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TITLE: Role of Sulfation Pharmacogenetics in Breast Cancer Treatment with 2-Methoxyestradiol

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Role of Sulfation Pharmacogenetics in Breast Cancer Treatment with 2-Methoxyestradiol

2-ME<sub>2</sub> is an endogenous estrogen metabolite that inhibits the proliferation of breast and other human cancer cell lines. 2-ME<sub>2</sub> also has potent anti-angiogenic and anti-tubulin properties, and it may inhibit estrogen-induced carcinogenesis in the mammary gland. We set out to test the hypothesis that 2-ME<sub>2</sub> might be a substrate for sulfate conjugation and, therefore, that individual variations in the sulfation of 2-ME<sub>2</sub> might contribute to individual differences in its metabolism, pharmacokinetics and therapeutic efficacy. As a first step, we tested 2-ME<sub>2</sub> as a substrate for 7 human sulfotransferase (SULT) isoforms -- as well as all of the common allozymes for SULT1Al and 1A2. Substrate kinetic studies were conducted in two stages -- starting with concentrations over 5 orders of magnitude, followed by determination of $K_m$ values over a narrow concentration range. 2-ME<sub>2</sub> was a sulfate acceptor substrate for SULT1A1*1, *2, *3; 1A2*1, *2, *3; 1A3; 1E1; 2A1; 2B1a and 2B1b, with apparent $K_m$ values of 2.5, 5.2, 1.6; 4.2, 111, 5.3; 91; 0.067; 8.3; 4.1 and 4.1 μM, respectively. These results suggest that individual pharmacogenetic variation in sulfate conjugation might contribute to individual differences in 2-ME, pharmacokinetics and therapeutic effect.
# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
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<tr>
<td>FRONT COVER</td>
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<td>2</td>
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<tr>
<td>TABLE OF CONTENTS</td>
<td>3</td>
</tr>
<tr>
<td>INTRODUCTION</td>
<td>4</td>
</tr>
<tr>
<td>BODY</td>
<td>4</td>
</tr>
<tr>
<td>KEY RESEARCH ACCOMPLISHMENTS</td>
<td>5</td>
</tr>
<tr>
<td>REPORTABLE OUTCOMES</td>
<td>5</td>
</tr>
<tr>
<td>CONCLUSIONS</td>
<td>5</td>
</tr>
<tr>
<td>REFERENCES</td>
<td>6</td>
</tr>
<tr>
<td>APPENDICES</td>
<td>8</td>
</tr>
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</table>
INTRODUCTION

The risk of estrogen-induced breast cancer is affected by the balance between activities of several enzymes involved in the biotransformation of estrogen and its metabolites. 2-methoxyestradiol (2-ME), an endogenous estrogen metabolite that inhibits the proliferation of many human cell lines in vitro and in vivo, is being developed for clinical testing as an anticancer agent. 2-ME has unique biological properties not shared with the other estrogen metabolites. It has potent anti-angiogenic activity in vitro and in vivo as well as antitubulin properties. While the exact mechanism of antiproliferative activity of 2-ME is unknown, emerging evidence suggests that 2-ME may inhibit estrogen-induced carcinogenesis in target tissues such as the mammary gland. Conjugation of 2-ME, catalyzed by sulfotransferase (SULT) enzymes may alter its anti-tumorigenic effects in the treatment of breast cancer. Many human SULTs are genetically polymorphic therefore, individual variations in SULT enzyme activity imply variations in the inactivation of 2-ME, with subsequent variations in antitumorigenic activity. We therefore hypothesized that SULT enzyme activities may play an active and important role in the response of breast cancer patients to treatment with 2-ME, through changes in catabolism. Hence, sulfation, may play a role in the therapeutic response of individuals to the treatment of breast cancer with 2-ME.

BODY

The main task was to characterize the SULT enzymes involved in the catabolism of 2-ME. This task was completed.

To initiate this work, cDNAs for human SULT1A1*1, SULT1A1*2, SULT1A1*3; SULT1A2*1, SULT1A2*2, SULT1A2*3; SULT1A3; SULT1B1; SULT1C1; SULT1E1, SULT2A1; SULT2B1a; SULT2B1b and SULT4A1 were each ligated into either the eukaryotic expression vector pCR3.1 or p91023B. Sequences of the cDNA inserts were confirmed by DNA sequencing prior to transfection of COS-1 cells using the DEAE-dextran or the Transfast method. Cytosol from transfected COS-1 cells served as a source of recombinant protein. The resulting recombinant SULT proteins were used for the biochemical characterization of 2-ME. Substrate kinetic studies for the sulfation of 2-ME was performed using the modified assay method of Foldes and Meek for sulfotransferases. Because of profound substrate inhibition displayed by SULTs, two sets of experiments were performed for each enzyme. The Km and Vmax values were then calculated using the method by Cleland. See appendix.

The long term goal of this project would be to identify the existence of functionally significant polymorphism(s) in the SULT isoform(s) responsible for catabolism of 2-ME in the target tissue. Genotyping of patients prior to treatment with 2-ME would be expected to predict response and/or toxicity, and allow for the tailoring of drug doses to individual patients.

In the appendix are:

Figure 1: Scheme showing the sulfate conjugation of 2-ME.
Figure 2: Substrate curves and double inverse plot for 2-ME catalyzed by SULT1E1.
Table 1: Substrate Kinetic results obtained from this study.

4

PROPRIETARY DATA
KEY RESEARCH ACCOMPLISHMENTS

The proposed task/work indicated in the concept was completed. A poster with this work was presented at the 102nd Annual meeting of the American Society for Clinical Pharmacology and Therapeutics (ASCPT), in March 2001, at Orlando, FL.

REPORTABLE OUTCOMES


CONCLUSIONS

- 2-ME₂ is an endogenous estrogen metabolite formed in vivo by the O-methylation of 2-hydroxyestradiol, a reaction catalyzed by COMT.
- 2-ME₂ is being tested as an antineoplastic agent because of its anti-proliferative, anti-angiogenic and anti-tubulin properties.
- Sulfate conjugation is one potential metabolic pathway for 2-ME₂.
- We found that seven of the ten known human SULT isoforms can catalyze the sulfation of 2-ME₂.
- Of the isoforms studied, SULT1E1 had the lowest apparent Kₘ value for 2-ME₂.
- The common allozymes for SULT1A1 also catalyzed the sulfation of 2-ME₂. Therefore, if this isoform contributes significantly to 2-ME₂ biotransformation in vivo, genetic variation in SULT1A1 might contribute to individual differences in 2-ME₂ metabolism, pharmacokinetics and therapeutic efficacy.
- The next step in these studies will require a determination of the relative importance of sulfate conjugation in the metabolism of 2-ME₂ when this agent is administered in a clinical setting.

"SO WHAT"

As a result of these studies, we have evidence that SULTs metabolize the anti-tumorigenic drug, 2-ME2. Since many of the human SULTs are genetically polymorphic, genotyping patients prior to treatment, perhaps, may predict response and/or toxicity and allow for tailoring of drug doses to individual patients.
REFERENCES


APPENDICES

Appendix I:


2-ME is an endogenous estrogen metabolite that inhibits the proliferation of breast and other human cancer cell lines. 2-ME also has potent anti-angiogenic and anti-tubulin properties, and it may inhibit estrogen-induced carcinogenesis in the mammary gland. We set out to test the hypothesis that 2-ME might be a substrate for sulfate conjugation and, therefore, that individual variations in the sulfation of 2-ME might contribute to individual differences in its metabolism, pharmacokinetics and therapeutic efficacy. As a first step, we tested 2-ME as a substrate for 7 human sulfotransferase (SULT) isoforms -- as well as all of the common allozymes for SULT1A1 and 1A2. Substrate kinetic studies were conducted in two stages -- starting with concentrations over 5 orders of magnitude, followed by determination of Km values over a narrow concentration range. 2-ME was a sulfate acceptor substrate for SULT1A1*1, *2, *3; 1A2*1, *2, *3; 1A3; 1E1; 2A1; 2B1a and 2B1b, with apparent Km values of 2.5, 5.2, 1.6; 4.2, 111, 5.3; 91; 0.067; 8.3; 4.1 and 4.1 μM, respectively. These results suggest that individual pharmacogenetic variation in sulfate conjugation might contribute to individual differences in 2-ME pharmacokinetics and therapeutic effect.

[Supported by DAMD Grant DAMD17-00-1-0684]

Appendix II: See attached Figures and Table on pages 9-11.

N.B. FIGURES AND TABLE IN APPENDIX II ARE PROPRIETARY DATA.
SULT Catalyzed 2-Methoxyestradiol Sulfation

SULT

PAPS

PAP

2-Methoxyestradiol

2-Methoxyestradiol-3-O-Sulfate
Sulfation of 2-Methoxyestradiol by SULT1E1

(A) Activity, CPM x 10^{-3}

(B) Activity, NET CPM x 10^{-3}

(C) 1/Activity, CPM^{-1} x 10^{3}
Table 1.

SUBSTRATE KINETICS FOR SULT ISOFORMS:
REACTION WITH 2-METHOXYESTRADIOL

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<th>Recombinant SULT Isoforms</th>
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<th>Vmax Units/B-Gal units</th>
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<td>2.5 ± 0.1</td>
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<td>1188</td>
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<tr>
<td>1A1*2</td>
<td>5.2 ± 0.4</td>
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<tr>
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<td>1.6 ± 0.2</td>
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<tr>
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<td>4.2 ± 0.3</td>
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<td>91.4 ± 23.0</td>
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<td>4.1 ± 0.3</td>
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FOR THE COMMANDER:

Encl

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