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# Reversal of 6-mercaptopurine and 6-methylmercaptopurine ribonucleoside cytotoxicity by amidoimidazole carboxamide ribonucleoside in Molt F4 human malignant T-lymphoblasts

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**Abstract**—Cytotoxicity of 6-mercaptopurine (6MP) and 6-methylmercaptopurine ribonucleoside (Me-MPR) was studied in Molt F4 human malignant lymphoblasts. Both drugs are converted into methyl-thioIMP (Me-tIMP), which inhibits purine *de novo* synthesis. Addition of amidoimidazole carboxamide ribonucleoside (AICAR) circumvented inhibition of purine *de novo* synthesis, and thus partly prevented 6MP and Me-MPR cytotoxicity. Purine nucleotides, and especially adenine nucleotides, were recovered by addition of AICAR. Under these conditions, Me-tIMP formation decreased. The results of this study indicate that formation of Me-tIMP may be important for 6MP cytotoxicity in Molt F4 cells. These data suggest that depletion of adenine nucleotides is the main cause for Me-tIMP cytotoxicity.

6-Mercaptopurine (6MP*), an analogue of the purine base hypoxanthine, is used in the treatment of children with acute lymphoblastic leukemia. The first step in 6MP cytotoxicity is conversion into thioIMP (tIMP) [1, 2]. tIMP can be metabolized by two pathways. First, it is converted into thioguanine nucleotides, which can be incorporated into the RNA and DNA of the cells, leading to DNA damage and delayed cytotoxicity [3, 4]. Second, it is methylated into methyl-thioIMP (Me-tIMP) by the enzyme thiopurine methyltransferase (EC 2.1.1.67) [1, 5]. Since Me-tIMP is a strong inhibitor of purine *de novo* synthesis [6–8], it induces cytotoxicity by depletion of purine ribonucleotides.

In the present study, the importance of Me-tIMP for 6MP cytotoxicity was examined in more detail. Amidoimidazole carboxamide ribonucleoside (AICAR), an intermediate of purine *de novo* synthesis distal to the site where Me-tIMP exerts its inhibiting effect, was used to circumvent inhibition of this route by Me-tIMP. AICAR is phosphorylated into AICAR monophosphate by adenosine kinase (AK, EC 2.7.1.20) [9, 10], and can enter purine *de novo* synthesis in this form. Me-tIMP cytotoxicity was examined more specifically using 6-methylmercaptopurine ribonucleoside (Me-MPR). This is also converted by AK [11] into Me-tIMP. Again AICAR was used to prevent inhibition of purine *de novo* synthesis.

* Abbreviations: 6MP, 6-mercaptopurine; Me-MPR, 6-methylmercaptopurine ribonucleoside; AICAR, amidoimidazole carboxamide ribonucleoside; MPA, mycophenolic acid; tIMP, thioIMP; Me-tIMP, methyl-thioIMP; AK, adenosine kinase.

## *Materials and Methods*

Mycophenolic acid (MPA), Me-MPR and AICAR were purchased from the Sigma Chemical Co. (St Louis, MO, U.S.A.); 6MP from Wellcome (The Netherlands). The experiments were performed with Molt F4, a human T-cell acute lymphoblastic leukemia cell line. Conditions for cell culture and experimental procedures have been described in detail previously [12].

Endogenous nucleotides (di- and triphosphates) were determined in $3 \times 10^6$ viable cells, according to the method described earlier [13], and were measured at a wavelength of 254 nm. Nucleotide concentrations were expressed as pmol/$10^6$ viable cells. Thionucleotides were extracted from $10^7$ viable cells, according to the procedure described earlier [12]. t-IMP and t-GMP were determined at 320 nm.

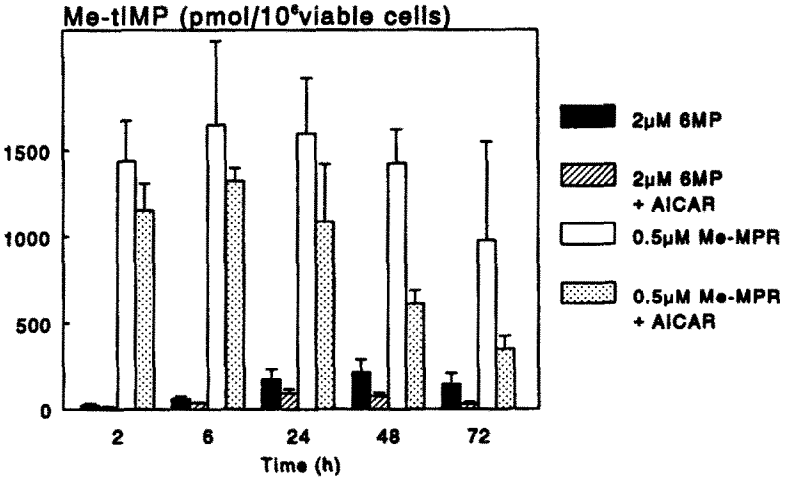


Fig. 1. Me-tIMP concentration of Molt F4 cells treated with 2 μM 6MP or 0.5 μM Me-MPR alone, or in combination with 50 μM AICAR (expressed as pmol/$10^6$ viable cells; mean with standard error of three independent experiments).

Table 1. Cell number and cell viability of Molt F4 cells after 48 hr exposure to 2 μM 6MP, 0.5 μM MPA or a combination of both, and to 0.5 μM Me-MPR with or without addition of 50 μM AICAR

| | Cell number AICAR | | Cell viability AICAR | |
|---|---|---|---|---|
| | − | + | − | + |
| Control | 1.64 ± 0.15 | 1.71 ± 0.24 | 97.5 ± 0.8 | 97.4 ± 1.0 |
| 2 μM 6MP | 0.65 ± 0.25 | 0.98 ± 0.15 | 78.0 ± 12.6 | 90.9 ± 4.4 |
| 0.5 μM MPA | 0.70 ± 0.1 | ND | 75.7 ± 2.6 | ND |
| 2 μM 6MP + 0.5 μM MPA | 0.27 ± 0.09 | 0.57 ± 0.06 | 47.4 ± 6.5 | 68.0 ± 1.8 |
| 0.5 μM Me-MPR | 0.27 ± 0.06 | 0.79 ± 0.12 | 60.6 ± 1.6 | 91.0 ± 3.1 |

Cell number is expressed as $10^6$ viable cells/mL. Cell viability is expressed in %; mean ± SD of three independent experiments.
ND, not done.

Me-tIMP was measured at 290 nm. Concentrations were expressed as pmol/$10^6$ viable cells.

*Results*

Treatment of Molt F4 cells with 2 μM 6MP induced cytotoxicity (Table 1), and resulted in intracellular depletion of purine nucleotides (Table 2). Depletion of guanine nucleotides occurred more rapidly than that of adenine nucleotides, but recovered earlier. Complete recovery of the guanine nucleotides could be observed after 48 hr. Maximal depletion of the adenine nucleotide pool occurred after 24 hr, and lasted longer. Simultaneous addition of AICAR to treatment with 2 μM 6MP partly prevented cytotoxicity, and resulted in less depletion of adenine nucleotides as compared to 6MP alone (Table 2). Depletion of guanine nucleotides could be partly prevented by AICAR during the first 2 hr. Decreased formation of tIMP and tGMP (data not shown) and of Me-tIMP (Fig. 1) was observed in cells treated with 6MP and AICAR as compared to 6MP alone. When 2 μM 6MP was combined with 0.5 μM MPA, an inhibitor of the conversion of tIMP into thioguanine nucleotides under these conditions, more Me-tIMP was formed, and cytotoxicity was potentiated [12]. Addition of 50 μM AICAR to this treatment resulted in cytotoxicity comparable to that of MPA alone (Table 1).

Inhibition of purine *de novo* synthesis by Me-tIMP was examined further using Me-MPR as its precursor. Me-MPR (0.5 μM) induced inhibition of cell growth and cell viability (Table 1), and resulted in depletion of purine nucleotides (Table 2). Addition of 50 μM AICAR to this treatment led to a complete recovery of cell viability, and a partial recovery of cell growth (Table 1). Under these conditions adenine nucleotide concentrations were partly recovered (Table 2), while guanine nucleotides were recovered only during the first 6 hr of the treatment. Moreover, the intracellular formation of Me-tIMP was reduced as a result of treatment of cells with a combination of 0.5 μM Me-MPR and AICAR as compared to 0.5 μM Me-MPR alone, especially after prolonged incubation (Fig. 1).

*Discussion*

In the present study the contribution of Me-tIMP formation to 6MP cytotoxicity is examined in Molt F4 cells. 6MP cytotoxicity is partly prevented by addition of AICAR to 6MP treatment (Table 1). Furthermore, AICAR ameliorates 6MP cytotoxicity in experiments where MPA is used to inhibit 6MP conversion into thioguanine nucleotides. Earlier research showed that with a combination of 2 μM 6MP and 0.5 μM MPA more Me-tIMP is formed, and 6MP cytotoxicity is potentiated [12]. Addition of AICAR to treatment with 0.5 μM MPA and 2 μM 6MP reduces cytotoxicity to a level obtained with MPA alone (Table 1).

Prevention of 6MP cytotoxicity by AICAR may be attributed to two mechanisms. First, as a result of addition of AICAR the effects of inhibition of purine *de novo* synthesis by Me-tIMP can be circumvented. This is demonstrated by the partial recovery of the adenine nucleotides (Table 2) after addition of AICAR. Second, Me-tIMP formation is decreased as a result of addition of AICAR to 6MP treatment (Fig. 1). This may be a consequence of phosphoribosylpyrophosphate consumption by the intracellular metabolism of AICAR [14], and may result in the availability of less phosphoribosylpyrophosphate for 6MP conversion into tIMP, and subsequently less intracellular thioguanine nucleotide formation. This complicates elucidation of the effects of addition of AICAR on 6MP cytotoxicity. Therefore, combinations of Me-MPR and AICAR were used to investigate the effect of AICAR on inhibition of purine *de novo* synthesis by Me-tIMP more thoroughly, as Me-MPR is converted directly into Me-tIMP by the enzyme AK [11]. This conversion is reflected by rapid formation of very high concentrations of Me-tIMP in the cells treated with 0.5 μM Me-MPR (Fig. 1). The more severe depletion of adenine nucleotides as compared to guanine nucleotides by 0.5 μM Me-MPR (Table 2) could be ascribed both to consumption of ATP by AK, during conversion of Me-MPR into Me-tIMP, and to inhibition of adenine nucleotide formation as a consequence of competitive inhibition of AK by Me-MPR. Addition of AICAR restored cell viability and growth of cells treated with Me-MPR (Table 1). This is in accordance with data reported on amidoimidazole carboxamide (AIC), which is also converted into AICAR monophosphate and is able to reverse cytotoxicity of Me-MPR [15]. Depletion of adenine nucleotides was partly prevented by addition of AICAR to Me-MPR treatment (Table 2). Simultaneously, a remarkable decrease in Me-tIMP concentration was observed (Fig. 1), probably as a result of competition for AK between AICAR and Me-MPR. However, since 500 pmol Me-tIMP/$10^6$ viable cells induce complete inhibition of purine *de novo* synthesis [16], the partial recovery of the adenine nucleotides observed here is still a result of circumvention of inhibition of purine *de novo* synthesis by AICAR, rather than of a suboptimal inhibition of purine *de novo* synthesis by a decreased Me-tIMP concentration. The partial recovery of adenine nucleotides by addition of AICAR (Table 2) was associated with an almost complete recovery of cell viability, and less inhibition of cell growth (Table 1). Therefore, depletion of adenine nucleotides by Me-tIMP must be the main cause of Me-tIMP cytotoxicity in Molt F4 cells.

In conclusion, 6MP and Me-MPR cytotoxicity can be prevented by AICAR. These data confirm that formation

Table 2. Purine nucleotide concentrations of Molt F4 cells treated with 2 μM 6MP or 0.5 μM Me-MPR alone, or in combination with 50 μM AICAR

| | Adenine nucleotides | | | | Guanine nucleotides | | | |
|---|---|---|---|---|---|---|---|---|
| Time (hr) | 6MP | 6MP + AICAR | Me-MPR | Me-MPR + AICAR | 6MP | 6MP + AICAR | Me-MPR | Me-MPR + AICAR |
| 2 | 83 (42–93) | 89 (88–109) | 83 (65–89) | 84 (71–100) | 66 (22–66) | 79 (50–83) | 68 (41–82) | 76 (61–93) |
| 6 | 78 (73–135) | 83 (77–102) | 50 (28–80) | 69 (67–85) | 52 (39–93) | 60 (56–69) | 56 (53–94) | 80 (63–81) |
| 24 | 52 (40–58) | 70 (61–86) | 43 (33–49) | 60 (52–95) | 75 (40–79) | 65 (50–86) | 88 (79–89) | 64 (60–110) |
| 48 | 54 (53–82) | 88 (82–166) | 40 (35–46) | 55 (34–55) | 102 (83–103) | 98 (54–236) | 78 (71–78) | 71 (45–72) |
| 72 | 83 (72–104) | 106 (101–112) | 30 (18–37) | 61 (50–80) | 101 (99–128) | 110 (109–122) | 57 (30–67) | 68 (59–70) |

Values are % of untreated cells; median and range (between brackets) of three experiments.
The purine nucleotide concentrations of untreated Molt F4 cells are 5502 ± 495 and 921 ± 61 pmol/$10^6$ viable cells for adenine and guanine nucleotides, respectively.

of Me-tIMP may be a second important route for 6MP cytotoxicity. Whether the methylation route of tIMP is important for the therapeutic effects of 6MP in patients remains to be elucidated. The bioavailability of orally administered 6MP is low, and under these conditions Me-tIMP formation may not be significant enough to inhibit purine *de novo* synthesis [12]. Under these conditions the methylation route may even function as a detoxification pathway, as described by Lennard *et al.* [17, 18]. However, when 6MP is administered intravenously very high levels are reached [19], and the methylation route may indeed contribute to 6MP cytotoxicity [12].

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