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Triamterene-induced elevation of dihydrofolate reductase activity in human leukocytes*

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TRIAMTERENE,† 2,4,7-triamino 6-phenylpteridine (Dyrenium), was synthesized as a diuretic,[1, 2] but has many of the structural characteristics associated with inhibitors of dihydrofolate reductase.[3] When tested as an inhibitor of dihydrofolate reductase, an inhibition constant of 1×10^{-8} M was observed with enzyme from rat liver[4] or from Ehrlich ascites carcinoma cells.[5] The inhibitory effect of this drug on cell cultures of L5178Y resembled the pattern observed with methotrexate.‡ An antifolate activity of triamterene on *Streptoccoccus faecalis* was reported by Rosenthale and Van Arman.[6]

The administration of methotrexate was accompanied by an elevation of dihydrofolate reductase in leukocytes from patients with nonhematological neoplastic disease and in patients with chronic myelogenous leukemia.[7, 8] This effect has also been observed in erythrocytes from the guinea pig[9] and from the dog and man.[7, 10] The elevation in leukocytes of the dog was dose related, and in leukocytes from the human was not blocked by the administration of actinomycin D.[11]

The present study describes the elevation of dihydrofolate reductase in the leukocytes of five normal human subjects after the administration of triamterene, and compares this effect with the response to methotrexate in two patients with nonhematological neoplastic disease.

METHODS

After the removal of a control specimen of blood, 100 mg triamterene was administered orally three times a day for 7 days to 5 normal human volunteers. Leukocytes were isolated by the method of Fallon *et al.*[12] and assayed for dihydrofolate reductase as previously described.[13] Blood was drawn immediately prior to administration of methotrexate, 0·2, 0·5 or 0·8 mg/kg, when treatment coincided with enzyme study. Protein content of homogenates was assayed by the method of Lowry *et al.*[14]

RESULTS

An elevation of dihydrofolate reductase activity was observed in leukocytes of normal subjects receiving triamterene (Fig. 1). The enzyme activity rose rapidly between the third and eight day. On the eight day, approximately 16 hr after the last dose of triamterene, a greater than 20-fold increase of enzyme activity was observed in all subjects. With the exception of one subject, a drop in activity was observed on the third day after discontinuation of the drug. By the eleventh to fourteenth day, enzyme activity was in the range generally observed for normal leukocytes, i.e. 3 ± 5 mμmole tetrahydrofolate/hr/g protein.[8]

A dihydrofolate reductase activity of 80 m μmole tetrahydrofolate/hr/g protein was observed in a patient with carcinoma of the adrenal, who was receiving 100 mg triamterene daily. Twelve days after discontinuation of therapy, the enzyme level had fallen to 2 mμmole/hr/g protein.

Patients with chronic lymphocytic leukemia were less responsive to methotrexate-induced elevation of enzyme activity than patients with normal hematology.[8] From an initial pretreatment level of 2 mμmole/hr/g protein, the enzyme activity of leukocytes from a patient with chronic lymphocytic leukemia was elevated to 46 mμmole/hr/g protein after 2 weeks of 100 mg triamterene three times a day; after discontinuation of treatment the enzyme activity returned in 6 days to the pretreatment level.

The pattern of elevation of dihydrofolate reductase after the administration of methotrexate, 0·2, 0·5 or 0·8 mg/kg, to two patients with nonhematological neoplastic disease is presented in Fig. 2.

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‡ Glenn L. Fischer and S. C. Joseph, unpublished observations.

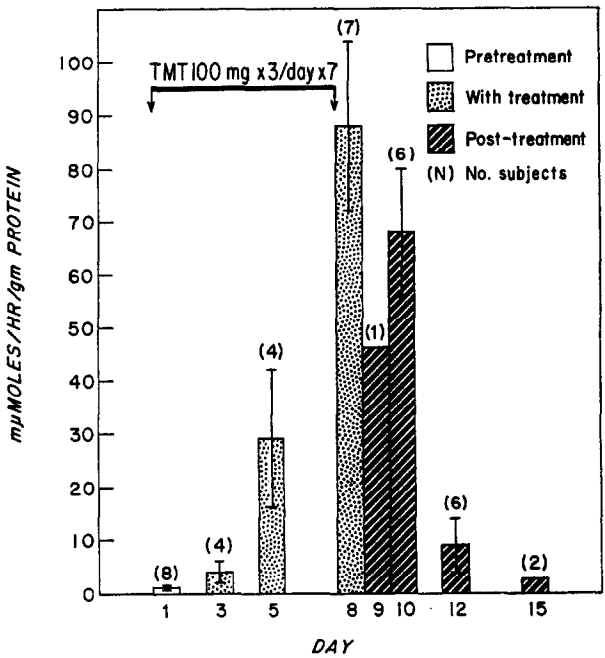

FIG. 1. The effect of triamterene on dihydrofolate reductase activity of human leukocytes from normal subjects.

The gaps in drug administration were required to allow for recovery from drug toxicity. Enzyme activity was elevated on the fourth day in both patients, and by 7 days reached the range of 30–40 mμmole tetrahydrofolate/hr/g protein. The reason for the delay in observing the elevation in patient B after the second course of therapy is unknown. The range of enzyme activity in patient B was the same after 0·2 mg/kg as with 0·8 mg/kg.

DISCUSSION

When triamterene was given according to the optimal clinical dosage schedule for a diuretic, a greater elevation of dihydrofolate reductase was observed with triamterene than with methotrexate given in usual clinical dose and schedule. Triamterene is a competitive inhibitor of dihydrofolate reductase,* and in the present study the extent of inhibition of the enzyme is not known. However, its inhibition *in vitro* of rat liver enzyme is approximately two magnitudes less effective than methotrexate. The molecular weights of the drugs are approximately the same and a dose of 300 mg triamterene is over 100 times greater than the daily oral dose of methotrexate. Although the drug is rapidly cleared from blood and leukocytes† and the blood samples were drawn 10 hr or more after the preceding dose of drug, these values must be considered a minimal estimate of the total enzyme activity. A negligible dissociation of the methotrexate–enzyme complex is considered to have occurred under the conditions of the assay,[8] and no estimate was made of the methotrexate–enzyme complex. The duration of the elevation of dihydrofolate reductase was much shorter after triamterene than after methotrexate.

The passage of 3–4 days with triamterene administration before elevation of enzyme activity suggests, as with methotrexate,[7] that triamterene acts on the marrow rather than on circulating leukocytes. The present evidence does not distinguish between a derepression of enzyme activity and a trapping of the enzyme by association with the drug which might interfere with enzyme turnover.[15] Methotrexate is cleared very slowly from the body,[16] and the prolonged elevation of enzyme activity may result from the trapping of enzyme activity by traces of the drug which remain in the body, or the prolonged elevation may be the result of the gradual release of a population of marrow cells which

* D. Roberts and T. C. Hall, unpublished observations.

† David Kessel, unpublished observations.

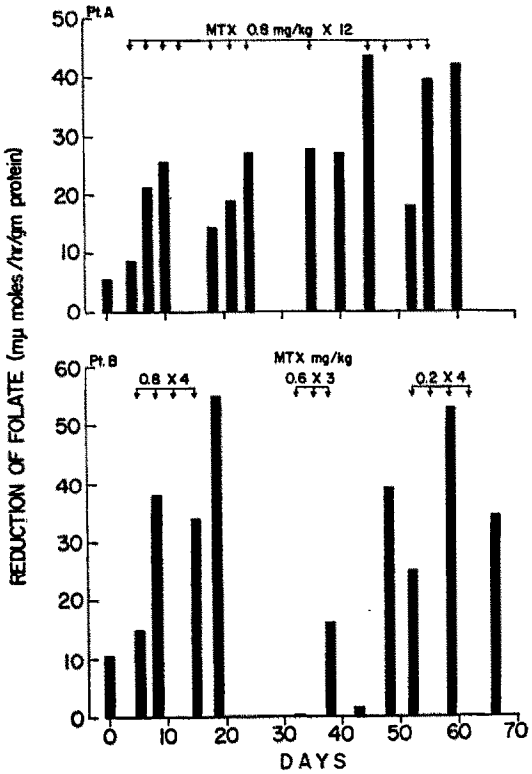

FIG. 2. The effect of methotrexate on dihydrofolate reductase activity of human leukocytes from patients with nonhematological neoplastic disease. Patient A had an undifferentiated carcinoma of the neck. Patient B had epidermoid carcinoma of the lung.

were altered by the drug. Rapid clearance of triamterene would possibly explain the more rapid return of dihydrofolate reductase activity to the normal range. If the elevation of enzyme activity occurs in the immature marrow cells, then the enzyme activity would be expected to remain elevated until the marrow had been cleared of the exposed sensitive cells, unless these cells had some method of inactivating the extra enzyme as dissociation of the drug occurred. The observations of normal or close to normal levels of enzyme activity 5 days after discontinuation of triamterene suggest that the cells do have a system, in addition to cell turnover, for reducing the level of elevated enzyme activity, or that the enzyme elevation occurs in that population of cells which are 5 days from discharge into blood from the marrow.

The observation of elevated levels of dihydrofolate reductase activity after the administration of triamterene indicates that the induction of this enzyme does not require a stoichiometric inhibitor[17] of the enzyme and may be studied in the human with a drug which is less toxic than methotrexate and under conditions where the recovery of normal levels of enzyme activity is more rapid than with methotrexate.

Triamterene, an inhibitor of dihydrofolate reductase, elevates the dihydrofolate reductase activity of normal human leukocytes. The greater elevation and shorter duration of the elevation of dihydrofolate reductase activity after triamterene is compared with the pattern observed with methotrexate administration.

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A colorimetric assay system for tetrahydrofolate dehydrogenase

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IT APPEARS that no chromogenic assay method for the demonstration of FH_4 dehydrogenase (EC 1.5.1.3) activity has so far been devised. Frequent reference has been made to the need for such a method which could possibly be applied to the demonstration of enzymic activity *in situ*.[1] This need arises because of the importance of the enzyme in cell replication and its association with the antifolate therapy of leukaemia.[2]

This study was carried out using the following systems:—

A. *A partially purified FH_4 dehydrogenase preparation, obtained from rat liver.* 5 g. Liver was homogenised in 0·1M Tris -HCl buffer, pH 7·4. This was then centrifuged at 100,000 *g* for 1 hr. Supernatant protein which precipitated at 40–80% saturation using solid ammonium sulphate was dissolved in 0·1M Tris-HCl buffer, pH 7·4, and then dialysed at 4° overnight. This preparation contained $NADPH_2$ diaphorase as well as FH_4 dehydrogenase activity. The sp. act. of the FH_4 dehydrogenase was 10 enzyme units/mg protein.[3]

B. *A highly purified FH_4 dehydrogenase preparation.* The partially purified FH_4 dehydrogenase preparation was further purified, according to the method of Mathews *et al.*[3] yielding a preparation with a sp. act. of 300 units/mg protein.

C. *Rat liver sections.* Tissue sections (15 μ in thickness) were cut from cylindrical blocks (6 mm dia.) of fresh frozen liver, using a Bright's Cryostat.