The Effects of Trimethadione and Its Metabolite on Human Liver Debrisoquine 4-Hydroxylase Activity In Vitro

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Abstract: The effects of trimethadione (TMO) and its metabolite, dimethadione (DMO), on human liver microsomal debrisoquine 4-hydroxylase (cytochrome P-450db1) activity were studied. Inhibition curves showed that there is no significant decrease in the activity of debrisoquine 4-hydroxylase by TMO or DMO (0.1 μM to 10 mM). These data suggest that debrisoquine 4-hydroxylase in human liver is not responsible for N-demethylation of TMO, nor are TMO or DMO bound to its active site.

Key words: trimethadione, debrisoquine 4-hydroxylase, human liver

Introduction

TMO is extensively metabolized to DMO by cytochrome P-450 dependent monooxygenase and is almost completely absorbed from the gastrointestinal tract when given orally. Neither TMO nor DMO is bound to plasma protein. From these properties we used the serum concentration ratio, DMO/TMO, as an index of hepatic drug oxidizing capacity in human1-3). Nevertheless, we do not know the isozyme of P-450 that is responsible for demethylation of TMO in human.

Debrisoquine 4-hydroxylase is a well-known isozyme of P-450 in humans. About 10% of the Caucasian population has an inherited deficiency of 4-hydroxylation of debrisoquine as the debrisoquine polymorphism4,5) and these poor metabolizers are at high risk to develop adverse responses to such drugs as β-blocker, antiarrhythmics, antidepressants, hallucinogens, opioids and others6). To avoid the clinical risk of these drugs, the urinary excretion ratio of 4-hydroxydebrisoquine to debrisoquine after oral administration of debrisoquine has been used to determine debrisoquine polymorphism in humans8). We have previously studied the activities of both TMO N-demethylase and debrisoquine 4-hydroxylase in vivo in humans after simultaneous oral administration of TMO and debrisoquine.

In the present study we investigated the possibility that TMO or DMO affects the activity of debrisoquine 4-hydroxylase in human liver in vitro, since, if TMO can be catalyzed to DMO by this P-450 isozyme, or if TMO or DMO can bind to an active site of debrisoquine 4-hydroxylase, its activity might be inhibited by addition of TMO or DMO in vitro. Additionally, the activity of TMO demethylase in human liver in vitro has not yet been determined.

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Materials and Methods

Debrisoquine hemisulfate and 4-hydroxydebrisoquine hemisulfate were generously provided by Roche Products Ltd. (Welwyn Garden City, England). Nonadueto-4-hydroxydebrisoquine was prepared biologically as described previously. Hexafluoroacetylacetone was purchased from Fluorochem Ltd. (Glosso, England). TMO was purchased from Dainippon Pharmaceutical Co., Ltd. (Osaka, Japan) and DMO from Tokyo Kasei (Tokyo, Japan). As previously reported, human liver samples, each of which was judged from its debrisoquine 4-hydroxylase activity in vitro to be an extensive metabolizer phenotype were obtained from renal transplant donors. The Local Ethics Committee (Hammersmith Hospital in London) granted permission for the use of such sample in these studies. Microsomal fractions from human liver samples were isolated by differential centrifugation and stored at -80°C as suspensions in 0.25 M potassium phosphate buffer, pH 7.25, containing 30% (v/v) glycerol until required. Debrisoquine 4-hydroxylase activity, as assayed by a modification of our previously described method, is shown in Fig. 1. In the present study, aqueous solutions of TMO or DMO to give final concentrations of 0.1 μM to 10 mM were freshly prepared on the day of use. Samples were preincubated for 2 min at 37°C in the presence of TMO or DMO before addition of the substrate, debrisoquine.

Results

Curves of inhibition of human liver microsomal debrisoquine 4-hydroxylase by TMO and DMO are shown in Fig. 2. Debrisoquine 4-hydroxylase activity was not inhibited from control values by TMO or DMO at concentrations in the range of 0.1 μM to 1 mM. However, at the concentration of 10 mM DMO, this activity was inhibited by about 30% from the control value (not significant), whereas TMO did not inhibit the activity even at this concentration.

Fig. 1. Determination of the activity of debrisoquine 4-hydroxylase.
Fig. 2. Inhibition curves of human liver microsomal debrisoquine 4-hydroxylase activity by TMO and DMO. TMO (●), DMO (○). Values plotted are activity, expressed as a percentage of control activity in the absence of TMO or DMO, of debrisoquine 4-hydroxylase in the presence of the indicated concentrations of TMO or DMO. Values are mean±S.D. of two different human samples determined in duplicate.

Discussion

TMO metabolism in rat liver was not changed significantly by 3-methylcholanthrene, which might increase the content of cytochrome P-448, and TMO was metabolized mainly by the cytochrome P-450 system\(^1\). In our previous study\(^1\), the serum ratio, DMO/TMO, was increased by phenobarbital and decreased by CCl\(_4\), in rat. Also the serum DMO/TMO was increased by phenobarbital treatment (unpublished data) in humans. Among the P-450 gene families\(^12\), the P-450 IIB and IIC subfamilies were indicated to be the most phenobarbital-inducible, and debrisoquine 4-hydroxylase was indicated to be the P-450 IID subfamily. TMO metabolism in both human and rat liver was increased significantly by phenobarbital, therefore, the P-450 II gene family presumably catalyzes N-demethylation of TMO. In the present study, no significant inhibition of debrisoquine 4-hydroxylase activity by TMO or DMO was observed in the concentration range of 0.1 μM to 10 mM. When we used 4 mg/kg TMO as an index to assess the capacity of the mixed-function oxidase system \textit{in vivo} in humans, the plasma concentration (C\(_{\text{max}}\)) of TMO and DMO usually reached 6.0 μg/ml and 12.8 μg/ml\(^1\), respectively. These plasma concentrations of TMO and DMO were calculated to be approximately 50 μM and 100 μM, respectively; these concentrations did not indicate inhibition of human liver debrisoquine 4-hydroxylase activity in this study. The effective clinical serum concentration of DMO was usually above 700 μg/ml\(^13\), a concentration calculated to be about 5.4 mM which seems to slightly inhibit debrisoquine 4-hydroxylase activity. At the highest concentration used in the present study, 10 mM DMO, a concentration calculated to be 1.3 mg/ml which is almost toxic, debrisoquine 4-hydroxylase activity was inhibited by as much as 30%.
Therefore, we conclude that neither TMO nor DMO inhibits the activity of debrisoquine 4-hydroxylase in human liver, that debrisoquine 4-hydroxylase is not responsible for N-demethylation of TMO, and neither TMO nor DMO is bound to its active site. Further studies are required to determine the isozyme of cytochrome P-450 which catalyzes N-demethylation of TMO, and whether or not debrisoquine affects the active site of TMO N-demethylase in human liver microsome.

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References


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