and spermidine to putrescine and providing acetylpolyamines for export from the cell (Wallace and Mackarel, 1998). This increase in catabolism was an unexpected bonus as it increases the potential of the cells to deplete intracellular polyamine content.

The type of cell death induced by the analogues is almost exclusively apoptosis with induction of caspases being commonly a part of the death pathway (Fraser et al., 2002).

The first generation polyamine analogues synthesized were simple symmetrically substituted *N*, *N*-bis(ethyl)polyamines which were terminally alkylated analogues of either spermidine or spermine. These agents were tested for their ability to inhibit cell growth, alter polyamine content and inhibit the biosynthetic enzymes, ODC and S-adenosylmethionine decarboxylase (SAMDC). Early work concentrated on spermidine analogues, such as N^1,N^8-bis(ethyl)spermidine (BES). This compound was shown to decrease ODC and SAMDC and to be cytotoxic to lung cancer cells (Casero et al., 1987). The equivalent spermine analogue N^1,N^{12}-bis(ethyl)spermine (BESpm; DESpm; BE-3-4-3) was found to be more effective in some cells and so further analogue development focussed on spermine derivatives. The importance of molecular charge

and the presence of terminal amines were found to be critical for transport of the analogues (Porter et al., 1985).

In addition to BESpm, the most studied and successful compounds have been N^1,N^{11}-bis(ethyl)-norspermine (BENSpm; DENSpm; BE-3-3-3) and N^1,N^{14}-bis(ethyl)-homospermine (BEHSpm; DEHSpm; BE-4-4-4) which deplete intracellular polyamine pools and exert cytotoxic effects in several cell lines (Bergeron et al., 1988; Bernacki et al., 1992; Casero et al., 1989; Chang et al., 1992). Although the analogues all resembled the natural polyamines in structure they do not substitute for them in terms of function (Bergeron et al., 1984, 1988). The most effective and the best tolerated of the three homologues in man was BENSpm which has undergone Phase I/II clinical trials (Streiff and Bender, 2001). In Phase I trials the drug was shown to be safe to administer on a once a day schedule for 5 days (Hahm et al., 2002) with minimal toxicity but, unfortunately, in Phase II trials it has shown little evidence of clinical activity (Wolff et al., 2003).

The second generation polyamine analogues are the unsymmetrically substituted analogues such as N^1-ethyl-N^{11}-((cycloheptyl)methyl)-4,8-diazaundecane (CHENSpm), N^1-ethyl-N^{11}-((isopropyl)methyl)-4,8-diazaundecane (IPENSpm) and N^1-ethyl-N^{11}-(cyclopropyl)-methyl-4,8-diazaundecane (CPENSpm) which were developed by Woster's group in Detroit in the 1990s (reviewed by Woster, 2006). These alkylpolyamines are almost exclusively spermine analogues with an alkyl group added to the C-terminus and larger substituent added to their N-terminus. Cell-type specific toxicity has been demonstrated for these analogues. In lung cancer cell lines, CHENSpm toxicity correlates with the ability to induce SSAT activity (Casero et al., 1995). However, in human leukaemic cells CHENSpm is the most toxic analogue but induces only minor changes in SSAT activity. CPENSpm, on the other hand, produces significant induction of SSAT in these cells but has only minimal effects on cell growth (Fraser et al., 2002). In general, these analogues have demonstrated lower toxicity and greater therapeutic efficacy than the first generation compounds.

From the success of the first unsymmetrically substituted compounds a family of analogues were produced with either a 3-3-3 or 3-7-3 carbon backbone. One of the interesting discoveries separating these two groups is their specificity: the 3-3-3 analogues tend to have antiproliferative activity and little anti-trypanosomal activity while the 3-7-3 compounds had anti-trypanosomal activity and minimal anticancer activity (Bellevue et al., 1996).

The latest generation of analogues, originally synthesized by the S'LIL Biomedical Corporation (now Cellgate), includes conformationally restricted, cyclic and long-chain oligoamine analogues. These compounds were based on the originally successful spermine analogues such as BESpm, BENSpm and BEHSpm and have been designed for wider use against human disease and several Cellgate compounds are currently being tested against conditions such as macular degeneration and vascular hyperplasia. Studies in our laboratory have shown that the Cellgate compounds fall into three clear categories in terms of toxicity to human leukaemic cells: non toxic ($IC_{50} > 100\,\mu M$ after 96 h exposure); moderately toxic (IC_{50} between 10–100 μM after 72 h exposure) and highly toxic ($IC_{50} < 10\,\mu M$ after 24 h exposure) (Fraser and Wallace, unpublished results).

With the introduction of new and more efficient analogues it is clear that the polyamine analogues divide effectively into two categories: the antimetabolites and the mimetics (Fraser et al., 2002; Seiler et al., 1998). The antimetabolites are readily transported into cells, decrease biosynthesis, increase catabolism and export and significantly deplete intracellular polyamine concentrations. The mimetics, on the other hand, enter the cell by the transport process and displace polyamines from their natural binding sites. However, they do not decrease the total intracellular polyamine content to any significant extent but still cause toxicity and cell death due to loss of intracellular function.

Other analogues

A range of other analogues targeting the polyamine pathway has been synthesised. Aminoxy analogues such as 1-aminooxy-3-aminopropane (APA), 1-aminooxy-3-N-[3-aminopropyl]-aminopropane (AP-APA) and N-[2-aminooxyethyl]-1,4-diaminobutane (AOE-PU) were produced by Khomutov's group in Moscow. While these compounds have been shown to be effective in inhibiting the activity of ODC and SAMDC they have generally been too reactive to be developed further (Eloranta et al., 1990). A second generation of these analogues is been developed currently and isoteric charge deficient analogues of spermine are the latest compounds under investigation (Khomutov et al., 2007).

Other compounds synthesised from the polyamine backbone include bis-naphthalimidopropyl-derivatives. These compounds have some cytotoxic activity but have only been tested to a limited extent (Lin and Pavlov, 2000).

Polyamine analogues in other diseases

In an attempt to understand the physiological role of SSAT a Finnish group have developed a number of transgenic animal models. One model, MT-SSAT rats which have the SSAT gene inserted under the control of the mouse metallothionein I promoter, develop acute pancreatitis in response to treatment with zinc (Alhonen et al., 2000). In this model the pancreatitis can be completely prevented by prior administration of the metabolically stable spermidine analogue, α-methylspermidine (Räsänen et al., 2002). When the same model is subjected to partial hepatectomy, there was massive decrease in the higher polyamine pools and no initiation of liver regeneration. Again, α-methylspermidine and also bis-α-methylspermine restored early liver regeneration when administered prior to the operation (Räsänen et al., 2002, Järvinen et al., 2005). Thus indicating critical roles for polyamines in these too situations. In these two cases, however, the analogues were acting in a positive manner mimicking the action of the natural polyamines as opposed to the previous examples where the analogues were acting as antagonists of polyamine function.

Conclusions

The concept of the polyamine analogues as multi-site inhibitors of the polyamine pathway remains viable with promising *in vitro* results being obtained, particularly with the new generations of analogues. It may be however that these agents will not be limited to anticancer trials and will have wider use against a range of human diseases which involve disrupted or dysfunctional cell growth. One area still to be developed in terms of the analogues is chemoprevention. DFMO is currently under investigation as a cancer chemopreventative and this may also be a possibility for the new analogues.

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