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EFFECT OF 1-BENZYLIMIDAZOLE ON CYTOCHROMES P-450 INDUCTION AND ON THE ACTIVITIES OF EPOXIDE HYDROLASES AND UDP-GLUCURONOSYLTRANSFERASES IN RAT LIVER

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Abstract—The influence of 1-benzylimidazole on the activities of hepatic monooxygenases cytochromes P-450 dependent, epoxide hydrolases and UDP-glucuronosyltransferases was investigated in male Wistar rats. Several doses (25, 75 and 100 mg/kg/day) were administered gastrically during 5 days in order to evaluate the dose-related induction. The treatment caused a dose-dependent hepatomegaly. 1-Benzylimidazole decreased the plasma level in triglycerides by 60–70%; by contrast the cholesterol content was not changed during the time course of the experiment. Lauric acid hydroxylase, benzphetamine *N*-demethylase, 7-ethoxyresorufin *O*-deethylase, 7-ethoxycoumarin *O*-deethylase activities were increased 3.5-, 4-, 13- and 46-fold, respectively with the highest dose. By immunoblotting, an enhancement in the protein bands corresponding to cytochromes P-450c and P-450b could be simultaneously observed, whatever the dose administered, thus suggesting an induction process. However, 1-benzylimidazole failed to bind with high affinity to the cytosolic *Ah* receptor. On the other hand, measurement of the activity of the microsomal epoxide hydrolase with benzo(*a*)pyrene-4,5-oxide as substrate and quantitation of the enzyme protein by immunoassay revealed that the increase in the activity after treatment with the compound was the result of enzyme activation only. By contrast, cytosolic epoxide hydrolase was not affected by 1-benzylimidazole. This compound also stimulated three distinct forms of UDP-glucuronosyltransferase. The activities towards 4-methylumbelliferone, 1-naphthol, morphine or a monoterpenoid alcohol, nopol, supported by two different isozymes were significantly increased only with the highest dose; meanwhile bilirubin glucuronidation was 2-fold enhanced, whatever the dose used. These observations emphasize the variety of the effects of 1-benzylimidazole on drug-metabolizing enzymes.

Imidazole- and benzimidazole-related compounds have large therapeutic applications. Clinical and experimental studies revealed the potency of compounds containing imidazole structure to induce or, on the contrary, inhibit mixed function oxidase activity [1]. Substituted imidazoles have been shown to bind strongly *in vitro* to hepatic cytochrome P-450 and thereafter inhibit hydroxylation reactions [2]. By using several classes of imidazole derivatives a relationship between lipophilicity, structural factors and inhibitory capacity could be established [3, 4]. On the other hand, induction of hepatic cytochrome P-450 by some of the most potent inhibitors of mixed-function oxidases such as clotrimazole, miconazole or ketoconazole has also been reported [5]. Characterization of their inducing properties revealed that each compound exhibited its own induction profile [6].

Induction of conjugation enzymes such as UDP-glucuronosyltransferases (EC, 2.4.1.17) by these drugs has been poorly investigated until now. The activity of these membrane-bound enzymes is selectively enhanced by various chemicals. 3-Methylcholanthrene increases specifically glucuronidation of planar hydroxylated compounds or group 1 substrates such as naphthol or 4-methylumbelliferone, while phenobarbital stimulates preferentially conjugation of bulkier molecules (monoterpenoid alcohols, morphine) or group 2 substrates [7]. Finally hypolipidaemic agents have been found to induce specifically glucuronidation of bilirubin [8]. These three groups of activities are believed to be supported by different enzyme proteins [9]. In that respect it was interesting to elucidate if 1-benzylimidazole (Fig. 1), which was reported to decrease plasma lipid levels [10], was able to increase a distinct form of UDP-glucuronosyltransferase.

This study was conducted to elucidate which molecular forms of hepatic cytochromes P-450, UDP-

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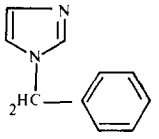

Fig. 1. Structure of 1-benzylimidazole.

glucuronosyltransferases and epoxide hydrolases, which are also sensitive to treatment with drugs are affected by different doses of 1-benzylimidazole in rats.

MATERIALS AND METHODS

Chemicals. 1-Benzylimidazole and lauric acid were purchased from Aldrich Chemicals (Strasbourg, France). (1-^{14}C) Lauric acid (9.25 MBq; 2.18 GBq/mmol) and (G-^{3}H) benzo(*a*)pyrene (185 MBq; 3.41 TBq/mmol were obtained from Amersham (Little Chalfont, Buckinghamshire, U.K.). Benzphetamine chlorhydrate was a gift from Upjohn Laboratory (Paris, France), and benzo(*a*)pyrene was purchased from Fluka (Buchs, Switzerland). 7-Ethoxyresorufin was purchased from Pierce (Interchim, Montluçon, France) and 7-ethoxycoumarin from Sigma (St Louis, MO). Silica gel plates (60 F254, 20 × 20 cm, thickness 0.25 mm) for thin-layer chromatography were obtained from Merck (Darmstadt, F.R.G.), and 1,2-diphenyl-1,3,5-hexatriene from Koch-Light (Colnbrook, U.K.).

Substrates for UDP-glucuronosyltransferase were 4-methylumbelliferone, a monoterpenoid alcohol nopol (Fluka, Buchs, Switzerland), 1-naphthol, bilirubin (Merck, Darmstadt, F.R.G.), and morphine (Coopérative Pharmaceutique Française, Melun, France). Triton X-100 was purchased from Merck (Darmstadt, F.R.G.) and digitonin from Sigma (St Louis, MO). Pyruvate kinase, NADH, lactate dehydrogenase, UDP-glucuronic acid (disodium salt), phosphoenolpyruvate, and NADPH were obtained from Boehringer (Mannheim, F.R.G.).

All the other reagents were of the best purity commercially available.

Animals, drug treatment, microsomes and plasma preparation. 1-Benzylimidazole, suspended in sucrose syrup, was given at a daily dose of 25, 75 and 100 mg/kg for 5 days by gastric intubation to groups of 3 male Wistar rats weighing 200–230 g (Iffa-Credo, St Germain sur l'Abresle, France). Control animals received the vehicle, only. All animals had free access to food (commercial diet, U.A.R. Villemoisson, France) and tap water. A daily determination of food intake revealed that food consumption was not modified by the treatments.

Rats were killed by decapitation 24 hr after the last ingestion and after 12 hr of starvation. Blood samples were collected in heparine tubes and centrifuged at 2500 *g* for 5 min. The livers were homogenized individually in a Potter-Elvejhem apparatus and the microsomal fraction was prepared according to Hogeboom [11]. Microsome pellets were finally suspended and homogenized in 0.25 M sucrose, 1 mM Tris–HCl buffer, pH 7.4 to give a microsomes-liver weight ratio of 2:1. Plasma, microsomes and the corresponding cytosolic fraction, were stored in small aliquots at −30° until use.

Plasma lipid levels, microsomal protein, fluorescence polarization and cytochrome P-450 *content*. Cholesterol and triglyceride levels in plasma were measured with enzymatic and colorimetric tests developed by Boehringer (Mannheim, F.R.G.). Protein concentration in microsomes and cytosol was determined by the method of Lowry *et al.* [12], with bovine serum albumin as standard. The anisotropy of the steady-state fluorescence emission of 1,6-diphenyl-1,3,5-hexatriene was recorded on a spectrofluorimeter Jobin-Yvon JY3 equipped with a polarization accessory and coupled to an Apple II computer for data processing. The incubation conditions of the fluorescent probe with the microsomal membranes have been already described [8]. Cytochrome P-450 content was estimated from CO difference spectra of dithionite-reduced samples using a value of 91 $mM^{-1}.cm^{-1}$ for the molar extinction increment between 450 and 490 nm [13].

Estimation of the binding of 1-benzylimidazole on the Ah *receptor*. The hepatic cytosolic fraction of male Wistar rats was prepared, as already indicated, in 25 mM Hepes buffer, pH 7.4, containing 1.5 mM EDTA, 1 mM dithiothreitol and 10% v/v glycerol. The rats were previously treated with phenobarbital in saline, at a single intraperitoneal dose of 100 mg/kg/body weight, and then given with drinking water (1 g/l) for 5 days. The binding of tritiated benzo(*a*)-pyrene and the competition of a 200 molar excess of 1-benzylimidazole for the cytosolic receptor was measured using the hydroxylapatite binding assay, described by Gasiewicz and Neal [14], and modified by Houser *et al.* [15].

Enzyme assays. Benzphetamine *N*-demethylase activity measurement was carried out by the method of Yang and Strickhart [16], that of 7-ethoxycoumarin deethylase and 7-ethoxyresorufin by the techniques of Ullrich and Weber [17], and Burke and Mayer [18], respectively. w-, w-1 Hydroxylations of lauric acid were followed according to Salaün *et al.* [19]; the hydroxylated products, which migrated as a single spot were separated from lauric acid by thin-layer chromatography with petroleum ether/ethyl ether/acetic acid 30:70:2 v/v as the mobile phase. The radioactivity associated to the metabolites was detected by an automatic TLC linear analyser (Berthold, LB 2832, Wildbad, F.R.G.). After scraping the plates, the content in radioactive products was calculated by liquid scintillation counting on a Beckman LS 1801 apparatus (Palo-Alto, CA).

The activity of the microsomal epoxide hydrolase was measured by the method of Dansette *et al.* [20] with benzo-(*a*)pyrene-4,5-oxide as fluorescent substrate (IIT, Chicago, IL); quantitation of the enzyme protein was achieved by immunoassay as previously described [21]. Cytosolic epoxide hydrolase activity was monitored with tritiated *trans*-stilbene oxide as substrate [22].

UDP-glucuronosyltransferase activities towards monohydroxylated aglycones (group 1 substrates, 4-methylumbelliferone, 1-naphthol; group 2 substrates, nopol, morphine) were measured in Triton X-100 activated microsomes by the Mulder and

Van Doorn's method [23], adapted on a Cobas fast analyser centrifuge (Roche Bioelectronic, Basel, Switzerland) [24]. Bilirubin UDP-glucuronosyltransferase activity was evaluated according to Heirwegh *et al.* [25].

Electrophoresis. Microsomes were submitted to sodium dodecylsulfate polyacrylamide gel electrophoresis [26], using 10% w/v acrylamide in the separating gel and 5% w/v in the stacking gel. Proteins were transferred electrophoretically with a Trans-Blot system (Bio-Rad, Richmond, CA) at room temperature for 90 min at 70 V in 20 mM Tris–HCl buffer (pH 8.3) containing 20% v/v methanol and 150 mM glycine [27].

For the immunochemical detection, nitrocellulose sheets containing protein samples, after electrophoretic transfer, were incubated for 30 min at room temperature in 150 mM sodium phosphate buffer containing 140 mM NaCl, 3 mM KCl, 10% v/v new born calf serum and 0.2% Triton X-100. IgG were purified from rabbit antibodies raised against cytochrome P-450c (P-448) or P-450b (P-450 induced by phenobarbital). The IgG were added after dilution and the incubation was carried out for 1 hr at room temperature. The sheets were washed four times with the phosphate buffer and then incubated in a 1/500 dilution of peroxidase-conjugated sheep anti rabbit IgG (Pasteur Institute, Paris, France) for 1 hr, followed by washing as described above. Peroxidase activity was detected with 3-3'-diaminobenzidine (Sigma, St Louis, MO). The reaction was quenched after 5 min by immersing the nitrocellulose into water.

Statistical determination. The data were reported as means ± SD for three to four animals and were compared by Student's *t*-test for small samples and non-paired series. A difference between groups of $P < 0.05$ was considered significant.

RESULTS

Morphological hepatic effects of 1-*benzylimidazole*

1-Benzylimidazole did not change the body weight but was responsible for an important dose-related hepatomegaly (30–40% over controls), thus increasing the liver/body weight ratio accordingly (Table 1). Microsomal proteins increased by 35% after a 1-benzylimidazole dose of 75 mg/kg/day; on the other hand, concentration in cytosolic proteins did not vary at all (data not shown). Alkaline phosphatase, transaminases and gamma-glutamyltransferase activities in plasma were not modified by 1-benzylimidazole treatment, even with the highest dose (data not shown). Modifications of the physicochemical properties of the microsomal membranes upon administration of 1-benzylimidazole were estimated from measurements of the mean anisotropy (r) of the fluorescent probe 1,6 diphenyl-1,3,5-hexatriene. Whatever the dose used, (r) was not significantly changed and ranged from 0.131 ± 0.008 for the controls to 0.124 ± 0.006 for rats treated at the dose 100 mg/kg.

Hypolipidaemic effect of 1-*benzylimidazole*

Table 1 also indicated the effect of 1-benzylimidazole on cholesterol and triglyceride levels in plasma. This compound caused important decreases in the content in triglycerides (60–70%); by contrast, no significant decrease on cholesterol concentration was observed, whatever the dose used.

Cytochrome P-450 *dependent monooxygenases*

The cytochrome P-450 content was increased by treatment with 1-benzylimidazole 0.6–2.6 times according to the dose (Table 2). In order to define the action of this drug on specific forms of cytochrome P-450, different monooxygenase activities were monitored. All the activities measured were strongly increased in a dose-dependent manner (Table 2). For example the 7-ethoxyresorufin *O*-deethylation (cytochrome P-448 dependent) and the 7-ethoxycoumarin *O*-deethylation (cytochromes P-450–448 dependent) were enhanced more than 13 and 46 times, respectively, after treatment of rats by the highest dose. Benzphetamine *N*-demethylation (cytochrome P-450 dependent) and w-, w-1 hydroxylations of lauric acid were increased by 4 and 3.5 times, respectively (Table 2).

The results of the immunoblotting of cytochromes P-450 are presented in Fig. 2. The specificity of the antibodies used was checked by their ability to recognize selectively cytochrome P-448 or P-450 from 3-methylcholanthrene or phenobarbital-treated rats. After treatment with 1-benzylimidazole, a 55 kDa and a 51 kDa band could be revealed using antibodies against cytochromes P-448 and P-450, respectively. The intensity of the staining corresponding to these two bands was increasing as a function of the dose administered. All together with the results of the different monooxygenases activities

Table 1. Effect of 1-benzylimidazole on morphological parameters and on cholesterol and triglyceride plasma levels

	Morphological parameters			Plasma lipids	
Treatment (dose)	Body weight (g)	Liver weight (g)	Liver weight/ body weight (× 100)	Cholesterol (mmol/l)	Triglycerides (mmol/l)
Controls	213 ± 4	6.50 ± 0.10	3.05 ± 0.12	1.79 ± 0.21	2.01 ± 0.53
25 mg/kg/day	228 ± 2	8.53 ± 0.29[a]	3.73 ± 0.09[a]	2.03 ± 0.10	0.83 ± 0.09[a]
75 mg/kg/day	220 ± 1	8.90 ± 0.27[a]	4.00 ± 0.13[a]	1.76 ± 0.18	0.56 ± 0.06[a]
100 mg/kg/day	205 ± 6	9.22 ± 0.74[a]	4.50 ± 0.36[a]	1.46 ± 0.32	0.55 ± 0.05[a]

Values are the means ± SD for 3 to 4 animals. [a] $P \leq 0.05$, significantly different from control group.

Table 2. Effect of 1-benzylimidazole on microsomal cytochrome P-450 concentration and monooxygenase activities

Treatment (dose)	Cytochrome P-450 content	Monooxygenase activities			
		Benzphetamine *N*-demethylation	7-Ethoxyresorufin *O*-deethylation	7-Ethoxycoumarin *O*-deethylation	Lauric acid hydroxylation
Controls	0.78 ± 0.04	2.45 ± 0.52	27 ± 1.0	0.13 ± 0.02	0.32 ± 0.03
25 mg/kg/day	1.25 ± 0.21[a]	4.54 ± 0.43[a]	40 ± 1.9[a]	2.54 ± 0.61[a]	0.30 ± 0.09
75 mg/kg/day	1.67 ± 0.11[a]	7.07 ± 1.04[a]	129 ± 1.5[a]	4.45 ± 0.95[a]	0.91 ± 0.07[a]
100 mg/kg/day	2.04 ± 0.53[a]	9.78 ± 1.17[a]	357 ± 75.0[a]	6.01 ± 1.05[a]	1.18 ± 0.01[a]

Cytochrome P-450 was expressed as nmol/mg protein; 7-ethoxyresorufin *O*-deethylase activity was expressed as pmol/min/mg protein; benzphetamine *N*-demethylase, 7-ethoxycoumarin *O*-deethylase, lauric acid hydroxylase activities were expressed as nmol/min/mg protein. Values are the means ± SD for 3 to 4 animals. [a] $P < 0.05$, significantly different from control group.

Table 3. Comparative binding of 1-benzylimidazole and benzo(*a*)pyrene on the *Ah* receptor

Ligand	Specific binding of (^{3}H)benzo(*a*)pyrene (cpm/mg cytosolic protein)
(1) (^{3}H)Benzo(*a*)pyrene	13907 ± 1330
(2) (^{3}H)Benzo(*a*)pyrene + 1-benzylimidazole	14073 ± 694
(3) (^{3}H)Benzo(*a*)pyrene + cold benzo(*a*)pyrene	4184 ± 700

Cytosol (2.4 mg/ml protein, 2 ml) was incubated for 2 hr at 20° in presence of either 2 pmoles tritiated benzo(*a*)pyrene alone (1), or the same amount of the labelled compound in presence of 400 pmoles of 1-benzylimidazole (2), or of 400 pmoles of cold benzo(*a*)pyrene (3). The proteins were thereafter adsorbed on hydroxylapatite by incubation for 30 min at 0°. The gel was washed 3 times with the Hepes buffer containing Triton X-100 0.5% v/v. Finally the radioactivity associated to the hydroxylapatite was determined by liquid scintillation spectrometry. Values are the mean ± SD for 4 to 6 independent determinations.

(Table 2), this study indicated that 1-benzylimidazole could induce both cytochromes P-450b and c.

The binding of 1-benzylimidazole on the cytosolic *Ah* receptor by comparison with that of the polycyclic hydrocarbon benzo(*a*)pyrene is illustrated in Table 3. Competition experiment, using a 200 molar excess of 1-benzylimidazole, revealed that this drug was unable to displace effectively the benzo(*a*)pyrene from the receptor.

Epoxide hydrolases

Microsomal epoxide hydrolase activity was also increased (2.6 times) by 1-benzylimidazole 75 or 100 mg/kg/day (Table 4). However, quantitation of the enzyme revealed no change in the amount of epoxide hydrolase in microsomes. This strongly suggests that the drug causes activation of membrane-bound epoxide hydrolase.

By contrast, cytosolic epoxide hydrolase was not affected by 1-benzylimidazole, whatever the dose used (Table 4).

UDP-glucuronosyltransferase activities

1-Benzylimidazole was also examined for its ability to increase the activity of distinct forms of UDP-glucuronosyltransferases. Figure 3 represents the

Table 4. Effect of 1-benzylimidazole on epoxide hydrolases

Treatment (dose)	Microsomal EH		Cytosolic EH
	Concentration[a]	Activity[b]	Activity[c]
Controls	2.66 ± 0.72	7.73 ± 1.30	36.9 ± 5.9
25 mg/kg/day	2.38 ± 0.45	9.42 ± 0.88	44.4 ± 10.5
75 mg/kg/day	3.56 ± 0.37	20.52 ± 2.18[d]	46.7 ± 13.2
100 mg/kg/day	3.56 ± 0.61	19.92 ± 4.48[d]	39.3 ± 8.0

The activity of microsomal and cytosolic epoxide hydrolases (EH) was measured with benzo(*a*)pyrene-4,5-oxide and *trans*-stilbene oxide as substrates, respectively. [a] mg microsomal epoxide hydrolase/mg microsomal total protein × 100; [b] nmol/min/mg protein; [c] pmol/min/mg protein. Values are the means ± SD for 3 to 4 animals. [d] $P < 0.05$, significantly different from control group.

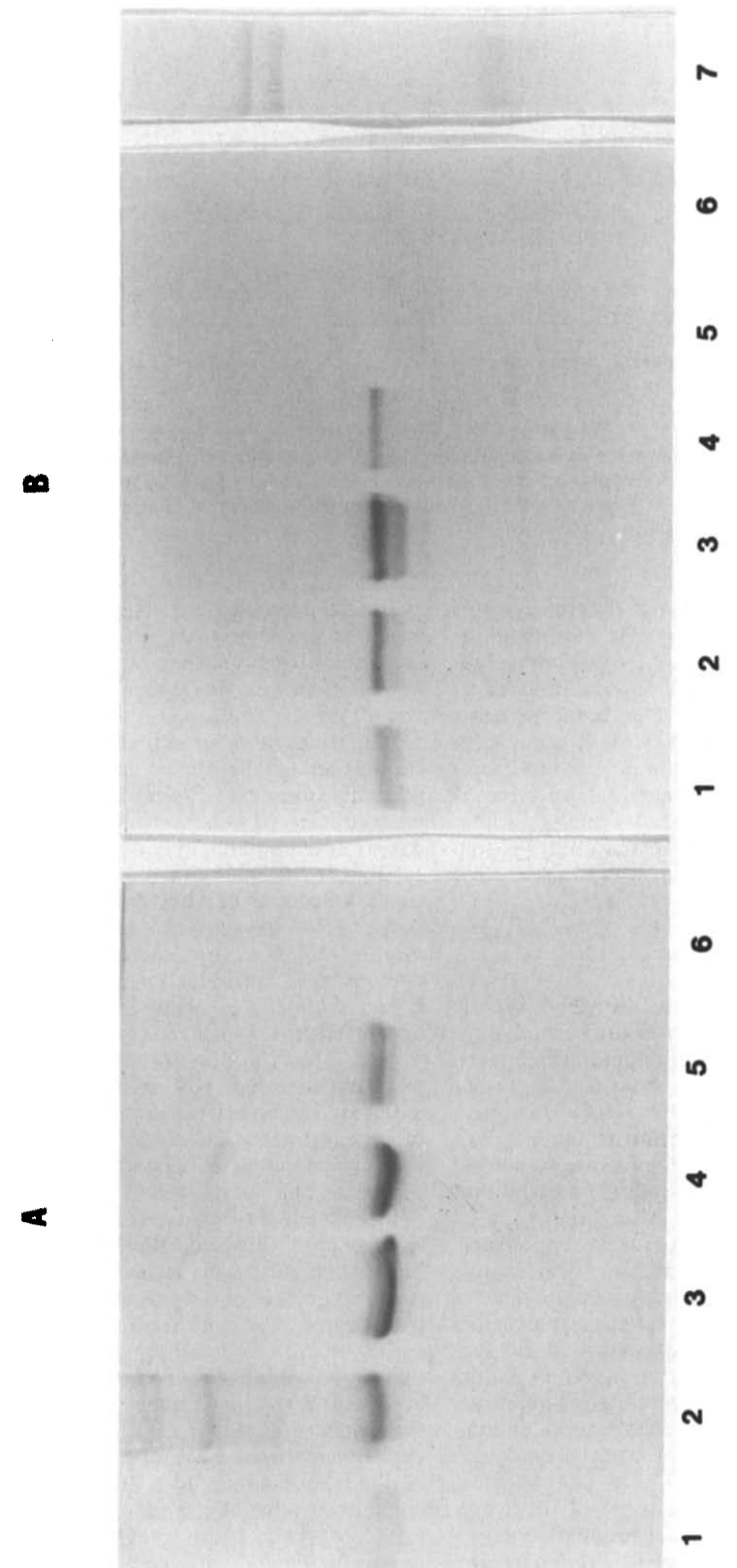

Fig. 2. Immunoblotting of rat liver cytochromes P-450 after induction by 1-benzylimidazole. (A) Antibodies against cytochrome P-450b (phenobarbital), dilution 1/700; lane 1, control; lanes 2,3,4,1-benzylimidazole 25, 75 and 100 mg/day, respectively; lanes 5 and 6, microsomes from rats induced by phenobarbital and 3-methylcholanthrene, respectively. (B) Antibodies against cytochrome P-450c (3-methylcholanthrene), dilution 1/1000; lanes 1, 2 and 3, 1-benzylimidazole 25, 75 and 100 mg/kg, respectively; lanes 4 and 5, microsomes from rats treated by 3-methylcholanthrene and phenobarbital, respectively; lane 6, control. In each lane 5 μg protein were loaded into the gel. Molecular weight markers are in lane 7.

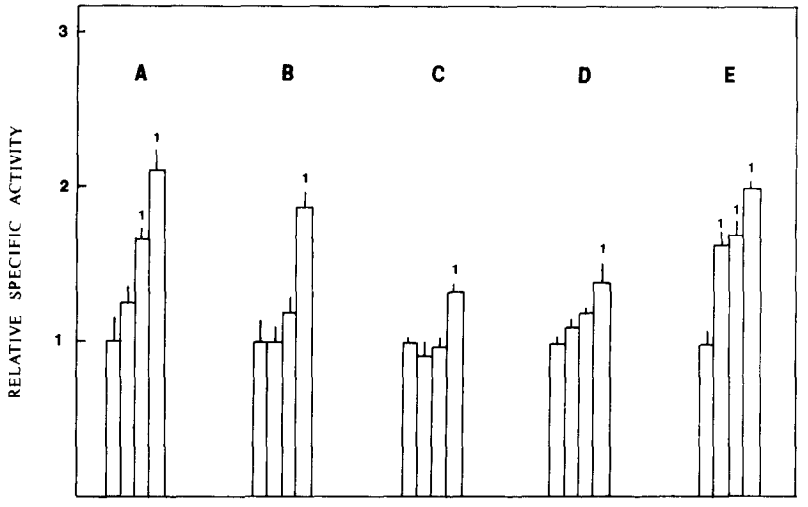

Fig. 3. Effect of 1-benzylimidazole on microsomal UDP-glucuronosyltransferases. The activities towards methylumbelliferone (A), 1-naphthol (B), nopol (C), morphine (D) and bilirubin (E) were reported as a ratio between control and treated rats. For each substrate bars correspond, from left to right, to treatments with 0 (control), 25, 75, 100 mg 1-benzylimidazole/kg/day, respectively. Specific activities for controls were (nmol/min/mg microsomal protein): 54.6 ± 8.0 for 4-methylumbelliferone, 20.2 ± 2.9 for 1-naphthol, 12.4 ± 0.5 for nopol, 4.5 ± 0.2 for morphine, 0.83 ± 0.08 for bilirubin. Values are the mean ± SD for 3 to 4 animals. $^1P \leq 0.05$, significantly different from control group.

values of specific activity towards different groups of substrates after treatment by this compound. 1-Benzylimidazole increased 1.6–2.1-fold conjugation of 4-methylumbelliferone and 1.6-fold that of 1-naphthol (group 1 substrates). Significant increases were found only with the highest dose used. Glucuronidation of morphine and nopol (group 2 substrates) was less enhanced than that of group 1 substrates (30–40% over controls). Finally bilirubin conjugation was significantly increased 1.7–2-fold over controls by this compound, whatever the dose (Fig. 3).

DISCUSSION

1-Benzylimidazole treatment disturbed greatly plasma triglycerides and hepatic drug-metabolism enzymes in Wistar rats. This compound, when given for a long time period (6 weeks) showed an important plasma cholesterol lowering effect [10]. This effect did not appear during our experiment, the length of treatment being too short to demonstrate major and lasting modifications in cholesterol metabolism. Nevertheless, imidazoles and structurally related compounds were responsible for morphological changes, lipid accumulation in liver and disturbances in hepatic metabolism [10]. Particularly, imidazoles were involved in accumulation of sterol precursors and probably inhibited a major step in sterol synthesis [28]. The lowering effect observed on plasma triglyceride level may be related either to inhibition of triacylglycerol secretion by imidazole-related compounds [29] or to an increase of triacylglycerol-rich lipoproteins catabolism [30].

Various drugs or chemicals, some of them presenting a hypolipidaemic effect, cause liver enlargement [31]. This is also the case of 1-benzylimidazole, even at the lowest dose. This hepatomegaly is generally followed in subchronic treatment by proliferation of endoplasmic reticulum, and by a large induction of enzymes especially those involved in xenobiotic metabolism.

Imidazoles and structurally related compounds showed antagonist effects on phase I enzymes and particularly on cytochrome P-450(s). Most of them were considered as inhibitors of mixed-function oxidases, and lipophilicity or structural factors were related to the inhibition potency [32–34]. By contrast, ketoconazole, miconazole and clotrimazole did not affect the activity of lauric acid hydroxylation *in vitro* [35]. Recently, these imidazole compounds demonstrated an induction capacity on liver cytochromes P-450, especially the *N*-substituted imidazoles such as clotrimazole which was able to induce several forms [6, 36] according to the amount of the drug administered. 1-Benzylimidazole, as indicated in this study, showed induction potency on two forms of cytochromes P-450 (b and c) and stimulation of the corresponding associated hydroxylase activities, whatever the dose tested. However, 1-benzylimidazole failed to displace benzo(*a*)pyrene from the *Ah* receptor, thus indicating that the drug did not bind with high affinity. If the fixation on the receptor is a prerequisite for induction of cytochrome P-450c, the isozyme most closely associated with aryl hydrocarbon hydroxylase activity, one must acknowledge that 1-benzylimidazole could bind to the receptor, even with very low efficiency in order to account for the induction of this type of cytochrome. This could explain why the compound caused a weak induction of 7-ethoxyresorufin *O*-deethylase (13-fold) when compared to that observed with methylcholanthrene (200-fold) [37]. Moreover 1-benzylimidazole also greatly increased hydroxylation of lauric acid. Induc-

tion of w hydroxylation activity (cytochrome P-452 dependent) occurred specifically with plasma lipid-lowering compounds such as clofibrate, whereas stimulation of w-1 hydroxylation activity was observed after clofibrate or phenobarbital treatments [38]. Although the induction of cytochrome P-452 was not the direct cause of the hypolipidaemic effect observed [39], one must acknowledge there is, nevertheless, a relationship between induction of this form of cytochrome and decrease in plasma lipids. Existence of an endogenous substrate more physiologically important than lauric acid itself, such as arachidonic acid, could be considered, since 1-benzylimidazole has been found to affect prostaglandin metabolism [40].

Imidazole and structurally related compounds have been reported to enhance greatly the activity of microsomal epoxide hydrolase [41, 42]. Immuno quantitation of the protein demonstrates that microsomal epoxide hydrolase was only activated and not induced by this compound. This result was in good accordance with that of Seidegard *et al.* [43]. On the other hand the weak increase in activity of cytosolic epoxide hydrolase after treatment with 1-benzylimidazole (1.8-fold) [42] was not observed in this study, indicating that no peroxisome proliferation occurred. This may be due to the fact that application of the drug, duration of treatment and rat strains were different.

The increase in activity of several forms of UDP-glucuronosyltransferases could be due to induction process rather than activation. This was supported by the fact that, when liver microsomes were pre-incubated, *in vitro*, with 1-benzylimidazole up to a concentration of 5 mM, there was no change in the enzyme activity (data not shown); thus suggesting that no activation was likely to occur during the treatments. Finally, as indicated from fluorescence polarization experiments, possible changes in the physicochemical properties of the microsomal membranes, which could affect the latency of the enzyme could also be ruled out. Until now, the induction of UDP-glucuronosyltransferases by imidazoles has not been extensively investigated. Glucuronidation of 4-nitrophenol by *N*-imidazoles was reported to increase [44]. On the other hand, Souhaili-El Amri *et al.* [45] have shown that methyl(5-(propylthio)-1*H*-benzimidazole-2yl)carbamate or Albendazole increased glucuronidation of group 1 substrates, only. 1-Benzylimidazole enhanced non-specifically all the activities tested, even if conjugation of group 2 substrates seemed less favoured than group 1 substrates. Interestingly, bilirubin glucuronidation was enhanced by 1-benzylimidazole even with the lowest dose (25 mg/kg). Few compounds are known to enhance activity of this enzyme form. This is particularly the case of clofibrate-related structures and some other arylcarboxylic acids [8].

In conclusion, 1-benzylimidazole administration causes a non specific induction of drug-metabolizing enzymes in the liver. The variety of these effects raises the question of the mechanism of action of this molecule.

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