Polyamines and DNA methylation in childhood leukaemia

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Abstract

Both polyamine metabolism and DNA methylation play an important role in normal and malignant growth. Specific enzyme inhibitors or drugs that interfere with these metabolic pathways have proven to be potential anticancer agents. Since DNA methylation and polyamine metabolism depend on a common substrate, i.e. S-adenosylmethionine, interaction between both pathways can be expected. Little is known about the relationship between these pathways but studies are available indicating that polyamines and DNA methylation are directly or indirectly interconnected, metabolically as well as physiologically with respect to the regulation of cell growth, differentiation and cancer development. These considerations give rise to the possibility that, by targeting both pathways, a more profound and effective inhibitory effect on the growth of malignant cells can be achieved. In previous studies we showed that 6-MP (6-mercaptopurine) as well as MTX (methotrexate), well-known drugs in the treatment of acute lymphoblastic leukaemia, inhibit DNA methylation and induce apoptosis in malignant blood cells. Our recent results show that combined treatment with 6-MP, MTX and drugs interfering with polyamine metabolism has additive/synergistic effects on the growth, cell viability and/or apoptotic death of leukaemic cells. Such a combination therapy could have great clinical value for patients in which therapy using inhibitors of thiopurines/purine metabolism has failed.

Introduction

ALL (acute lymphoblastic leukaemia) is a heterogeneous disease with distinct biological and prognostic groupings [1]. The overall prognosis of ALL has improved significantly over the last few decades with current long-term disease-free survival rates of 75%. MTX (methotrexate) and 6-MP (6-mercaptopurine) is one of the crucial combinations in maintenance treatment of childhood ALL. Still, a substantial number of patients succumb to disease progression. For these patients, either new strategies or refinements of old ones are needed to improve the long-term prognosis.

The control of DNA methylation may be an important target of interference with cancer development, including leukaemia (see [2–4] for reviews). It has been found that primary and cultured neoplastic cells have methylation patterns which differ from the patterns of normal cells. These changes are apparent from measurements of the methylcytosine content of the DNA, which is reduced in many tumours [5–8]. DNA regions are often hypomethylated in hepatomas and leukaemias, including those of the c-myc, Ha-ras, Ki-ras, fos, Erb-A1 and bcl-2 proto-oncogenes [9,10]. On the other hand, specific chromosome regions have been detected that are frequently hypermethylated in neoplastic cells, e.g. Ecad, CALCA, ER, MDR1, THBS2, MYF3, p15, p16, p73, p57, CDH1, RARβ [11–18]. Moreover, methylation patterns may have clinical implications in ALL in that CALCA, p13, p57 and p21 methylation were found to be associated with dismal outcome. The occurrence of AML (acute myelocytic leukaemia), ALL and progressed stages of CML (chronic myelocytic leukaemia) is strongly correlated with hypermethylation of the calcitonin gene region on chromosome 11p [19–22].

Methylation processes by SAM (S-adenosylmethionine)

The methylation status of cytosine residues of genomic CpG islands (repetitive pairs of cytosine and guanine) affects gene expression. Regions with elevated levels of 5mC (5-methylcytosine) are in general associated with down-regulated genes. DNA methylation plays a role in diverse genetic processes such as imprinting, X-chromosome inactivation and developmental processes. The methylation state of individual CpG sites remains constant in most normal cell populations. This is due to the fact that DNA MTase (DNA–cytosine–methyltransferase) preferentially acts on double-stranded CpG sites that are already methylated in one strand, probably shortly after DNA replication. It is not known how the methylation state changes in a controlled manner.

SAM (also known as AdoMet) is the methyl donor for DNA methylation reactions [23]. SAM serves as the methyl donor for DNA methylation reactions. In addition, SAM is a substrate for decarboxylation to S-adenosylhomocysteine (AdoHcy), which is further metabolized by AdoHcy hydrolase to S-adenosylhomocysteine (AdoHcy). The balance between these reactions is crucial for the cell, as a decrease in SAM levels can lead to decreased DNA methylation and subsequent changes in gene expression.

Key words: childhood leukaemia, DNA methylation, 6-mercaptopurine, methotrexate, polyamines.

Abbreviations used: ALL, acute lymphoblastic leukaemia; 5mC, 5-methylcytosine; dSAM, decarboxylated S-adenosylmethionine; DFMAS, di fluoromethylaminothidiane; DHF, dhydrofolate; DNA MTAse, DNA-cytosine methyltransferase; 6-MP, 6-mercaptopurine; MTX, methotrexate; ODC, ornithine decarboxylase; PAI, palmitic acid; SAM, S-adenosylhomocysteine; SAMH, S-adenosylmethionine; SARDX, S-adenosylmethionine decarboxylase; SSpd, spermidine; SSpm, spermine; TdH, tetrahydrofolate; BUMP, thio IMP.
Interconnection of the pathways of DNA methylation and the polyamine interconversion cycle

These pathways are dependent on a common substrate: SAM. Possibilities of interfering with DNA methylation or polyamine metabolism are indicated. Polyamine analogues (e.g. SL-11093) interfere with polyamine homeostasis not only by down-regulation of biosynthetic enzymes or polyamine uptake but often also by up-regulation of catabolic enzymes, both leading to polyamine depletion. See the text for details.

Polyamines

SAM is also used as a source of aminopropyl groups in the synthesis of polyamines. Polyamines have a low molecular mass, a simple chemical structure (aliphatic amines) and are highly charged cations under physiological conditions [32,33]. Evidence from in vitro as well as in vivo experiments indicates that polyamines are intimately involved in cell kinetic behaviour, i.e. cell proliferation/growth, cell differentiation and apoptosis [32–34]. Transfection as well as inhibition studies showed that ODC (ornithine decarboxylase) activity and polyamines are essential for transformation [35]. Various polyamine biosynthesis inhibitors, e.g. 4-aminooindan-1-one 2′-amidinohydrazone (SAM486A), DFMO (difluoromethylornithine) and polyamine analogues have growth-inhibitory effects on several tumour types, including leukaemia. Their potential as antineoplastic agents has been investigated and extensively reviewed [36,37].

It is evident that polyamine metabolism is an inviting target for chemotherapy of different types of cancers. Since polyamines are required for malignant cell growth and function, interference with polyamine metabolism could provide a possible target for chemotherapy of leukaemia.

The compound SAM486A is of particular interest since it acts on the conversion of SAM, the branching point between DNA methylation and polyamine synthesis. In vitro studies have shown that SAM486A has growth-inhibitory effects on several types of human cancers, including leukaemia [38]. Preclinical studies suggest that CGP486A has additive and/or synergistic activity in combination with currently available cytostatic agents [39]. Phase I, II and pharmacological studies of SAM486A in patients with solid tumours showed that biologically relevant levels were achievable at doses that were well tolerated in patients [40–43].
Figure 2 | Effect of 5 μM 6-MP on ODC activity and intracellular polyamine levels in MOLT-F4 cells

Treatment with 5 μM 6-MP resulted in a strong inhibition of ODC activity. Note that by 6-MP treatment polyamine levels are decreased compared with the untreated cells. If 1 mM putrescine was added to 6-MP-treated cells putrescine levels increased; however, spermidine and spermine levels remained unchanged, implying a block in the synthesis of these polyamines presumably elicited by depletion of SAM.

Polyamine homoeostasis may affect the process of DNA methylation. Heby [44] and Frotesjö et al. [45] found that inhibition of putrescine production by DFMO caused hypomethylation in F9 teratocarcinoma stem cells. The observed hypomethylation could be counteracted by an inhibitor of SAMDC (S-adenosylmethionine decarboxylase). They hypothesized that DFMO increases the levels of deSAM (decarboxylated S-adenosylmethionine) by reducing the levels of putrescine, which serves as the acceptor for the aminopropyl group of deSAM. Accumulation of deSAM leads to inhibition of DNA MTase and subsequent loss of DNA methylation. By inhibiting SAMDC the accumulation of deSAM can be prevented and the degree of methylation can be restored.

Studies of DNA methylation in fungal differentiation and in growth and differentiation of human colon cancer cells suggest that polyamines are directly involved in DNA hypomethylation-mediated developmental processes by selective inhibition of DNA MTases [46–49].

To investigate the interconnection between the DNA methylation pathway and polyamine metabolism, we studied the effect of 6-MP on ODC and polyamine levels in a lymphoblastic cell line (MOLT-F4). Treatment with 5 μM 6-MP resulted in a strong inhibition of ODC activity (Figure 2). Comitantant to the decrease in ODC, putrescine, spermidine and spermine levels decreased. It is not yet clear by which mechanism ODC activity is inhibited by 6-MP. Studies with purified ODC enzyme showed that 6-MP had no direct effect on the ODC protein (results not shown). The decrease in the higher polyamines spermidine and spermine is most probably caused by the decrease in SAM induced by 6-MP, since SAM, after decarboxylation by SAMDC, is a precursor for the synthesis of spermidine and spermine. This is substantiated by experiments in which putrescine was added to 6-MP-treated cells (Figure 2). Although putrescine levels increased, spermidine and spermine levels remained unchanged impling a block in the synthesis of these polyamines elicited by depletion of SAM. Since spermidine/spermine levels are important cellular regulators of cell growth/survival, the decrease in ODC activity and/or spermidine/spermine levels may be attributed to the deleterious effects of 6-MP on leukaemic cells.

In vitro studies of inhibitors of thiopurines/purine metabolism and polyamine antimetabolites in childhood leukaemia

MTX and 6MP inhibit thiopurine/purine metabolism and are well-known drugs in the treatment of childhood acute lymphoblastic leukaemia. Our earlier work indicated that 6-MP inhibits DNA methylation at the methionine adenosyltransferase level, since methionine is increased, SAM is depleted and the rate of DNA methylation is decreased in in vitro experiments with MOLT F4 lymphoblasts treated with 6-MP [50,51]. The first step in 6-MP metabolism is conversion into thio-IMP (tIMP) which can be metabolized by two pathways: first, tIMP is converted into thioguanine nucleotides which are predominantly incorporated into DNA, inducing DNA-damage that results in delayed cytotoxicity [52]. Secondly, tIMP is methylated into me-tIMP. Me-tIMP is a strong inhibitor of purine de novo synthesis and therefore formation of me-tIMP contributes to cytotoxicity by depleting purine ribo- and deoxyribo-nucleotides, including ATP [53,54]. ATP is required to convert methionine into SAM and the level of SAM is also reduced in 6-MP-treated cells.

We have examined the induction of cell death by combinations of 6-MP and MTX in MOLT-F4 cells [55]. We observed that MTX and 6-MP induced apoptosis and that sequential exposure to MTX for 24 h followed by 4 h exposure of 6-MP showed a synergistic induction of apoptosis compared with single drug exposure.

We studied the effect of 6-MP and SAM486A, an inhibitor of SAMDC, on the viability of MOLT-F4 cells. In the study of a possible interconnection between the DNA methylation pathway and polyamine metabolism, these compounds are of particular interest since they both act on conversions of SAM, the branching point between DNA methylation and polyamine synthesis. 6-MP and SAM486A have an ED50 (effective dose of 50% viable cells) in the range 1–5 μM and 0.1–0.2 μM respectively. A combination of
6-MP and SAM486A increased the cytotoxicity in MOLT-F4. Using isobologram analysis (for example, after an incubation period of 48 h; see Figure 3), an additive effect of a combination of 6-MP with SAM486A was found in the range 0.7–0.9 µM and 0.3–0.15 µM. SAM486A also had an additive effect on apoptosis induced by exposure to a combination of MTX and 6-MP (results not shown).

Concluding remarks

Both DNA methylation and polyamine metabolism play an important role in normal and malignant growth. Specific enzyme inhibitors or drugs that interfere with these metabolic pathways have proven to be potential anticancer agents. Since DNA methylation and polyamine metabolism depend on a common substrate, i.e. SAM, interaction between both pathways can be expected. Little is known about the relationship between these pathways, but studies are available indicating that polyamines and DNA methylation are directly or indirectly interconnected, metabolically as well as physiologically with respect to the regulation of cell growth.

Our pilot studies showed that 6-MP and MTX have profound effects on the viability of MOLT-F4 cells, even in the submicromolar range, and that SAM486A has an additive effect on the cytotoxicity. These considerations give rise to the possibility that, by targeting both pathways, a more profound and effective inhibitory effect on the growth of malignant cells can be achieved. Given the good tolerability profile of SAM486A and the promising anti-tumour activity observed in pre-clinical models, further investigation of the compound as a single agent or for use in combination therapy of acute lymphoblastic leukaemia is clearly warranted.

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References


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