ABSTRACT:

S-Methyl N,N-diethyldithiocarbamate (MeDDC), a metabolite of the alcohol deterrent disulfiram, is converted to MeDDC sulfine and then S-methyl N,N-diethyldithiocarbamate sulfoxide, the proposed active metabolite in vivo. Several isoforms of CYP450 and to a lesser extent flavin monoxygenase (FMO) metabolize MeDDC in the liver. The human kidney contains FMO1 and several isoforms of CYP450, including members of the CYP3A, CYP4A, CYP2B, and CYP4F subfamilies. In this study the metabolism of MeDDC by the human kidney was examined, and the enzymes responsible for this metabolism were determined. MeDDC was incubated with human renal microsomes from five donors or with insect microsomes containing human FMO1, CYP4A11, CYP3A4, CYP3A5, or CYP2B6. MeDDC sulfine was formed at 5 μM MeDDC by renal microsomes at a rate of 210 ± 50 pmol/min/mg of microsomal protein (mean ± S.D., n = 5) and by FMO1 at 7.6 ± 0.2 nmol/min/nmol (n = 3). Oxidation of 5 μM MeDDC was negligible by all CYP450 tested (≤0.03 nmol/min/nmol). Inhibition of FMO by methimazole or heat diminished MeDDC sulfine formation 75 to 89% in renal microsomes. Inhibition of CYP450 in renal microsomes by N-benzylimidazole or antibody to the CYP450 NADPH reductase had no effect on MeDDC sulfine production. Benzylamine N-oxidation, a probe for FMO activity, correlated with MeDDC sulfine formation in renal microsomes (r = 0.951, p = 0.013). The Kₘ values for MeDDC sulfine formation by renal microsomes and recombinant human FMO1 were 11 and 15 μM, respectively. These results demonstrate a role for the kidney and FMO1 in the metabolism of MeDDC in humans.

The alcohol deterrent disulfiram is rapidly reduced in vivo to N,N-diethyldithiocarbamate, which is methyalted to form S-methyl N,N-diethyldithiocarbamate (MeDDC) (Cobby et al., 1977; Glauzer et al., 1993). MeDDC is oxidized primarily to the intermediate metabolite MeDDC sulfine, which is ultimately converted to S-methyl N,N-diethyldithiocarbamate sulfoxide, the proposed active metabolite of disulfiram in vivo, and a small amount of MeDDC sulfide (Fig. 1) (Hart and Faiman, 1992; Madan and Faiman, 1995; Madan et al., 1998). Inhibition of mitochondrial aldehyde dehydrogenase by the active metabolite(s) of disulfiram diminishes the metabolism of acetaldehyde, a product of ethanol metabolism (Hald and Jacobsen, 1948). Accumulation of acetaldehyde leads to the unpleasant effects of the “disulfiram-ethanol reaction”, which deters patients from consuming alcohol (Kitson, 1977).

The metabolism of disulfiram and its metabolites has been studied in subcellular fractions of liver and with recombinant hepatic enzymes. The oxidation of MeDDC in human liver microsomes is catalyzed by at least four isoforms of CYP450 (Madan et al., 1998). Recently, it was reported that recombinant human flavin monoxygenase (FMO) 3, the major isoform found in adult liver, oxidizes MeDDC to MeDDC sulfine (Pike et al., 1999). However, this reaction in human liver microsomes is catalyzed primarily by CYP450 (90%) and only to a minor extent by FMO3 (10%) (Pike et al., 1999). MeDDC has been measured in human plasma over a concentration range of 2 to 4 μM after oral administration of disulfiram (Faiman et al., 1984) and therefore is accessible to extrahepatic organs. The kidney contributes to the elimination of drugs and contains phase I
drug-metabolizing enzymes (Krishna and Klotz, 1994; Lohr et al., 1998), but it has not been considered as a site of metabolism for disulfiram. Oxidation of nucleophile sulfur heteroatoms of drugs in vivo is catalyzed by both CYP450 and FMO (Ziegler, 1988; Nebert and McKinnon, 1994; Cashman, 1995). Several isozymes of CYP450 (members of the CYP2B, CYP3A, CYP4A, and CYP4F subfamilies) have been identified in the human kidney (Haehner et al., 1996; Powell et al., 1998; Gervot et al., 1999). FMO1 and low levels of FMO2 have also been detected in the human kidney, but there is little information regarding the contribution of these enzymes to drug metabolism (Cashman, 1995; Yeung et al., 2000). In this article we report for the first time the extrahepatic metabolism of a disulfiram metabolite and a role for renal FMO1 in the metabolism of MeDDC in human tissues.

Materials and Methods

Reagents. Diethylenetraminepentaacetic acid (DTPA), NADPH, 7-ethoxycoumarin, 7-hydroxycoumarin, methimazole, benzydamine hydrochloride, and potassium phosphate monobasic and dibasic were purchased from Sigma Chemical Co. (St. Louis, MO). N-Benzylimidazole (NBI), platinum black, and maleic acid were obtained from Aldrich Chemical Co. (Milwaukee, WI). Phosphoric acid was received from J.T. Baker (Phillipsburg, NJ), and methanol from EM Science (Gibbstown, NJ). Insect microsomes (Supersomes) containing recombinant human FMO1 (lot 2), CYP2B6, CYP4A11, CYP3A4, or CYP3A5 from a baculovirus expression system and antibody to the NADPH CYP450 reductase were purchased from GENTEST (Woburn, MA). Human NADPH CYP450 reductase, which stimulates the activity of several isozymes of CYP450 in this insect cell expression system, was coexpressed with the CYP450 used in these studies. Human renal microsomes from five male donors, ages 19 to 64 years, were purchased from the International Institute for the Advancement of Medicine (IIAM, Exton, PA). MeDDC (Fulman et al., 1983), MeDDC sulfine (Mays et al., 1998), and MeDDC sulfoxide (Mays et al., 1998) were synthesized as previously described.

Synthesis of Benzydamine N-Oxide Hydrogen Maleate. Benzydamine N-oxide hydrogen maleate was synthesized by oxidizing benzydamine with hydrogen peroxide (Cope and Ciganek, 1963; Kataoka et al., 1973). One equivalent of a 10 mM solution of NaOH (145 mmol dissolved in 750 equivalent of a 10 mM solution of NaOH (145 hydrogen peroxide (Cope and Ciganek, 1963; Kataoka et al., 1973). One N-oxide hydrogen maleate was synthesized by oxidizing benzydamine with Two equivalents (330 mmol) of 30% hydrogen peroxide were added, and the reaction was monitored by high performance liquid chromatography (HPLC).

HPLC Conditions. Analyses were performed on a Waters (Milford, MA) HPLC system, with a model 996 photodiode array detector, 717 autosampler, and 600 pump controller with in-line degasser. Data were collected from 200 to 400 nm at 1.0 spectrum/s with 4.8-nm resolution. For quantitation of MeDDC sulfine and 7-hydroxycoumarin, mobile phase consisting of 5 mM phosphoric acid and methanol was run over a Phenomenex (Torrance, CA) Hypersil BDS C18 column (250 × 4.6 mm, 5-μm particles) in a linear gradient from 40 to 55% methanol over 7 min, 55 to 100% methanol from 7 to 9 min, and then maintained at 100% methanol until 19 min. The column was equilibrated with 40% methanol for 10 min following the gradient. MeDDC, MeDDC sulfine, and 7-ethoxycoumarin and 7-hydroxycoumarin were quantitated with a Sciex API 365 triple quadrupole analyzer mass spectrometer by ion spray ionization (Applied Biosystems, Foster City, CA). Benzydamine HCl produced a molecular ion (MH+) at m/z 310 (100% relative abundance) and fragment ions at m/z 86 (99%), 174 (8%), and 265 (24%). Mass spectrometry produced a molecular ion (MH+) at m/z 326 (100% relative abundance) and fragment ions at m/z 102 (19%) and 265 (6%) for benzydamine N-oxide. Benzydamine N-oxide hydrogen maleate produced a molecular ion (MH+) at m/z 326 (100% relative abundance) and fragment ions at m/z 102 (52%), 174 (10%), and 265 (30%).
A single product peak, identified as MeDDC sulfine by HPLC-UV, was detected in incubations of the disulfiram metabolite MeDDC with human renal microsomes in the presence of NADPH. MeDDC sulfine was formed by human renal microsomes at a rate of 210 ± 50 pmol/min/mg (mean ± S.D. for five donors) at 5 μM MeDDC and 570 ± 150 pmol/min/mg (mean ± S.D. for five donors) at 50 μM MeDDC. MeDDC sulfoxide was not detected in incubations with MeDDC and FMO1 or human renal microsomes (<50 pmol/min/mg).

The two most likely enzymes responsible for the formation of MeDDC sulfine in human renal microsomes were FMO and CYP450. At 5 μM MeDDC, recombinant FMO1 produced MeDDC sulfine at a rate of 7.6 ± 0.2 nmol/min/nmol (Table 1). We examined the potential for formation of MeDDC sulfine by CYP2B6, CYP3A4, CYP3A5, and CYP4A11, four isozymes of CYP450 found in human kidney at 5 μM MeDDC (Haehner et al., 1996; Powell et al., 1998; Gervot et al., 1999). In contrast to FMO1, human recombinant CYP2B6, CYP3A4, and CYP3A5, expressed in insect cells, produced MeDDC sulfine at rates that were 2 to 3 orders of magnitude slower (22 ± 2, 32 ± 2, and 7 ± 1 pmol/min/nmol P450, respectively; Table 1). CYP4A11 did not catalyze this reaction at a detectable level. No product was detected in incubations of MeDDC with control insect microsomes (data not shown).

Inhibition studies were performed to determine whether FMO or CYP450 was responsible for the metabolism of MeDDC in human renal microsomes. CYP450 activity was inhibited by NBI, a general chemical inhibitor of CYP450 (Grothuesen et al., 1996), or by antibody to the CYP450 NADPH reductase. In our experiments, NBI completely blocked the formation of MeDDC sulfine by recombinant CYP3A4 and CYP3A5 at 5 μM MeDDC (Table 1), but not CYP2B6, indicating NBI would inhibit CYP3A4 and CYP3A5 in the kidney microsomes. NBI moderately inhibited recombinant FMO1 in a concentration-dependent manner (14 and 37% for 1 and 4 mM NBI at 5 μM MeDDC; Table 2). In human renal microsomes, NBI also moderately inhibited MeDDC oxidation (mean of 11 and 35% for 1 and 4 mM NBI at 5 μM MeDDC). Similar results were seen for incubations with 50 μM MeDDC. Inhibitory antibody to the CYP450 NADPH reductase did not reduce the formation of MeDDC sulfine, indicating that CYP450 did not contribute to the metabolism of MeDDC in the renal microsomes (Table 2). The O-deethylation of 7-ethoxycoumarin, a substrate for CYP450 (including CYP1A1/2, -2A6, -2B6, -2C8/9, -2E1, and -3A4/5) (Chang et al., 1994) but not FMO1 (Table 2) or FMO3 (data not shown), was not detected in our human renal microsomes, suggesting the levels of these CYP450 were very low.

FMO activity was inhibited in the human renal microsomes by either adding methimazole to the incubation or by heating the microsomes (Sadequee et al., 1992; Tugnait et al., 1997). Heating human liver microsomes at 45°C for 5 min in the absence of NADPH has been shown to inactivate approximately 90% of the CYP450 activity, while having no effect on the CYP450 activity (McManus et al., 1987). In human renal microsomes, MeDDC sulfine formation was reduced 75 to 85% by heating and ≥81% by methimazole, a competitive inhibitor for FMO, at 5 μM MeDDC (Table 2). Methimazole also caused a ≥86% decrease in MeDDC metabolism by recombinant human FMO1 at 5 μM MeDDC. Heat inactivation of recombinant human FMO1 reduced MeDDC sulfine formation 43%. Similar results were seen when 50 μM MeDDC was used. For reasons that are unclear, the inhibition with heat inactivation of recombinant FMO1 in insect microsomes was highly variable. Nevertheless, inhibition of human renal microsomes and recombinant human FMO1 with methimazole was quite similar, and heat inactivation, a known inactivator of FMO but not of CYP450, inhibited product formation in both human renal microsomes and recombinant FMO1. These results indicate FMO is metabolizing MeDDC in human renal microsomes.

The FMO-specific metabolism of benzydamine to its N-oxide was used as a probe for FMO activity in the renal microsomes (Kawaji et al., 1993; Lang et al., 1998). The rate of benzydamine N-oxide formation varied over a range of 460 ± 10 to 870 ± 20 pmol/min/mg in the five samples of renal microsomes used in our study (Table 2). MeDDC sulfine formation correlated well with benzydamine N-oxide formation (r = 0.951; p = 0.013), providing additional evidence that FMO is responsible for MeDDC oxidation in human renal microsomes (Fig. 2).

The dependence of the rate of MeDDC sulfine formation on the concentration of MeDDC for recombinant human FMO1 and pooled human renal microsomes is presented in Fig. 3. The K_M of 15 μM for recombinant FMO1 was similar to the K_M of 11 μM for human renal microsomes, suggesting that similar enzymes are metabolizing MeDDC in both systems. The V_max of 11 nmol/min/mg for recombinant FMO1 is equivalent to a turnover number of 29 min⁻¹.

**Discussion**

In this study, MeDDC was metabolized to MeDDC sulfine in human renal microsomes. In human liver, this reaction is catalyzed mainly by isoforms of CYP450 (Madan et al., 1998) and to a minor extent by FMO (Pike et al., 1999). Our subsequent experiments were designed to determine the relative contributions of CYP450 and FMO to the formation of MeDDC sulfine in human kidney microsomes. Much less is known about the isoform expression of CYP450 in human liver compared with that in the liver. Several isoforms of CYP450, including CYP3A4, CYP3A5, CYP2B6, CYP4A11, and CYP4F2, have been detected in human kidney microsomes (Haehner et al., 1996; Powell et al., 1998; Gervot et al., 1999). Many other
Metabolism of MeDDC, 7-ethoxycoumarin, and benzydamine by human renal microsomes and recombinant human FMO1

MeDDC (5 or 50 μM) was incubated for 5 min. benzydamine (0.5 mM) for 10 min, or 7-ethoxycoumarin (1 mM) for 30 min, with 0.8 mg/ml human renal microsomes or 0.056 mg/ml FMO1 Supersomes. Values are the mean ± S.D. (percentage of control), n = 3.

### TABLE 2

<table>
<thead>
<tr>
<th>MeDDC Sulfine Formation</th>
<th>FMO1</th>
<th>Donor 1</th>
<th>Donor 2</th>
<th>Donor 3</th>
<th>Donor 4</th>
<th>Donor 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>MeDDC, 5 μM</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>2870 ± 60 (100)</td>
<td>200 ± 20 (100)</td>
<td>160 ± 0 (100)</td>
<td>270 ± 10 (100)</td>
<td>260 ± 30 (100)</td>
<td>170 ± 10 (100)</td>
</tr>
<tr>
<td>Methimazole</td>
<td>&lt;410 (&lt;14)</td>
<td>&lt;30 (&lt;15)</td>
<td>&lt;30 (&lt;19)</td>
<td>30 ± 10 (11)</td>
<td>&lt;30 (&lt;12)</td>
<td>&lt;30 (&lt;18)</td>
</tr>
<tr>
<td>Heat</td>
<td>1640 ± 90 (57)</td>
<td>30 ± 10 (15)</td>
<td>40 ± 0 (25)</td>
<td>40 ± 10 (15)</td>
<td>40 ± 0 (15)</td>
<td>30 ± 0 (18)</td>
</tr>
<tr>
<td>NBI, 1 mM</td>
<td>2470 ± 160 (86)</td>
<td>190 ± 10 (95)</td>
<td>130 ± 0 (81)</td>
<td>250 ± 20 (93)</td>
<td>N.D.</td>
<td>150 ± 10 (88)</td>
</tr>
<tr>
<td>NBI, 4 mM</td>
<td>1810 ± 50 (63)</td>
<td>140 ± 0 (70)</td>
<td>100 ± 0 (63)</td>
<td>180 ± 10 (67)</td>
<td>170 ± 10 (65)</td>
<td>100 ± 10 (59)</td>
</tr>
<tr>
<td>No NADPH</td>
<td>&lt;410 (&lt;14)</td>
<td>&lt;30 (&lt;15)</td>
<td>&lt;30 (&lt;19)</td>
<td>30 ± 10 (11)</td>
<td>&lt;30 (&lt;12)</td>
<td>&lt;30 (&lt;18)</td>
</tr>
<tr>
<td>Control serum</td>
<td>2850 ± 170 (100)</td>
<td>200 ± 10 (100)</td>
<td>150 ± 10 (100)</td>
<td>270 ± 20 (100)</td>
<td>220 ± 10 (100)</td>
<td>150 ± 10 (100)</td>
</tr>
<tr>
<td>Anti-reductase</td>
<td>3050 ± 130 (107)</td>
<td>210 ± 10 (105)</td>
<td>130 ± 10 (87)</td>
<td>250 ± 10 (93)</td>
<td>220 ± 20 (100)</td>
<td>150 ± 10 (100)</td>
</tr>
<tr>
<td>MeDDC, 50 μM</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>7320 ± 230 (100)</td>
<td>530 ± 20 (100)</td>
<td>390 ± 20 (100)</td>
<td>770 ± 30 (100)</td>
<td>660 ± 40 (100)</td>
<td>480 ± 10 (100)</td>
</tr>
<tr>
<td>Methimazole</td>
<td>1340 ± 190 (18)</td>
<td>120 ± 0 (23)</td>
<td>100 ± 0 (26)</td>
<td>190 ± 20 (25)</td>
<td>140 ± 10 (21)</td>
<td>140 ± 0 (29)</td>
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<tr>
<td>Heat</td>
<td>4270 ± 200 (58)</td>
<td>160 ± 10 (30)</td>
<td>140 ± 0 (36)</td>
<td>210 ± 20 (27)</td>
<td>170 ± 0 (26)</td>
<td>150 ± 10 (31)</td>
</tr>
<tr>
<td>NBI, 1 mM</td>
<td>7210 ± 230 (99)</td>
<td>510 ± 10 (96)</td>
<td>380 ± 10 (97)</td>
<td>730 ± 50 (95)</td>
<td>N.D.</td>
<td>450 ± 20 (94)</td>
</tr>
<tr>
<td>NBI, 4 mM</td>
<td>6550 ± 340 (90)</td>
<td>480 ± 10 (91)</td>
<td>360 ± 20 (92)</td>
<td>680 ± 10 (88)</td>
<td>590 ± 20 (89)</td>
<td>420 ± 10 (88)</td>
</tr>
<tr>
<td>No NADPH</td>
<td>1610 ± 150 (22)</td>
<td>120 ± 10 (23)</td>
<td>100 ± 0 (26)</td>
<td>190 ± 10 (25)</td>
<td>160 ± 20 (24)</td>
<td>120 ± 10 (25)</td>
</tr>
<tr>
<td>Control serum</td>
<td>7750 ± 70 (100)</td>
<td>550 ± 0 (100)</td>
<td>380 ± 10 (100)</td>
<td>770 ± 20 (100)</td>
<td>600 ± 0 (100)</td>
<td>440 ± 20 (100)</td>
</tr>
<tr>
<td>Anti-reductase</td>
<td>7950 ± 420 (103)</td>
<td>520 ± 20 (98)</td>
<td>370 ± 20 (97)</td>
<td>710 ± 30 (92)</td>
<td>610 ± 20 (102)</td>
<td>460 ± 10 (105)</td>
</tr>
</tbody>
</table>

| Benzydamine N-oxide Formation |       |         |         |         |         |         |
| Benzydamine, 0.5 mM         |       |         |         |         |         |         |
| Control                    | 15,600 ± 210 | 660 ± 10 | 460 ± 10 | 780 ± 10 | 870 ± 20 | 550 ± 0 |
| No NADPH                   | <540  | <40     | <4      | <4      | <4      | <4      |

| 7-Hydroxycoumarin Formation |       |         |         |         |         |         |
| 7-Ethoxycoumarin, 1 mM     |       |         |         |         |         |         |
| Control                    | <4    | <4      | <4      | <4      | <4      | <4      |

N.D., not determined.

Compared with the liver, the overall contribution of the CYP450 system in the kidney to the oxidation of chemicals, perhaps with the exception of fatty acid hydroxylation by CYP4A4/4F, is thought to be relatively minor (Lohr et al., 1998). The rate of MeDDC sulfine formation was below our detectable limit for CYP4A11 and very low for CYP2B6, CYP3A4, and CYP3A5. These low velocities and the apparently low levels of CYP3A4 and CYP3A5 expression in kidney can be demonstrated by using a nominal value of 47 pmol of FMO1/mg in human renal microsomes (Amet et al., 1997). The rate of MeDDC sulfine formation observed in human renal microsomes is too low to contribute significantly to MeDDC sulfine formation in renal microsomes.

The minor role of CYP450 toward MeDDC sulfine formation in the kidney can be demonstrated by assuming a level of renal CYP3A4 of 42 pmol/mg [equivalent to the total renal CYP450 content (Amet et al., 1997)] and a rate of sulfine formation of 0.032 pmol/min/pmol CYP450 (Table 1). The predicted rate of sulfine formation due to CYP3A4 would be 1.3 pmol/min/mg, which is <1% of the total rate of 210 pmol/min/mg for sulfine formation observed in human renal microsomes at 5 μM MeDDC. A similar calculation for FMO1 can be made using a nominal value of 47 pmol of FMO1/mg in human renal microsomes determined by immunoquantification (Yeung et al., 2000) and a sulfine formation rate of 7.6 pmol/min/pmol FMO1 (Table 1). This gives a predicted velocity for FMO1 of 357 pmol/min/mg (170% of the observed velocity in human renal microsomes). This higher predicted velocity could be due to a higher content of FMO1 Supersomes.

![Fig. 2. Correlation of MeDDC sulfine formation with benzydamine N-oxidation.](image-url)

MeDDC sulfine formation at 5 μM MeDDC as described in Table 2 is plotted against benzydamine N-oxide formation at 0.5 mM benzydamine, a probe for FMO activity, in human renal microsomes.

Isoforms are apparently absent from human kidney, including CYP1A2 (De Waziers et al., 1990), CYP2A6, CYP2A7, and CYP2A13 (Koskela et al., 1999); CYP2C8, CYP2C9, CYP2C10, and CYP2D6 (De Waziers et al., 1990); and CYP2E1 (Amet et al., 1997). The substrate specificity of members of the CYP3A family is very broad and includes the hydroxylation of endogenous and exogenous steroids (Thummel and Wilkinson, 1998). Members of the CYP4A and CYP4F families catalyze the oxidation of lipids, including the hydroxylation of medium- and long-chain fatty acids, such as lauric acid and arachidonic acid (Amet et al., 1997; Powell et al., 1998).

Compared with the liver, the overall contribution of the CYP450 system in the kidney to the oxidation of chemicals, perhaps with the exception of fatty acid hydroxylation by CYP4A4/4F, is thought to be relatively minor (Lohr et al., 1998). The rate of MeDDC sulfine formation was below our detectable limit for CYP4A11 and very low for CYP2B6, CYP3A4, and CYP3A5. These low velocities and the apparently low levels of CYP3A4 and CYP3A5 expression in kidney may be as high as 40 pmol/mg of protein (Powell et al., 1998), remarkable because it is so near the level of 42 pmol/mg reported for total renal CYP450 (Amet et al., 1997), the activity of CYP4A11 is too low to contribute significantly to MeDDC sulfine formation in renal microsomes.
FMO1 in the renal microsomal samples studied by Yeung and co-workers (2000). Alternatively, the reported FMO1 protein levels determined by immunoquantitation in renal tissue may be an overestimation of catalytically competent enzyme due to the recognition of inactive enzyme (e.g., apoenzyme) by the antibody. Regardless, our data indicate that the contribution of FMO1 to the oxidation of MeDDC in human kidney microsomes is 2 to 3 orders of magnitude greater than that of CYP450.

Deethylation of 7-ethoxycoumarin was measured as a general indicator of CYP450 activity because this reaction has been attributed to several isoforms of CYP450 in humans, including CYP1A1, -1A2, -2A6, -2B6, -2E1, -3A3, and -3A4 (Chang et al., 1994). The formation of 7-hydroxycoumarin could not be detected in human renal microsomes in our study (<4 pmol/min/mg), but it has been reported once previously at 10 pmol/min/mg (Pacifici et al., 1988). We selected 7-hydroxycoumarin formation as a general probe for CYP450 in human renal microsomes because in our previous studies the velocity of this reaction in human hepatic microsomes was relatively high and easily measured by HPLC-UV [420 ± 10 pmol/min/mg hepatic microsomal protein, mean ± S.D., n = 11, (Pike et al., 1999)]. Thus, in our current study, 7-hydroxycoumarin formation in renal microsomes was <1% of the hepatic rate. These results are consistent with previous reports of low or nondetectable activities for several CYP450 substrates in renal microsomes (Haehner et al., 1996; Amet et al., 1997). For example, midazolam-1'-hydroxylation, an activity attributed to CYP3A, ranged from 0.2 to 3 pmol/min/mg in kidney compared with 350 pmol/min/mg in liver (Haehner et al., 1996). Our results are consistent with the notion that several isoforms of the CYP450 superfamily are much less important overall for drug metabolism in the kidney compared with the liver. We cannot rule out the possibility that CYP450 was preferentially degraded in our samples of kidney microsomes. However, this seems unlikely in view of the general consensus that CYP450 is much more stable than FMO in tissues (Ziegler, 1988; Tugnait et al., 1997; Cashman, 1999).

MeDDC sulfine formation in renal microsomes was effectively inhibited by methimazole, a competitive inhibitor of FMO, and by heat inactivation (Table 2). NBI caused a moderate, concentration-dependent inhibition of MeDDC metabolism in renal microsomes and in insect microsomes containing recombinant FMO1. Anti-NADPH CYP450 reductase had no effect on MeDDC oxidation. MeDDC sulfine formation correlated well with the FMO-specific oxidation of benzamidine (r = 0.951, p = 0.013). Furthermore, the similarity of the K_M values for MeDDC sulfine formation in renal microsomes (K_M = 11 μM) and recombinant FMO1 (K_M = 15 μM) is consistent with the reaction being catalyzed by the same enzyme.

Little is known about the metabolism of drugs by FMO1 in human kidney. The stereoselective sulfoxidation of p-tolyl methyl sulfide (Sadeque et al., 1992) and sulindac (Hamman et al., 2000) and the N-oxidation of imipramine (Lemoine et al., 1990) in human renal microsomes have been attributed to FMO activity. As mentioned previously, the primary isoform of FMO expressed in human kidney is FMO1, but low levels of FMO2 expression have been reported (Cashman, 1995). The contribution of FMO2 to the oxidation of MeDDC in human renal microsomes could not be ascertained by our experiments. However, it is improbable that MeDDC is oxidized by FMO2, because the human gene for FMO2 encodes a truncated inactive form of the enzyme (Dolphin et al., 1998).

The clinical significance of MeDDC oxidation in the kidney is uncertain. Given that the liver and kidneys, respectively, contain approximately 32 and 5.3 mg of microsomal protein/g of tissue (Pacifici et al., 1988) and weigh approximately 1.4 and 0.6 kg, the liver contains 14 times more microsomal protein than do the kidneys. Thus, based on the metabolic capacity of each organ, formation of MeDDC sulfine is probably quantitatively much less important in the kidney than it is in the liver of an otherwise healthy patient. Of course, the relative contribution of the metabolism in the kidney may become greater in an individual whose hepatic metabolism is impaired by disease or by inhibition of CYP450. Production of an active metabolite in a tissue may have local pharmacological or toxicological implications. For example, although MeDDC sulfine has not been detected in plasma of humans taking disulfiram, our data indicate it is probable that the kidney is exposed to locally formed MeDDC sulfine. Possibly more important is that FMO1 is the predominant isoform in fetal liver (Yeung et al., 2000). Thus, formation of active metabolites in this organ potentially could have deleterious effects on the fetus.

In summary, MeDDC is oxidized in human kidney to MeDDC sulfine, a proposed necessary intermediate metabolite for the in vivo inhibition of aldehyde dehydrogenase by disulfiram. MeDDC sulfine
formation in kidney is catalyzed primarily by FMO1, which is in sharp contrast to the reaction in the liver, in which CYP450 is the major catalyst.

Acknowledgment. We thank Frank Crow in the Biomedical Mass Spectrometry and Functional Proteomics department at the Mayo Clinic for providing the mass spectrometry characterization of benzamidine N-oxide.

References