\textbf{\textsuperscript{14}C-PROPOXYPHENE DEMETHYLATION IN THE RAT}

An example of differences between liver and intestinal drug-presystemic metabolism

YVES HORSMANS, ALAIN SALIEZ, VÉRONIQUE VAN DEN BERGE, JEAN-PIERRE DESAGER, ANDRÉ P. GEUBEL, STANISLAS PAUWELS, AND LUC LAMBOTTE

Department of Gastroenterology (Y.H., A.P.G., S.P.), Experimental Surgery (A.S., L.L.), and Pharmacotherapy (Y.H., V.V.D.B., J.-P.D.) Laboratories, Louvain Medical School, Cliniques Universitaires Saint Luc

(Received August 6, 1996; accepted July 15, 1997)

ABSTRACT:
Presystemic metabolism is believed to occur mainly in the liver with some minor intestinal participation. The aim of this study was to investigate the respective part of each of these two organs in the metabolism of the analgesic d-propoxyphene (DP). Pharmacological doses of DP were given in the duodenum (ID), the portal vein (IP), and the femoral vein (IV) of male Wistar rats. A tracer dose of \textsuperscript{14}C-DP was also administered either in IV, IP, or ID as well as in hepatectomized rats or rats with bile duct diversion. In vitro demethylation occurring in liver and intestinal microsomes was also studied. Absolute DP bioavailability obtained after oral administration was two times higher than that observed after portal administration (48.9\% vs. 23.2\%, respectively), an result opposite (i.e. a lower bioavailability) of that expected on the basis of the existence of a liver enzyme saturation phenomenon. The \textsuperscript{14}CO\textsubscript{2} cumulative excretion after \textsuperscript{14}C-DP administration was significantly lower after IV or ID administration than after injection in the portal vein as a bolus or within 20 min. The biliary excretion of the labeled compound varied in the opposite direction, being greater after IV or ID than after IP administration, suggesting that the metabolism of DP in the liver is influenced by an extrahepatic transformation. This most likely occurs in the gut since the production of \textsuperscript{14}CO\textsubscript{2} after IV administration was similar to that after IP administration. This transformation did not prohibit DP detection in the systemic blood but was sufficient to increase the part eliminated with bile and to decrease the part demethylated into NP. Demethylation mainly occurs in the liver since the production of \textsuperscript{14}CO\textsubscript{2} was nearly abolished in hepatectomized rats. Furthermore, microsomes of hepatic but not of intestinal origin were able to demethylate DP. Our data suggest that the transformation of DP occurring in gut after oral administration is responsible for changes in the hepatic metabolism of the drug.

After oral administration in the rat, d-propoxyphene (DP) is subjected to a major first-pass effect and is metabolized through various pathways (1, 2). The major pathway involves demethylation to norpropoxyphene (NP) (2, 3). NP is detected in the plasma after portal administration of DP but was not found after systemic injection by Oguma and Levy (3). This difference, which was attributed to the saturation of enzymatic pathways in the liver, might indicate that the hepatic and extrahepatic metabolisms of the drug are not identical. We compared the absolute bioavailability of DP obtained after oral and portal administration. We also examined differences in DP metabolism when tracer doses of (dimethylamino-\textsuperscript{14}C) DP were administered in the duodenum, the portal vein, or a peripheral vein. The demethylation of DP to NP was evaluated by the measurement of exhaled \textsuperscript{14}CO\textsubscript{2}. With both pharmacological and tracer doses, it was shown that DP is metabolized differently in the intestine and in the liver, suggesting that the route of administration may play a critical role in its transformation.

\textsuperscript{1} Abbreviations used are: DP, d-propoxyphene; ID, intraduodenal; IP, intraportal; NP, norpropoxyphene.

Send reprint requests to: Y. Horsmans, M.D., Ph.D., Department of Gastroenterology, Cliniques Universitaires Saint Luc, Avenue Hippocrate, 10, 1200 Brussels, Belgium.

Materials and Methods

\textbf{Animals.} Wistar male rats weighing 230–270 g were purchased from the University Catholic of Louvain animal facilities. All animals were deprived of food 16 hr prior to drug administration. They were handled in accordance with Catholic University of Louvain regulations.

\textbf{Methods.} In experiments using pharmacological doses of DP, DP and NP determinations in plasma were performed by capillary gas chromatography as previously described (4) or by GC-MS following the method described by Kintz and Mangin (courtesy of Prof. Gielen, University of Liège, Belgium) (5). Low cut-off value for plasma NP was 50 and 10 ng/ml, respectively.

In experiments using tracer dose of DP, a dose (1 ml; specific activity: 7.9 mCi/mmol; 126 nmol) of (+)-(dimethylamino-\textsuperscript{14}C) propoxyphene hydrochloride (Amersham, UK) was administered by bolus in 200-\textmu l saline solution. Exhaled \textsuperscript{14}CO\textsubscript{2} was continuously trapped over a 2-hr period following the method described by Lauterburg and Bircher (6) and as previously performed by our group (7). After adding a scintillation mixture, exhaled radioactivity was counted in a liquid scintillator spectrometer (Wallac 1409, Pharmacia, Sweden). The counts per minute were converted to percentage of administered doses. In some experiments, continuous breath collection was prolonged up to 24 hr. Cumulative \textsuperscript{14}CO\textsubscript{2} was expressed as the percentage of the administered dose collected during these time periods.

\textbf{In Vivo Study Design.} To elucidate the importance of the route of administration and hepatic metabolism, either pharmacological doses of DP or \textsuperscript{14}C-DP tracer doses were administered by bolus in 200-\textmu l saline solution, except if otherwise specified.

\textbf{Route of administration.} To take into account the intravenous toxicity of 20 mg/kg DP dose and data from Oguma and Levy (3), various pharmacological doses of DP were administered by gavage (4, 8, 12, and 20 mg/kg), in the...
portal vein (12 mg/kg), or in the femoral vein (4 mg/kg). Blood samples were collected from the jugular vein, and plasma samples were stored at −20°C until assay.

To determine the DP AUC after each mode of administration, plasma samples (N = 5) were obtained in three groups of rats at various times after DP administration. The DP AUC0–2hr value was determined using the trapezoidal method. The absolute bioavailability was calculated by comparing the AUC after duodenal or systemic administration with that obtained after portal administration. In other series of rats, a tracer dose of 14C-DP was administered into the duodenum, into the portal vein, or into the femoral vein. In the portal vein, 14C-DP was administered not only by bolus injection but also by a 20-min infusion with a pump (Perfusor VI, Braun, Germany). In two other rats, a 14C-DP or a pharmacological dose of DP was directly injected in the left ventricle through a catheter inserted via the carotid artery.

**Role of the liver.** 14C-DP was administered in rats that had undergone, either a total hepatectomy, a surgical portacaval shunt, or a bile duct diversion. A 95% hepatectomy completed by total ligature of the hepatic pedicule was performed 24 hr after confection of a surgical portacaval anastomosis (8). 14C-DP was administered in rats that had undergone a 95% hepatectomy immediately after the hepatectomy. 14C-DP production was also measured after duodenal administration in a group of animals with surgical portacaval shunt. Implementation of a duodenal canula and shunt operation were performed 24 hr before drug administration. 14C-DP breath test was also performed in animals in which a catheter was introduced in the cholecocysto 90 min before drug administration.

**In Vitro Study Design.** Liver and intestinal microsomes were prepared as previously described (9) and kept frozen at −80°C until used. DP (2.5 nmol in 600-µl final incubation volume) was incubated for 3 min at 37°C with 70 µmol of MgCl2, 0.7 U glucose 6-phosphate dehydrogenase, and 15 µmol of NADP, 25 µmol of MgCl2, 0.7 U glucose 6-phosphate dehydrogenase, and 15 µmol of glucose-6-phosphate. The reaction was stopped by the addition of ZnSO4 (15%). Extraction procedure was similar to that used for plasma, and NP determinations were further performed using capillary gas chromatography. With these optimal conditions, linear reaction rates were ensured and intra- and interday coefficients of variation were lower than 5%.

Statistical analysis was performed, using an unpaired Student t test. Results are expressed as mean ± SD.

**Results**

**Pharmacological Doses.** DP AUC values were 255, 220, and 538 ng · hr/ml after portal, systemic, and duodenal administration, respectively. Absolute DP bioavailability obtained after duodenal administration was two times higher than that observed after portal administration (48.9% vs. 23.2%, respectively).

NP was detected after portal administration and after oral administration of 12 and 20 mg/kg of the test substance but not, even using the GC-MS technique, after administration in the femoral vein or in the left ventricle.

**Tracer Doses (Table 1).** After duodenal administration, breath 14CO2 2 hr cumulative excretion (CE) reached 13.6 ± 5.3%. In preliminary experiments in which 14C-DP was also administered through a duodenal canula in animals with external biliary diversion, only 4.1% of total radioactivity was found in the gut 24 hr after drug administration. In the presence of portacaval shunt, 14C-DP 2 hr CE fell to 5.4 ± 4.1% (p = 0.066) in comparison with that in normal rats.

After systemic injection, 14CO2 2 hr CE (11.8 ± 1.3%) was similar to that observed after duodenal administration. A similar result has also been observed in one rat after injection in the left ventricle (14C-DP 2 hr CE: 11.2%). 14C-DP injection directly in the portal system was associated with a significantly greater 14C-DP 2 hr CE than that observed after duodenal or systemic administration (p = 0.001). This difference was present after bolus injection as well as after a 20 min infusion (20.1 ± 3.1% and 20.5%, respectively) and was also observed after 24 hr (18.8 ± 0.6% in femoral group; 28.3 ± 5.5% in portal group; p < 0.001).

In hepatomectomized rats, CE after 2 hr was reduced to 1.7 (0.3%)%.

In three groups of animals with bile duct diversion in which 14C-DP was administered in the duodenum, in the portal vein, or in the femoral vein, the 14CO2 production was similar to that previously obtained in animals without bile duct diversion. The radioactivity found in a 24-hr bile collection was significantly higher after femoral injection than after portal administration (64.9 ± 4.75% vs. 40.5 ± 4.4%; p < 0.001), whereas the level of radioactivity found after duodenal administration was intermediate between the level found in the two other groups (51 ± 3.6%).

In *In vitro* incubation of hepatic microsomes with DP generated a quantifiable amount of NP (897 ± 56 ng/nmol P450/min; N = 8). In contrast, when intestinal microsomes were incubated with similar amount of cytochrome P450 and experimental conditions, no NP was detected despite the fact that intestinal microsomes were catalytically active as demonstrated by the level of 7-ethoxyresorufin-O-deethylase activity (mean activity: 9.5 ± 2 pmol/mg protein/min) (10).

**Discussion**

In the rat doses of DP lethal when given intravenously are well tolerated after oral administration, a fact in agreement with an important first-pass effect reducing the systemic availability of the drug and its central toxic effect. The liver is still considered the major site of drug transformation. Surprisingly, however, DP absolute bioavailability was much greater after duodenal administration than after direct injection in the portal vein. As shown by others (1), the intestinal absorption of DP is complete, a feature confirmed by our tracer studies performed in the presence of biliary diversion. Moreover, the existence of an incomplete absorption could not explain the greater bioavailability after duodenal administration. Saturation of an hepatic metabolic pathway, an hypothesis which is not supported by our tracer

**TABLE 1**

<table>
<thead>
<tr>
<th>Route of Administration</th>
<th>Models</th>
<th>N</th>
<th>14CO2, CE* 2 hr</th>
<th>14CO2, CE 24 hr</th>
<th>Bile 2 hr</th>
<th>Bile 24 hr</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intraduodenal Normal</td>
<td>10</td>
<td>13.6 ± 5.3%</td>
<td>21.5 ± 4.8%</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Intraduodenal PCS**</td>
<td>5</td>
<td>5.4 ± 4.1%</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intraduodenal Bile duct diversion</td>
<td>4</td>
<td>14.1 ± 3.5%</td>
<td>21.7 ± 2.2%</td>
<td>32.3 ± 4.3%</td>
<td>51 ± 3.6%</td>
<td></td>
</tr>
<tr>
<td>Femoral vein Normal</td>
<td>8</td>
<td>11.8 ± 1.3%</td>
<td>18.8 ± 0.6%</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Femoral vein Bile duct diversion</td>
<td>4</td>
<td>9.5 ± 0.9%</td>
<td>18.8 ± 3.05%</td>
<td>27.4 ± 6.8%</td>
<td>64.9 ± 4.75%</td>
<td></td>
</tr>
<tr>
<td>Femoral vein Hepatectomy</td>
<td>4</td>
<td>1.7 ± 0.3%</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Portal vein Normal</td>
<td>9</td>
<td>20.1 ± 3.1%</td>
<td>28.3 ± 5.5%</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Portal vein Bile duct derivation</td>
<td>4</td>
<td>24.3 ± 6.45%</td>
<td>28.7 ± 5%</td>
<td>35.4 ± 3.5%</td>
<td>40.5 ± 4.4%</td>
<td></td>
</tr>
</tbody>
</table>

*CE, cumulative excretion.
**Portacaval shunt.

14CO2 cumulative excretion and amount of radioactivity found in bile after administration of tracer dose of 14C-DP by various routes.
experiments, is also unlikely. Such a phenomenon would indeed lead to the opposite results since sinusoidal DP concentration would be much higher after portal than after oral administration. This observation suggests that DP is transformed into the gut in a metabolite which largely escapes transformation by the liver and remains recognized as DP by the analytical method used. This could explain the greater bioavailability obtained after duodenal administration.

Data concerning the specific of NP after administration of pharmacological doses of DP are in agreement with those of Sullivan et al. (11) and Oguma and Levy (3), although a more sensitive method to measure NP concentration of 10 ng/ml was used. To explain the absence of NP after systemic injection, two hypotheses based on saturation mechanisms have been proposed by Oguma and Levy (3). First, NP is very rapidly transformed and is only detected when the enzyme responsible for its metabolism is saturated. Second, NP is only produced in significant amounts when another major metabolic pathway of DP is saturated. The fact that $^{14}$CO$_2$ production was still greater after IP than after ID or systemic administration tends to rule out the second hypothesis. Not being able to selectively measure NP at these concentrations, we instead relied on an indirect method to evaluate its production, the measurement of $^{14}$CO$_2$ resulting from the demethylation of $^{14}$C-DP. The $^{14}$CO$_2$ breath elimination and thus likely the demethylation of DP into NP was greater after portal than after systemic or duodenal administration. This difference persisted at the end of the 24-hr period of collection, indicating that it was not because of an initial dilution in a peripheral reservoir after duodenal or systemic administration. The saturation of an enzymatic pathway is unlikely to explain our results obtained using tracer doses of the drug. Furthermore, the level of $^{14}$CO$_2$ elimination was not influenced by the rate of administration in the portal vein either as a bolus or by a continuous infusion within 20 min. The fact that the production of $^{14}$CO$_2$ mainly varied according to the route of DP administration indicates that it might be related to the amount of the drug reaching the liver prior to any transformation. This hypothesis is further supported by the 50% reduction in $^{14}$CO$_2$ elimination observed after portacaval shunting, a procedure that is likely to increase DP extrahepatic elimination.

Presystemic extrahepatic transformation of DP should have two consequences. First, it may reduce the amount of DP demethylated to NP which only occurs when sufficient amounts of untransformed DP reach the liver (such as that obtained after direct IP administration or after administration of very high doses in the duodenum). Second and when compared with IP administration, it may increase the relative amount of the label excreted into bile. The total amounts of excreted labeled material were indeed similar after duodenal or after portal administration, but there was an inverse relationship between the amount of excreted in bile and that excreted in the exhaled air, respectively (Table 1).

As further shown by experiments performed in hepatectomized animals, the liver is the main site of $^{14}$CO$_2$ production. This observation is in agreement with the current knowledge about the demethylation of the drug, even if it is difficult to exclude that only the final transformation of the drug, i.e., the transformation of formaldehyde into $^{14}$CO$_2$, occurs in the organ. As supported by our in vitro studies with isolated microsomes, it is likely that the gut contributes little to the production of $^{14}$CO$_2$ and to in vivo demethylation of DP. On the other hand, various arguments suggest that the extrahepatic transformation of DP mainly occurs in the gut. $^{14}$CO$_2$ excretion was indeed very similar after duodenal and systemic administrations, but in the two instances significantly lower than after portal administration. A major role for the lung has been excluded on the basis of results obtained following intracardiac or systemic injection.

Our experiments thus suggest that DP is transformed in the gut in such a way that its further handling by the liver is modified, an observation well in agreement with the data described by Giacomini et al. (12) who showed that portacaval shunting is not able to suppress completely the first-pass effect. The nature of this prehepatic metabolism was however not investigated. Prehepatic metabolism of various drugs has already been described for drugs such as cyclosporine, propranolol, and verapamil (9, 13, 14). Our work indicates that the prehepatic transformation of DP is likely to influence not only quantitatively but also qualitatively its metabolism. This new concept may be of potential pharmacological importance since it may be anticipated that organs responsible for various biotransformations exhibit different metabolic behaviors in terms of pharmacological induction (14) or response to various pathological conditions.

In conclusion, it is proposed that DP is transformed in the gut wall in such a way that its hepatic metabolism is modified.

References