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Inhibitors of polyamine metabolism: Review article

H. M. Wallace and **A. V. Fraser**

Departments of Medicine and Therapeutics and Biomedical Sciences, University of Aberdeen, Polwarth Building, Foresterhill, Aberdeen, Scotland, United Kingdom

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Summary. The identification of increased polyamine concentrations in a variety of diseases from cancer and psoriasis to parasitic infections has led to the hypothesis that manipulation of polyamine metabolism is a realistic target for therapeutic or preventative intervention in the treatment of certain diseases.

The early development of polyamine biosynthetic single enzyme inhibitors such as α-difluoromethylornithine (DFMO) and methylglyoxal bis (guanylhydrazone) showed some interesting early promise as anticancer drugs, but ultimately failed *in vivo*. Despite this, DFMO is currently in use as an effective anti-parasitic agent and has recently also been shown to have further potential as a chemopreventative agent in colorectal cancer.

The initial promise *in vitro* led to the development and testing of other potential inhibitors of the pathway namely the polyamine analogues. The analogues have met with greater success than the single enzyme inhibitors possibly due to their multiple targets. These include down regulation of polyamine biosynthesis through inhibition of ornithine decarboxylase and S-adenosylmethionine decarboxylase and decreased polyamine uptake. This coupled with increased activity of the catabolic enzymes, polyamine oxidase and spermidine/spermine N^1-acetyltransferase, and increased polyamine export has made the analogues more effective in depleting polyamine pools. Recently, the identification of a new oxidase (PAO-h1/SMO) in polyamine catabolism and evidence of induction of both PAO and PAO-h1/SMO in response to polyamine analogue treatment, suggests the analogues may become an important part of future chemotherapeutic and/or chemopreventative regimens.

Keywords: Polyamines – Putrescine – Spermidine – Spermine – Cancer – Apoptosis – Analogues – Enzyme inhibitors – Difluoromethylornithine – Disease

Inhibition of polyamine biosynthesis and depletion of intracellular polyamine content has been a strategy for inhibiting inappropriate cell growth for the last 25 years. This has resulted from the positive link between polyamine concentrations and cell growth. It is well recognised that high polyamine concentrations are required for rapid cell growth and low polyamine content is typical of quiescent cells (Wallace, 1996). There are two main lines of evidence that show the essential role of the polyamines in cell growth. The first is from studies where polyamine production has been limited by the presence of an inhibitor of biosynthesis (Marton and Pegg, 1995) and the second is where polyamine synthesis has been altered due to a mutation in one or more of the genes responsible for the synthesis of the metabolic enzymes (Tabor et al., 1982). As a result of these observations the polyamine pathway has become a popular target for therapeutic intervention. The ultimate aim of these interventions is to limit or prevent excessive cell growth by depletion of intracellular polyamine content.

Target sites

The metabolic pathways involving the polyamines comprise 6 independent but interrelated reactions (Fig. 1). Each reaction is a potential target site for therapeutic intervention. Inhibitors of varying selectivity and specificity are available against almost every reaction (Table 1).

In terms of therapeutic intervention, the most obvious targets for inhibition are the two decarboxylases required for the first and second steps in biosynthesis. Inhibition of these enzymes would be expected to deplete all three polyamines in the case of inhibitors of ODC, and only spermidine and spermine in the case of SAMDC inhibitors. Although inhibitors of SAMDC do decrease the content of spermidine and spermine significantly the accumulation of putrescine can confound results obtained (Byers et al., 1992). Inhibitors of ODC, on the other hand, have been found to deplete putrescine and spermidine effectively but often spermine content is not affected (Gerner and Mamont, 1986).

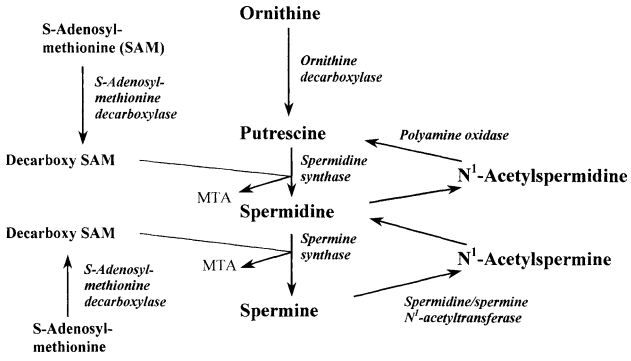

Fig. 1. Polyamine metabolic pathway (MTA – 5′-methylthioadenosine)

Ornithine decarboxylase (ODC; EC 4.1.1.17) and S-adenosylmethionine decarboxylase (SAMDC; EC 4.1.1.50) are interesting enzymes in their own right with some novel regulatory features. For example, they are highly regulated and rapidly turned over in mammalian cells (Pegg, 1986), however, this makes long term inhibition of activity difficult to achieve.

The next two enzymes involved in biosynthesis, spermidine and spermine synthase, are more stable proteins and as such are potentially ideal targets for manipulation, but inhibition of these enzymes has, in general, been less influential in on cell growth (Coward and Pegg, 1987).

Since the therapeutic aim is to deplete polyamine content, with the catabolic reactions it is induction rather than inhibition that is required. The combination of spermidine/spermine N^1-acetyltransferase (SSAT; EC 2.3.1.57), polyamine oxidase (PAO; EC 1.5.3.11) and polyamine export appears to be responsible for the removal of polyamines from the cell, while spermine oxidase (hPAO-1/SMO) seems to be used for the recycling of spermine (Wang et al., 2001; Vujcic et al., 2002). With the further identification of several hPAO homologues (Murray-Stewart et al., 2002), it seems highly likely that one of these may turn out to be responsible for the recycling of spermidine. Several inducers of SSAT and PAO exist including a variety of polyamine analogues (Casero et al., 1990; Wang et al., 2001; Wallace and Fraser, 2003), hydrogen peroxide (Chopra and Wallace, 1998) and some anticancer drugs such as etoposide (Lindsay and Wallace, 1999).

Disease

As a consequence of the link between polyamines and cell growth, conditions where excessive cell growth is part of the disease aetiology have been particular targets for inhibitors of polyamine biosynthesis. Development of cancer, for example, has been closely associated with alterations in polyamine metabolism. Increased concentrations of polyamines have been found in many solid tumours and similar increases in urinary and serum polyamine content are common features of malignancy (Wallace and Caslake, 2001). ODC activity is also increased in tumour tissue (Narisawa et al., 1989; Nishioka et al., 1991) making it an attractive target for the design of antiproliferative drugs. A discussion of all the changes occurring in the various cancers is beyond the scope of this review however a summary of the major alterations in a range of malignancies is provided in Table 2.

Although the majority of inhibitors of polyamine metabolism have been designed as antiproliferative agents directed towards cancer or trypanosomes the inhibitors are also effective in other diseases. Development of branched polyamines have found success in the treatment of prion-related diseases (Supattapone et al., 2002). These compounds are believed to bind directly to PrpSc (the protease resistant form of the prion protein), and promote their clearance (Supattapone et al., 2001).

The involvement of polyamines in tissue regeneration is also important. Recovery of such organs as the pancreas after acute pancreatitis (Jurkowska et al., 1997), the liver after partial hepatactomy (Luk, 1986) and intestinal resections (Luk and Baylin, 1983) have all shown improvements with the presence of polyamines.

Although there are many inhibitors of polyamine metabolism (see Table 1) this review will concentrate on a few of the most effective compounds.

Inhibitors of the decarboxylases

α-Difluoromethylornithine (DFMO)

Perhaps the best-known and most useful of the inhibitors of polyamine biosynthesis is α-difluoromethylornithine (DFMO), an enzyme-activated, irreversible, suicide inhibitor of ornithine decarboxylase (ODC; Metcalf et al., 1978). DFMO was one of the first rationally designed anticancer drugs and was directed against ODC, an enzyme that was known to be upregulated in cancer cells. DFMO is a substrate for ODC and acts by binding within the active site at lys 69 and lys 360. It is cleaved by ODC but the product is not released, remaining in the active site and rendering the enzyme irreversibly inactive (Poulin et al., 1992). A recent study has shown that although both the L- and the D-enantiomers of DFMO can irreversibly inactivate ODC, the L-form has a 20 times greater probability of forming an enzyme-inhibitor complex than the D-form (Qu et al., 2003). Many studies using DFMO

Table 1. Polyamine metabolic enzyme inhibitors and their effects on polyamine metabolism

Inhibitor		Enzyme(s) affected	Effect on polyamine metabolism	Reference
AbeAdo (MDL 73811)	(5′-{[(*Z*)-4-amino-2-butenyl] methylamino}-5′-deoxyadenosine)	SAMDC	↑ putrescine, ↓ spermidine and spermine	Byers et al., 1993
AdoMac	(*S*-[5′-deoxy-5′-adenosyl]-1-ammonio-4-[methylsulfonio]-2-cyclopentene)	SAMDC		Wu and Woster, 1992
AdoMao	(*S*-[5′-deoxy-5′-adenosyl]-1-aminoxy-4-[methylsulfonio]-2-cyclopentene)	SAMDC	↔ putrescine, spermidine and spermine	Gao et al., 1995
AdoDATO	(*S*-adenosyl-1,8-diamino-3-thiooctane)	Spermidine Synthase	↑ putrescine and spermine, ↓ spermidine	Holm et al., 1989
AdoDATAD	(*S*-adenosyl-1,12-diamino-3-thio-9-azadodecane)	Spermine Synthase	↑ spermidine, ↓ putrescine and spermine	Pegg et al., 1989
AMA	(*S*-[5′-deoxy-5′-adenosyl] methylthioethylhydroxylamine)	SAMDC	↑ putrescine, ↓ spermidine and spermine	Kramer et al., 1989
APA	(1-aminooxy-3-aminopropane)	ODC, SAMDC, Spermidine Synthase	↓ ODC activity	Khomutov et al., 1985
DFMO	(α-difluoromethylornithine)	ODC	↓ putrescine and spermidine, ↔ spermine	Metcalf et al., 1978
CGP 48664 (SAM 364A)	(4-amidinoindanon-1-[2′amidino] hydrazone)	SAMDC	↑ putrescine, ↓ spermidine and spermine ↑ ODC activity	Regenass et al., 1994
CGP 39937	([2,2-bipyridine]-6,6′-dicarboximidamide)	SAMDC		Stanek et al., 1993
EGBG	(ethylglyoxal bis[guanylhydrazone])	SAMDC	↑ putrescine, ↓ spermine ↑ ODC activity	Sjoholm et al., 1994
MGBG	(methylglyoxal bis[guanylhydrazone])	SAMDC	↑ putrescine, ↓ spermidine ↑ ODC and SAMDC activity	Porter et al., 1980
Berenil	(1,3-tris-[4′-amidinophenyl]triazine)	SSAT	↑ polyamine accumulation, esp. spermine	Libby and Porter, 1992
Pentamidine	(*p*,*p*′-[pentamethylenedioxy] dibenzamidine)	ODC, SAMDC,	↓ putrescine, ↔ spermidine ↓ ODC activity	Libby and Porter, 1992

(*continued*)

Table 1 (*continued*)

Inhibitor		Enzyme(s) affected	Effect on polyamine metabolism	Reference
MDL 72527	(N^1,N^2-bis[2,3-butadienyl]-1,4-butanediamine)	PAO		Bolkenius et al., 1985
AP-APA	(1-aminooxy-3-*N*-[3-aminopropyl]-aminopropane)	ODC, SAMDC, Spermine Synthase		Eloranta et al., 1990
DCHA	(dicyclohexylamine sulfate)			Ito et al., 1982
4MCHA	(*trans*-4-methylcyclohexylamine)	Spermidine Synthase	↑ putrescine and spermine, ↓ spermidine ↑ SAMDC activity	Beppu et al., 1995
APCHA	(*N*-[3-aminopropyl]cyclohexylamine)	Spermine Synthase	↑ putrescine and spermine, ↓ spermidine ↑ SAMDC activity	Beppu et al., 1995
AOE-PU	(*N*-[2[aminooxyethyl]-1,4-diaminobutane)	ODC, SAMDC, Spermine Synthase		Eloranta et al., 1990
MAOEA	(5′-deoxy-5′-{*N*-methyl-*N*-[2-(aminooxy)ethyl]amino}adenosine)	SAMDC	↑ putrescine, ↓ spermidine and spermine	Pegg et al., 1988
MHZPA	(5′-deoxy-5′-[*N*-methyl-*N*-[(3-hydrazinopropyl)amino]adenosine)	SAMDC	↑ putrescine, ↓ spermidine and spermine	Pegg et al., 1988
MHZEA	(5′-deoxy-5′-[(2-hydrazinoethyl)-methylamino]adenosine)	SAMDC		Tekwani et al., 1992
N^1OSSpm	(N^1-[*n*-octanesulphonyl]spermine)	Spermine oxidase (SMO)		Vujcic et al., 2002

Table 2. Alterations in polyamines and their metabolism in disease

Disease	Alteration in polyamines/enzymes	Reference
Psoriasis	Increased polyamine biosynthesis	Lauharanta and Kapyaho, 1983
	Elevated spermidine and spermine in blood	Proctor et al., 1975
	Increased ODC activity	Lowe et al., 1982
	Increased polyamine content	Russell et al., 1978
Systemic Lupus Erythematous (SLE)	Increased polyamine expression and susceptibility to induce Z form of DNA	Thomas et al., 1990
Chronic Renal Failure	Impairment of ornithine metabolism and polyamine biosynthesis	el-Marjou et al., 1997
	Increased spermidine and spermine in erythrocytes, increased putrescine in urine	Swendseid et al., 1980
Uremia	Decreased SSAT activity, increased ODC activity and gene expression	Imanishi et al., 1996
	Elevated polyamines in patient serum	Saito et al., 1983
Liver Cirrhosis	Elevated polyamine content	Nishiguchi et al., 2002
	Increased free, monoacetylated and total polyamines	Cecco et al., 1992
	Increased polyamine content	Moulinoux et al., 1985
	Elevated ODC activity	Kubo et al., 1998
Cystic Fibrosis	Elevated polyamines in urine	Russell et al., 1979
	Increased spermidine and spermine associated with erythrocytes	Cohen et al., 1976
Insulin Dependent Diabetes Mellitus (IDDM)	Elevated polyamine content in erythrocytes	Seghieri et al., 1997
Duchenne Muscular Dystrophy (DMD)	Increased urinary polyamine levels	Russell and Stern, 1981
Alzheimer's Disease	Increased ODC activity in temporal cortex	Morrison et al., 1998
	Increased spermidine, decreased putrescine in temporal cortex decreased spermine in occipital cortex	Morrison and Kish, 1995
Parkinson's Disease	Elevated spermidine and spermine, decreased putrescine	Gomes-Trolin et al., 2002
Rheumatoid Arthritis	Excessive polyamines contribute to IL-2 deficiency	Flescher et al., 1989, 1992
	Elevated in activated lymphocytes (including peripheral blood lymphocytes)	Nesher et al., 1997
	Increased urinary polyamine levels	Furumitsu et al., 1993
	Polyamines elevated in blood and synovial fluid	Talal et al., 1988

in vitro have shown effective depletion of putrescine and spermidine while spermine content is often unaffected (Gerner and Mamont, 1986). This polyamine depletion leads to cell growth arrest, an effect that can be reversed with the addition of exogenous putrescine. Growth arrest of cells in response to DFMO occurs predominantly at the G_1 phase of the cell cycle (Monti et al., 1999; Ray et al., 2001; Li et al., 2002), but increases the length of S phase in CHO cells have also been reported (Fredlund and Oredsson, 1996).

In cancer therapy, DFMO has been less successful, resulting in cytostatic rather than cytotoxic effects *in vivo* (Pegg et al., 1982). This is due, in part, to the incomplete depletion of spermine and its retroconversion to spermidine and putrescine (Gerner and Mamont, 1986), and partly due to compensatory increases in uptake of polyamines from the circulation or extracellular medium induced by polyamine depletion (Alhonen-Hongisto et al., 1980). Despite the lack of success of DFMO as a global antitumour agent, some positive results have been achieved in recurrent anaplastic gliomas where a decrease in the tumour burden or arrest of tumour growth for up to 1 year was observed in 45% of patients after treatment with DFMO (Levin et al., 1992).

More recently, an alternative role has emerged for DFMO as a chemopreventative agent (for review see Meyskens and Gerner, 1999). Chemoprevention is the use of chemical agents to prevent tumour development and, ideally, to cause regression of existing tumours. Perhaps the greatest potential for DFMO and chemoprevention is in colorectal cancer where decreases in ODC activity

Table 3. Alterations in polyamines and their metabolism in cancer

Disease	Alteration in polyamines	Reference
Respiratory Tract Cancers	Elevated spermidine and spermine	Dreyfuss et al., 1975
Lung Cancer	Elevated polyamines in urine	Lipton et al., 1975a
Bronchogenic Carcinoma	Increased putrescine and spermidine excretion	Waalkes et al., 1975
Breast Cancer	Elevated tumour polyamine content	Kingsnorth et al., 1984a
Gastro-intestinal Cancers	Elevated polyamine content	Dreyfuss et al., 1975
Gastro-intestinal Malignancies	Increased polyamine excretion (particularly spermidine and spermine)	Lipton et al., 1975b
Stomach Cancer	Increased urinary polyamines	Kubota et al., 1985
Colon Carcinoma	Increased spermine excretion	Waalkes et al., 1975
Colorectal Cancer	Elevated spermidine and spermine tumour content	Kingsnorth et al., 1984b
	Increased urinary polyamines	Lipton et al., 1975a
	Increased urinary and serum polyamines	Nishioka and Rhomsdahl, 1974
Kidney/Genitourinary Cancers	Increased putrescine and spermidine	Dreyfuss et al., 1975
Kidney Adenocarcinoma	Increased urinary polyamines	Sanford et al., 1975
Bladder Carcinoma	All 3 polyamines elevated, particularly putrescine (correlated with degree of tumour infiltration)	Pastorini et al., 1981
	Increased urinary polyamines	Sanford et al., 1975
Childhood Leukaemia	Polyamine:creatinine ratios increased in patients with the active disease	Garnica et al., 1981
Acute Myelocytic Leukaemia	Increased excreted urinary polyamines	Russell et al., 1971
	Increased urinary polyamines	Fujita et al., 1976
Chronic Lymphocytic Leukaemia	Increased urinary polyamine levels	Russell et al., 1971
Hodgkin's Lymphoma	Elevated urinary polyamines	Russell et al., 1971
Haematological Neoplasms	All 3 urinary polyamines elevated	Durie et al., 1977
Prostatic Carcinoma	Increased spermidine urinary excretion	Fair et al., 1975
Prostatic Adenocarcinoma	Elevated urinary polyamines	Sanford et al., 1975
Testicular Carcinoma	Elevated urinary polyamine content	Sanford et al., 1975
Sarcoma	Increased urinary and serum polyamine content	Nishioka and Rhomsdahl, 1974
Lymphosarcoma	Elevated urinary polyamine content	Russell et al., 1971
Melanoma	Elevated serum and urinary polyamines	Nishioka and Rhomsdahl, 1974
Malignant Melanoma	Elevated urinary polyamines	Lipton et al., 1975a
Metastatic Adenocarcinoma	Elevated urinary polyamines	Lipton et al., 1975a
Metastatic Squamous Cell Carcinoma	Increased urinary polyamines	Lipton et al., 1975a
Multiple Myelomas	Increased spermidine and spermine	Dreyfuss et al., 1975
Transitional Cell Carcinoma	Increased urinary polyamine levels	Sanford et al., 1975

and polyamine content limit the formation of tumours. Meyskens' group (1994) has shown that frequent, low dose DFMO can suppress polyamine concentrations in the colon for prolonged periods thus potentially limiting tumour growth. DFMO is thought to act late in tumour progression (Nigro et al., 1987). Other types of epithelial cancers in animal models can also be prevented by daily administration of DFMO (Carbone et al., 1998; Meyskens and Gerner, 1998). Chemoprevention regimens are being developed currently for a number of human tumours. These trials look promising as there are few side effects associated with DFMO usage, only a reversible ototoxicity at high doses (Love et al., 1998).

In other diseases there have also been some positive outcomes from the use of DFMO. In murine lupus, the survival rate of female MRL-*lpr*/*lpr* mice was increased by 29% by treatment with DFMO. This effect was the result of the inhibitory action of DFMO on cell proliferation and the prevention of DNA from forming an immunogenic left-handed Z-DNA conformation (Thomas and Messner, 1989).

However, the greatest success of DFMO has been in the treatment of parasitic infections. DFMO has cured acute infections of *Trypanosoma brucei brucei* in mammals (Giffin et al., 1986) and is active in the late stage of *Trypanosoma brucei gambiense* (Bacchi et al., 1980). It is also useful against infections caused by *T. b rhodesiense* although it is less useful here due to the faster turnover rate of ODC of 3–4 h, compared with 20 h for *T. b gambiense* (Iten et al., 1997). DFMO was approved by the FDA in 1990 for use in parasitic infections (Nightingale, 1991). Its administration causes the trypanosomes to convert to their non-dividing stumpy forms, which prevents

them from changing their surface glycoproteins, thus making them more vulnerable to attack from the host immune system (Wang, 1995). Treatment with DFMO also leads to decreased spermidine content and, as spermidine is used to form trypanothione, a compound parasites use to resist cellular oxidative stress (Fairlamb et al., 1987), the parasites are more susceptible to stress effects. A comprehensive review of this area is available (Bacchi and Yarlett, 2002).

Methylglyoxal bis(guanylhydrazone) MGBG

Interest in methylglyoxal bis(guanylhydrazone) or MGBG began with two key observations. The first was that MGBG inhibited the growth of L1210 leukaemia in mice (Freedlander and French, 1958) and the second was that MGBG was similar in structure to spermidine and therefore may interfere with polyamine production (French et al., 1960). MGBG is thought to bind to DNA but as it is not fully protonated at physiological pH, the binding is likely to be weaker than that of the polyamines (Mihich, 1975). The drug is a potent competitive inhibitor of SAMDC and depletes spermidine and spermine content but causes significant accumulation of putrescine (Porter et al., 1980). Treatment of cells with MGBG results in inhibition of cell growth, an effect that can be reversed by exogenous spermidine (Pegg, 1973). Use of MGBG in man was however limited by extreme toxicity despite the use of a variety of treatment and dosage regimens (Warrell and Burchenal, 1983).

A number of reports indicated that MGBG caused significant damage to mitochondria with ultrastructural damage such as loss of cristae and matrix components as well as metabolic damage that is indicative of loss of mitochondrial integrity (Pleshkewych et al., 1980).

MGBG uses the polyamine transport system to enter cells and depletion of spermidine and spermine by treatment with DFMO will enhance the uptake of MGBG (Seppanen et al., 1980). It has been suggested that cancer cells accumulate more MGBG than normal cells due to the unregulated nature of the growth of the tumour cells. Comprehensive reviews of MGBG action and pharmacokinetics are available (Williams-Ashman and Seidenfeld, 1986; Warrell and Burchenal, 1983).

In combination with other inhibitors of polyamine biosynthesis such as DFMO, MGBG showed synergistic responses in childhood leukaemia and in P388 leukaemia in mice (Nakaike et al., 1988; Siimes et al., 1981). These promising observations led to the development of other SAMDC inhibitors based on the structure of MGBG such as EGBG and CGP 48664 (SAM486A) (Igarashi et al., 1984; Stanek et al., 1993). Treatment with SAM486A inhibits SAMDC by 90% and decreased spermidine and spermine pools (Regenass et al., 1994), as well as exhibiting decreased antimitochondrial activity when compared with MGBG (Pathak et al., 1977). SAM486A also stabilised SAMDC (Svensson et al., 1997). SAM486A has recently undergone a pharmacokinetic study in patients with solid malignancies to determine its toxicity profile (Siu et al., 2002). In addition to being well tolerated in patients, analysis of tumour tissue found decreased SAMDC activity, and increases in both ODC activity and putrescine content, consistent with the known effects induced by SAMDC inhibitors.

SAM analogues

Structural analogues of SAM have also been tested, and shown to decrease putrescine and spermidine pools (Hyvonen and Eloranta, 1990) while increasing the half-life of SAMDC due to enzyme stabilisation (Shirahata and Pegg, 1985). SAM analogues are site-directed irreversible inhibitors of SAMDC synthesised for use as anti-parasitic agents against *T. brucei.* They include MAOEA (5′-deoxy-5′-[(2-aminooxyethyl)-methylamino]adenosine) and MHZEA (5′-deoxy-5′-[(2-hydrazinoethyl)methylamino]adenosine; Pegg et al., 1988). These analogues contain a tertiary nitrogen atom in place of the sulfonium ion with MHZEA being more potent that MAOEA. In *T. brucei brucei* SAMDC was irreversibly inactivated by both of these analogues. This suggests that these analogues bind to the active site and form a covalent bond with SAMDC (Tekwani et al., 1992). As with DFMO, there is great potential for the therapeutic use of these analogues in trypanosomiasis and other parasitic infections.

Two other agents that can inhibit SAMDC are berenil and pentamidine (trypanocidal diamidines). These are effective anti-trypanosomal agents but they are non-specific, being able to inhibit SSAT (Karvonen et al., 1985; Libby and Porter, 1992). In addition, pentamidine shows effective inhibition of PAO.

The majority of these inhibitors, although effective at depleting spermidine and spermine, often fail to deplete putrescine sufficiently to induce cell growth arrest and cell death thus limiting their utility.

Other enzyme inhibitors

PAO

Originally, PAO was thought to be a constitutively expressed enzyme and therefore not important in terms of regulating polyamine content (Seiler et al., 1980).

However, several studies have shown that PAO activity can be increased (Flayeh and Wallace, 1990; Lindsay and Wallace, 1999), and, interestingly, PAO activity is low in several types of cancer (Quash et al., 1979), leading to the suggestion that induction of PAO in response to a chemotherapeutic agent may be an alternative approach in the treatment of cancer (Wallace et al., 2000).

Two forms of PAO have been identified, and these were originally proposed to be a cytosolic and a peroxisomal form of the same enzyme, as their separation by chromatography revealed them to be isozymes (Libby and Porter, 1987). Recent work in this area has led to the cloning of PAO (Wang et al., 2001) and the suggestion has been made that this isozyme may in fact be a spermine oxidase (PAO-h1/SMO). The enzyme was inducible, and found to preferentially cleave spermine rather than the recognised preferred substrate of PAO, N^1-acetyl spermine (Vujcic et al., 2002). The resurgence in interest in this area, and the possibility of an increasingly important role for PAO and SMO has led to further identification of at least 4 isozymes/isoforms of PAO-h1/SMO, each with different substrate specificities (Murray-Stewart et al., 2002).

The most successful inhibitor of PAO is MDL 72527 (N^1,N^4-bis(2,3-butadienyl)-1,4-butanediamine), that specifically inhibits PAO without having affecting either mono or di- amine oxidases (Bey et al., 1985). MDL 72527 has shown cytotoxic effects in some leukaemic cell lines, resulting in the formation of lysosomally-derived vacuoles. These effects could not be reversed by the addition of putrescine or spermidine, and the rapid apoptosis induced suggested that MDL 72527 could override survival factors (Dai et al., 1999). However, the concentration of MDL 72527 used to induce such effects was more than 10 times that required to inhibit PAO (Wallace and Duthie, unpublished observations).

SSAT

Although a specific inhibitor for SSAT has yet to be developed, some other agents have been found to affect SSAT activity. Berenil and pentamidine, both effective SAMDC inhibitors, have been shown to inhibit other polyamine metabolic enzymes, including SSAT (Karvonen et al., 1985). In most instances, however SSAT is induced by antiproliferative agents; for example, the polyamine analogues.

Transport

The net positive charge on polyamines requires that cells have a transport system for polyamine uptake and export (Wallace, 1987). Although the mammalian transport system has yet to be isolated, it is believed to be energy-dependent, carrier-mediated (Seiler and Dezeure, 1990), and highly adaptive, in that it can transport not only polyamines but other polyamine-like molecules including MGBG (Byers et al., 1987) and many polyamine analogues (Porter et al., 1984; Fraser et al., 2002). More recently, polyamine transport has been suggested to occur through receptor-mediated endocytosis, an event that has been demonstrated in both yeast and mammalian cells (Soulet et al., 2002).

The significance of polyamine transport in maintaining polyamine homeostasis was first recognised when DFMO failed to be effective *in vivo* (Alhonen-Hongisto et al., 1980). The upregulation of polyamine transport to compensate for blockade of biosynthesis, produced no net loss of polyamines and no significant decreases in cell growth. Several studies have shown increased polyamine uptake in cases where biosynthesis is either defective, or suppressed by an enzyme inhibitor (Kramer et al., 1989; Pilz et al., 1990). The importance of polyamine transport has led to development of several types of transport inhibitors, (Aziz et al., 1995; Weeks et al., 2000). These have demonstrated polyamine uptake inhibition, but the inhibitory effect was found to decrease with continued exposure (Fraser et al., 2002).

Polyamine analogues

Polyamine analogues are multisite inhibitors of polyamine metabolism and developed from the lack of *in vivo* success obtained with single enzyme inhibitors. The initial success of polyamine homologues in terms of their recognition by cells and support of cell growth (Porter and Bergeson, 1983), led to further modification that resulted in a selection of molecules, based on the structures of spermidine and spermine, that were recognised as polyamine-like but failed to substitute for function (Porter and Sufrin, 1986). Comprehensive reviews on many of the analogues and their structures are available (Frydman and Valasinas, 1999; Casero and Woster, 2001; Wallace and Fraser, 2003).

Examination of the intracellular effects resulting from analogue exposure revealed that these molecules could effectively deplete intracellular polyamine pools by having a similar mechanism to that induced by a high natural polyamine content. The outcome resulted in down regulation of the biosynthetic enzymes ODC and SAMDC (Porter et al., 1990), increased polyamine export and in some cases, upregulation of the catabolic enzyme SSAT (Casero et al., 1989a).

The range of polyamine analogues studied to date has led to their division into 2 main categories, based on their ability to affect changes in polyamine content. The first group is the anti-metabolites, where the cellular uptake of polyamine analogue leads to active catabolism and export of natural polyamines, cell growth arrest and cell death (Seiler et al., 1998). The other group that has emerged more recently, is that of the mimetics, analogues that maintain the ability to displace natural polyamines at intracellular sites so causing cytotoxicity, but fail to effect any significant change in polyamine content (Fraser et al., 2002).

The best studied of the analogues are the bis(ethyl) polyamines, bis(ethyl)norspermine (BENSpm), bis (ethyl)homospermine (BEHSpm), and bis(ethyl)spermine (BESpm). These symmetrically substituted analogues are all based on the structure of spermine, and have shown effective cytotoxicity in several tumour cell lines (Bergeron et al., 1988; Casero et al., 1989b; Chang et al., 1992). Most notable from these studies is that the analogues induced differential effects in different cell types, indicating there are cell type-specific effects induced by these analogues. The recognition of this may mean it can be exploited to treat particular types of disease. In particular, the differential induction of the catabolic enzyme SSAT was found to vary from 2-1000 fold in 2 lung cancer cell lines (Casero et al., 1989a, 1992) further supporting the cell type-specificity of polyamine analogues. This discovery also provided a mechanism for analogue toxicity, namely through SSAT induction and polyamine catabolism and export.

The success of these 'first-generation' analogues has since led to the development of second and third generation analogues, the unsymmetrically-substituted and S'Lil analogues respectively (Saab et al., 1993; Reddy et al., 1998). Both of these classes of analogues also exhibit cell type-specific effects. Several of the unsymmetrically-substituted analogues have demonstrated effective cytotoxicity and polyamine depletion in HL-60 leukaemic cells (Nairn et al., 2000; Fraser et al., 2002), leading to apoptotic cell death. The administration of these analogues also causes decreased ODC activity and SSAT induction (McCloskey et al., 1996, 2000).

Summary

The polyamine metabolic pathway remains a valid target for therapeutic intervention. Success of the single enzyme inhibitors has been variable with the most positive results in parasitic diseases such as trypanasomiasis. In cancer therapy, the single enzyme inhibitors have shown promise but now seem more likely to be useful as chemopreventative agents rather than standard anticancer drugs. The concept of multisite inhibition with the analogues is showing significant potential for therapeutic use by exploiting the cell's own regulatory mechanisms to deplete polyamine content and induce cell death by apoptosis.

References

Alhonen-Hongisto L, Seppanen P, Janne J (1980) Intracellular putrescine and spermidine deprivation induces increased uptake of the natural polyamines and methylglyoxal bis(guanylhydrazone). Biochem J 192: 941–945

Aziz SM, Gosland MP, Crooks PA, Olson JW, Gillespie MN (1995) A novel polymeric spermine conjugate inhibits polyamine transport in pulmolary artery smooth muscle cells. J Pharmacol Exp Ther 274: 181–186

Bacchi CJ, Yarlett N (2002) Polyamine metabolism as chemotherapeutic target in protozoan parasites. Mini-Rev Med Chem 2: 553–563

Bacchi CJ, Nathan HC, Hutner SH, McCann PP, Sjoerdsma A (1980) Polyamine metabolism: a potential therapeutic target in trypanosomes. Science 210: 332–334

Beppu T, Shirahata A, Takahashi N, Hosoda H, Samejima K (1995) Specific depletion of spermidine and spermine in HTC cells treated with inhibitors of aminopropyltransferases. J Biochem (Tokyo) 117: 339–345

Bergeron RJ, Neims AH, McManis JS, Hawthorne TR, Vinson JRT, Bortell R, Ingeno RJ (1988) Synthetic polyamine analogues as antineoplastics. J Med Chem 31: 1183–1190

Bey P, Bolkenius FN, Seiler N, Casara P (1985) N-2,3-butadienyl-1,4-butanediamine derivatives: potent irreversible inactivators of mammalian polyamine oxidase. J Med Chem 28: 1–2

Bolkenius FN, Bey P, Seiler N (1985) Specific inhibition of polyamine oxidase *in vivo* is a method for the elucidation of its physiological role. Biochim Biophys Acta 838: 69–76

Byers TL, Kameji R, Rannels DE, Pegg AE (1987) Multiple pathways for uptake of paraquat, methylglyoxal bis(guanylhydrazone) and polyamines. Am J Physiol 252: C663–C669

Byers TL, Ganem B, Pegg AE (1992) Cytostasis induced in L1210 murine leukaemia cells by the S-adenosyl-L-methionine decarboxylase inhibitor 5′([(Z)-4-amino-2-butenyl]methylamino)-5′-deoxyadenosine may be due to hypusine depletion. Biochem J 287: 717–724

Byers TL, Wiest L, Wechter RS, Pegg AE (1993) Effects of chronic 5′-([(Z)-4-amino-2-butenyl]methylamino)-5′-deoxy-adenosine (AbeAdo) treatment on polyamine and eIF-5A metabolism in AbeAdo-sensitive and -resistant L1210 murine leukaemia cells. Biochem J 290: 115–121

Carbone PP, Douglas JA, Larson PO, Verma AK, Blair AI, Tutsch K (1998) Phase I chemoprevention study of piroxicam (PXM), and α-difluoromethylornithine (DFMO). Cancer Epidemiol Biomark Prev 7: 907–912

Casero RA Jr, Woster PM (2001) Terminally alkylated polyamine analogues as chemotherapeutic agents. J Med Chem 44: 1–26

Casero RA Jr, Celano P, Ervin SJ, Porter CW, Bergeron RJ, Libby PR (1989a) Differential induction of spermidine/spermine N^1-acetyltransferase in human lung cancer cells by the bis(ethyl)polyamine analogues. Cancer Res 49: 3829–3833

Casero RA Jr, Ervin SJ, Celano P, Baylin SB, Bergeron RJ (1989b) Differential response to treatment with the bis(ethyl)polyamine analogues between human small cell lung carcinoma and undifferentiated large cell lung carcinoma in culture. Cancer Res 49: 639–643

Casero RA Jr, Celano P, Ervin SJ, Weist L, Pegg AE (1990) High specific induction of spermidine/spermine N^1-acetyltransferase in a human large cell lung carcinoma. Biochem J 270: 615–620

Casero RA Jr, Mank AR, Xiao L, Smith J, Bergeron RJ, Celano P (1992) Steady-state messenger RNA and activity correlates with sensitivity to N^1,N^{12}-bis(ethyl)spermine in human cell lines representing the major forms of lung cancer. Cancer Res 52: 5359–5363

Cecco L, Antoniello S, Auletta M, Cerra M, Bonelli P (1992) Pattern and concentration of free and acetylated polyamines in urine of cirrhotic patients. Intl J Biol Markers 7: 52–58

Chang BK, Bergeron RJ, Porter CW, Vinson JRT, Liang Y, Libby PR (1992) Regulatory and antiproliferative effects of N-alkylated polyamine analogues in human and hamster pancreatic adenocarcinoma cell lines. Cancer Chemother Pharmacol 30: 183–188

Chopra S, Wallace HM (1998) Induction of spermidine/spermine N1-acetyltransferase in human cancer cells in response to increased production of reactive oxygen speices. Biochem Pharmacol 55: 1119–1123

Cohen LF, Lundgren DW, Farrell PM (1976) Distribution of spermidine and spermine in blood from cystic fibrosis patients and control subjects. Blood 48: 469–475

Coward JK, Pegg AE (1987) Specific multisubstrate adduct inhibitors of amino-propyltransferases and their effect on polyamine biosynthesis in cultured cells. Adv. Enz. Regul. 26: 107–113

Dai H, Kramer DL, Yang C, Murti KG, Porter CW, Cleveland JL (1999) The polyamine oxidase inhibitor MDL 72527 selectively induces apoptosis of transformed hematopoietic cells through lysosomotropic effects. Cancer Res 59: 4944–4954

Dreyfuss F, Chayen R, Dreyfuss G, Dvir R, Ratan J (1975) Polyamine excretion in the urine of cancer patients. Israel J Med Sci 11: 785–795

Durie BG, Salmon SE, Russell DH (1977) Polyamines as markers of response and disease activity in cancer chemotherapy. Cancer Res 37: 214–221

el-Marjou A, Bankir L, Pierrat D, Trinh-Trang-Tan MM (1997) Messenger RNA for enzymes of ornithine and polyamine metabolism are selectively underexpressed in kidney of 5/6 nephrectomized rats. Exp Nephrol 5: 285–294

Eloranta TO, Khomutov AR, Khomutov RM, Hyvonen T (1990) Aminooxy analogues of spermidine as inhibitors of spermine synthase and substrates of hepatic polyamine acetylating activity. J Biochem (Tokyo) 108: 593–598

Fair WR, Wehner N, Brorsson U (1975) Urinary polyamine levels in the diagnosis of carcinoma of the prostate. J Urol 114: 88–92

Fairlamb AH, Blackburn P, Uhlrich P, Chait BT, Cerami A (1987) *In vivo* effects of difluoromethylornithine on trypanothione and polyamine levels in bloodstream forms of *Trypanosoma brucei*. Mol Biochem Parasitol 24: 185–191

Flayeh KA, Wallace HM (1990) Polyamine oxidase activity in a human colonic carcinoma cell line. Biochem Soc Trans 18: 1225

Flescher E, Bowlin TL, Talal N (1992) Regulation of IL-2 production by mononuclear cells from rheumatoid arthritis synovial fluids. Clin Exp Immunol 83: 435–437

Flescher E, Bowlin TL, Ballester A, Houk R, Talal N (1989) Increased polyamines may downregulate interleukin 2 production in rheumatoid arthritis. J Clin Invest 83: 1356–1362

Fraser AV, Woster PM, Wallace HM (2002) Induction of apoptosis in human leukaemic cells by IPENSpm, a novel polyamine analogue and anti-metabolite. Biochem J 367: 307–312

Fredlund JO, Oredsson SM (1996) Impairment of DNA replication within one cell cycle after seeding of cells in the presence of a polyamine-biosynthesis inhibitor. Eur J Biochem 237: 539–544

Freedlander BL, French FA (1958) Carcinostatic action of polycarbonyl compounds and their derivatives. II. Glyoxal bis(guanylhydrazone) and derivatives. Cancer Res 18: 360–363

French FA, Freedlander BL, Hasking A, French J (1960) Acta Un Int Cancer 16: 614–616

Frydman B, Valasinas A (1999) Polyamine-based chemotherapy of cancer. Expert Opin Ther Pat 9: 1055–1068

Fujita K, Nagatsu T, Maruta K, Ito M, Senba H (1976) Urinary putrescine, spermidine, and spermine in human blood and solid cancers in an experimental gastric tumor of rats. Cancer Res 36: 1320–1324

Furumitsu Y, Yukioka K, Kojima A, Yukioka M, Shichikawa K, Ochi T, Matsui-Yuasa I, Otani S, Nishizawa Y, Morii H (1993) Levels of urinary polyamines in patients with rheumatoid arthritis. J Rheumatol 20: 1661–1665

Garnica A, Benton T, Slanina P, Miale T, Stenke AL (1981) Changes in urine polyamines in childhood leukemias. Annals Clin Lab Sci 11: 109–114

Gerner EW, Mamont PS (1986) Restoration of polyamine contents in rat hepatoma (HTC) cells after inhibition of polyamine biosynthesis: relationship with cell proliferation. Eur J Biochem 156: 31–35

Giffin BF, McCann PP, Bitonti AJ, Bacchi CJ (1986) Polyamine depletion following exposure to DL-alpha-difluoromethylornithine both *in vivo* and *in vitro* initiates morphological alterations and mitochondrial activation in a monomorphic strain of *Trypanosoma brucei brucei*. J Protozool 33: 238–243

Gomes-Trolin C, Nygren I, Aquilonius SM, Askmark H (2002) Increased red blood cell polyamines in ALS and Parkinson's disease. Exp Neurol 177: 515–520

Guo J, Wu YQ, Rattendi D, Bacchi CJ, Woster PM (1995) S-(5′-deoxy-5′-adenoxyl)-1-aminoxy-4-(methylsulfonio)-2-cyclopentene (AdoMao): an irreversible inhibitor of S-adenosylmethionine decarboxylase with potent *in vitro* antitrypanosomal activity. J Med Chem 38: 1770–1777

Holm I, Persson L, Pegg AE, Heby O (1989) Effects of S-adenosyl-1,8-diamino-3-thio-octane and S-methyl-5′-methylthioadenosine on polyamine synthesis in Ehrlich ascites-tumour cells. Biochem J 261: 205–210

Hyvonen T, Eloranta TO (1990) Regulation of *S*-adenosyl-L-methionine decarboxylase by 1-aminooxy-3-aminopropane: enzyme kinetics and effects on the enzyme activity in cultured cells. J Biochem 107: 339–342

Igarashi K, Porter CW, Morris DR (1984) Comparison of specificity of inhibition of polyamine synthesis in bovine lymphocytes by ethylglyoxal bis(guanylhydrazone) and methylglyoxal bis(guanylhydrazone). Cancer Res 44: 5326–5331

Imanishi Y, Koyama H, Inaba M, Okuno S, Nishizawa Y, Morii H, Otani S (1996) Phosphorous intake regulated intestinal function and polyamine metabolism in uremia. Kidney Int 49: 499–505

Iten M, Mett H, Evans A, Enyaru JC, Brun R, Kaminsky R (1997) Alterations in ornithine decarboxylase characteristics account for tolerance of Trypanosoma brucei rhodesiense to D,L-alpha-difluoromethylornithine. Antimicrob Ag Chemother 41: 1922–1925

Ito H, Hibasami H, Shimura K, Nagai J, Hidaka H (1982) Antitumor effect of dicyclohexylammonium sulfate, a potent inhibitor of spermidine synthase against P388 leukemia. Cancer Lett 15: 229–235

Jurkowska G, Rydzewska G, Andrzejewska A (1997) The influence of polyamines synthesis inhibition on pancreas regeneration and phospholipase D activity after acute caerulein induced pancreatitis in rats. Biochemical and ultrastructural study. J Physiol Pharmacol 48: 789–804

Karvonen E, Kauppinen L, Partanen T, Poso H (1985) Irreversible inhibition of putrescine-stimulated *S*-adenosyl-L-methionine decarboxylase by berenil and pentamidine. Biochem J 231: 165–169

Khomutov RM, Hyvonen T, Karvonen E, Kauppinen L, Paalanen T, Paulin L, Eloranta TO, Pajula RL, Andersson LC, Poso H (1985) 1-Aminooxy-3-aminopropane, a new and potent inhibitor of polyamine biosynthesis that inhibits ornithine decarboxylase, adenosylmethionine decarboxylase and spermidine synthase. Biochem Biophys Res Comm 130: 596–602

Kingsnorth AN, Wallace HM, Bundred NJ, Dixon JMJ (1984a) Polyamines in breast cancer. Br J Surg 71: 352–356

Kingsnorth AN, Lumsden AB, Wallace HM (1984b) Polyamines in colorectal cancer. Br J Surg 71: 791–794

Kramer DL, Khomutov RM, Bukin YV, Khomutov AR, Porter CW (1989) Cellular characterization of a new irreversible inhibitor of S-adenosylmethionine decarboxylase and its use in determining the relative abilities of individual polyamines to sustain growth and viability of L1210 cells. Biochem J 259: 325–331

Kubo S, Tamori A, Nishiguchi S, Kinoshita H, Hirohashi K, Kuroki T, Omura T, Otani S (1998) Effect of alcohol abuse on polyamine metabolism in hepatocellular carcinoma and noncancerous hepatic tissue. Surgery 123: 205–211

Kubota S, Yamasaki Z, Yoshimoto M, Murata N, Wada T, Ohsawa N, Takaku F (1985) The value of urinary polyamine assay in stomach cancer. Comparison with serum carcinoembryonic antigen. Cancer 56: 1630–1635

Lauharanta J, Kapyaho K (1983) Arginase activity and polyamine biosynthesis in psoriasis. Acta Derm Venereol 63: 277–282

Levin VA, Prados MD, Yung WKA, Gleason MJ, Ictech S, Malec M (1992) Treatment of recurrent gliomas with eflornithine. J Natl Cancer Inst 84: 1432–1437

Li L, Liu L, Rao JN, Esmaili A, Strauch ED, Bass BL, Wang J (2002) JunD stabilization results in inhibition of normal intestinal epithelial cell growth through p21 after polyamine depletion. Gastroenterology 123: 764–779

Libby PR, Porter CW (1987) Separation of two isozymes of polyamine oxidase from murine L1210 leukemia cells. Biochem Biophys Res Comm 144: 528–535

Libby PR, Porter CW (1992) Inhibition of enzymes of polyamine back-conversion by pentamidine and berenil. Biochem Pharmacol 44: 830–832

Lindsay GS, Wallace HM (1999) Changes in polyamine catabolism in HL-60 human promyelogenous leukaemic cells in response to etoposide-induced apoptosis. Biochem J 337: 83–87

Lipton A, Sheehan LM, Kessler GF (1975a) Urinary polyamine levels in human cancer. Cancer 35: 464–468

Lipton A, Sheehan LM, Harvey HA (1975b) Urinary polyamine levels in patients with gastrointestinal malignancy. Cancer 36: 2351–2354

Love RR, Jacoby R, Newton MA, Tutsch KD, Simon K, Pomplun M, Verma AK (1998) A randomized, placebo-controlled trial of low-dose alpha-difluoromethylornithine in individuals at risk for colorectal cancer. Cancer Epidemiol Biomark Prev 7: 989–992

Lowe NJ, Breeding J, Russell D (1982) Cutaneous polyamines in psoriasis. Brit J Dermatol 107: 21–25

Luk GD (1986) Essential role of polyamine metabolism in hepatic regeneration. Inhibition of deoxyribonucleic acid and protein synthesis and tissue regeneration by difluoromethylornithine in the rat. Gastroenterology 90: 1261–1267

Luk GD, Baylin SB (1983) Polyamines and intestinal growth – increased polyamine biosynthesis after jejunectomy. Am J Physiol 245: G656–G660

Marton LJ, Pegg AE (1995) Polyamines as targets for therapeutic intervention. Annu Rev Pharmacol Toxicol 35: 55–91

McCloskey DE, Yang J, Woster PM, Davidson NE, Casero RA Jr (1996) Polyamine analogue induction of programmed cell death in human lung tumor cells. Clin Cancer Res 2: 441–446

McCloskey DE, Woster PM, Casero RA Jr, Davidson NE (2000) Effects of the polyamine analogues N^1-ethyl-N^{11}-((cyclopropyl)methyl)-4,8-diazaundecane and N^1-ethyl-N^{11}-((cycloheptyl)methyl)-4,8-diazaundecane in human prostate cancer cells. Clin Cancer Res 6: 17–23

Metcalf BW, Bey P, Danzin C, Jung MJ, Casara P, Vevert JP (1978) Catalytic irreversible inhibition of mammalian ornithine decarboxylase (E.C. 4.1.1.17) by substrate and product analogs. J Am Chem Soc 100: 2551–2553

Meyskens FL Jr, Gerner EW (1999) Development of difluoromethylornithine (DFMO) as a chemoprevention agent. Clin Cancer Res 5: 945–951

Meyskens FL Jr, Emerson SS, Pelot D, Meshkinpour H, Shassetz LR, Einspahr J, Alberts DS, Gerner EW (1994) Dose de-escalation chemoprevention trial of alpha-difluoromethylornithine in patients with colon polyps. J Natl Cancer Inst 86: 1122–1130

Meyskens FL Jr, Gerner E, Emerson S, Pelot D, Durbin T, Doyle K, Lagerberg WA (1998) A randomized double-blind placebo controlled Phase IIb trial of difluoromethylornithine for colon cancer prevention. J Natl Cancer Inst 90: 1212–1218

Mihich E (1975) Handbook of experimental pharmacology. In: Sartorelli AC, Johns DG (eds) Springer, New York, pp 766–788

Monti MG, Ghiaroni S, Barbieri D, Franceschi C, Marverti G, Moruzzi MS (1999) 2-Deoxy-D-ribose-induced apoptosis in HL-60 cells is associated with the cell cycle progression by spermidine. Biochem Biophys Res Comm 257: 460–465

Morrison LD, Kish SJ (1995) Brian polyamine levels are altered in Alzheimer's disease. Neurosci Lett 197: 5–8

Morrison LD, Cao XC, Kish SJ (1998) Ornithine decarboxylase in human brain: influence of aging, regional distribution, and Alzheimer's disease. J Neurochem 71: 288–294

Moulinoux JP, Delamaire D, Beau B, Quemener V, Brissot P, Le Calve M, Deugnier Y, Chambon Y, Bourel M (1985) Diagnostic value of erythrocyte-free polyamines and histaminemia in malignant hepatic tumors and in liver cirrhosis. Clin Chim Acta 145: 77–87

Murray-Stewart T, Wang Y, Devereux W, Casero RA Jr (2002) Cloning and characterisation of multiple human polyamine oxidase splice variants that code for isoenzymes with different biochemical characteristics. Biochem J 368: 673–677

Nairn LM, Lindsay GS, Woster PM, Wallace HM (2000) Cytotoxicity of novel unsymmetrically substituted inhibitors of polyamine biosynthesis in human cancer cells. J Cell Physiol 182: 209–213

Nakaike S, Kashiwagi K, Terao K, Iio K, Igarashi K (1988) Combined use of alpha-difluoromethylornithine and an inhibitor of S-adenosylmethionine decarboxylase in mice bearing P388 leukemia or Lewis lung carcinoma. Japan J Cancer Res 79: 501–508

Narisawa T, Takahashi M, Niwa M, Koyama H, Kotanagi H, Kusaka N, Yamazaki Y, Nagasawa O, Koyama K, Wakizaka A, Fukaura Y (1989) Increased mucosal ornithine decarboxylase activity in large bowel with multiple tumors, adenocarcinoma and adenoma. Cancer 63: 1572–1576

Nesher G, Osborn TG, Moore TL (1997) Effect of treatment with methotrexate, hydroxychloroquine and prednisone on lymphocyte polyamine levels in rheumatoid arthritis: correlation with the clinical response and rheumatoid factor synthesis. Clin Exp Rheumatol 15: 343–347

Nightingale SL (1991) Drug for sleeping sickness approved. JAMA 265: 1229

Nigro ND, Bull AW, Boyd ME (1987) Importance of the duration of inhibition on intestinal carcinogenesis by difluoromethylornithine in rats. Cancer Lett 35: 153–158

Nishiguchi S, Tamori A, Koh N, Fujimoto S, Takeda T, Shiomi S, Oka H, Yano Y, Otani S, Kuroki T (2002) Erythrocyte-binding polyamine as tumor growth marker for human hepatocellular carcinoma. Hepato-Gastroenterol 49: 504–507

Nishioka K, Grossie VB, Chang TH, Ajani JA, Ota DM (1991) Colorectal ornithine decarboxylase activity in human mucosa and tumors: elevation of enzymatic activity in distal mucosa. J Surg Oncol 47: 117–120

Nishioka K, Romsdahl MM (1974) Elevation of putrescine and spermidine in sera of patients with solid tumors. Clin Chim Acta 57: 155–161

Pastorini P, Milano G, Toubol J, Raymond G, Cambon P, Lalanne CM (1981) The diagnostic and prognostic value of urinary polyamine measurement in bladder cancer. Urol Res 9(1): 13–16

Pathak SN, Porter CW, Dave C (1977) Morphological evidence for an antimitochondrial action by methylglyoxal bis(guanylhydrazone). Cancer Res 37: 2246–2250

Pegg AE (1973) Inhibition of spermidine formation in rat liver and kidney by methylglyoxal bis(guanylhydrazone). Biochem J 132: 537–540

Pegg AE (1986) Recent advances in the biochemistry of polyamines in eukaryotes. Biochem J 234: 249–262

Pegg AE, Tang K, Coward JK (1982) Effects of *S*-Adenosyl-1,8-diamino-3-thiooctane on polyamine metabolism. Biochem Stry 21: 5082–5089

Pegg AE, Jones DB, Secrist JA III (1988) Effect of inhibitors of S-adenosylmethionine decarboxylase on polyamine content and growth of L1210 cells. Biochem 27: 1408–1415

Pegg AE, Wechter R, Poulin R, Woster PM, Coward JK (1989) Effect of S-adenosyl-1,12-diamino-3-thio-9-azadodecane, a multisubstrate adduct inhibitor of spermine synthase, on polyamine metabolism in mammalian cells. Biochem Stry 28: 8446–8453

Pilz RB, Steglich C, Scheffler IE (1990) Molecular and genetic characterisation of an ornithine decarboxylase-deficient chinese hamster cell line. J Biol Chem 265: 8880–8886

Pleshkewych A, Kramer DL, Kelly E, Porter CW (1980) Independence of drug action on mitochondria and polyamines in L1210 leukemia cells treated with methylglyoxal-bis(guanylhydrazone). Cancer Res 40: 4533–4540

Porter CW, Bergeron RJ (1983) Spermidine requirement for cell proliferation in eukaryotic cells: structural specificity and quantitation. Science 219: 1083–1085

Porter CW, Sufrin JR (1986) Interference with polyamine biosynthesis and/or function by analogs of polyamines or methionine as a potential anticancer chemotherapeutic strategy. Anticancer Res 6: 525–542

Porter CW, Dworaczyk D, Ganis B, Weiser MM (1980) Polyamines and biosynthetic enzymes in the rat intestinal mucosa and the influence of methylglyoxal bis(guanylhydrazone). Cancer Res 40: 2330–2335

Porter CW, Miller J, Bergeron RJ (1984) Aliphatic chain length specificity of the polyamine transport system in ascites L1210 leukemia cells. Cancer Res 44: 126–128

Porter CW, Pegg AE, Ganis B, Madhabala R, Bergeron RJ (1990) Combined regulation of ornithine and S-adenosylmethionine decarboxylases by spermine and the spermine analogue N^1,N^{12}-bis(ethyl)spermine. Biochem J 268: 207–212

Poulin R, Lu L, Ackermann B, Bey P, Pegg AE (1992) Mechanism of the irreversible inactivation of mouse ornithine decarboxylase by α-difluoromethylornithine. J Biol Chem 267: 150–158

Proctor MS, Fletcher HV Jr, Shukla JB, Rennert OM (1975) Elevated spermidine and spermine levels in the blood of psoriasis patients. J Invest Dermatol 65: 409–411

Qu N, Ignatenko NA, Yamauchi P, Stringer DE, Levenson C, Shannon P, Perrin S, Gerner EW (2003) Inhibition of human ornithine decarboxylase activity by enantiomers of difluoromethylornithine. Biochem J 375: 465–470

Quash G, Keolouangkhot T, Gazzolo L, Ripoll H, Saez S (1979) Diamine oxidase and polyamine oxidase activities in normal and transformed cells. Biochem J 177: 275–282

Ray RM, McCormack SA, Johnson LR (2001) Polyamine depletion arrests growth of IEC-6 and Caco-2 cells by different mechanisms. Am J Physiol 281: G37–G43

Reddy VK, Valasinas A, Sarkar A, Basu HS, Marton LJ, Frydman B (1998) Conformationally restricted analogues of ^1N,^{12}N-bisethylspermine: synthesis and growth inhibitory effects on human tumor cell lines. J Med Chem 41: 4723–4732

Regenass U, Mett H, Stanek J, Mueller M, Kramer D, Porter CW (1994) CGP 48664, a new S-adenosylmethionine decarboxylase inhibitor with broad spectrum antiproliferative and antitumor activity. Cancer Res 54: 3210–3217

Russell DH, Stern LZ (1981) Altered polyamine excretion in Duchenne muscular dystrophy. Neurology 31: 80–83

Russell DH, Levy CC, Schimpff SC, Hawk IA (1971) Urinary polyamines in cancer patients. Cancer Res 31: 1555–1558

Russell DH, Combest NL, Duell EA, Stawiski MA, Anderson TF, Voorhees JJ (1978) Glucocorticoid inhibits elevated polyamine biosynthesis in psoriasis. J Invest Dermatol 71: 177–181

Russell DH, Rosenblum MG, Beckerman RC, Durie BG, Taussig LM, Barnett DR (1979) Altered polyamine metabolism in cystic fibrosis. Ped Res 13: 1137–1140

Saab NH, West EE, Bieszk NC, Preuss CV, Mank AR, Casero RA Jr, Woster PM (1993) Synthesis and evaluation of unsymmetrically substituted polyamine analogues as modulators of human spermidine/spermine N1-acetyltransferase (SSAT) and as potential antitumor agents. J Med Chem 36: 2998–3004

Saito A, Takagi T, Gi Chung T, Ohta K (1983) Serum levels of polyamines in patients with chronic renal failure. Kidney Int 24 [Supp 16]: 5234–5237

Sanford EJ, Drago JR, Rohner TJ, Kessler GF, Sheehan L, Lipton A (1975) Preliminary evaluation of urinary polyamines in the diagnosis of genitourinary tract malignancy. J Urol 113: 218–221

Seghieri G, Anichini R, Ciuti M, Gironi A, Bennardini F, Franconi F (1997) Raised erythrocyte polyamine levels in non-insulin-dependent diabetes mellitus with great vessel disease and albuminuria. Diabetes Res Clin Pract 37: 15–20

Seiler N, Dezeure F (1990) Polyamine transport in mammalian cells. Int J Biochem 22: 211–218

Seiler N, Atanassov CL, Raul F (1998) Polyamine metabolism as a target for cancer chemoprevention (Review). Intl J Oncol 13: 993–1006

Seiler N, Bolkenius FN, Knogden B, Mamont P (1980) Polyamine oxidase in rat tissue. Biochim Biophys Acta 615: 480–488

Seppanen P, Alhonen-Hongisto L, Janne J (1980) Relation of the antiproliferative action of methylglyoxal-bis(guanylhydrazone) to the natural polyamines. Eur J Biochem 110: 7–12

Shirahata A, Pegg AE (1985) Regulation of *S*-adenosylmethionine decarboxylase activity in rat liver and prostate. J Biol Chem 260: 9583–9588

Siimes M, Seppanen P, Alhonen-Hongisto L, Janne J (1981) Synergistic action of two polyamine antimetabolites leads to a rapid therapeutic response in childhood leukemia. Intl J Cancer 28: 567–570

Siu LL, Rowinsky EK, Hammond LA, Weiss GR, Hidalgo M, Clark GM, Moczygemba J, Choi L, Linnartz R, Barbet NC, Sklenar IT, Capdeville R, Gan G, Porter CW, Von Hoff DD, Eckhardt SG (2002) A Phase I and pharmacokinetic study of SAM486A, a novel polyamine biosynthesis inhibitor, administered on a daily-times-five every-three-week schedule in patients with advanced solid malignancies. Clin Cancer Res 8: 2157–2166

Sjoholm A, Bucht E, Theodorsson E, Larsson R, Nygren P (1994) Difluoromethylornithine and ethylglyoxal bis(guanylhydrazone) as inhibitors of human renal carcinoma cell proliferation and polyamine metabolism. Anticancer Res 13: 979–983

Soulet D, Covassin L, Kaouass M, Charest-Gaudreault R, Audette M, Poulin R (2002) Role of endocytosis in the internalisation of spermidine-C(2)-BODIPY, a highly fluorescent probe of polyamine transport. Biochem J 367: 347–357

Stanek J, Caravatti G, Frei J, Furet P, Mett H, Schneider P, Regenass U (1993) 4-Amidinoindan-1-one 2′-amidinohydrazone: a new potent and selective inhibitor of S-adenosylmethionine decarboxylase. J Med Chem 36: 2168–2171

Supattapone S, Wille H, Uyechi L, Safar J, Tremblay P, Szoka FC, Cohen FE, Prusiner SB, Scott MR (2001) Branched polyamines cure prion-infected neuroblastoma cells. J. Virol. 75: 3453–3461

Supattapone S, Nishina K, Rees JR (2002) Pharmacological approaches to prion research. Biochem Pharmacol 63: 1383–1388

Svensson F, Mett H, Persson L (1997) CGP 48664, a potent and specific *S*-adenosylmethionine decarboxylase inhibitor: effects on regulation and stability of the enzyme. Biochem J 322: 297–302

Swendseid M, Panaqua M, Kopple JD (1980) Polyamine concentrations in red cells and urine of patients with chronic renal failure. Life Sci 26: 533–539

Tabor CW, Tabor H, Tyagi AK, Cohn MS (1982) The biochemistry, genetics, and regulation of polyamine biosynthesis in Saccharomyces cerevisiae. Fed Proc 41: 3084–3088

Talal N, Tovar Z, Dauphinee MJ, Flescher E, Dang H, Galarza D (1988) Abnormalities of T cell activation in the rheumatoid synovium detected with monoclonal antibodies to CD3. Scand J Rheumatol [Suppl] 76: 175–182

Tekwani BL, Bacchi CJ, Secrist JA 3rd, Pegg AE (1992) Irreversible inhibition of S-adenosylmethionine decarboxylase of *Trypanosoma brucei brucei* by S-adenosylmethionine analogues. Biochem Pharmacol 44: 905–911

Thomas TJ, Messner RP (1989) Beneficial effects of a polyamine biosynthesis inhibitor on lupus in MRL-lpr/lpr mice. Clin Exp Immunol 78: 239–244

Thomas TJ, Meryhew NL, Messner RP (1990) Enhanced binding of lupus sera to the polyamine-induced left-handed Z-DNA form of polynucleotides. Arth Rheum 33: 356–365

Vujcic S, Diegelman P, Bacchi CJ, Kramer DL, Porter CW (2002) Identification and characterization of a novel flavin-containing spermine oxidase of mammalian cell origin. Biochem J 367: 665–675

Waalkes TP, Gehrke CW, Tormey DC, Zumwalt RW, Hueser JN, Kuo KC, Lakings DB, Ahmann DL, Moertel CG (1975) Urinary excretion of polyamines by patients with advanced malignancy. Cancer Chemother Rep Pt 1 59: 1103–1116

Wallace HM (1987) Polyamine catabolism in mammalian cells: excretion and acetylation. Med Sci Res 15: 1437–1440

Wallace HM (1996) Polyamines in human health. Proc Nut Soc 55: 419–431

Wallace HM, Caslake R (2001) Polyamines and colon cancer. Eur J Gastroenterol Hepatol 13: 1033–1039

Wallace HM, Fraser AV (2003) Polyamine analogues as anticancer drugs. Biochem Soc Trans 31: 393–396

Wallace HM, Duthie J, Evans DM, Lamond S, Nicoll KM, Heys SD (2000) Alterations in polyamine catabolic enzymes in human breast cancer tissue. Clin Cancer Res 6: 3657–3661

Wang CC (1995) Molecular mechanisms and therapeutic approaches to the treatment of African trypanosomiasis. Annu Rev Pharmacol Ther 35: 93–127

Wang Y, Devereux W, Murray-Stewart T, Casero RA Jr (2001) Cloning and characterisation of a human polyamine oxidase that is inducible by polyamine analogue exposure. Cancer Res 61: 5370–5373

Warrell RP Jr, Burchenal JH (1983) Methylglyoxal-bis(guanylhydrazone) (Methyl-GAG): current status and future prospects. J Clin Oncol 1: 52–65

Weeks RS, Vanderwerf SM, Carlson CL, Burns MR, O'Day CL, Cai F, Devens BH, Webb HK (2000) Novel lysine-spermine conjugate inhibits polyamine transport and inhibits cell growth when given with DFMO. Exp Cell Res 261: 293–302

Williams-Ashman HG, Seidenfeld J (1986) Aspects of the biochemical pharmacology of methyl glyoxal bis(guanylhydrazone). Biochem Pharmacol 35: 1217–1225

Wu Y, Woster PM (1992) S-(5′deoxy-5′-adenosyl)-1-ammonio-4-(methylsulfonio)-2-cyclopentene: a potent enzyme-activated irreversible inhibitor of S-adenosylmethionine decarboxylase. J Med Chem 35: 3196–3201

Authors' address: Dr. Heather M. Wallace, Senior Lecturer, Department of Medicine & Therapeutics and Biomedical Sciences, University of Aberdeen, Polwarth Building, Foresterhill, Aberdeen AB25 2ZD, Scotland, UK,
Fax: +44 1224 554761, E-mail: h.m.wallace@abdn.ac.uk