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Hypothesis

Methotrexate Hepatotoxicity

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An inhibitor of folate metabolism, amethopterin (methotrexate) has been successfully used in the treatment of psoriasis and neoplastic disease. This drug produces several dangerous side effects of both an acute and chronic nature which have widely curtailed its use. A serious chronic side effect of the drug is its hepatotoxicity, which may culminate in hepatic cirrhosis and death. To date the underlying mechanism of methotrexate in producing liver damage is unknown. Results of three studies conducted in this laboratory on the nutritional effects of methotrexate offer some evidence that the hepatotoxicity may possibly be incurred through the effect of the drug on methionine biosynthesis and methylation processes. This thesis is discussed in the light of methylating agents vital to the synthesis of methionine.

Key words: hepatotoxicity, methionine, folate, betaine, methotrexate, homocysteine

Methotrexate (amethopterin) is a folate antagonist that has been widely used in cancer chemotherapy [1–5] and in the treatment of psoriasis [6–8]. This substance, owing to its structural similarity to folic acid, binds to active sites on the enzyme dihydrofolate reductase and hence by competitive inhibition blocks the formation of folate coenzymes needed in the biosynthesis of DNA and RNA. Benefit from the drug appears to result from the inhibition of nucleic acid synthesis, as is apparent also in its restriction of rapidly growing normal tissue.

Methotrexate (MTX) causes both acute and chronic side effects, which have limited its clinical use in humans. Some of the early signs of MTX toxicity are characterized by dermatitis, loss of hair, defective spermatogenesis, and fatigue, but the most feared are bone marrow depression and gastrointestinal bleeding and ulceration.

The major chronic side effects of MTX administration reported in humans are nephrotoxicity and hepatotoxicity, the latter being the principal effect [9]. Although it is realized that MTX does not selectively attack the liver, long-term administration of this drug promotes fatty infiltration, fibrosis, and cellular necrosis, and may culminate in hepatic cirrhosis [10–13]. Despite all that is known about MTX and its effect on

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folate mechanism and nucleic acid synthesis, the underlying mechanism by which this drug induces liver damage is still unknown.

It has recently been pointed out by Roenigk et al [14] that MTX-induced hepatotoxicity is influenced by preexisting toxic or metabolic disease. For example, arsenic exposure and alcohol abuse, as well as diabetes mellitus or renal impairment, increase the risk factor of MTX administration.

Recent reports in the literature suggest that chronic MTX toxicity may be related to the depletion of tissue folates. Barford et al [15] have demonstrated that in MTX-treated rats liver folate levels are decreased and urinary and fecal losses are increased. Also, Kamen et al [16] measured levels of MTX and folates in liver biopsy and red blood cells of a psoriatic patient receiving MTX and in MTX-treated children with leukemia. These workers showed that MTX polyglutamates accumulated in the liver and red blood cells with MTX treatment and the folate polyglutamates were depleted. Both of these studies indicate strongly that MTX and folate compete for mechanisms of storage in cells. If this is true, and MTX dominates in the competition, it could lead to impairment of folate-dependent biosynthetic reactions.

In recent years, work in this laboratory has proceeded on the premise that, if MTX interferes with folate-dependent one-carbon metabolism in the liver, damage to this organ might be a consequence of the lack of certain vital lipotropes normally synthesized by way of one-carbon metabolism. In addition to their role in nucleic acid formation, tetrahydrofolic acid coenzymes function in one-carbon transfers which are essential for methyl group generation (methylneogenesis) needed in the formation of lipotropes—ie methionine, choline, and S-adenosylmethionine (SAM). Since Mudd and Poole [17] have shown that the endogenous formation of these methylated compounds must normally exceed the dietary intake of labile methyl groups to meet body requirements, this process is essential to liver membrane structure and function [18].

In the total scheme of methionine metabolism, the conversion of homocysteine to methionine is a highly important reaction from the standpoint of conserving methionine, detoxifying accumulated homocysteine, and the production of SAM, the principal methylating agent in the various vital transmethylation reactions. The biochemical step between homocysteine and methionine occurs through two reactions [19]. In the first reaction, through the medium of 5-methyltetrahydrofolate-homocysteine methyltransferase, a methyl group is transferred from 5-methyltetrahydrofolate to vitamim B_{12} to form methylcobalamine. Methylcobalamine then transfers the methyl group to homocysteine to form methionine. In the second reaction, a methyl group is transferred from betaine to homocysteine to form methionine through the medium of betaine-homocysteine methyltransferase. Finkelstein et al [20] have suggested that the folate reaction is the primate reaction in the maintenance of methionine levels in tissue. Hence, if MTX impairs folate-dependent one-carbon metabolism and production of 5-methyltetrahydrofolate, it is entirely feasible that MTX hepatotoxicity may be a consequence of an imposed lipotrope deficiency due to slowed biosynthesis of methionine.

A past study [21] conducted in this laboratory using the rat as the experimental model has lent support to this hypothesis. This work tested the effect of MTX administration on hepatic fatty infiltration in rats fed a lipotrope-deficient diet and the effect of MTX administration on similarly fed animals when the diet was individually supplemented with the lipotropes methionine, choline, and vitamin B_{12}. Control

animals were fed their respective diets for 10 days while the experimental animals were injected with MTX at the level of 0.1 mg/kg/day for the same period while consuming the same diets. After the animals were killed, hepatic triglyceride and cholesterol ester levels were measured as an index of liver injury. Triglycerides and cholesterol esters were highly elevated in the rats fed the basal diet and considerably lower in the diets supplemented with choline, methionine, and vitamin B_{12}. In those animals receiving MTX along with the lipotropes, the drug did not effect the triglyceride and cholesterol ester levels in the livers of animals supplemented with either choline or methionine, but the drug negated the lipotropic effect of vitamin B_{12}. The results of these studies indicate that MTX does not interfere with the lipotropic action of methionine or choline, which are products of one-carbon metabolism beyond involvement of folate. The fact that MTX blocks the lipotropic effect of vitamim B_{12} would suggest that this drug exerts its deleterious effects on the folate-dependent reaction involved in the methylation of homocysteine in the de novo synthesis of methionine, where B_{12} plays a vital role. This probably occurs through the lack of production of 5-methyltetrahydrofolate, the prime methylating agent for homocysteine in the reaction.

A recent study [22] in this laboratory sought to determine whether MTX administration affected the hepatic pool size of 5-methyltetrahydrofolate. In that study male rats were fed a complete rat diet for 4 months during which time the experimental rats were injected intraperitoneally daily with MTX at a dose of 0.1 mg/kg. Control rats were injected with saline. During the experimental period, the drug did not influence the growth rate of the animals, nor did it cause any fatty changes in the liver despite the fact that it inhibited hepatic dihydrofolate reductase by 90%. Hepatic levels of 5-methyltetrahydrofolate were reduced by an average of 52% by treatment with the drug; the pool size of betaine, the secondary methylating agent utilized in methionine biosynthesis, was decreased by 25%. In another study [23] it was shown that doubling the dosage of MTX to 0.2 mg/kg produced an even greater lowering of hepatic betaine (39.5%) in a quarter of the time. These data suggest that betaine may be used by the liver to compensate for the loss of 5-methyltetrahydrofolate when folate metabolism is antagonized.

Although the above studies were conducted on the rat, they offer some clues as to the reasons for the hepatotoxic side effects of MTX. In the presence of the drug, preformed methionine appears to perform its function in methylation processes. According to these studies, as well as those of Freeman-Narrod et al [9], choline also retains its lipotropic activity in the presence of MTX.

However, because of the drug-induced lack of 5-methyltetrahydrofolate and subsequent incapacity of vitamin B_{12} to function as a lipotrope with MTX, it is feasible that the hepatotoxicity of this drug may be at least partially implemented through impairment of methylneogenesis and methionine biosynthesis. Since Mudd and Poole [17] have emphasized the importance of methylneogenesis in meeting methylation requirements, these studies have shown that the one salving feature in rats, not seen in humans, is the ease in converting choline to betaine [24]. Hence when challenged with MTX, the rat may overcome the lack of methylneogenesis by involving betaine-homocysteine methyltransferase and produce needed methionine through betaine utilization. Since studies by Freeman et al [25] indicate that betaine-homocysteine methyltransferase is active in human liver, it is possible that humans do have the ability to utilize betaine. This fact may prove to be significant in the future

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