Effects of Allopurinol on the Therapeutic Efficacy of Methotrexate

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SUMMARY

The antitumor effects of methotrexate against early leukemia L1210 were partially reversed by the coadministration of allopurinol in vivo, even though allopurinol did not alter the growth-inhibitory effects of methotrexate against L1210 cells in culture. These data suggest that this alteration in antitumor activity results from a decreased catabolism of preformed systemic purines by allopurinol, a potent inhibitor of xanthine oxidase. On the other hand, the therapeutic effect of methotrexate against the P288 leukemia was not significantly altered by allopurinol treatment. In addition, coadministration of allopurinol did not significantly alter the toxicity of methotrexate in normal mice. The data are consistent with the hypothesis that, in the mouse, the antileukemic effects of methotrexate are more related to a purineless rather than a thymineless death.

INTRODUCTION

The studies by Hakala and Taylor (8, 9) demonstrated that inhibition of the growth of mouse Sarcoma 180 cells in culture by MTX could be completely prevented by the addition of the end products of folate metabolism to the culture medium, i.e., by a source of preformed purine such as Hx, as well as exogenous TdR and the otherwise nonessential amino acid glycine. When Eagle’s medium was supplemented with Hx, the concentration of MTX required to bring about half-maximal inhibition of the growth of Sarcoma 180 cells was twice that observed in the absence of Hx; the addition of TdR alone had no such sparing effect. In a subline of Sarcoma 180 selected in the presence of TdR for catabolism of preformed systemic purines by allopurinol, a potent inhibitor of xanthine oxidase. On the other hand, the therapeutic effect of methotrexate against the P288 leukemia was not significantly altered by allopurinol treatment. In addition, coadministration of allopurinol did not significantly alter the toxicity of methotrexate in normal mice. The data are consistent with the hypothesis that, in the mouse, the antileukemic effects of methotrexate are more related to a purineless rather than a thymineless death.

In the present study, the relevancy of such effects to the therapeutic selectivity of MTX in vivo was approached. Hughes et al. (13) and Christine et al. (4) reported that the level of TdR in normal mouse serum is 1 to 2 μM. Such levels of TdR suggest that, in the mouse, an increased availability of preformed purines might be all that is required to alter the toxicity and/or the therapeutic efficacy of MTX. As has been shown by Pomales et al. (18, 19), HPP, a potent inhibitor of xanthine oxidase, markedly increased the utilization of exogenous Hx for synthesis of cellular nucleic acids in vivo. Hence, the effects of the concurrent administration of HPP on the toxicity and antitumor efficacy of MTX were examined.

MATERIALS AND METHODS

Female DBA/2 Ha-DD mice (18 to 21 g) were obtained from the breeding colony of the Roswell Park Memorial Institute, while the female DBA/2J mice were purchased from The Jackson Laboratory, Bar Harbor, Maine. The leukemias L1210 and P-288 used were as described previously (15, 20). In all experiments, 1 x 10⁶ tumor cells were inoculated i.p. into recipient mice and treatments were begun, except where indicated, 24 hr after tumor cell inoculation. In all experiments, HPP was administered 1 hr prior to MTX. The mean survival was counted from the day of inoculation, which was considered Day 0. Drugs were obtained from Sigma Chemical Co., St. Louis, Mo., or from the pharmacy of the Roswell Park Memorial Institute. S.D. and t tests were as described by Snedecor (21).

Leukemia L1210 cells (7) were grown in culture in Roswell Park Memorial Institute Medium 1640 supplemented with 10% dialyzed fetal calf serum (Grand Island Biological Co., Grand Island, N. Y.), which was further dialyzed for 3 days after purchase, and with N-2-hydroxyethylpiperazine-N°-2-ethanesulfonic acid and 2-(N-morpholino)ethanesulfonic acid (Sigma Chemical Co.) at a final concentration of 16 and 8 mM, respectively. Cell number was determined with a Coulter Model B particle counter (Coulter Electronics, Hialeah, Fla.) with a 70-μm window. Under these conditions, L1210 grew logarithmically for about 48 hr with a doubling time of 10 to 12 hr. Leukemia P-288 cells grown in culture originated from a 6-day ascites tumor in female DBA/2J mice. The cells were washed and suspended in Roswell Park Memorial Institute Medium 1640, supplemented as above at an initial density of 2 to 2.5 x 10⁶ cells/ml. Such primary cultures, when incubated at 37°, grew logarithmically for about 72 hr with a mean doubling time of about 18 hr after a slight lag.
RESULTS

The ability of various concentrations of TdR and Hx to support the growth of cultures of L1210 cells in the presence of a high concentration of MTX is shown in Chart I. Under culture conditions that allowed the continuous logarithmic growth of control cultures, the optimal concentration of TdR required for the reversal of MTX toxicity was 3 to 6 µM in the presence of adequate Hx (32 µM). The optimal concentration of Hx in the presence of adequate TdR (5.6 µM) was 18 to 50 µM (Chart 1). The same range of concentrations of both TdR and Hx were also found to be adequate for the reversal of the toxicity of 10⁻⁴ M MTX for mouse leukemia P-288 cells in culture; such levels of TdR and Hx have previously been shown to protect Sarcoma 180 cells in culture from the effects of MTX (9).

As shown in Chart 2, HPP, given daily for 6 days at 20 mg/kg, partially reversed the antitumor effects of various doses of MTX against early leukemia L1210 in DBA/2 H-DD mice. HPP alone on this schedule had no effect on the survival of tumor-bearing animals, nor was it toxic to normal mice, as indicated by both weight loss and mortality. MTX alone at the optimal dosage (3 mg/kg) on this schedule increased the life-span of leukemic mice by 73%, while only a 33% increase in life-span was observed in mice treated with the combination of MTX and HPP. The difference in survival time due to HPP coadministration was significant at p < 0.001. The effects of varying doses of HPP on the therapeutic activity of MTX given every other day for 3 days (3 mg/kg) are shown in Table 1. While HPP at 5 mg/kg afforded a slight reversal of MTX activity, maximum effects were observed at 20 mg/kg (p < 0.01). HPP alone at 30 mg/kg had no effect on tumor lethality.

The effects of administration of HPP on the lethal potency of MTX were examined in normal DBA/2Ha-DD mice. The results of such a study, in which MTX was administered daily for 5 days and HPP was administered daily for 6 days, are shown in Chart 3. When these data were analyzed by the method of Litchfield and Wilcoxon (14), the lethal doses for 50% of the mice for MTX alone (4.0 mg/kg) and for MTX plus HPP (3.2 mg/kg) were found not to differ significantly (p = 0.05). In addition, no definite effects of HPP on the lethality of MTX were observed when both drugs were administered daily for 15 days.
The above data demonstrate that the coadministration of HPP alters the therapeutic efficacy of MTX against mouse leukemia L1210 in vivo. When the effect of HPP on the antiproliferative effects of MTX against L1210 cells growing in culture was examined, the presence of noninhibitory or even toxic concentrations of HPP did not alter the potency of MTX (Chart 4). These data constitute evidence against a direct reversal of the effects of MTX by HPP at the level of the target cell and suggest that an indirect effect of HPP is responsible for the decreased antitumor efficacy of MTX seen in vivo (Chart 2).

In an attempt to completely reverse the therapeutic activity of MTX against leukemia L1210 in DBA/2 Ha-DD mice, HPP was administered daily for 11 days starting 3 days prior to tumor inoculation. This was followed by the administration of MTX every other day for 3 days starting 24 hr after tumor inoculation. However, this intensive schedule of HPP still afforded only a partial reversal of the therapeutic activity of MTX against leukemia L1210. On the other hand, administration of HPP only very slightly reversed the therapeutic effects of MTX against leukemia P-288 (Chart 5). At no dose of MTX was this reversal significant (p > 0.05). Thus, depending on the type of tumor, treatment with HPP can result in either no effect or a partial reversal of the antitumor activity of MTX.

**DISCUSSION**

Murray (16) has proposed that, in the normal mouse, the presence of significant levels of Hx would be unlikely, due to the high xanthine oxidase activity in serum (1). This is consistent with the observations by Pomales et al. (18, 19) that administration of HPP, a potent inhibitor of xanthine oxidase, dramatically increases the incorporation of both Hx and xanthine into cellular nucleic acids in the mouse. Thus the decreased therapeutic activity of MTX observed following concurrent administration of HPP (Chart 2), although apparently not attributable to a direct antagonism at the level of the target cell (Chart 4), might well be the result of increased levels of circulating Hx.

As described by Hakala and Taylor (8, 9), the growth-inhibitory effects of MTX against mammalian cells in culture can be completely reversed by the addition of TdR and Hx to the culture medium. The concentration of TdR in normal mouse serum (1 to 2 μM), as reported by Hughes et al. (13) and Christine et al. (4), is sufficient for substantial reversal of the growth-inhibitory effects of MTX in the presence of adequate preformed purine (Chart 1). Thus, in mice, an increased availability of preformed purine might be all that is required to reduce the therapeutic effectiveness of MTX. As shown in Chart 2, concurrent administration of HPP reduced the therapeutic effectiveness of MTX against leukemia L1210. On the other hand, treatment with HPP neither reduces the antitumor effect of MTX against leukemia P-288 (Chart 5) nor significantly alters the toxicity of MTX in normal mice (Chart 3). It is difficult to attribute the partial reversal of the cytotoxic effects of MTX by HPP strictly on the basis of the exogenous supply of the end products of folate metabolism, since the same concentrations of TdR and Hx will reverse MTX toxicity against both L1210 and P-288 in culture. In addition, it is difficult to evaluate the effects of HPP on host immunity to these transplanted tumors in decreasing the therapeutic activity of MTX (15).

It would seem impossible to predict a priori the effect of MTX therapy on the circulating levels of TdR or Hx following HPP treatment. Such levels might increase due to gross cellular cytotoxicity, with resultant release of salvage metabolites, or they might decrease due to a decreased overall rate of purine and thymidylate synthesis in tissues that normally would release such compounds into the serum. The partial reversal of MTX effects by HPP might also be due not to end product reversal by TdR and Hx but rather to the presence of circulating Hx by itself. In mouse fibroblasts in culture, it has been reported that the addition of purine to the culture medium increased the lethality of MTX (3). On the other hand, Hryniiuk and Bertino (11, 12) have reported that, in L5178Y murine lymphoblasts in culture, the addition of Hx to the medium partially protected the cells against the lethal effects of MTX.

In preliminary studies (6) we found that injection of levels of TdR phosphorylase, partially purified from *Escherichia coli* B, which suppressed the incorporation of TdR into tumor >90%, had little effect on the therapeutic efficacy of MTX towards mouse leukemia L1210. However, the toxicity of
MTX towards the host was substantially increased by this enzyme therapy. This suggests that the normal levels of circulating TdR in the mouse partially spare those tissues critical for survival from the toxicity of MTX. In addition, a report has recently appeared (22) that administration of additional TdR further decreases the host toxicity of MTX in the mouse, while not affecting the response of an early L1210 tumor to MTX therapy.

While these results support the concept that, in the mouse, the therapeutic index of MTX might well be improved by coadministration of TdR, it is clear that this concept cannot be directly applied in the clinic without further studies. Whereas the high levels of xanthine oxidase in mouse serum make significant levels of circulating Hx unlikely in the mouse (1, 16), substantial concentrations of Hx (> 40 \(\mu\)M) have been found in human serum (17), which lacks xanthine oxidase activity (1). In the presence of TdR, these concentrations of Hx are adequate to reverse the growth-inhibitory effects of MTX against 2 human malignant cell lines (HeLa and J-111) in culture (9). Recently, Then and Angehrn (23), utilizing a biological assay, reported that the concentration of TdR in human blood was extremely low (<2 \(\times\) 10\(^{-7}\) M). They concluded that the concentration of TdR in human blood is too low to prevent the bactericidal action of the antifolate, Trimethoprim. Thus, coadministration of MTX and TdR to human subjects (who may already have sufficient levels of circulating purines) might well result in a total product reversal of MTX and may compromise or negate the therapeutic effects of this agent.

In summary, while the therapeutic effects of MTX in the mouse may be more related to a purineless death, efficacy may be dependent on a thymineless death in the clinic. Further studies are needed to document the levels of TdR and Hx in plasma in cancer patients and to evaluate the role of various salvage metabolites in clinical MTX therapy.

REFERENCES


Interaction of HPP and MTX
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