Distribution and Elimination of Trimethoprim in Pregnant and Newborn Rats

R. SCHULZ*

Institut für Veterinär-Pharmakologie und -Toxikologie der Freien Universität Berlin

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Summary. After a single i.v. administration of 50 mg/kg of Trimethoprim (TM) to rats in late pregnancy, a diffusion equilibrium of the folic acid inhibitor in the foetal organs is reached after 30--60 min. The TM levels in maternal as well as in foetal organs can have an inhibitory effect on folic acid reductase activity.

The elimination of TM is lower in pregnant than in non-pregnant animals. The TM elimination of the foeti is dependent on the elimination capacity of the mother.

Three-days-old rats eliminate TM some four times slower than mature animals owing to their reduced kidney development.

Key words: Trimethoprim — Placental Transfer — Distribution — Elimination — Rat.

Trimethoprim (TM)\(^1\) influences the dihydrofolate reductase in Escherichia coli to a considerably greater extent than it does the corresponding enzyme in rat liver. Whereas the rat liver enzyme is only inhibited to 50\(^\circ\) in the presence of a TM concentration of \(2.6 \times 10^{-4}\) M, the enzyme derived from E. coli is already inhibited to this degree in the presence of only \(5 \times 10^{-9}\) M TM (Hitchings, 1969).

In spite of the poor affinity of TM for folic reductase of animal origin, the administration of TM to the mother during pregnancy or to the newborn during the first weeks of life, is contraindicated. This recommendation is based on the findings of Thiersch (1963) and Udall (1969) who reported a high prenatal death rate and foetal malformations following oral administration of large doses of TM to pregnant rats and rabbits.

Hitherto, nothing has been known concerning the placental transfer of TM. It is important to know further the manner in which this folic acid antagonist is distributed throughout the foetal organs. It is also necessary to clarify whether or not a sufficiently high concentration of TM is attained in the foetal organs to achieve an inhibitory effect on folic acid reductase, and how long TM is retained in the organism.

* Present address: Stanford University, Medical Center, Stanford, Calif. 94305, U.S.A.

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Methods

1. Experimental Procedure

The studies were conducted on female conventional Wistar rats weighing between 280—300 g, provided by Messrs. Bruenger (Halle, Westfalen). The animals received 0.1 ml of Proluton® (1 ml = 10 mg Progesterone) i.m. on three consecutive days. Following the gestagen administration, the animals were paired singly with males for a period of 12 h. The first day of pregnancy was defined as the succeeding 24 h.

On the 20th day of pregnancy they were each given 50 mg/kg TM as a 5%/ solution (pH 6.5) by injection into the saphenous vein. At specific times following the injections, the pregnant animals were decapitated and their blood collected. The placentae and foeti were then removed as rapidly as possible. The foeti were decapitated, the blood collected and the organs, as well as these of the mother, subjected to TM quantitative analysis.

Analogous to this determination, a number of non-pregnant rats, as well as 3-days old animals, were given 50 mg/kg TM and were also sacrificed at specific times and blood and organs analysed.

For the evaluation of these findings, the half-lifes ($t_{1/2}$), and the elimination constants ($k_{e}$) in the serum and organs as well as the serum distribution coefficient ($A'$) of the TM were determined. In addition, the distribution quotient of TM throughout the extravascular tissue at any time following diffusion equilibrium was determined by the method of Dost (1968).

The Mann-Whitney Test (U-Test) was used to compare two independent samples.

2. Determination of TM in Serum and Tissues

TM concentrations in serum and in various organs were measured by the slightly modified method of Schwarz et al. (1969), using a Zeiss Spectrofluorometer (ZF 46). In nearly all instances there was sufficient material for duplicate determinations to be made.

8 ml of a 0.1 M Na$_2$CO$_3$ solution and 10 ml of chloroform were pipetted into 0.5—1.0 g of the tissue, which was then homogenized with an Ultra-Turrax homogenizer in an ice-bath for extraction of the TM. 0.5—1.0 ml of serum was also treated with the Na$_2$CO$_3$ solution and chloroform, and shaken for 10 min. After centrifugation of the samples, the aqueous layer was rejected and 6 ml of the chloroform phase were shaken for 10 min with 4 ml 0.01 N H$_2$SO$_4$ and briefly centrifuged. 3 ml of the sulfuric acid extract were then incubated with 2 ml of an alkaline KMnO$_4$ solution (0.1 M KMnO$_4$ into 0.1 N NaOH) for 15 min at 60°C in a water bath. 0.3 ml of a 35%/ formaldehyde solution were then pipetted into each sample. The samples were kept in the water bath until completion of the reduction. After cooling to room temperature the resulting trimethoxy-benzoic acid (TMBA) was extracted into 2 ml chloroform by shaking for 10 min. Its fluorescence was measured setting the activation wavelength at 275 nm and the fluorescence wavelength at 350 nm (layer 1 cm thick). TMBA in chloroform was used as the standard in concentrations equimolar to 10 and 50 μg of TM per ml. These standard solutions were used for standardisation between values of 0.5—10.0 μg TM/ml and 10—50 μg TM/ml respectively. Concentrations higher than 50 μg per ml inhibited the fluorescence.

Preliminary experiments have shown that this procedure resulted in a general 5%/ substrate loss determined by addition of TM to the assayed tissues.

Additional investigations served to clarify the questions whether, as a result of the preparatory method used, TM metabolites were interfering with the TM mea-