Effect of triamterene on nucleic acid synthesis

Triamterene is a diuretic with activity in vitro as a folic acid antagonist inhibiting dihydrofolate reductase. Megaloblastosis in patients on triamterene was suspected to be due to inhibition of DNA synthesis secondary to triamterene inhibition of dihydrofolate reductase. We studied the effect of triamterene on nucleic acid synthesis with the use of L1210 mouse ascites leukemia. Cells were harvested 5 days after intraperitoneal inoculation of 2×10^5 cells and the incorporation of ^{14}C-thymidine (TdR), ^{3}H-deoxyuridine (UdR), or ^{14}C-uridine (UR) was measured. In vitro triamterene (10^{-1} μM per milliliter) resulted in 40 per cent suppression of UdR incorporation into DNA as compared to 73 per cent suppression with methotrexate in the same concentration. Triamterene minimally inhibited and methotrexate moderately stimulated TdR into DNA. Neither triamterene nor methotrexate influenced UR incorporation into RNA. In contrast to methotrexate, in vivo treatment of ascites mice with triamterene showed no inhibition of UdR incorporation. The lack of triamterene effect on DNA synthesis in vivo suggests rapid dissociation of drug-enzyme complex in vivo, which would be desirable for a diuretic nonmyelosuppressive action.

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Triamterene (2,4,7-triamino-6-phenylpteridine) is a diuretic which was designed to exploit the diuretic renal effects of methotrexate yet avoid bone marrow toxicity.[13] It is known that triamterene results in dehydrofolate reductase (DHFR) "induction" to an extremely high level in human white blood cells.[7] Recently it has been postulated that triamterene may cause megaloblastosis of the bone marrow in patients with cirrhosis of the liver.[6] Because of these observations and the structural similarity of triamterene to folic acid (Fig. 1), the possibility of triamterene as a folic acid antagonist which inhibits DHFR has been suspected. Roberts and Hall[8] reported that triamterene was a potent inhibitor in vitro of DHFR. Corcino and associates[4] studied the in vitro effect of triamterene with the use of short-term human bone marrow cultures and showed interference by triamterene on de novo DNA-thymine synthesis from deoxyuridine. They also found that the mechanism of action is similar to that of methotrexate by the

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inhibition of DHFR (Fig. 2). However, the rapid reversibility of triamterene inhibition of DHFR suggests that in vitro effects may not occur in vivo and other explanations, for example, diet, may be involved in cirrhotic patients.

Although triamterene has been successfully used as a diuretic for the past several years, no case of megaloblastosis due solely to triamterene has been recognized. We designed this study to investigate the effect of triamterene on nucleic acid synthesis in vitro and in vivo with the use of L1210 mouse ascites leukemic cell line. We chose the L1210 system because they are rapidly proliferating cells with a high rate of nucleic acid synthesis.[11] This characteristic might be a sensitive indicator of triamterene effect on the changing rate of DNA synthesis. In addition, it was felt that the in vitro suggestion of DNA inhibition needed to be examined by an in vivo system more comparable to that occurring during the clinical use of triamterene.

Materials and Methods

In vitro. L1210 mouse ascites leukemic cells were harvested 5 days after the intraperitoneal inoculation of 2×10^5 cells into male CDF_1 mice. A one per cent cell suspension was made in Eagle's medium containing 10 per cent horse serum with either triamterene* or methotrexate at various concentrations. A control was also studied without triamterene or methotrexate. The rate of DNA and RNA synthesis was measured by a modification of the method of Roberts and co-workers.[9] ^{14}C-labeled thymidine (TdR) of specific activity 30 mc. per millimole, ^{3}H-labeled deoxyuridine (UdR) of specific activity 13 C. per millimole or ^{14}C-labeled uridine (UR) of specific activity 54.1 mc. per millimole was added and incubated at 37° C. with continuous agitation. Thirty minutes later the incubation was terminated by the addition of 10 per cent cold trichloracetic acid. After 3 washes with trichloracetic acid, the precipitate was washed again with ether-ethanol mixture and then ether alone. The precipitated nucleic acid was hydrolyzed with 2M ammonium hydroxide. The incorporation of radioisotope-labeled nucleic acid precursors into DNA and RNA was determined by liquid scintillation counting. Changes in DNA and RNA synthesis resulting from exposure to various concentrations of triamterene and methotrexate were plotted as per cent of control values.

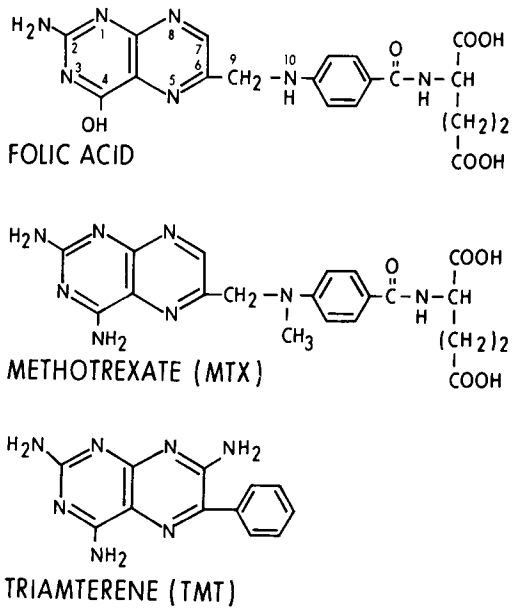

Fig. 1. Structural formulas of folic acid (pteroyl glutamic acid), methotrexate (4-amino-N^{10} methyl-pteroyl glutamic acid), and triamterene (2,4,7-triamino-6-phenylpteridine)

In vivo. Twenty male CDF_1 mice were intraperitoneally inoculated with 2×10^5 L1210 leukemic cells. At Day 5, triamterene suspension made in 0.9 per cent saline was injected intraperitoneally on the basis of 40 mg. per kilogram to 10 mice. The 10 control mice were injected with the same volume of the diluent. At 4 hours, 5 triamterene-treated and 5 control mice were put to death and L1210 cells were harvested for TdR, UdR, and UR incorporation into DNA and RNA. The other 5 triamterene-treated and 5 control mice were put to death at 24 hours for TdR, UdR,

*Triamterene was supplied by Dr. Alfred R. Maass, Smith Kline & French Laboratories.

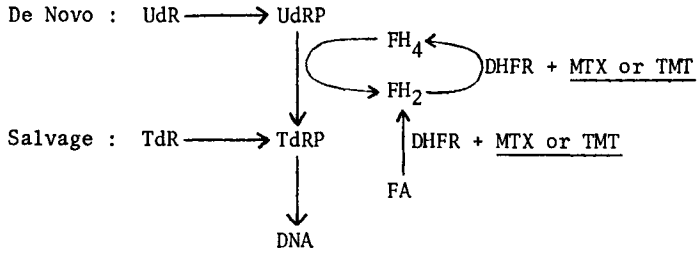

Fig. 2. Metabolic pathways for the synthesis of deoxyribonucleic acid (*DNA*) and mechanism of action of methotrexate (*MTX*) and triamterene (*TMT*). Deoxyuridine (*UdR*), deoxyuridylate (*UdRP*), tetrahydrofolate (FH_4), thymidine (*TdR*), thymidylate (*TdRP*), dehydrofolate (FH_2), folic acid (*FA*), dihydrofolate reductase (*DHFR*).

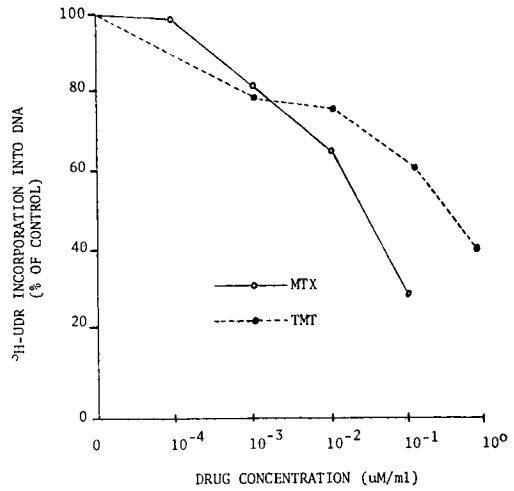

Fig. 3. In vitro effect of methotrexate (*MTX*) and triamterene (*TMT*) on deoxyuridine (*UdR*) incorporation into DNA. Mouse L1210 leukemic cells were harvested for the study of de novo DNA synthesis using deoxyuridine under the influence of various concentrations of methotrexate or triamterene.

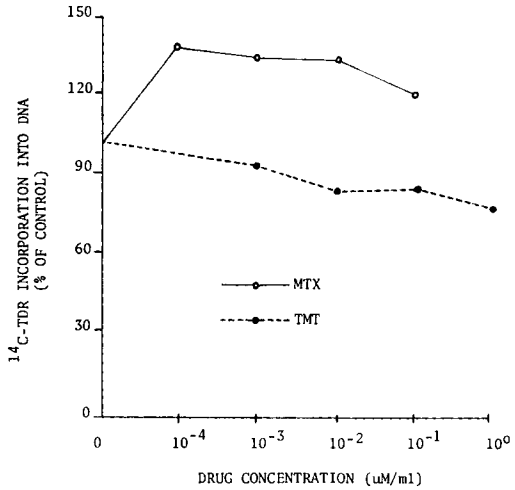

Fig. 4. In vitro effect of methotrexate (*MTX*) and triamterene (*TMT*) on thymidine (*TdR*) incorporation into DNA.

and UR incorporation. The result of incorporation into nucleic acid was expressed as per cent of control values.

Results

In vitro (Figs. 3, 4, and 5). Fig. 3 shows the effects of triamterene on UdR incorporation into DNA in comparison with methotrexate. It is well known that methotrexate results in marked inhibition of UdR incorporation into DNA of mouse L1210 leukemic cells.[10] In our study, both methotrexate and triamterene inhibited de novo DNA synthesis. At a concentration of triamterene of 10^{-1} μM per milliliter UdR incorporation was inhibited to about 60 per cent of control; on the other hand, methotrexate in the same concentration inhibited incorporation to about 27 per cent of control. Triamterene and methotrexate had characteristic and different effects upon TdR incorporation into DNA. In contrast to moderate stimulation by methotrexate, up to 140 per cent of control, triamterene resulted in slight inhibition of TdR incorporation. Increases of exogenous TdR incorporation have been reported when an ineffective agent inhibits the de novo pathway for DNA-thymine synthesis.[14] The effect of triamterene on RNA synthesis is summarized in Fig. 5, which shows no significant changes in either methotrexate or triamterene on RNA synthesis over control. These drugs resulted

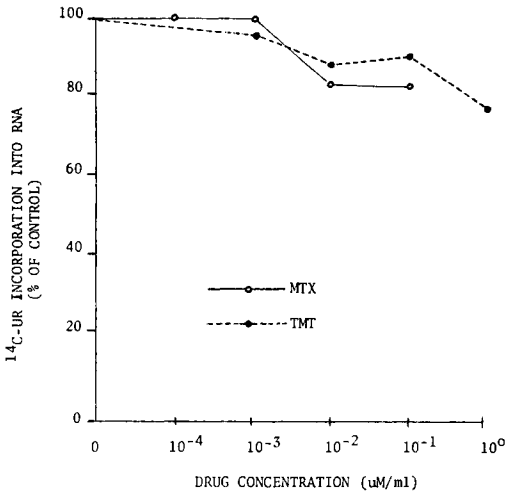

Fig. 5. In vitro effect of methotrexate (*MTX*) and triamterene (*TMT*) on uridine (*UR*) incorporation into RNA.

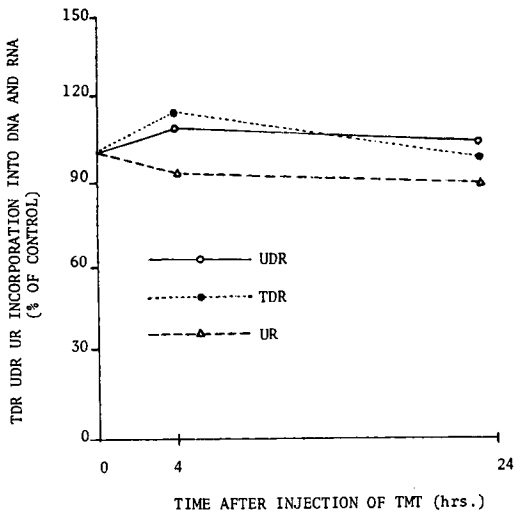

Fig. 6. In vivo effect of triamterene (*TMT*) on nucleic acid synthesis. Mice bearing L1210 leukemia received 40 mg. per kilogram of triamterene. At 4 hours and 24 hours after triamterene, mouse L1210 cells were studied for deoxyuridine (*UdR*), thymidine (*TdR*), and uridine (*UR*) incorporation into nucleic acids.

in slight inhibition of RNA synthesis only in concentration of 10^{-2} μM per milliliter or more.

In vivo (Fig. 6). There is no demonstrable triamterene effect on DNA and RNA synthesis of mouse L1210 leukemic cells after in vivo injection. At 4 hours and 24 hours, no significant changes of TdR and UdR incorporation into DNA were noted in either control or triamterene-treated mice. Minimal inhibition of RNA synthesis has resulted in triamterene-treated mice at both 4 hours and 24 hours after in vivo injection.

Discussion

Triamterene has been a widely used diuretic with a renal effect similar to that of spironolactone. This drug has been more consistently effective as a diuretic in cirrhosis of the liver than in congestive heart failure.[5] Recently reported cases of possible triamterene-induced megaloblastic anemia and the study of Corcino and associates on DNA inhibition in vitro of triamterene have brought attention to the use of this drug in patients who may have borderline folate stores, such as pregnant women and alcoholics.[4, 6] This raises the question of the advisability of triamterene use in cirrhosis of the liver as a diuretic. The study of Corcino and co-workers made use of an in vitro technique which may not have accurately represented the conditions prevailing during human clinical in vivo treatment.

Our in vitro study indicates that triamterene acts much like methotrexate in inhibition of UdR incorporation into DNA, although the degree of inhibition is less than that of methotrexate. This finding is compatible with triamterene interaction with an enzyme, DHFR (Fig. 2). In the de novo pathway, UdR is converted to thymidylate, in which process tetrahydrofolate is required. Tetrahydrofolate is formed from folic acid or dihydrofolate by the enzyme, DHFR. Under the influence of methotrexate, the enzyme, DHFR, is a limiting factor in the de novo pathway, since methotrexate combines with DHFR to form a pseudo-irreversible methotrexate-DHFR complex. Triamterene also interacts with DHFR to block the conversion of deoxyuridylate to thymidylate.[1, 4, 8] In our study, methotrexate induced a moderate stimulation of TdR incorporation into DNA in comparison with

slight inhibition after triamterene. This suggests that the effect of methotrexate is pharmacologically significant and reduces the effective pool size of deoxyuridylate, which in turn results in greater TdR utilization. However, the absence of increased TdR incorporation following triamterene suggests that the degree of pharmacologically important DHFR inhibition is much less with this drug. Neither triamterene nor methotrexate showed significant changes in RNA synthesis. In our in vivo study of mouse L1210 leukemic cells, no inhibition of UdR or TdR incorporation was noted in triamterene-treated mice compared with control mice. These studies indicate that the in vivo effect of triamterene is less apparent than that in vitro. This suggests rapid in vivo dissociation of drug-enzyme complex, which would be desirable for a diuretic nonmyelosuppressive action. Such dissociation is also suggested by the observation that the plasma of patients on a therapeutic level of triamterene did not inhibit DNA synthesis of mouse L1210 ascites leukemic cells.[2]

Megaloblastosis in vivo has been reported with several drugs, including cytosine arabinoside.[12] The mechanism of action is thought to be an inhibition of ribotide reductase,[3] a mechanism similar to that by which vitamin B_{12} deficiency is also considered to create in vivo megaloblastosis. Thus, the possibility that triamterene-induced megaloblastosis is due to mechanisms other than inhibition of DNA synthesis needs investigation.

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