Methotrexate Levels in the Interstitial Space and Seminiferous Tubule of Rat Testis

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INTRODUCTION

Significant improvement in the therapy for acute lymphoblastic leukemia has increased the number of patients experiencing long-term remissions (12). With increasing survival, an increased incidence of localized testicular relapse has been reported to occur in up to 30% of patients (2, 4, 7, 9-11, 13-17) and is often followed by systemic relapse (9, 14-16). It is unknown why the testis represents a privileged site for leukemic relapse in the interstitium of the testes in boys with acute lymphocytic leukemia.

MATERIALS AND METHODS

Animals

Sexually mature male Wistar rats (Walter Reed strain) weighing between 380 and 540 g were used in these experiments. Sexually mature animals were chosen because tubular fluid cannot be obtained in those that are sexually immature. These animals were anesthetized with sodium pentobarbital (60 mg/kg i.p.) and cannulae were inserted into the contralateral jugular and femoral veins for blood sampling and drug infusion, respectively.

Intravenous Infusions

MTX was diluted with sterile water to a concentration of 1, 10, or 100 mg/0.5 ml. Following a priming dose of 1, 10, or 100 mg/kg, a constant infusion rate of 0.5 ml/hr of the appropriate solution was maintained utilizing a Harvard Model 940 infusion pump (Harvard Apparatus Co., Inc., Dover, Mass.). Six to 27 animals were used at each dose level.

Sampling

Plasma. At 1-, 2-, 3-, and 4-hr intervals from the start of infusion, 0.5 ml of heparinized blood was collected via the jugular vein cannula and promptly centrifuged. Aliquots of plasma were immediately placed in the dark and frozen at -28° until MTX concentrations were determined.

Testis. The testis was exposed by an incision of the scrotal sac and placed on a glass warming basin which maintained the testis at its normal temperature (32°) throughout the experiment. Seminiferous tubules were exposed by incision of the tunica albuginea. Tubular micropuncture was performed at x10 to 20 using a technique described previously which uses a glass micropipet sharpened to a diameter of 120 µm (18). A small drop of water-equilibrated mineral oil colored with Sudan black was injected to confirm the intraluminal location of the pipet tip. By aspirating fluid from 5 to 10 tubules, about 1 µl of tubular fluid can be collected over a period of 20 to 30 min. Tubular fluid samples were centrifuged at 12,000 x g for 30 min at 0° in a Beckman RC2B centrifuge to obtain a cell-free sample. The volume of each sample was measured, diluted with 0.9% NaCl solution up to 100 µl and stored in the dark at 20° prior to MTX assay. Interstitial fluid (the fluid between tubules that was devoid of RBC as determined by light microscopy) was obtained by aspirating from multiple extraluminal sites in areas different from those used for intratubular samples. One to 2 µl of interstitial fluid were obtained at each time point.

Peritoneal and Scrotal Fluid. In 4 separate experiments, the peritoneal and scrotal cavities were opened following 3 hr of MTX continuous infusion, and 5 µl of fluid were aspirated immediately.

MTX Assay

MTX concentrations were measured by the dihydrofolate reductase inhibition assay (1) in which the lower limit of sensitivity was 0.4 to 10-10 M.

RESULTS

The plasma MTX levels achieved with the 3 different doses are shown in Chart 1. A constant level was achieved in plasma at each dose level for the duration of the experiment.

A typical experiment at the 10-mg/kg/hr dose is shown in...
Chart 2. During the infusion, plasma MTX levels remained constant and were about 30 times higher than the simultaneously obtained tubular levels and 2 times higher than the interstitial levels.

The MTX levels achieved in the plasma, interstitial, and tubular fluids with the 3 different dose infusions are shown in Chart 3. Each column represents the mean ± S.E. of the data obtained at all time points throughout the infusion. Ten rats were studied at the 100-mg/kg/hr infusion rate, 27 rats were studied at the 10-mg/kg/hr infusion rate, and 6 rats were studied at the 1-mg/kg/hr infusion rate. At the 1-mg/kg/hr infusion rate, MTX levels were undetectable in the seminiferous tubule. The interstitial space MTX levels were one-half those of plasma. At the 10-mg/kg/hr infusion rate, the MTX levels were about 4-fold lower than plasma in the interstitial testicular fluid and 18 times lower than plasma in the seminiferous tubule. At the 100-mg/kg/hr infusion rate, a 4-fold decrease of MTX concentration was also found in the interstitial space and about a 50-fold decrease was found in the seminiferous tubule.

The MTX levels in the s.c. fluid of the scrotum and in the peritoneal fluid were evaluated after 3 hr at the 10-mg/kg/hr infusion rate in 4 animals and found to be $2.02 ± 0.02 \times 10^{-5}$ M and $1.75 ± 0.6 \times 10^{-5}$ M, respectively (Chart 4). These levels are indistinguishable from those obtained from the testicular interstitial space in animals given the same dose.

DISCUSSION

The existence of a physiological blood-testis barrier which prevents the entry of large-molecular-weight compounds into the seminiferous tubule has been well established both in animals and in humans (3, 6). Anatomically, this barrier is believed to comprise the tight junctions of the Sertoli cells.
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cross the blood-testis barrier. Using radioactive cyclophospha-
anineptical agent which has been analyzed for its ability to
exists is unclear. One or both of these barriers could potentially
modify the therapeutic efficacy and/or toxicity of the commonly
used chemotherapeutic agents. Cyclophosphamide is the only
antineoplastic agent which has been analyzed for its ability to
cross the blood-testis barrier. Using radioactive cyclophospha-
mainde injected directly into the spermatic artery and measuring
the radioactivity in the tubular fluid via direct micropuncture,
Forrest et al. (5) showed that it readily crosses the blood-testis
barrier. The significance of this finding is not clear since the
drug was injected into the testicular artery, thus probably by-
passing the hepatic conversion of cyclophosphamide into its
active form. However, the clinical observation that men treated
with cyclophosphamide have abolition of spermatogenesis sug-
gests that the active metabolites of cyclophosphamide cross
the blood-testis barrier (8).

However, in the present study, we have shown that, in
contrast to cyclophosphamide, a tubular barrier to MTX exists.
Drug concentrations were 18 to 50 times lower in the seminif-
erous tubules than in corresponding plasma levels.

Spread of leukemia into the seminiferous tubules is believed
to occur only in the late stages of leukemic infiltration into the
tests. In most cases, the leukemia is localized in the intersti-
tium. A pharmacological barrier between the blood and inter-
stitium has been proposed to explain this finding. However,
although we detected a substantial barrier to MTX at the level
of the seminiferous tubule, our data revealed only a 2- to 4-fold
decrease in the interstitial fluid MTX levels compared to plasma.
Notably, the peritoneum and scrotum, rare sites of leukemic
relapse, had levels of MTX that were similar to those of the
interstitial space of the testsis.

Therefore, it does not appear that a unique pharmacological
barrier for MTX exists between the blood and interstitium in the
tests. If these results can be extrapolated to humans, the
leukemic infiltration which occurs in the testes does not appear
to be due to a pharmacological barrier between the plasma and
the interstitial space.

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