MECHANISM OF THIOAMIDE ANTITHYROID DRUG ASSOCIATED HYPOPROTHROMBINEMIA

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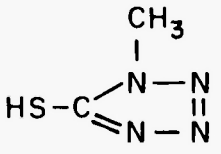
SUMMARY

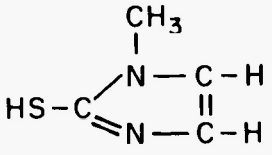
The thioamide class of antithyroid drugs has been associated with the development of hypoprothrombinemia. Two drugs in this class, propylthiouracil and methimazole, resemble the methyltetrazole-thiol leaving group of certain cephalosporin antibiotics. Both were found *in vitro* to inhibit the vitamin K dependent step in clotting factor synthesis, the gamma-carboxylation of glutamic acid with 50 per cent inhibitory concentrations of 2 mM for propylthiouracil and 0.1 mM for methimazole. Methimazole was also found to inhibit vitamin K epoxide reductase, an enzyme related to the carboxylation reaction, with a 50 per cent inhibitory concentration of 25 uM. *In vivo* methimazole, administered twice at a dose of 500 mg/kg to rats on a vitamin K deficient diet produced hypoprothrombinemia. These results suggest that the mechanism of hypoprothrombinemia associated with thioamide antithyroid drugs may be similar to the mechanism of hypoprothrombinemia associated with cephalosporins which contain the methyltetrazole-thiol leaving group.

I. INTRODUCTION

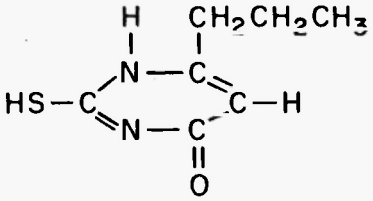
The thioamide class of antithyroid drugs, which includes propylthiouracil and methimazole, has been occasionally associated with the development of hypoprothrombinemia in humans /1-5/. The mechanism of this adverse effect has not been elucidated. Recently, the methyltetrazole-thiol (MTT) group, which is structurally similar to these antithyroid drugs (Figure 1), has been implicated in the production of hypoprothrombinemia associated with antibiotics which contain this moiety. The MTT group has been shown *in vitro* to inhibit the vitamin K-dependent step in clotting factor synthesis, the gamma-carboxylation of glutamic acid /6,7/, and *in vivo* to inhibit a related step in vitamin K metabolism, the reduction of vitamin K epoxide /8/. Additionally MTT has been shown to produce hypoprothrombinemia when administered to the rat /9/. Since methimazole and propylthiouracil are structurally similar to MTT, the potential of these drugs to inhibit the gamma-carboxylation of glutamic acid as well as the reduction of vitamin K epoxide *in vitro* was examined. Additionally, the ability of methimazole to produce hypoprothrombinemia in the rat was determined.



1-Methyltetrazole-5-thiol



Methimazole



Propylthiouracil

Fig. 1: The structures of methimazole, propylthiouracil, and methylthiotetrazole.

II. MATERIALS AND METHODS

2.1 Carboxylation Assay

Rat liver microsomes were prepared and the carboxylation of glutamic acid was determined by the method of Houser /10/ with the previously described modifications /7/. In brief, reactions were run using a microsomal system in 25 mM imidazole and 250 mM sucrose buffer, pH 7.4, which contained the substrates: L-phenyl-alanyl-L-leucyl-L-glutamyl-L-glutamylisoleucine, 1.45 mM, and 10 uCi of $NaH^{14}CO_2$ (43 mCi/mM; final concentration, 0.4mM); 500 ul of microsomal preparation, and 2 mM NADH. Ten microliters of drug

were added at the concentration given in the text to give a final volume of 580 ul. The pentapeptide, NADH and drugs were added as solutions in the imidazole buffer. Reactions were initiated by the addition of 25 ug of vitamin Kl, phylloquinone (used as Aquamephyton, Merck Sharp and Dohme), and terminated 30 minutes later by the addition of trichloroacetic acid. Following centrifugation, the supernatant solution was flushed with unlabeled CO_2 and the incorporation of radioactive carbon dioxide into the pentapeptide was determined by liquid scintillation counting of an aliquot of the supernatant solution. In vitamin K reversal experiments 28 nanomoles of vitamin Kl were added at the start of the incubation and an additional 28 nanomoles were added at fifteen minutes later.

2.2 Vitamin K epoxide reductase assay

The activity of vitamin K epoxide reductase was assayed in a rat liver microsomal fraction which was prepared by a modification of the method which is described above. After the first 100,000 g centrifugation the resulting pellet was homogenized in buffer which contained 8.0mM 3-[(3-cholamidopropyl)-dimethylammonio]-l-propanesulfonate (CHAPS) and was diluted with this buffer to a final concentration of 10mg protein/ml. The activity of vitamin K epoxide reductase was assayed by a modification of the method of Wallin and Martin /11/ in the microsomal preparation which contained 5.0mg of microsomal protein, and 40 uM vitamin K epoxide in ethanol which had been prepared by the method of Tischler, et al. /12/. The reaction was initiated by the addition of dithiothreitol (DTT) to give a final concentration of 0.1 mM. Incubations were carried out at 21°C for 30 minutes in the presence of various concentrations of methimazole. The reaction was terminated by the addition of 2 ml of a 1:1 isopropanol:hexane mixture. Tubes were then vortexed for 20 seconds and centrifuged for 5 min at 1300g. One ml of the resulting upper layer was removed and evaporated under a nitrogen atmosphere. The resulting solid was redissolved in 0.1 ml of methanol and was stored at -70°C until analysis could be performed. Vitamin K-epoxide reductase activity, defined as the degree of conversion of vitamin K epoxide to vitamin K quinone, was measured with a C-18 HPLC column with methanol as the mobile phase.

2.3 In Vivo Studies

The rat model used to test the ability of methimazole to produce hypoprothrombinemia was developed as a modification of a previously described method /9/. Two month-old Sprague-Dawley rats, housed in cages designed to prevent coprophagy, were maintained on vitamin K-deficient rat chow (catalog no. 960037, ICN Pharmaceuticals Inc. Cincinnati, Ohio) for 10 days before receiving drug. On day 11 two doses of methimazole 500 mg/kg was administered intraperitoneally eight hours apart. This large dose was used because it had been determined that large doses of MTT were required to produce hypoprothrombinemia in the rat /9/. Control animals received sterile saline intraperitoneally. Prothrombin times on blood obtained from cardiac puncture were measured on day 11 just prior to the first dose of methimazole and again on day 13.

III. RESULTS

The potential of methimazole and propylthiouracil to inhibit the gamma-carboxylation of glutamic acid in a microsomal system was examined, and compared to the inhibitory effect of MTT in the same system. Both methimazole and propylthiouracil were able to inhibit the carboxylation system (Figure 2). The fifty per cent inhibitory concentration for methimazole was approximately 0.1 mM as compared to 0.7 mM for MTT and 2mM for propylthiouracil. Previous studies with MTT indicate that the inhibition of the carboxylation system is not reversible by vitamin K /7/. Therefore we questioned whether the inhibition by methimazole was reversible by vitamin K. In the presence of 0.1 mM methimazole, an additional complement of 28 nanomoles of vitamin K added fifteen minutes into the incubation, resulted in 79 per cent inhibition. Control experiments in which additional vitamin K was not added, inhibition was 75 per cent. Thus the inhibition was not reversed by vitamin K.

The ability of methimazole to inhibit vitamin K epoxide reductase is shown in Figure 3. Methimazole was found to be a potent inhibitor of this enzyme with a fifty per cent inhibitory concentration of approximately 25 micromolar.

The administration of methimazole to rats resulted in the development of hypoprothrombinemia within 48 hours after ad-

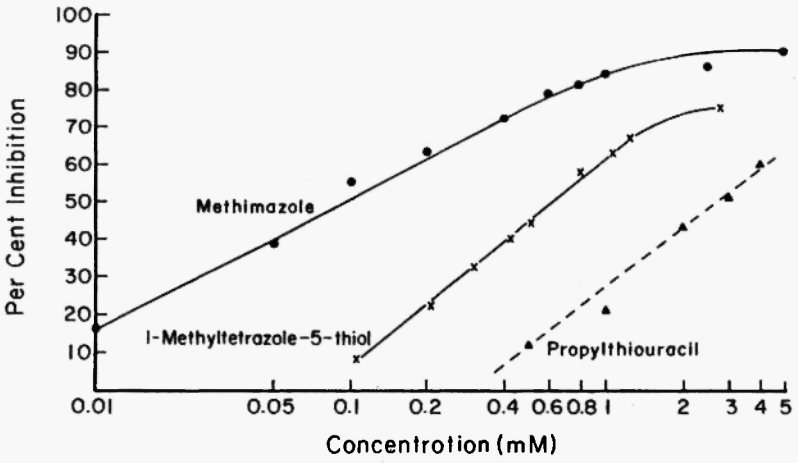


Fig. 2: Dose response of the inhibition of the gamma-carboxylation of glutamic acid by methimazole, propylthiouracil, and methyltetrazole-5-thiol. Incubations were carried out in the microsomal system as described in the text.

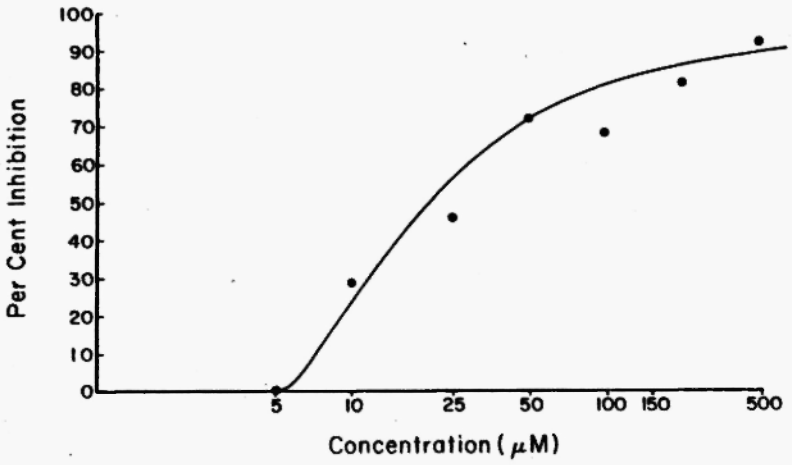


Fig. 3: Dose response of the inhibition of vitamin K epoxide reductase by methimazole. The conditions of the incubations are described in the text.

TABLE 1

Effect of Methimazole on the Prothrombin Time

	Prothrombin Time (seconds + SD)	
Day	Control	Methimazole
11	16.1 ± 1.3 (n=12)	16.2 ± 1.8 (n=12)
12	17.8 ± 6.1 (n=12)	27.0 ± 6.2* (n=6)[1]

* Statistically significantly different from control ($p < .01$)

[1] Six animals died, of five rats which were examined, all had evidence of bleeding into the intestines

ministration (Table 1). Control animals who received water did not show a prolongation of the prothrombin time. In addition, three animals in the methimazole group died with signs of intestinal hemorrhage before prothrombin times could be obtained.

IV. DISCUSSION

Although hypoprothrombinemia has been recognized as a consequence of the administration of antithyroid drugs, the mechanism underlying this effect was unknown. The results of the studies described in this report now suggest a mechanism for this effect, that is, that the thioamide antithyroid drugs have the ability to inhibit the vitamin K dependent step in clotting factor synthesis, the gamma-carboxylation of glutamic acid. Since the structurally similar compound, MTT, has also been shown to inhibit this reaction /6,7/, it is likely that the affected steps leading to the hypoprothrombinemia associated with MTT containing-antibiotics and antithyroid drugs are the same.

Methimazole was also found to inhibit vitamin K epoxide reductase. Inhibition of this enzyme may also lead to hypo-

prothrombinemia in that this enzyme is part of a metabolic cycle of vitamin K. Vitamin K is ingested in the quinone form. In order to serve as a cofactor in the carboxylation reaction, vitamin K must be reduced to the hydroquinone form. During the carboxylation reaction, vitamin K epoxide is formed. The epoxide is then reduced to the quinone form to complete the cycle. Since the body stores of vitamin K are low /13/, the cycle must be intact in order for the carboxylation reaction to continue. Therefore inhibition of the reductase by methimazole would prevent the cycling of vitamin K and lead to an inhibition of the gamma-carboxylation of glutamic acid and subsequent hypoprothrombinemia. Whether inhibition of the reductase or the direct inhibition of the carboxylation reaction is the primary cause of the clinical hypoprothrombinemia observed with methimazole is not known at the present.

However the finding that the direct inhibition of the gamma-carboxylation of glutamic acid by methimazole was not reversed by vitamin K might indicate that this effect of methimazole may be the cause of the hypoprothrombinemia. These results may provide for an explanation of the clinical observation which was noted in the first case report of propylthiouracil associated hypoprothrombinemia, that is, that the hypoprothrombinemia was not rapidly corrected by the administration of vitamin K /1/. Subsequent case reports have confirmed this finding. However there are not enough reported cases of hypoprothrombinemia with antithyroid drugs to be certain if this is always the case.

Only rarely do patients develop hypoprothrombinemia on antithyroid drugs. Therefore certain risk factors must be involved which make a patient more or less susceptible to this side effect. The requirement for certain risk factors in the development of hypoprothrombinemia on MTT-containing antibiotics has been noted /14/. These include decreased renal function, lack of oral intake of food, cancer, and the post operative state. It would be of interest to determine what these factors are for the antithyroid drugs and whether or not they are similar to those for the antibiotics.

V. ACKNOWLEDGEMENTS

We wish to thank Michael D. Hall and Svetlana Zalivansky for technical assistance. Dr. Lipsky is a Burroughs Wellcome Scholar in Clinical Pharmacology. This work was supported in part by a gift from Hoechst-Roussel Pharmaceuticals and NIH grant GM 37121. Experiments were performed in the Alan Bernstein Laboratories of Clinical Pharmacology.

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