FORMATION OF GUANOXABENZ FROM GUANABENZ IN HUMAN LIVER

A new metabolic marker for CYP1A2

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ABSTRACT:
The in vitro N-hydroxylation of guanabenz as well as the corresponding N-dehydroxylation of guanoxabenz has been previously detected in biotransformation studies with microsomal fractions of different species including human hepatic microsomes. Furthermore, the N-hydroxylation of guanabenz was found to be catalyzed by enriched cytochrome P450 (P450) fractions in reconstituted systems. Strong correlations between 7-ethoxyresorufin O-deethylation (r = 0.96; p < 0.001), caffeine N-demethylation (r = 0.92; p < 0.001), respectively, and guanabenz N-hydroxylation activities were demonstrated in 10 human liver microsomal preparations. Studies with microsomes from human B-lymphoblastoid cell lines expressing human cytochrome P450 enzymes proved that CYP1A2 is the major isozyme responsible for this metabolic pathway. Further, P450 isozymes did not show any detectable conversion rates. The reaction was inhibited in presence of the potent CYP1A2 inhibitors α-naphthoflavone (7,8-benzoflavone) and furafylline. The N-reduction of guanoxabenz to guanabenz exhibits a significant correlation to the benzamidoxime N-reduction after incubation with 10 human liver microsomal preparations (r = 0.97; p < 0.001). The formation of benzamidine from benzamidoxime was described previously to be catalyzed by the benzamidoxime reductase. These results suggest that the guanbenz N-hydroxylation is mediated via CYP1A2, whereas the corresponding guanoxabenz N-reduction is catalyzed by an enzyme system composed of cytochrome b<sub>5</sub> NADH cytochrome b<sub>5</sub>-reductase, and benzamidoxime reductase. The high affinity of guanabenz to CYP1A2 and the distinct selectivity of this P450 isozyme toward guanabenz confirms the in vitro formation of guanoxabenz N-hydroxylation to be a suitable metabolic marker for CYP1A2 in biotransformation studies.

Guanabenz 1 (1-(2,6-dichlorobenzylideneamino)guanidine; Wyten-sin, Rexitene, Hipiten) (1, 2) and guanoxabenz 2 (1-(2,6-dichloroben-zylideneamino)-3-hydroxyguanidine; Benze´rial) (3) are known to be centrally acting α<sub>2</sub>-adrenoceptor agonists with antihypertensive activities. Both derivatives belong to the amidinohydrazone (aminoguanidine) class of compounds, and guanoxabenz is the N-hydroxylated derivative of guanabenz. As well, the in vitro N-hydroxylation of guanabenz to guanoxabenz as the corresponding reduction could be previously demonstrated by the use of microsomal fractions from livers of rabbits, pigs, and humans (fig. 1). The metabolic cycle (bioreversible reaction) was characterized, and the apparent kinetic parameters were determined. The guanabenz N-hydroxylation was shown to be catalyzed by enriched cytochrome P450 (P450)<sup>1</sup> fractions in reconstituted systems (4). As the participation of single P450 isozymes concerning the N-hydroxylation of guanabenz, as well as the guanoxabenz N-dehydroxylation, was unclear, it seemed reasonable to investigate the catalytic mechanism of this metabolic cycle.

In the present study, both substrates were incubated with each sample of a bank of 10 human liver microsomal preparations, characterized for several known human cytochrome P450 marker activities. The observed conversion rates were then correlated with the activities of each of the marker reactions through linear regression. Experiments with microsomes from human B-lymphoblastoid cell lines expressing human cytochrome P450 isozymes were conducted to underline the obtained results. Furthermore, incubations in the presence of the cytochrome P4501A2 (CYP1A2) inhibitor probes α-naphthoflavone (7,8-benzoflavone) (5, 6) and furafylline (7), as well as studies with liver microsomal fractions from rats pretreated with 3-methylcholanthrene, a well known CYP1A inducer (8), were carried out. To confirm the results obtained, several P450 inhibitor probes such as sulfaphenazole (CYP2C9), quinidine (CYP2D6), TAO (CYP3A4), and coumarin (CYP2A6) were tested for their influence on the formation of guanoxabenz from guanabenz.

CYP1A2, which is constitutively expressed in human liver (9), has been reported to play an important role in the metabolic activation of numerous chemical carcinogens, including aflatoxin B<sub>1</sub>, various heterocyclic and aromatic amines, and a large number of nitroaromatic compounds in humans (10). The metabolism of many heterocyclic amines by human hepatic microsomes to their highly mutagenic N-OH derivatives has been proved to be catalyzed primarily by CYP1A2 (11, 12). Furthermore the biotransformation of several common drugs and dietary constituents such as caffeine and phenacetin have been shown to be apparently mediated largely by CYP1A2 (13).

Considering the marked interindividual differences in CYP1A2 expression (14, 15), an effective means of quantifying the in vivo formation of guanoxabenz 2.

<sup>1</sup> Abbreviations used are: P450, cytochrome P450; TAO, triacetylolendomycine; HPLC, high performance liquid chromatography.

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activation of promutagens in man is necessary to enable the potential risk, posed by these compounds, to be assessed effectively. Consequently, a reliable isoenzyme-specific metabolic marker activity for CYP1A2 represents a suitable tool to elucidate the metabolism of countless xenobiotics. Presently used CYP1A2 substrate probes are phenacetin O-deethylation (13, 16), caffeine N-demethylation (17), theophylline N-demethylation (18), and 7-ethoxyresorufin O-deethylation (19). However, a common feature of many of these CYP1A2 substrates is that other competing metabolic pathways, catalyzed by different P450 isoenzymes, have to be considered (20).

Materials and Methods

Chemicals. Guanabenz acetate was kindly supplied by Wyeth-Pharma GmbH (Muenster, Germany). Guanoxabenz-HCl was a generous gift from Laboratoires Houdé (Paris, France). α-Naphthoflavone (7,8-benzoflavone), 3-methylcholanthrene sulfaphenazole, quinidine, TAO, coumarin, 7-ethoxyresorufin, and resorufin were purchased from Sigma Chemical Co. (Deisenhofen, Germany). Furafylline was provided by Salford Ultrafine Chemicals Ltd. (Manchester, England). NADPH (tetrasodium salt) and NADH (disodium salt) as well as all other chemicals and solvents were obtained from E. Merck (Darmstadt, Germany). All chemicals were of analytical grade.

Microsomal Preparations. Pooled human liver microsomes (Pooled HepatoSomes) as well as microsomal preparations from 10 different human donors (HepatoScreen Test Kit) were obtained from Human Biologics, Inc. (Phoenix, AZ). All samples were from otherwise healthy donors, and in all cases, the cause of death was not due to any known biochemical deficiency in the liver. Individual samples of HBI 2, 3, 5, 6, 7, 9, 10, 11, 12, and 13, characterized for the following activities, were used: NADPH-cytochrome c reductase, 7-ethoxyresorufin O-dealkylation, caffeine N-demethylation, coumarin 7-hydroxylation, tolbutamide methyl-hydroxylation, S-mephenytoin 4'-hydroxylation, dextromethorphan O-demethylation, chlorozoxazine 6-hydroxylation, testosterone 6β-hydroxylation, lauric acid 12-hydroxylation, lauric acid 11-hydroxylation, benzphetamine N-demethylation, as well as the content of cytochrome P450 and cytochrome b5. Microsomes from characterized human B-lymphoblastoid cell lines (AHH-1 TK"± cells), which after transfection with human P450 cDNA exhibit stable expression of human cytochrome P450 isoenzymes (CYP1A1, CYP1A2, CYP2A6, CYP2B6, CYP2C8, CYP2C9, CYP2D6, CYP2E1, and CYP3A4), were purchased from Gentest Corp. (Woburn, MA). Microsomal fractions from rats pretreated with 3-methylcholanthrene and liver microsomal fractions from untreated rats were prepared as described previously (21).

Incubations. The usual incubation mixture (0.3 ml) contained 50 mM Tris-HCl buffer (pH 7.4), 0.5 mM substrate (guanabenz/guanoxabenz), 0.5 mM cofactor (NADPH for guanabenz N-hydroxylation; NADH for guanoxabenz N-reduction), and 0.3 mg of protein from the enzyme source. The substrate concentration of 500 µM lies above the Km (48.4 µM). It was chosen for a better evaluation of the HPLC analytic method, which is based on UV detection. After 1 min of preincubation, the reaction was started by addition of the cofactor. The samples were incubated for 30 min at 37°C in a shaking water bath under aerobic conditions. Sample work-up was performed as described previously (4).

HPLC Analysis. The HPLC analysis was performed as described in more detail in ref. 4. Briefly, the HPLC system consisted of a prepacked reversed phase column (125 × 4 mm i.d., particle size 5 µm; Lichrospher RP-select B, E. Merck, Darmstadt, Germany). An isocratic solvent system consisting of methanol/ammonium acetate buffer (50 mM), pH 4.0 (guanabenz 30:70 v/v; guanoxabenz, 25:75 v/v), respectively, at a flow rate of 1 ml/min was used to isolate the metabolites. The injected sample volume was 20 µl. Solvents used in the analysis were filtered through a Sartolon membrane filter (0.45 µm, Sartorius AG, Goettingen, Germany) and degassed by bubbling with helium or sonication.

A high performance liquid chromatograph (Waters 510, Milford, CT) was equipped with a variable wavelength UV detector (Waters 486) set at 272 nm (guanabenz) and 274 nm (guanoxabenz) and an autosampler (Waters 710 WISP). The areas under the peaks were integrated with a chromatointegrator (Waters 746 or Merck Hitachi D-2500).

Guanabenz. For the determination of the recovery rate and the detection limit of the metabolite guanabenz, incubation mixtures with defined concentrations of synthetic reference substance (1.0, 5.0, 10.0, 20.0, 40.0, 60.0, 80.0, or 100.0 µM) were incubated and worked up under the same conditions as the experimental samples but without adding cofactor. The standard curves were linear over this range with correlation coefficients >0.9999. The signals obtained (peak areas) were compared with those of the same amount of guanabenz dissolved in the mobile phase. The recovery rate after incubation and sample work-up amounted to 102.8 ± 3.9% (N = 32). The detection limit was about 0.5 µM, which corresponds to a rate of N-reduction of 0.038 nmol guanabenz/min/mg protein. The retention times were 22.5 ± 0.5 min for guanoxabenz and 33.0 ± 0.5 min for guanabenz.

Guanoxabenz. Standard curves at the levels of 0.25, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 5.0, 7.0, and 10.0 µM guanoxabenz were constructed and found to be linear over this range with correlation coefficients >0.9992. The recovery rates of guanoxabenz from incubated mixtures was 96.7 ± 6.1% (N = 40) of that obtained using samples that contained the same amount of guanoxabenz dissolved in the mobile phase. The detection limit was represented by a 0.25 µM solution, which corresponds to a rate of N-hydroxylation of 0.019 nmol guanoxabenz/min/mg protein. The retention times were 16.5 ± 0.5 min for guanoxabenz and 23.5 ± 0.5 min for guanabenz.

Inhibition Experiments. Specific inhibition of CYP1A2 was achieved using α-naphthoflavone (7,8-benzoflavone) and furafylline [10 µM]. Other P450 isoform-selective inhibitors (sulfaphenazole, TAO, quinidine, coumarin) were added at concentrations of 100 µM. Incubation and sample work-up were performed as described above.

7-Ethoxyresorufin O-Deethylation Assay. The 7-ethoxyresorufin O-deethylation activity assay was performed as described previously (22, 23) with slight modifications.

Results

N-Hydroxylation of Guanabenz. The NADPH-dependent in vitro guanabenz N-hydroxylation was determined across 10 human liver microsomal preparations. The metabolic rates of guanoxabenz formation from guanabenz [500 µM] were found to vary substantially (11–404 nmol/min × nmol P450) (table 1). The activity obtained from each of the microsomal suspensions was then correlated with the metabolic rates determined for several known isoenzyme-specific marker reactions across the same set of human hepatic microsomes (table 2). The conversion rates for the guanabenz N-hydroxylation independently plotted against those observed for the two CYP1A2-specific marker activities 7-ethoxyresorufin O-deethylation (19) (fig. 2) and caffeine N-demethylation (17) (fig. 3) lead to strong correlations (r = 0.96/√r = 0.92; p < 0.001).

Microsomes from transfected human B-lymphoblastoid cell lines (AHH-1 TK"± cells) overexpressing CYP1A2 catalyzed the guana-
benz N-hydroxylation to high conversion rates (2.35 ± 0.23 nmol guanoxabenz/min × nmol P450), whereas cell microsomes containing CYP1A1, CYP2A6, CYP2B6, CYP2C9, CYP2D6, CYP2E1, or CYP3A4 did not show any detectable formation of guanoxabenz (data not shown). Thus, only the cell lines that expressed human CYP1A2 were conclusively shown to be capable of catalyzing the guanabenz N-hydroxylation.

The addition of α-naphthoflavone [10 μM] and furafylline [10 μM], two potent CYP1A2 inhibitors (5–7) almost completely inhibited the formation of guanoxabenz in human hepatic microsomes (table 3). Other P450 isoform-selective inhibitors (sulfaphenazole, TAO, quinidine, coumarin) did not significantly inhibit the in vitro guanabenz N-hydroxylation (data not shown). Microsomal fractions from rats pretreated with the CYP1A inducer 3-methylcholanthrene (8) yielded significantly higher conversion rates for guanabenz N-hydroxylation than liver microsomal fractions from untreated rats (table 4). Guanabenz [100 μM] caused 50% inhibition of 7-

TABLE 2
Correlation of the in vitro N-hydroxylation of guanabenz to guanoxabenz with known isoenzyme-specific P450 marker activities in 10 different samples of human hepatic microsomes

For incubation procedure, sample work-up, and analysis see Materials and Methods.

<table>
<thead>
<tr>
<th>Marker Reaction</th>
<th>Responsible P450 Isoenzyme</th>
<th>r</th>
</tr>
</thead>
<tbody>
<tr>
<td>7-Ethoxyresorufin O-deethylation</td>
<td>CYP1A2</td>
<td>0.96*</td>
</tr>
<tr>
<td>Caffeine N-demethylation</td>
<td>CYP1A2</td>
<td>0.92*</td>
</tr>
<tr>
<td>Coumarin 7-hydroxylation</td>
<td>CYP2A6</td>
<td>0.45</td>
</tr>
<tr>
<td>Tolbutamide-methylhydroxylation</td>
<td>CYP2C9/10</td>
<td>0.19</td>
</tr>
<tr>
<td>(S)-Mephenytoin 4'-hydroxylation</td>
<td>CYP2C19</td>
<td>0.10</td>
</tr>
<tr>
<td>Dextrometorphan O-demethylation</td>
<td>CYP2D6</td>
<td>0.16</td>
</tr>
<tr>
<td>Chloroxazone 6-hydroxylation</td>
<td>CYP2E1</td>
<td>0.24</td>
</tr>
<tr>
<td>Testosterone 6β-hydroxylation</td>
<td>CYP3A4</td>
<td>0.07</td>
</tr>
<tr>
<td>Lauric acid 12-hydroxylation</td>
<td>CYP4A</td>
<td>0.13</td>
</tr>
</tbody>
</table>

* p < 0.001.

Fig. 2. Correlation of guanabenz N-hydroxylation with 7-ethoxyresorufin O-deethylation across a bank of 10 human liver microsomal preparations.

For incubation procedure, sample work-up, and analysis see Materials and Methods.

TABLE 3
In vitro N-hydroxylation of guanabenz to guanoxabenz in human liver microsomal fractions in the presence of CYP1A2 selective inhibitors

For incubation procedure, sample work-up, and analysis see Materials and Methods.

<table>
<thead>
<tr>
<th>Inhibitor</th>
<th>Guanoxabenz [nmol/min × nmol P450]</th>
</tr>
</thead>
<tbody>
<tr>
<td>α-Naphthoflavone [10 μM]</td>
<td>ND</td>
</tr>
<tr>
<td>Furafylline [10 μM]</td>
<td>0.039 ± 0.009*</td>
</tr>
<tr>
<td>Control</td>
<td>0.301 ± 0.023</td>
</tr>
</tbody>
</table>

Values are presented as the mean ± SE of a minimum of four different determinations.

* Value statistically different from control with p < 0.01 (Student’s t test).

Table 3

FIG. 3. Correlation of guanabenz N-hydroxylation with caffeine N-demethylation across a bank of 10 human liver microsomal preparations.

For incubation procedure, sample work-up, and analysis see Materials and Methods.

TABLE 4
In vitro N-hydroxylation of guanabenz to guanoxabenz in liver microsomal fractions of rats pretreated with 3-methylcholanthrene (3-MC)

For incubation procedure, sample work-up, and analysis see Materials and Methods.

<table>
<thead>
<tr>
<th>Inducer</th>
<th>Guanoxabenz [nmol/min × nmol P450]</th>
</tr>
</thead>
<tbody>
<tr>
<td>3-MC</td>
<td>0.36 ± 0.02*</td>
</tr>
<tr>
<td>Control</td>
<td>0.21 ± 0.01</td>
</tr>
</tbody>
</table>

Values are presented as the mean ± SE of a minimum of four different determinations.

* Value statistically different from control with p < 0.01 (Student’s t test).

ethoxyresorufin O-deethylation at substrate concentrations of 10 μM (0.78 ± 0.13 nmol/min × nmol P450 vs. 1.57 ± 0.34 nmol/min × nmol (control)).

N-Dehydroxylation of Guanoxabenz. The in vitro formation of guanabenz detected in incubation mixtures from a bank of 10 human liver microsomal samples showed an appreciable interindividual variation, ranging from 1.13 to 7.47 nmol/min × mg of protein (table 5). The rates of guanoxabenz N-dehydroxylation, determined in this assay, were correlated with several known isoenzyme-specific cytochrome P450 marker activities obtained, utilizing microsomes from
activities in the 10 human liver samples only revealed a significant liver microsomal preparations. Correlation experiments with marker research as well as for pharmaceutical drug development. The formation studies is of increasing importance concerning toxicological activities and metabolically competent cell lines in xenobiotic biotransformation individual differences concerning the extent of drug actions and drug-drug interactions, respectively. The use of isoenzyme-specific marker activities and metabolically competent cell lines in xenobiotic biotransformation studies is of increasing importance concerning toxicological research as well as for pharmaceutical drug development. The results presented in this study provide evidence for the specificity of CYP1A2 toward the in vitro guanabenz N-dehydroxylation in human liver microsomal preparations. Correlation experiments with marker activities in the 10 human liver samples only revealed a significant correlation with 7-ethoxyresorufin O-deethylase and caffeine N³-demethylation, respectively. Inhibition experiments with the potent CYP1A2 inhibitors α-naphthoflavone and furafylline supported these results. They were substantiated by incubations with microsomes from human B-lymphoblastoid cell lines, which, after transfection with human P450 cDNA, exhibit stable expression of human P450 isozymes. Additionally, pretreatment of rats with the specific inducer for the CYP1A family in rodents (3-methylcholanthrene) resulted in an increasing turnover of guanoxabenz formation. Furthermore, known P450 isofrom-selective inhibitors, such as sulfaphenazole, TAO, quinidine, and coumarin, did not influence the in vitro guanoxabenz formation, whereas the 7-ethoxyresorufine O-deethylase activity assay, a CYP1A2-selective substrate probe, was inhibited in presence of guanabenz.

In summary, the present work clearly illustrates that the human in vitro guanabenz N-dehydroxylation is predominantly mediated by CYP1A2. The conversion of guanabenz to guanoxabenz can thus be employed as a marker activity to determine CYP1A2 in human liver samples. The HPLC analytical method of detecting guanoxabenz in enzyme preparations is robust and established easily. In contrast to presently used CYP1A2 substrate probes, the HPLC system is isocratic, and for the sample work-up, no extraction step, which might influence the recovery rate, is necessary.

The selectivity of this metabolic reaction was proved for human liver preparations in this study. Previous data have shown that the majority of human CYP1A xenobiotic substrate probes are nonspecific in their recognition of CYP1A1 and CYP1A2, although selectivity is apparent for some compounds (6). In contrast to these findings, the in vitro guanabenz N-dehydroxylation has been proved not to be catalyzed by CYP1A1 containing cell microsomes, whereas incubations in presence of microsomal preparations from human B-lymphoblastoid cell lines overexpressing human CYP1A2 resulted in high turnover rates for guanoxabenz formation. The reaction is not influenced by the corresponding N-dehydroxylation of guanoxabenz under the incubation conditions selected for this assay, although the

### Table 5

<table>
<thead>
<tr>
<th>Human Liver Sample</th>
<th>nmol Guanabenz/min x mg protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>HBI 2</td>
<td>3.13 ± 0.09</td>
</tr>
<tr>
<td>HBI 3</td>
<td>3.23 ± 0.08</td>
</tr>
<tr>
<td>HBI 5</td>
<td>3.40 ± 0.20</td>
</tr>
<tr>
<td>HBI 6</td>
<td>2.04 ± 0.13</td>
</tr>
<tr>
<td>HBI 7</td>
<td>1.99 ± 0.94</td>
</tr>
<tr>
<td>HBI 9</td>
<td>7.47 ± 0.32</td>
</tr>
<tr>
<td>HBI 10</td>
<td>1.85 ± 0.14</td>
</tr>
<tr>
<td>HBI 11</td>
<td>1.13 ± 0.05</td>
</tr>
<tr>
<td>HBI 12</td>
<td>3.46 ± 0.12</td>
</tr>
<tr>
<td>HBI 13</td>
<td>2.72 ± 0.15</td>
</tr>
</tbody>
</table>

For incubation procedure, sample work-up, and analysis see Materials and Methods.

Values are presented as the mean ± SE of a minimum of four different determinations.

A subsequent correlation between rates of formation of guanabenz from guanoxabenz and benzamidoxime N-dehydroxylation activities obtained, using the same set of human hepatic microsomes (24), showed a high degree of conformity between both metabolic reactions (r = 0.97; p < 0.001) (fig. 4). This was already described before for the reduction of sulfamethoxazole hydroxylamine independently plotted against the formation of benzamidine from benzamidoxime (r = 0.98) (24).

### Table 6

<table>
<thead>
<tr>
<th>Marker Reaction</th>
<th>Responsible P450</th>
<th>r</th>
</tr>
</thead>
<tbody>
<tr>
<td>7-Ethoxyresorufin O-deethylation</td>
<td>CYP1A2</td>
<td>0.33</td>
</tr>
<tr>
<td>Caffeine N-demethylation</td>
<td>CYP1A2</td>
<td>0.36</td>
</tr>
<tr>
<td>Coumarin 7-hydroxylation</td>
<td>CYP2A6</td>
<td>0.36</td>
</tr>
<tr>
<td>Tolbutamide-methylhydroxylation</td>
<td>CYP2C9/10</td>
<td>0.48</td>
</tr>
<tr>
<td>(S)-Mephentoin 4'-hydroxylation</td>
<td>CYP2C19</td>
<td>0.06</td>
</tr>
<tr>
<td>Dextrometorphan O-demethylation</td>
<td>CYP2D6</td>
<td>0.14</td>
</tr>
<tr>
<td>Chloroxazone 6-hydroxylation</td>
<td>CYP2E1</td>
<td>0.03</td>
</tr>
<tr>
<td>Testosterone 6β-hydroxylation</td>
<td>CYP3A4</td>
<td>0.43</td>
</tr>
<tr>
<td>Lauric acid 12-hydroxylation</td>
<td>CYP4A</td>
<td>0.04</td>
</tr>
</tbody>
</table>

For incubation procedure, sample work-up, and analysis see Materials and Methods.

**Discussion**

The objective of these investigations was to provide information regarding human P450 microsomal metabolism of guanabenz and guanoxabenz. Identification of the enzyme responsible for the oxidative metabolism of drugs is required to predict and explain interindividual differences concerning the extent of drug actions and drug-drug interactions, respectively. The use of isoenzyme-specific marker activities and metabolically competent cell lines in xenobiotic biotransformation studies is of increasing importance concerning toxicological research as well as for pharmaceutical drug development. The results presented in this study provide evidence for the specificity of CYP1A2 toward the in vitro guanabenz N-dehydroxylation in human liver microsomal preparations. Correlation experiments with marker activities in the 10 human liver samples only revealed a significant correlation with 7-ethoxyresorufin O-deethylase and caffeine N³-demethylation, respectively. Inhibition experiments with the potent CYP1A2 inhibitors α-naphthoflavone and furafylline supported these results. They were substantiated by incubations with microsomes from human B-lymphoblastoid cell lines, which, after transfection with human P450 cDNA, exhibit stable expression of human P450 isozymes. Additionally, pretreatment of rats with the specific inducer for the CYP1A family in rodents (3-methylcholanthrene) resulted in an increasing turnover of guanoxabenz formation. Furthermore, known P450 isofrom-selective inhibitors, such as sulfaphenazole, TAO, quinidine, and coumarin, did not influence the in vitro guanoxabenz formation, whereas the 7-ethoxyresorufine O-deethylase activity assay, a CYP1A2-selective substrate probe, was inhibited in presence of guanabenz.

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**Fig. 4. Correlation of guanoxabenz N-dehydroxylation with benzamidoxime N-dehydroxylation across a bank of 10 human liver microsomal preparations.**

For incubation procedure, sample work-up, and analysis see Materials and Methods.
produg function of guanoxabenz has already been discussed (4). In particular, NADPH was the preferred cosubstrate for the guanabenz N-dehydroxylation. The required substrate (guanabenz) is a commercially available drug.

The distinct correlation between the rates of guanoxabenz N-dehydroxylation and benzamidoxime N-dehydroxylation in human liver microsomal samples clearly illustrates the conformity of both metabolic reactions. The previously documented characteristics of the guanoxabenz N-dehydroxylation (4) show a high degree of correspondence with several known reductive biotransformation reactions of N-hydroxyaminodihydropyridines (25), N-hydroxyguanidines (26), amidoximes (27), N-hydroxyisothiouraees (28), and hydroxylamines (29, 30). Conclusively, these findings strongly suggest that the N-dehydroxylation under investigation is catalyzed predominantly by the same enzyme system as the one reported by Kadlubar and Ziegler (30) and detected in our previous investigations (24), consisting of cytochrome b$_6$, NADH cytochrome b$_6$-reductase, and a third protein (benzamidoxime reductase), showing a close relationship to the P450 family. All components were isolated from pig liver.

In conclusion, our results suggest that the metabolic cycle (bioreversible reaction) under investigation represents a useful tool for the elucidation of CYP1A2 involvement in biotransformation processes as well as for the description of N-reductive metabolic reactions in vitro. CYP1A2, which has been reported to be polymorphically distributed in man (31), continuously remains the object of biotransformation studies concerning metabolic activation of countless mutagenic and carcinogenic xenobiotics. Useful tools for better prediction of possible drug interactions or mutagenic potential of drugs and dietary constituents are needed.

Basic nitrogen-containing functional groups such as amidines, guanidines, and amidino/hydrazones have not been reported previously to be metabolized by CYP1A2. Guanoxabenz itself has been found to show mutagenic activity toward Salmonella typhimurium, whereas rat liver microsomal N-dehydroxylation resulted in a decrease of mutagenicity (4).

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