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# Differential induction of midazolam metabolism in the small intestine and liver by oral and intravenous dexamethasone pretreatment in rat

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1. Midazolam is metabolized in the rat by CYP3A enzymes to 4-OH-midazolam (4-OH-MDZ) and 1′-OH-midazolam (1′-OH-MDZ). The induction of midazolam metabolism was studied in male Wistar rats treated with dexamethasone ($50\,mg\,kg^{-1}\,day^{-1}$) during 4 days via the oral or intravenous routes. Microsomes were prepared from the liver and the proximal small intestine and *in vitro* metabolism of midazolam was determined. In addition, CYP3A1- and CYP3A2-like protein levels were measured by gel electrophoresis and immunoblotting.

2. The $V_{max}$'s (mean SEM) for 4-OH-MDZ and 1′-OH-MDZ formation were much lower in intestinal ($0.078 \pm 0.002$ and $0.074 \pm 0.002\,\mu M\,min^{-1}\,mg^{-1}$ protein, respectively) compared with hepatic microsomes prepared from the uninduced rat ($0.870 \pm 0.007$ and $0.310 \pm 0.020\,\mu M\,min^{-1}\,mg^{-1}$ protein, respectively). Induction by oral or intravenous dexamethasone pretreatment led to significant increases in $V_{max}$ for 4-OH-MDZ and 1′-OH-MDZ by both intestinal and hepatic microsomes. Oral dexamethasone pretreatment via the oral route resulted in a more pronounced increase in $V_{max}$ compared with intravenous administration of the inducer.

3. CYP3A1 and CYP3A2 protein levels in liver microsomes were significantly increased following oral (3.7- and 3.2-fold, respectively) or intravenous (2.6- and 2.1-fold, respectively) pretreatment with dexamethasone. On the contrary, only oral dexamethasone pretreatment resulted in a significant change in intestinal CYP3A2-like protein (7.3-fold). A slight difference in the migration distance of the immunoreactive band for CYP3A2 was also observed for intestinal microsomes.

4. These results suggest that intestinal CYP3A enzymes in the rat differ from hepatic CYP3A1 and CYP3A2. They also demonstrate that systemic dexamethasone administration can induce intestinal microsome activity.

## Introduction

Cytochrome P450 (CYP) enzymes play an important role in the metabolism of a large number of both endogenous and exogenous substances (Lu and West 1979, Porter and Coon 1991). Multiple forms of CYP exist and several enzymes can be expressed simultaneously in different tissues such as the liver, gut, lung, kidney and brain (Kaminsky and Fasco 1992, Krishna and Klotz 1994). It is now recognized that members of the CYP3A subfamily are among the most important of all drug-metabolizing enzymes and catalyse the metabolism of many drugs including cyclosporin, terfenadine, nifedipine, erythromycin and midazolam (Rendic and Di Carlo 1997). Certain members of this CYP subfamily, e.g. CYP3A4 in man and CYP3A1/3A2 in rat, are present in intestinal epithelial cells and have been shown to contribute significantly to the so-called first-pass

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effect following oral administration of a number of drugs (Wu *et al*. 1995, Thummel *et al*. 1996). More recently, Gushchin *et al*. (1999) demonstrated the existence of new CYP enzymes in the rat intestine. In addition, the activity of these CYP3A enzymes is modulated by inducers (e.g. dexamethasone, phenobarbital, rifampicin) and inhibitors (ketoconazole, grapefruit juice) and this may lead to clinically significant drug interactions (Thummel and Wilkinson 1998).

The induction of intestinal CYP3A enzymes could be expected to be influenced by the route of administration of the inducing agent (Zhang *et al*. 1996). Indeed, the exposure of the enterocytes to an orally administered inducing agent may be very different from the enterocyte exposure to the same agent administered systemically. The primary objective of the current study was to evaluate the effect of the route of administration, i.e. oral (p.o.) versus intravenous (i.v.), of the inducing agent dexamethasone on the expression and activity of CYP3A enzymes in the rat intestinal epithelium and liver. The rate of midazolam metabolism by intestinal and hepatic microsomes was used as a marker of CYP3A activity (Ghosal *et al*. 1996). In addition, CYP3A1 and CYP3A2 protein levels in control and dexamethasone-induced intestinal and hepatic microsomes were measured by immunoblot analysis.



## Material and methods

*Chemicals*

Flunitrazepam, midazolam (MDZ), 1′-hydroxymidazolam (1′-OH-MDZ) and 4-hydroxymidazolam (4-OH-MDZ) were kindly donated by Dr T. W. Guentert, Hoffman-La Roche Ltd (Basel, Switzerland). $NADP^+$, NADPH, isocitric acid, isocitrate dehydrogenase and dexamethasone 21-phosphate disodium salt were purchased from ICN (Costa Mesa, CA, USA). Rat CYP3A1 and CYP3A2 supersomes were purchased from Gentest Corp. (Woburn, MA, USA). Polyclonal antibodies against CYP3A1 and CYP3A2 were obtained from Chemicon International, Inc. (Temecula, CA, USA). Reagents used for sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) and Western blotting were obtained from Bio-Rad Laboratories (Watford, UK). The ECL kit used for the detection of immunoreactive bands was obtained from Amersham. HPLC-grade acetonitrile and methanol were purchased from Baker (Deventer, The Netherlands). All other chemicals used were of the highest purity available from standard commercial sources.

*Animals*

Male Wistar rats (Iffa Credo, Belgium), 8 weeks old (approximately 250 g) at the time of sacrifice, were housed in an environmentally controlled room at 20–22 °C with a 12-h light/dark cycle and had free access to food (type AO4, U.A.R., Espinay-sur-Orge, France) and tap water. Rats were always fasted overnight before sacrifice.

*Study protocol*

The experimental protocol was approved by the Animals Ethics Committee of the Catholic University of Louvain. The animals were divided into four groups of 12 rats each: (1) control-receiving vehicle p.o., (2) dexamethasone p.o., (3) control i.v. and (4) dexamethasone i.v. Rats were treated with dexamethasone ($50\,mg\,kg^{-1}\,day^{-1}$) for 4 days via the oral (gavage) or i.v. route (jugular vein). Control rats were treated with vehicle only. Rats receiving dexamethasone by the i.v. route, as well as their controls, were fitted with jugular vein cannulae. They were operated under Thalamonal®(Janssen Pharmaceutica, Beerse, Belgium) anaesthesia on the day before the start of dexamethasone treatment and a piece of silastic tubing (0.94 mm o.d., 0.51 mm i.d., Intramedic, The Netherlands) was implanted in the right and left jugular veins. The control p.o. and dexamethasone p.o. treatment rats were killed by decapitation. The control i.v. and dexamethasone i.v. treatment rats were killed by an i.v. injection of air. The liver was removed as well as the small intestine, which was cut in two equal parts: the proximal and distal small intestine. In each treatment group, the liver and proximal part of the small intestine of three rats were pooled for the preparation of microsomes.

*Preparation of microsomes*

Liver microsomes were prepared by the method of Leclercq *et al.* (1997). Briefly, livers were homogenized in microsomal buffer (0.1 M phosphate buffer, pH 7.4, 0.1 M KCl, 1 mM EDTA) using a Potter Elvehjem homogenizer. The homogenate was centrifuged at 9000*g* for 10 min at 4 °C. The supernatant was transferred to a clean tube and centrifuged at 11 000*g* for 60 min at 4 °C. The microsomal pellet corresponding to 3.5 g liver was resuspended in 10 ml microsomal buffer. Enterocytes were isolated by the method of Keelan *et al.* (1994). Subsequently, intestinal microsomes were prepared by the same method as the one used for the preparation of liver microsomes. Microsomal fractions were stored at −80 °C until used. The protein concentration of the microsomal preparations was determined by the method of Lowry *et al.* (1951).

*Immunoquantification of CYP3A1 and CYP3A2*

A total of 0.4–6.2 µg intestinal microsomal protein and 0.5–3.1 µg hepatic protein was used for the immunoquantification of CYP3A1 and CYP3A2. Electrophoresis was performed using a 10% acrylamide gel under 125 V for 90 min. Proteins were then electrophoretically transferred to polyvinylidene difluoride membranes under 30 V overnight. The membranes were blocked for 60 min in TBS (20 mM Tris-HCl, pH 7.6, 137 mM NaCl) containing 5% (w/v) defatted milk and incubated for 2 h with anti-CYP3A1 (1:16 000) or anti-CYP3A2 (1:8000). The membranes were washed five times in TBS containing 0.1% Tween 20 before incubation for 1 h with horseradish peroxidase-conjugated anti-rabbit IgG (1:10 000) diluted in blocking buffer. After washing as described above, the membranes were rinsed in TBS and the immunoreactive bands detected using the ECL kit. The optical density (OD) of the immunoreactive bands was determined using the Image Master 1.10 program (Pharmacia Biotech, Roosendaal, The Netherlands). The results are expressed as $OD\,mm^2\,mg^{-1}$ protein.

*Microsomal incubations*

The incubation mixture contained (in a total volume of 1 ml): intestinal or hepatic microsomes ($0.6\,mg\ protein\,ml^{-1}$), 0.1 m sodium phosphate buffer, pH 7.4, 0.1 M KCl, 1 mM EDTA, 6 mM $MgCl_2.6H_2O$, 10 mM isocitric acid, 0.3 units isocitric dehydrogenase and MDZ at concentrations ranging from 5 to 370 µM. The mixture was incubated in a shaking water bath at 37 °C. After a pre-incubation period of 5 min, the reaction was started by the addition of 0.2 µM $NADP^+$ and 0.2 µM NADPH. After 16 min, the reaction was terminated by adding 40 µl NaOH (2%) and placing the samples on ice. The internal standard (IS) flunitrazepam was added to the incubation mixture (30 µl of $106\,\mu g\,ml^{-1}$ for hepatic microsomal incubations, 50 µl of $5\,\mu g\,ml^{-1}$ for intestinal microsomal incubations). MDZ metabolites were quantified by capillary HPLC by using a slight modification of a previously described method (Eeckhoudt *et al.* 1998). Briefly, the incubation mixtures were extracted with 4.5 ml diethyl ether:cyclohexane (69:31 v/v). After centrifugation, the organic layer was transferred to a clean tube and evaporated to dryness at 40C under a stream of nitrogen. The residue was dissolved in 100–1000 µl of a mixture of water:acetonitrile (95:5 v/v) and 20 µl were injected into the capillary HPLC system. The three MDZ metabolites and flunitrazepam (IS) eluted from the column with retention times of approximately 7 min (di-OH-MDZ), 9 min (4-OH-MDZ), 10 min (1′-OH-MDZ) and 12 min (flunitrazepam), respectively.

Supersomes ($0.21\,mg\ protein\,ml^{-1}$) were incubated following the same method as described above for hepatic and intestinal microsomes, but in the presence of a high concentration of MDZ (400 µM). The incubation mixture was extracted and analysed by HPLC as described above.

*Data analysis*

Untransformed enzyme kinetic data were analysed by non-linear regression analysis using PCNonlin (SCI Software, Lexington, KY, USA). A simple Michaelis–Menten model or Hill equation was fitted to the metabolite formation rates versus the substrate concentration. The choice between the two models was based on the Akaike Information Criterion, the Schwartz Criterion and the *F*-test (Gorski *et al.* 1994). Values in the text, tables and graphs are given as mean ± SEM. Means of $V_{max}$, $K_m$ and OD obtained in the four treatment groups were compared by ANOVA followed by the Newman–Keuls test. The results of the *post-hoc* test were taken into account for the following comparisons: control p.o. versus dexamethasone p.o., control i.v. versus dexamethasone i.v. control p.o. versus control i.v. The last comparison allowed us to evaluate the effect of surgery on the enzyme kinetic parameters. Independent sample *t*-tests were carried out to compare mean $V_{max}$ for the formation of 4-OH-MDZ and 1′-OH-MDZ between control p.o. control i.v. microsomes. $p \leq 0.05$ was considered as significant.

## Results

*Induction of hepatic MDZ metabolism by p.o. and i.v. dexamethasone treatment*

Incubation of midazolam with liver microsomes from control p.o. and control i.v. rats resulted in the formation of two metabolites (figure 1). $K_m$ and $V_{max}$ were estimated for 1′-OH-MDZ and 4-OH-MDZ using the Hill equation (table 1).

In the p.o. and i.v. control rats, $V_{max}$ for the formation of 4-OH-MDZ by hepatic microsomes were substantially higher (2–3-fold) than for the formation of 1′-OH-MDZ.

Induction by p.o. or i.v. pretreatment of rats with dexamethasone resulted in a substantial increase in the formation of all three metabolites. $V_{max}$ for the formation of 1′-OH-MDZ was on average 4.6 and 3.3 times higher after p.o. and i.v. dexamethasone pretreatment, respectively. Similarly, statistically significant increases in $V_{max}$ for the formation of 4-OH-MDZ, i.e. 4.5 times for the p.o. dexamethasone group and 3.7 times for the i.v. dexamethasone treatment group, were observed. Whereas $V_{max}$ for the formation of 1′-OH-MDZ and 4-OH-MDZ were not significantly different between the i.v. and p.o. control rats, $V_{max}$ for the formation of both metabolites were significantly higher following pretreatment with dexamethasone p.o. compared with the i.v. route (table 1). Pretreatment of the rats by p.o. or i.v. dexamethasone was also associated with statistically significant increases in the $K_m$ for the formation of both MDZ metabolites (table 1). For both 1′-OH-MDZ and 4-OH-MDZ, the mean $K_m$ following p.o. administration of dexamethasone ($34.1 \pm 2.6$ and $44.0 \pm 3.0\,\mu M$, respectively) were statistically significantly higher than the mean $K_m$ observed following i.v. administration of dexamethasone ($25.9 \pm 1.6$ and $37.3 \pm 1.1\,\mu M$, respectively).

*Induction of intestinal MDZ metabolism by p.o. and i.v. dexamethasone*

Following incubation of MDZ in the presence of proximal intestinal microsomes of control p.o. and control i.v. rats, the formation rates of 1′-OH-MDZ and

Table 1. $V_{max}$, $K_m$ and the Hill coefficient (Hill equation) for the formation of 4-OH-midazolam and 1′-OH-midazolam in liver microsomes of the control and dexamethasone treated rat. The statistical significance for the comparisons oral (p.o.) control versus intravenous (i.v.) dexa and p.o. dexa versus i.v. control are not shown.

| Parameter | Treatment | 1′-OH-midazolam | 4-OH-midazolam |
|---|---|---|---|
| $V_{max}$ ($\mu M\,min^{-1}\,mg^{-1}$ protein) | p.o. control | $0.310 \pm 0.020^{a}$ | $0.870 \pm 0.007^{d}$ |
| | p.o. dexa | $1.438 \pm 0.127^{a,c}$ | $3.928 \pm 0.316^{d,f}$ |
| | i.v. control | $0.253 \pm 0.026^{b}$ | $0.773 \pm 0.085^{e}$ |
| | i.v. dexa | $0.840 \pm 0.047^{b,c}$ | $2.840 \pm 0.114^{e,f}$ |
| $K_m$ ($\mu M$) | p.o. control | $13.1 \pm 1.4^{g}$ | $14.4 \pm 0.5^{j}$ |
| | p.o. dexa | $34.1 \pm 2.6^{g,l}$ | $44.0 \pm 3.0^{j,l}$ |
| | i.v. control | $13.3 \pm 1.1^{h}$ | $15.0 \pm 1.8^{k}$ |
| | i.v. dexa | $25.9 \pm 1.7^{h,i}$ | $37.3 \pm 1.1^{k,l}$ |
| Hill coefficient | p.o. control | $1.58 \pm 0.27$ | $1.34 \pm 0.18$ |
| | p.o. dexa | $2.12 \pm 0.48$ | $2.03 \pm 0.35$ |
| | i.v. control | $1.67 \pm 0.25$ | $1.52 \pm 0.27$ |
| | i.v. dexa | $2.42 \pm 0.84$ | $2.35 \pm 0.52$ |

Common superscripts are statistically different ($p < 0.05$, ANOVA)[a-l]
Data are the mean ± SEM of $n = 4$ determinations.

Table 2. $V_{max}$ and $K_m$ (Michaelis–Menten equation) for the formation of 4-OH-midazolam and 1′-OH-midazolam in intestinal microsomes of the control and dexamethasone treated rat. The statistical significance for the comparisons oral (p.o.) control versus intravenous (dexa) and p.o. dexa versus i.v. control are not shown.

| Parameter | Treatment | 1′-OH-midazolam | 4-OH-midazolam |
|---|---|---|---|
| $V_{max}$ (μM min$^{-1}$ mg$^{-1}$ protein) | p.o. control | $0.074 \pm 0.002^{a}$ | $0.078 \pm 0.002^{d}$ |
| | p.o. dexa | $0.439 \pm 0.039^{a,c}$ | $0.435 \pm 0.041^{d,f}$ |
| | i.v. control | $0.076 \pm 0.001^{b}$ | $0.078 \pm 0.001^{e}$ |
| | i.v. dexa | $0.159 \pm 0.010^{b,c}$ | $0.180 \pm 0.007^{e,f}$ |
| $K_m$ (μM) | p.o. control | $7.3 \pm 0.9$ | $23.0 \pm 1.3^{h}$ |
| | p.o. dexa | $6.3 \pm 0.6^{g}$ | $28.9 \pm 0.9^{h}$ |
| | i.v. control | $4.7 \pm 0.1$ | $21.6 \pm 1.7^{I}$ |
| | i.v. dexa | $3.7 \pm 0.8^{g}$ | $30.0 \pm 0.9^{i}$ |

Common superscripts are statistically different ($p < 0.05$, ANOVA).
Data are the mean ± SEM of $n = 4$ determinations.



4-OH-MDZ were determined (figure 1). $V_{max}$ and $K_m$ were estimated using a simple Michaelis–Menten equation. No statistically significant difference was observed in $V_{max}$ for the formation of 1′-OH-MDZ and 4-OH-MDZ between the control p.o. and control i.v. rats (table 2). Compared with the results obtained with control liver microsomes, mean $V_{max}$ for 1′-OH-MDZ and 4-OH-MDZ formation by intestinal microsomes were approximately three and 10 times lower, respectively.

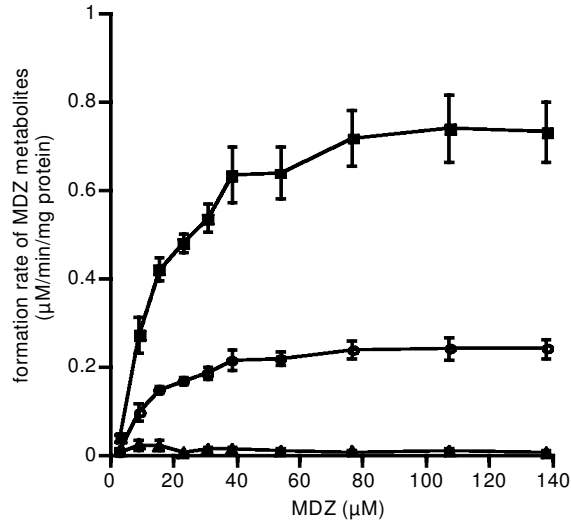
Induction by p.o. or i.v. dexamethasone pretreatment led to a significant increase in the formation rates of both 1′-OH-MDZ and 4-OH-MDZ. $V_{max}$ for the formation of 1′-OH-MDZ were on average 5.9 and 2.1 times higher following p.o. and i.v. dexamethasone pretreatment, respectively (table 2). Similarly, statistically significant increases in $V_{max}$ were observed for the formation of 4-OH-MDZ, i.e. 5.6 times for the p.o. dexamethasone group and 2.3 times for the i.v. dexamethasone treatment group. Whereas dexamethasone pretreatment did not lead to significant changes in the $K_m$ for the formation of 1′-OH-MDZ, it slightly but significantly increased the $K_m$ for the formation of 4-OH-MDZ (table 2).

### *MDZ metabolism by supersomes expressing CYP3A1 and CYP3A2*

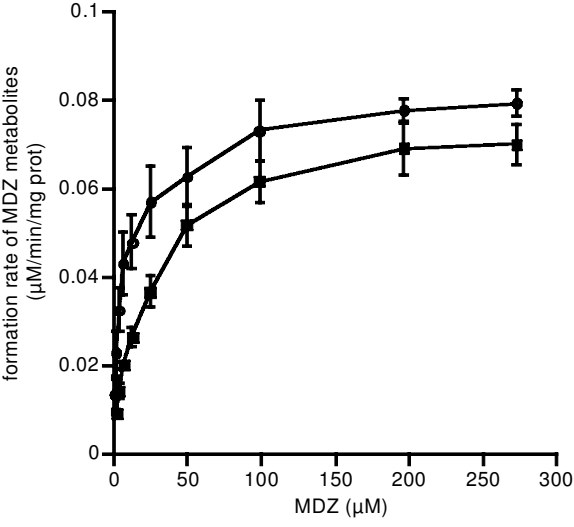
Incubation of MDZ (at a concentration of 400 μM) in the presence of supersomes expressing CYP3A1 resulted in comparable formation rates for 1′-OH-MDZ and 4-OH-MDZ. In the case of CYP3A2 supersomes, incubation with 400 μM MDZ also resulted in the formation of both MDZ metabolites, but the formation rate of 4-OH-MDZ was approximately twice as high compared with the 1′-OH-MDZ formation rate (data not shown).

### *Immunoquantification of CYP3A1 and CYP3A2 in control and induced hepatic and intestinal microsomes*

CYP3A1 and CYP3A2 were expressed in hepatic and intestinal microsomes of control rats, and p.o. and i.v. pretreatment with dexamethasone led to an increase in their expression. No statistical differences in CYP3A1 and CYP3A2




(A)




(B)

Figure 1. (A) Kinetics of formation of midazolam (MDZ) metabolites in rat liver microsomes (control i.v. treatment): —, 4-OH-midazolam; ○, 1′-OH-midazolam; △, 1′,4-diOH-midazolam. (B) Kinetics of formation of MDZ metabolites in rat intestinal microsomes (control i.v. treatment): —, 4-OH-midazolam; ○, 1′-OH-midazolam.

levels were observed between the p.o. and i.v. control rats (figure 2a, b). Our results suggest that CYP3A2 levels in liver microsomes seems to be higher (approximately 3–5-fold in p.o. and i.v. control rats, respectively) than CYP3A1 levels (figure 2a). Expression of both CYP3A1 and CYP3A2 in liver microsomes was significantly increased following p.o. (3.7- and 3.2-fold, respectively) or i.v. (2.6- and 2.1-fold, respectively) pretreatment with dexamethasone (figure 2a). In intestinal microsomes of both groups of control rats, CYP3A1 and CYP3A2 like

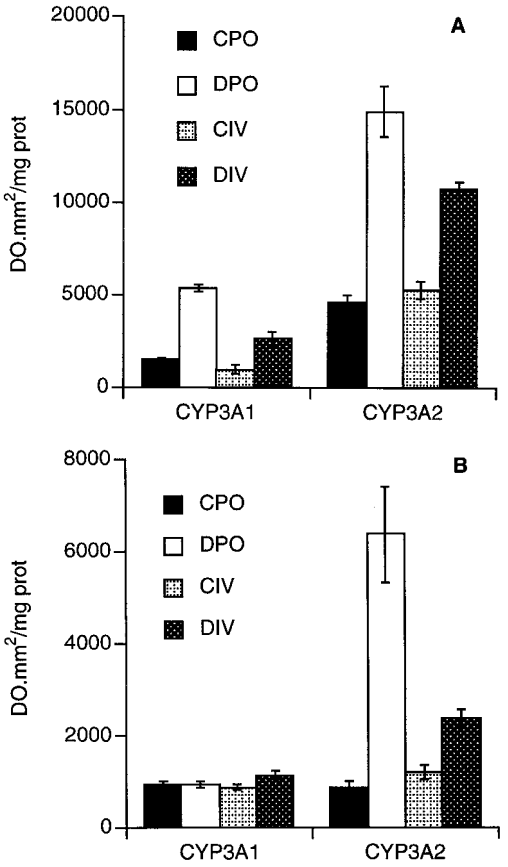



Figure 2. (A). CYP3A1 and CYP3A2 protein levels in rat liver microsomes: CPO, control oral (p.o.) rat; DPO, dexamethasone p.o.-treated rat; CIV, control intravenous (i.v.) rat; DIV, dexamethasone i.v.-treated rat. (B) CYP3A1 and CYP3A2 protein levels in rat intestinal microsomes: CPO, control p.o. rat; DPO, dexamethasone p.o.-treated rat; CIV, control i.v. rat; DIV, dexamethasone i.v.-treated rat.

protein expression seems to be similar (figure 2b). Intravenous or p.o. dexamethasone treatment did not result in a significant change in CYP3A1 levels in intestinal microsomes. On the contrary, treatment with dexamethasone led to an increase in CYP3A2-like protein levels in intestinal microsomes, but this increase was only statistically significant (7.3-fold) in the case of p.o. dexamethasone pretreatment (figure 2b). In microsomes of the control rat, expression of CYP3A1 was slightly lower (n.s.) in hepatic compared with intestinal microsomes, but CYP3A2 levels were significantly higher ($p \leq 0.001$) in hepatic microsomes.

As expected, no immunoreactive band was observed when the CYP3A1 supersomes were incubated with anti-CYP3A2 antibodies nor when the CYP3A2 supersomes were incubated with anti-CYP3A1 antibodies (figure 3). A slight difference in the migration distance of the immunoreactive band was observed for CYP3A2-like protein in intestinal microsomes compared with liver microsomes and supersomes (figure 3).


Figure 3. Western blot analysis showing the formation of protein–antibody complexes when CYP3A1 supersomes, CYP3A2 supersomes, control liver microsomes and control intestinal microsomes were incubated with CYP3A1 antibodies (lanes 1–4) or CYP3A2 antibodies (5–8). Samples of supersomes or microsomes were applied as follows: lanes 1, CYP3A2 supersomes; 2, CYP3A1 supersomes; 3, control liver microsomes; 4, control intestinal microsomes; 5, CYP3A1 supersomes; 6, CYP3A2 supersomes; 7, control liver microsomes; 8, control intestinal microsomes.



## Discussion

Biotransformation of midazolam to 1′-OH-MDZ and 4-OH-MDZ is catalysed in man as well as in rat by enzymes of the CYP3A subfamily (Gorski *et al*. 1994, Ghosal *et al*. 1996). The results of the present study show that pretreatment with dexamethasone, a known inducer of CYP3A enzymes in rat (Telkada *et al*. 1992, Higashikawa *et al*. 1995), resulted in significant increases in $V_{max}$ for the formation of both primary midazolam metabolites by hepatic and intestinal microsomes. In terms of the quantity of dexamethasone reaching the liver or intestine, oral administration was more effective than i.v. administration to induce both intestinal and hepatic CYP3A activity, but the difference was particularly pronounced in the small intestine.

The induction of intestinal CYP450 enzymes could be expected to be influenced by the route of administration of the inducing agent. Following p.o. administration of an enzyme inducer, the enterocytes are exposed to high concentrations of the inducer during its absorption across the intestinal epithelium. Exposure of the hepatic enzymes may also be high, although substantial intestinal first-pass metabolism of the inducer may limit its access to the hepatic enzymes. Zhang *et al*. (1996) reported that intestinal CYP1A1 was more sensitive to orally administered $\beta$-naphthoflavone, whereas induction of hepatic CYP1A1 was more sensitive to intraperitoneally administered $\beta$-naphthoflavone. Following systemic administration of an enzyme inducer, however, exposure of the intestinal CYP enzymes may be more limited. Indeed, CYP3A enzymes are most abundant at the tips of the villi, with lower levels found in the crypt cells (Kolars *et al*. 1992, Watkins *et al*. 1997). Consequently, an inducer present in the systemic circulation can only reach these enzymes by diffusion or transport across the intestinal epithelium from the basal to the apical side. The results of the present study show that there is clear induction of intestinal CYP3A activity following i.v. administration of dexamethasone indicating that this inducer has access from the systemic circulation to the CYP3A enzymes at the tips of the enterocyte villi. However, the degree of intestinal CYP3A induction is more pronounced when dexamethasone is administered orally. The inducibility of intestinal CYP3A activity following systemic administration of an enzyme inducer will depend upon the diffusion/transport characteristics of the inducer.

That induction of hepatic CYP3A activity is more pronounced following p.o. than i.v. administration of dexamethasone is not unexpected assuming that dexamethasone is not metabolized to a very high extent during its first pass through the intestinal epithelium to metabolites lacking enzyme induction properties. All dexamethasone absorbed from the gastrointestinal tract and which escapes the first-pass effect to 'inactive' metabolites, i.e. metabolit

capacity, arrives in the portal circulation and passes through the liver, whereas following i.v. administration only approximately 25% of the cardiac output passes through the liver. Consequently, concentrations of dexamethasone in the portal circulation, and therefore induction of hepatic enzymes, may be higher following p.o. compared with i.v. administration.

CYP3A1 and CYP3A2 expression in the liver was significantly increased by dexamethasone pretreatment. This finding is consistent with previously published results showing that both hepatic CYP3A enzymes are induced by dexamethasone (Chung *et al*. 1998, Watanabe *et al*. 1998, Wong *et al*. 1999). However, the results related to the induction by dexamethasone of CYP3A enzymes in rat enterocytes are more difficult to interpret. First, the CYP3A1 protein levels in enterocytes did not significantly increase after dexamethasone treatment. These results are not consistent with previously published data demonstrating the inducibility by dexamethasone of CYP3A1 in the rat small intestine (Kolars *et al*. 1992, Schmiedlin-Ren *et al*. 1993). In addition, our results indicate that the intestinal CYP3A2-like protein is different from the hepatic CYP3A2 protein. Indeed, the commercially available CYP3A1 and CYP3A2 antibodies showed specificity for the CYP3A1 and CYP3A2 supersomes. However, the CYP3A2 antibodies reacted with a protein in enterocyte microsomes, which showed a different migration distance in the SDS-PAGE compared with the migration distance of the antibody–CYP3A2 protein complex as revealed in the CYP3A2 supersomes and the liver microsomes. This indicates that the intestinal CYP3A2-like protein is very likely another CYP3A enzyme showing cross-reactivity with the commercial CYP3A2 antibody. Guschin *et al*. (1999) recently demonstrated the presence of a new CYP3A enzyme in rat intestinal microsomes. The gene for this intestinal CYP3A isoenzyme shows 97% identity with rat olfactory *CYP3A9*. Based on their results, Guschin *et al*. suggest that rat intestinal CYP3A differs from CYP3A1 and CYP3A2.

In conclusion, the results of the present study show that i.v. administration of dexamethasone leads to significant induction of both intestinal and hepatic CYP3A activity towards midazolam. Oral administration of dexamethasone, however, is more efficient than i.v. administration inducing CYP3A activity in both these tissues. Hepatic CYP3A1 and CYP3A2 protein levels were increased by dexamethasone pretreatment. Intestinal CYP3A1 protein levels, however, were not affected by dexamethasone pretreatment. In addition, the CYP3A2 antibodies reacted with a protein in the intestinal microsomes, which was different from the hepatic CYP3A2 as demonstrated by immunoblotting. These results support the finding of Gushchin *et al*. (1999) that intestinal CYP3A enzymes in rat differ from hepatic CYP3A1 and CYP3A2.



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