JPP Journal of Pharmacy and Pharmacology

JPP 2003, 55: 773–781

Received October 29, 2002
Accepted February 12, 2003
DOI 10.1211/0022357021044
ISSN 0022-3573

The effects of mebendazole on P4501A activity in rat hepatocytes and HepG2 cells. Comparison with tiabendazole and omeprazole

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Abstract

Mebendazole is a benzimidazole anthelmintic widely used in veterinary and human therapy. Among benzimidazole derivatives, several drugs with inducing effect on cytochromes P450 can be found. However, the induction capacity of mebendazole on P450s has not been explored yet. In this study, the effects of mebendazole on P4501A activity was tested in primary cultures of rat hepatocytes and in human hepatoma HepG2 cell line. Two known P4501A inducers with benzimidazole structure, tiabendazole and omeprazole, were also included in the experiments with the aim of studying structure–induction relationships. After 24-, 48- and 72-h incubation of rat hepatocytes and HepG2 cells with drugs in various concentrations (0.1–100 μM), enzyme activity associated with P4501A1/2 (EROD, MROD) was measured. In addition, the P4501A1/2 protein levels in both in-vitro systems were determined by Western-blotting. Mebendazole provoked a significant increase in P4501A1/2 protein expression and P4501A activity in both in-vitro systems. Omeprazole caused a significant dose-dependent increase of P4501A activity only in HepG2 cells. Although tiabendazole treatment led to significant increase of P4501A protein level, no effect on P4501A activity was observed in either system. The results demonstrate that mebendazole possesses the ability to significantly induce P4501A. Thus, pharmacological and toxicological consequences of P4501A induction should be taken into account in human therapy. The structure–induction relationships and differences between in-vitro systems used are discussed.

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Acknowledgements and funding: Financial support for this research was provided by the Department of Pharmacology, Pharmacy and Toxicology of Faculty of Veterinary Medicine, Utrecht University and partly by the Grant Agency of the Czech Republic, Grant no. 524/00/0514. Special thanks go to Y. Simarro Doorten, N. Treijtel, S. M. Nijmeijer and C. W. M. van Holsteijn for their skilful technical assistance. The authors are also grateful to P. Klemera and H. Kučerová for their assistance with statistical analysis and graphics software.

Introduction

Frequent administration of a number of pharmacologically active substances can result in the induction of biotransformation enzymes (Barry & Feely 1990; Okey 1990; van't Klooster et al 1993; Testa 1995; Nebbia 2001). Induction causes an increase in activity of metabolic enzymes in response to the presence of a xenobiotic substance in an organism. This increase in activity is mostly a consequence of an increased expression of the respective gene or lowering of protein degradation. Induction of biotransformation enzymes can have significant pharmacological and toxicological consequences (lowering the plasma concentration of effective substance below the threshold of therapeutic effect, increased risk of undesirable side effects related to metabolites). Moreover, parasitic resistance, which represents a worldwide problem in current anthelmintic therapy, can be facilitated. In addition, the modulation of biotransformation enzyme activity changes the intensity and time of action of the inductive agent, of drugs simultaneously or successively administered, and of other xenobiotics (environmental contaminants) (Pelkonen et al 1998). With regard to all these facts the induction potency should represent a carefully monitored parameter in all drugs used (Boobis et al 1990; Parke et al 1990).

Mebendazole belongs to the benzimidazole group of anthelmintics widely used in medicine. It is indicated in the treatment of acute parasital diseases as well as their prevention. Recently, several benzimidazole drugs have been found to induce cytochromes P450 in man and rodents (Masubuchi & Okazaki 1997; Backlund et al 1999).

However, the effects of mebendazole on biotransformation enzymes activity has not been studied yet.

Tiabendazole is another benzimidazole anthelmintic registered in human medicine (Joint Formulary Committee 2000) and it is also extensively used as a fungicidal agent in vegetable and fruit preservation (Nakagawa & Moore 1995). Tiabendazole was described as an inducer of cytochrome P4501A1 in studies using primary cultures of rabbit hepatocytes (Aix et al 1994; Rey-Grobellet et al 1996b), on rat cell cultures H4IIE (Backlund et al 1999), on cultures of mouse liver Hepa-1 cells and human liver HepG2 cells (Kikuchi et al 1998; Delescluse et al 2001). Tiabendazole is still widely used in man despite being metabolised by P4501A into toxic metabolites that covalently bind to tissue proteins (Coulet et al 2000). The later-developed benzimidazole drug mebendazole is considered to be less toxic than tiabendazole, as it does not result in toxic tissue residues.

Another benzimidazole derivate, omeprazole, is an important inhibitor of gastric acid secretion and is widely indicated for treatment of gastric and duodenal ulcers in man. This drug causes an increase in the expression of P4501A in rat hepatocytes (Masubuchi & Okazaki 1997) and both P4501A and P4503A in human hepatocytes (Lu & Li 2001; Curi-Pedrosa et al 1994; Masubuchi et al 1998). Increased expression of P4501A1 in human hepatoma HepG2 cells (Krusekopf et al 1997; Kikuchi et al 1998) and in rat hepatoma H4IIE cells (Backlund et al 1999) by omeprazole has also been described.

In this study, the effects of mebendazole on P4501A were tested using two in-vitro model systems — primary cultures of rat hepatocytes and HepG2 cells. Rat hepatocytes represent the model system commonly used to evaluate toxicity and metabolism, as well as the induction effects of drugs used clinically. However, rodents and man are known to have considerable differences in P450 isoenzymes. Thus, human-derived HepG2 cells were included for prediction of P4501A induction in patients (Doostdar et al 1993; Krusekopf et al 1997). Besides mebendazole, two known P4501A inducers with benzimidazole structure, tiabendazole and omeprazole, were included in the study and structure–induction relationships were also discussed.

Materials and Methods

Chemicals

Tiabendazole was obtained from Riedel de Haën (Praha, Czech Republic). Omeprazole was purchased from Sigma-Aldrich (Praha, Czech Republic). Mebendazole was obtained from Janssen (Praha, Czech Republic). Ethoxyresorufin, methoxyresorufin, dicoumarol, Hanks' balanced salt modified solution, hydrocortisone, insulin, powdered Williams E medium containing phenol red, bovine serum albumin (BSA), gentamicin sulfate, glutamine, dimethylthiazol diphenyl tetrazolium bromide (MTT), collagenase, Tris, glycine, glucose, HEPES, igepal CA-630, PMSF, aprotinin, sodium orthovanadate, sodium dodecylsulfate (SDS), ammonium persulfate, TEMED, glycerol, Trypan blue and bicinchoninic acid (BCA) were all obtained from Sigma-Aldrich (Zwijndrecht, the Netherlands). Dulbecco's Modified Eagles Medium (DMEM) and newborn calf serum were purchased from Life Technologies (Breda, The Netherlands). Rat tail collagenase, Type I, was obtained from Beckton Dickinson (Bedford, MA). Cell culture disposals were purchased from Corning Costar (Badhoevedorp, the Netherlands). Resorufin was purchased from Eastman Kodak (Rochester, NY). BCA protein assay reagent was obtained from Pierce (Rockford, The Netherlands). All other chemicals were of HPLC or analytical grade.

Isolation and culture of hepatocytes

The isolation of rat hepatocytes was carried out three times (three independent studies). In each study, all experiments were performed in triplicate ($n = 9$) and the incubations for Western blotting were carried out in duplicate (on two Petri dishes, protein was scraped together into the one sample for blot). Hepatocytes were obtained from male Wistar rats, 230–240 g, allowed free access to food and water. The cells were isolated from rat liver using the two-step collagenase perfusion technique described by Seglen (1976) with minor modifications. Hepatocytes were cultured using William's medium E supplemented with 3% (v/v) newborn calf serum, 1 μM insulin, 1 μM hydrocortisone, 2 mM glutamine and antibiotics (100 U mL^{-1} penicillin, 100 μg mL^{-1} streptomycin) in a humidified atmosphere of air (95%) and CO_2 (5%) at 37 °C. The cells were plated on 6-cm tissue culture dishes at a density of 3.2×10^6 cells/culture dish in 4 mL of medium (for Western blotting) and on 96-wells plates at a density of 4×10^4 cells/well in 50 μL of medium (for enzyme assays) and were incubated until attached. After 4–5 h, the medium was replaced with fresh serum-free medium (4 mL of medium per dish and 100 μL of medium/well, respectively) containing positive controls (50 μM β-naftoflavone and 1 nM 2,3,7,8-tetrachlorodibenzo-*p*-diooxine (TCDD)) or the benzimidazoles at various concentrations (dissolved in dimethyl sulfoxide (DMSO)). The concentration of benzimidazole was chosen according to the solubility of the drug and its plasma levels (0.1, 0.5, 1, 5 and 15 μM mebendazole; 0.1, 0.5, 1, 5, 15, 50 and 100 μM tiabendazole; 0.1, 0.5, 1, 5, 15, 50 and 100 μM omeprazole). The concentration of DMSO in the medium was 0.5%. Control media also contained 0.5% (v/v) DMSO. The hepatocytes were incubated with drugs for 24, 48 or 72 h. The medium was not changed during the incubation.

HepG2 cell cultures

HepG2 cells (ATCC number HB-8065) were grown in DMEM supplemented with 10% newborn calf serum, 2 mM glutamine, 1% non-essential amino acids and antibiotics (100 U mL^{-1} penicillin, 100 μg mL^{-1} streptomycin) at 37 °C in a humidified atmosphere of 5% CO_2 in air. Cells were seeded in 75-cm^2 flasks at a density of 2.5×10^4 cells/flask in 25 mL DMEM and subcultured twice a week.

The complete study was carried out with three individual cell generations (three independent studies). In each study, all experiments were performed in triplicate (n = 9) and the incubations for Western blotting were carried out in duplicate (on two Petri dishes; protein was scraped together in one sample for blot). Cells for enzymatic assays were seeded in 96-well plates at a density of 2×10^4 cells/well in 100 μL of DMEM. HepG2 cells were seeded in 6-cm tissue culture dishes at a density of 5×10^5cells/culture dish in 5 ml of DMEM for Western blotting. After 24 h, the medium was replaced by DMEM (5 mL per dish and 100 μL/well, respectively) containing either positive controls (50 μM β-naftoflavone and 1 nM TCDD) or the benzimidazoles at several concentrations (dissolved in DMSO). The concentration of benzimidazole was chosen according to the solubility of the drug and its plasma levels (0.1, 0.5, 1 and 5 μM mebendazole; 0.1, 0.5, 1, 5, 50 and 100 μM tiabendazole; 0.1, 0.5, 1, 5, 50 and 100 μM omeprazole). The concentration of DMSO in the medium was 0.5%. Control media also contained 0.5% (v/v) DMSO. The cells were incubated with drugs for 24, 48 or 72 h. The medium was not changed during the incubation.

Enzyme assays

Measurement of ethoxyresorufin O-deethylation (EROD) and methoxyresorufin O-demethylation (MROD) activity was performed as described by Wortelboer et al (1990), after 24, 48 and 72 h of incubation with benzimidazoles. Briefly, cells were incubated in 96-well plates with 10 μM ethoxyresorufin or 10 μM methoxyresorufin, and 20 μM dicoumarol in Krebs buffer, pH 7.4 (50 μL of incubation medium/well) for 1 h at 37 °C in a humidified atmosphere of 5%CO_2 in air. After incubation, the buffer was diluted 1:1 with 0.1 M NaOH (50 μL/well) and cells were lysed for 30 min at room temperature in the dark. Samples (50 μL) were taken from each well and fluorescence was measured with a Cytofluor 2300 (Millipore Corp., Bedford, MA) at $\lambda_{ex} = 530$ nm and $\lambda_{em} = 590$ nm. Cellular protein was assayed in 10 wells from each plate according to the BCA method using BSA as a standard. All incubations were performed in triplicate.

Cytotoxicity assay

Cytotoxicity was assayed by using the MTT test as described by Denizat & Lang (1986). Cells, seeded in 96-well plates, were incubated with the test compounds at different concentrations (rat hepatocytes — 0.1, 0.5, 1, 5 and 15 μM mebendazole; 0.1, 0.5, 1, 5, 15, 50 and 100 μM tiabendazole; 0.1, 0.5, 1, 5, 15, 50 and 100 μM omeprazole; HepG2 cells — 0.1, 0.5, 1 and 5 μM mebendazole; 0.1, 0.5, 1, 5, 50 and 100 μM tiabendazole; 0.1, 0.5, 1, 5, 50 and 100 μM omeprazole) and with positive controls (50 μM β-naftoflavone and 1 nM TCDD) for 24, 48 or 72 h, followed by incubation with MTT for 2.5 h (25 μL of MTT solution in PBS, 3 mg mL^{-1}, was applied per well without removal of the medium). The medium was discarded and cells were lysed by acetic isopropanol (0.08 M HCl in isopropanol; 50 μL/well). The absorbance of product of reaction, formazan, was measured at 595 nm and the absorbance of treated cells was compared with that of control cells exposed to medium with 0.5% DMSO alone.

Western blotting

The cells were treated with benzimidazoles at the highest concentrations used in the enzymatic assays (rat hepatocytes – 15 μM mebendazole, 100 μM tiabendazole and 100 mM omeprazole; HepG2 cells — 5 μM mebendazole, 100 μM tiabendazole and 100 μM omeprazole) and with positive controls (50 μM β-naftoflavone and 1 nM TCDD) and were harvested after 48 h of incubation. Microsomal proteins were isolated from the cells as described by Wortelboer et al (1991). Separation of microsomal proteins was carried out on a BioRad mini Protean II cell using the sodium dodecylsulfate polyacrylamide gel electrophoresis (SDS-PAGE) discontinuous system as described by Laemmli (1970). The resolved proteins were blotted on polyvinylidenfluoride (PVDF) sheets (Millipore, Etten-Leur, The Netherlands) according to the method of Towbin et al (1979). Antibody raised against rat P4501A1/1A2 was used for immunochemical staining. Absorbance of protein bands corresponding to P4501A1/2 was related to the background.

Statistical analysis

In the study of enzyme activity, a one-way analysis of variance was performed for comparison of the three benzimidazoles tested and the effects of time and drug concentration were analysed using a two-way analysis of variance. In addition, Student's *t*-test was used to quantify the difference between the data obtained from hepatocytes treated with benzimidazoles and the data from control hepatocytes. In the statistical methods mentioned, data from three individual rat hepatocyte cultures and three cell line generations, each in triplicate (n = 9), were compared. In the Western blotting assay, a one-way analysis of variance was performed for comparison of the three benzimidazoles tested after their treatment with rat hepatocytes (data from three individual rat hepatocyte cultures).

Results

Cytotoxicity assay

The concentration range of mebendazole had to be limited to 0.1–15 μM, as higher concentrations were insoluble in the culture medium. Moreover, 15 μM mebendazole was excluded from the experiments in HepG2 cells, as it exhibited significant toxic effects (decrease of cell viability by more than 50%; data not shown). At the highest concentrations used, 1 and 5 μM, mebendazole significantly decreased the cell viability of HepG2 cells, up to 30% compared with the control. In contrast, toxicity was not detected in rat hepatocytes treated with mebendazole (data are not shown) at any of the concentrations tested (0.1–15 μM). No cytotoxicity was observed with tiabendazole

or omeprazole at the concentration range tested (0.1–100 μM) in primary cultures of rat hepatocytes or in HepG2 cells (data not shown).

Basic values of EROD and MROD activity in rat hepatocytes and HepG2 cells

The P4501A2 expression was considerably lower in HepG2 than in primary cultures of rat hepatocytes (Table 1). After 24 h, MROD and EROD activity in HepG2 cells was 7 and 5.5 times, respectively, lower than in rat hepatocyte cultures. The P4501A activity in HepG2 cells was approximately 4 or 5 times lower than in rat hepatocytes after 48 and 72 h.

Effects of mebendazole on EROD and MROD activity and protein content

Mebendazole caused a concentration- and time-dependent induction of EROD and MROD activity in rat hepatocytes (Figure 1A, C). After 72 h of incubation, hepatocytes that had been exposed to 15 μM mebendazole showed EROD and MROD activity that was induced 10- and 2.5-times, respectively, which represented approximately 50% of β-naftoflavone or TCDD (data not shown). In HepG2 cells, however, 5 μM mebendazole only weakly increased EROD and MROD activity (approximately 2 fold) after 24 and 48 h incubation (Figure 1B, D). In hepatocyte cultures, mebendazole caused a 13.5-fold increase in P4501A1 protein level compared with the control response. In addition, an 11-fold enhancement of P4501A2 level was found in rat hepatocytes incubated with mebendazole (Figure 2). Mebendazole significantly increased P4501A1 as compared with P4501A2. A 15-fold increase in P4501A1/2 level in HepG2 cells following mebendazole exposure was detected (Figure 2), which was comparable to the increse in P4501A1/2 level in HepG2 cells treated with β-naftoflavone or TCDD (data not shown).

Effects of tiabendazole on EROD and MROD activity and protein content

Almost no induction effects of tiabendazole on enzyme activity (maximum 1.5-fold enhancement over control) in rat hepatocytes or HepG2 cells was observed (data not shown). Tiabendazole caused a 4.5-fold enhancement of P4501A2 protein level over the control response in rat hepatocytes cultures; the P4501A1 content only was weakly raised following tiabendazole treatment (2.5 fold). In HepG2 cells, tiabendazole caused a 14-fold increase in P4501A1/2 protein level (compared with control; Figure 2).

Effects of omeprazole on EROD and MROD activity and protein content

No inducing effect of omeprazole on the enzyme activity tested was found in rat hepatocytes (data not shown), whereas in HepG2 cells, a prominent concentration-dependent enhancement of EROD and MROD activity was observed (Figure 3A, B). After 24 h incubation of HepG2 cells with 50 μM omeprazole and 48 h incubation with 100 μM omeprazole, the increase in EROD activity was 15 and 14 fold, respectively. MROD activity was also increased by 13.5 and 5 fold in HepG2 culture incubated for 24 and 48 h, respectively. This enhancement of EROD and MROD activity reached as much as 75% of β-naftoflavone or TCDD (data are not shown). P4501A1 protein content remained unaffected in hepatocytes incubated with omeprazole. The omeprazole treatment of hepatocytes only weakly affected the amount of P4501A2 (2-fold increase over the control response; Figure 2). In HepG2 cells, omeprazole caused a 14-fold increase in P4501A1/2 level (compared with control; Figure 2), which was comparable with the increase in P4501A1/2 level in HepG2 cells treated with β-naftoflavone or TCDD (data not shown).

Statistical analysis

The structure–induction dependence was proved using the one-way analysis of variance for EROD activity measured in rat hepatocytes, as well as in HepG2 cells, at all concentrations of benzimidazoles tested and all times of incubation ($P < 0.001$). For MROD activity, analysis of variance showed the structure–induction dependence in HepG2 cells at all concentrations of benzimidazoles tested (except 0.1 μM) and all incubation times, and in rat hepatocytes treated with all concentrations of benzimidazoles

Table 1 Basic values of EROD and MROD activity in rat hepatocytes and HepG2 cells after 24, 48 and 72 h incubation.

	EROD		MROD	
	Rat hepatocytes	HepG2 cells	Rat hepatocytes	HepG2 cells
24 h	37.2 ± 3.6	7.0 ± 2.0	20.2 ± 5.3	2.9 ± 0.8
48 h	28.6 ± 4.9	6.3 ± 1.3	16.4 ± 4.3	2.9 ± 0.7
72 h	25.0 ± 1.8	6.7 ± 2.8	12.3 ± 3.2	3.4 ± 1.4

Data are from three individual rat hepatocytes cultures and three cell line generations, respectively. Each incubation was performed in triplicate (n = 9) and data are expressed as the average ± s.d. of activity (pmol mg^{-1} min^{-1}) measured in control incubations with 0.5% DMSO in medium only.

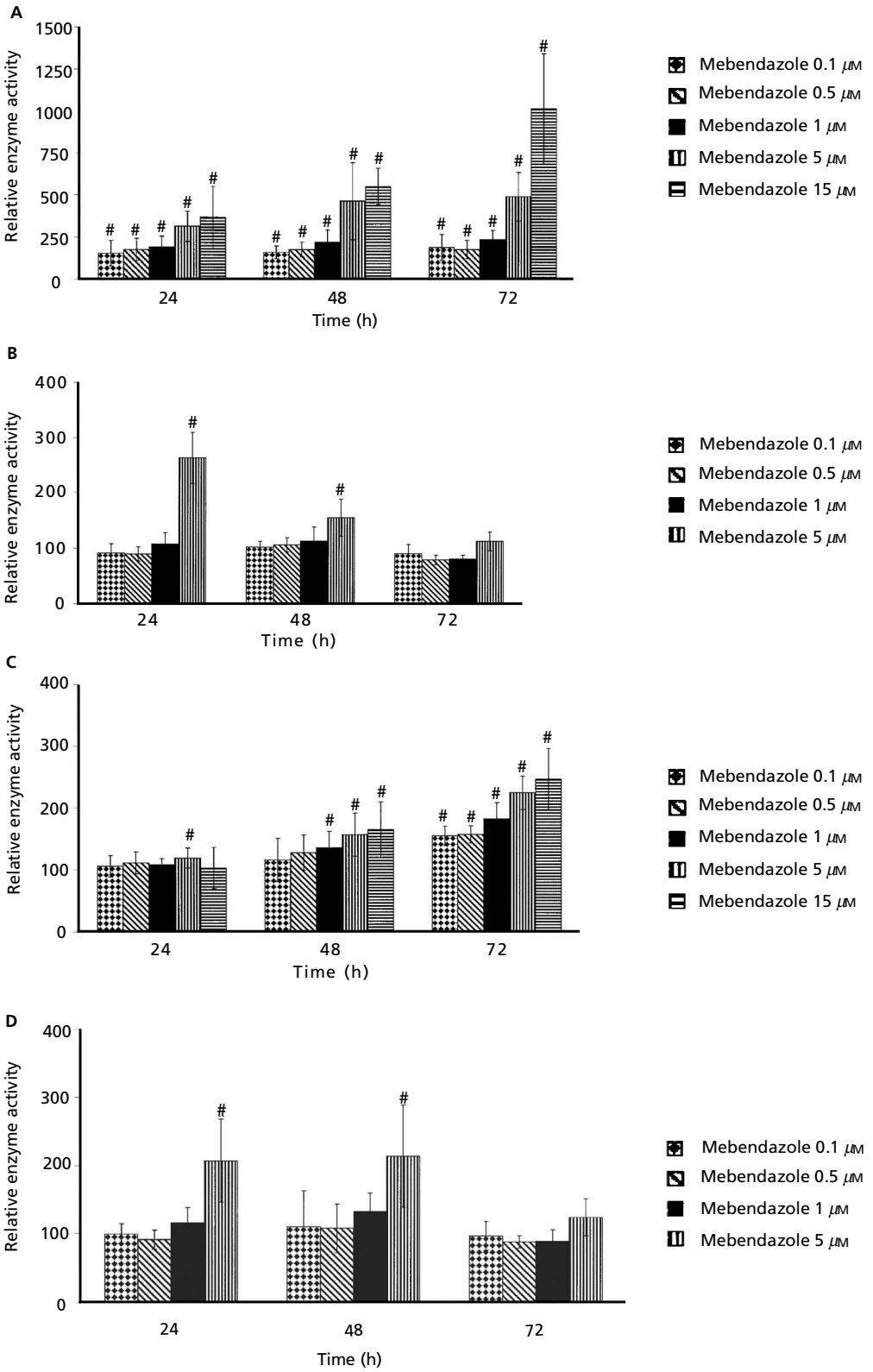

Figure 1 EROD (A, B) and MROD activity (C, D) in rat hepatocyte cultures (A, C) and HepG2 cells (B, D) treated with various concentrations of mebendazole (0.1–15 μM) for 24, 48 and 72h. Data from three individual rat hepatocyte cultures and three cell line generations, each in triplicate (n = 9) are expressed as percentage ± s.d. of control incubations with 0.5% DMSO in medium only (equals 100%). $^{\#}P < 0.01$, compared with control (Student's *t*-test)

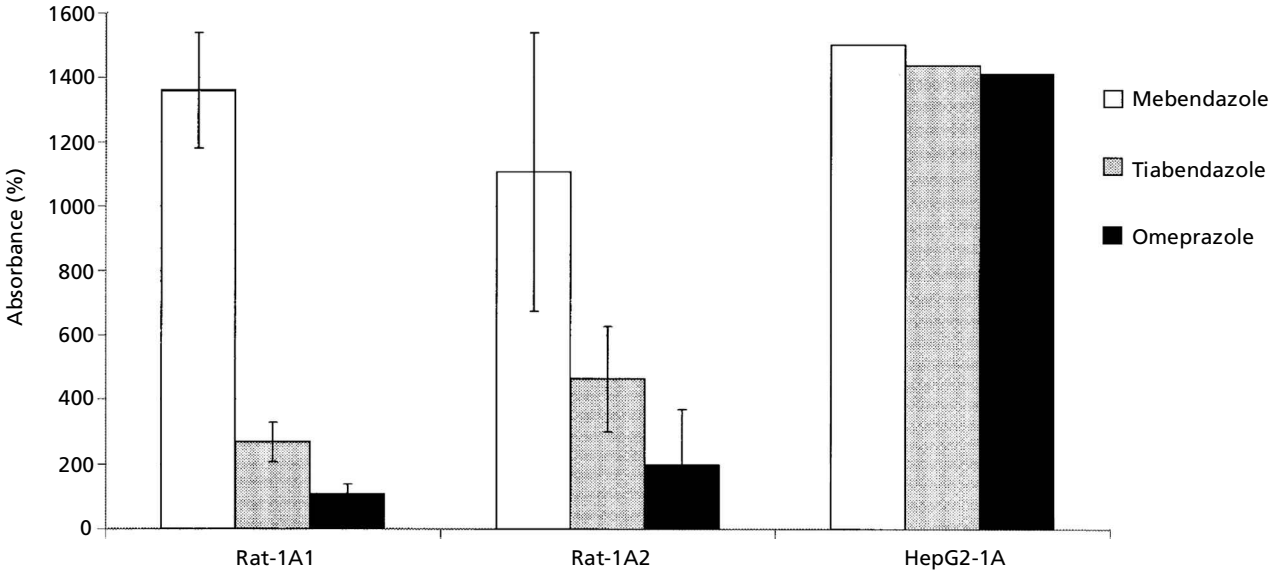

Figure 2 P4501A protein levels in rat hepatocyte cultures and HepG2 cells treated with 15 μM or 5 μM mebendazole, respectively, and with 100 μM tiabendazole and 100 μM omeprazole for 48 h. Data from three individual rat hepatocyte cultures and one cell line generation are expressed as percentage ± s.d. of control (absorbance of control = 100%). All incubations were performed in duplicate (on two Petri dishes, protein from these two dishes was scraped together into the one sample for blot).

for 72 h ($P < 0.001$), with 1 μM benzimidazoles for 48 h ($P < 0.05$) and with 5 and 15 μM benzimidazoles for 48 h ($P < 0.01$). The effects of incubation time and drug concentration on EROD activity were proved using the two-way analysis of variance in HepG2 cells, as well as in rat hepatocytes, treated with mebendazole ($P < 0.05$) or omeprazole ($P < 0.001$ and $P < 0.05$, respectively). For MROD activity, these effects were proved only in rat hepatocytes treated with mebendazole ($P < 0.01$) and in HepG2 cells treated with omeprazole ($P < 0.001$).

Discussion

Mebendazole, tiabendazole and omeprazole are benzimidazole drugs widely used in both man and animals. The capacity of tiabendazole and omeprazole to induce cytochrome P450 enzymes, especially P4501A, has been recognized (Masubuchi & Okazaki 1997; Backlund et al 1999). However, the effect of mebendazole on P450s has not been explored yet. In our study we described the effects of mebendazole, as well as tiabendazole and omeprazole in two different in-vitro models: primary cultures of rat hepatocytes and HepG2 cells. EROD and MROD activity, as P4501A1/2 enzyme assays (Doostdar et al 1993; Burke et al 1994), and immunodetection of protein levels by Western blotting technique were used to evaluate the induction effect of benzimidazoles on cytochrome P4501A1/2.

The two different in-vitro model systems were selected to study the differences between rat hepatocytes and HepG2 cells in response to inducers. Rat hepatocytes in primary culture represent the most widely used in-vitro model system applied to study biotransformation, including induction and inhibition experiments (Paine 1990), despite the fact that significant interspecies differences exist. As the availability of primary human hepatocytes is more limited, human hepatoma HepG2 cell line was included in our study. HepG2 cells have been recognized as a suitable model system to predict P4501A induction in man (Doostdar et al 1993; Krusekopf et al 1997). However, HepG2 cells are known to express only P4501A with little or no expression of other P450 enzymes. This limits their use in studying the biotransformation of P4501A substrates (Kikuchi et al 1998). In our experiments (see Table 1), the P4501A2 expression was considerably lower in HepG2 than in primary cultures of rat hepatocytes (after 24 h, MROD and EROD activity in rat hepatocytes was 7- and 5.5-times that in HepG2 cells). Table 1 exhibits gradual decrease in enzyme activity in rat hepatocyte cultures during the incubation time. This phenomenon does not occur in HepG2 cells or in other cell lines.

As it is generally accepted that the induction response depends on the concentration of potential inducers, a wide range of benzimidazole concentrations were used. The concentration range for individual compounds was chosen according to solubility, plasma concentrations and cytotoxic potential. Mebendazole exhibited significant toxic effects on HepG2 cells at concentrations above 0.5 μM. In contrast, no cytotoxicity was observed in rat hepatocytes, even at high concentrations. Mebendazole has already been reported to inhibit cell growth and cell differentiation through the prevention of mammalian tubulin polymerization (Higa et al 1992; Russell et al 1992; Whittaker & Faustman 1992). Thus, dividing cells may be more sensitive to mebendazole cytotoxicity than hepatocytes, which do not readily proliferate under normal culture conditions. As HepG2 cells exhibit low metabolic activity, the parent drug mebendazole may be

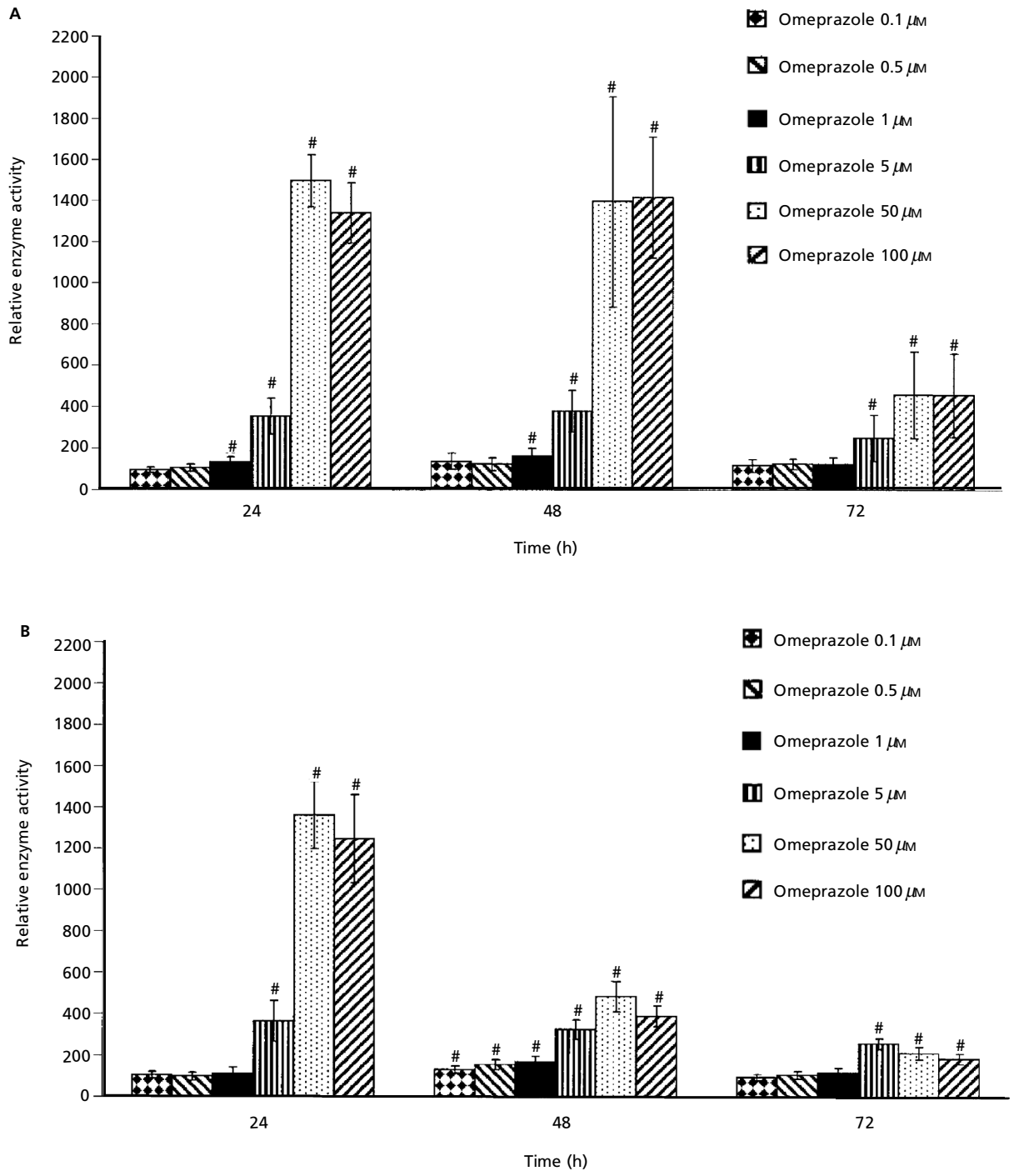

Figure 3 EROD (A) and MROD activity (B) in HepG2 cells treated with various concentrations of omeprazole (0.1–100 μM) for 24, 48 and 72 h. Data from three cell line generations, each in triplicate (n = 9) are expressed as percentage ± s.d. of control incubations with 0.5% DMSO in medium only (equals 100%). $^{\#}P < 0.01$, compared with control (Student's *t*-test).

responsible for the toxicity detected following mebendazole treatment.

Mebendazole produced a less extensive increase of P4501A1/2 activity in HepG2 cells, when considering the increase in corresponding protein. To explain this phenomenon, the possible inhibitory effect of the parent drug mebendazole, or its metabolites, on P4501A enzymes in HepG2 cells may be proposed. In addition, the possible participation of other metabolic enzymes in EROD and MROD activity, as well as the cytotoxicity, observed in HepG2 cell cultures treated with the two highest concentrations of mebendazole should also be taken into account.

In our experiments, almost no induction effect of tiabendazole on P4501A-associated activity (EROD, MROD) was observed in either rat hepatocytes or HepG2 cells. However, tiabendazole caused significant EROD induction in rabbit hepatocytes (Aix et al 1994; Rey-Grobellet et al 1996b). Kikuchi et al (1998) and Backlund et al (1999) detected the induction potency of tiabendazole by immunohistological techniques in rat hepatoma H4IIE cells and in HepG2 cells. In our experiments, a slight increase in the P4501A protein level (Western blotting) was observed in rat hepatocytes treated with tiabendazole and there was also a considerable protein increase in HepG2 cells. As tiabendazole metabolites were reported to bind covalently to tissue proteins (Yoneyama et al 1985; Rey-Grobellet et al 1996a; Coulet et al 2000), including microsomal proteins (Yoneyama & Ichikawa 1986), the hypothesis that tiabendazole inhibits EROD and MROD activity through the binding of tiabendazole metabolites to P4501A enzymes may be proposed. Thus, induction of P4501A by tiabendazole could be masked by the inhibition of the corresponding enzymes.

Omeprazole exhibited almost no induction effect in rat hepatocytes at the concentration range used. However, the same concentrations of omeprazole caused a significant induction of P4501A in HepG2 cells. This provides confirmation of limitations in extrapolating biotransformation studies in the rat to man.

Various relationships between benzimidazole structures and P4501A induction have been investigated (Rey-Grobellet et al 1996b; Backlund et al 1999). The hypothesis that only sulfur-containing benzimidazoles are capable of inducing P4501A1 has been reported (Gleizes-Escala et al 1991; Curi-Pedrosa et al 1994). Our results disproved this hypothesis, as mebendazole, a sulfur-free benzimidazole anthelmintic, caused a concentration-dependent induction of P4501A in hepatocytes, as well as in HepG2 cells. The presence of the sulfur atom is not an essential structural requirement of benzimidazoles for P4501A1 induction.

Taken together, the presented results suggest that the benzimidazole drug mebendazole exhibited a significant induction effect on cytochrome P4501A. A possible consequence of P4501A induction could be a loss of therapeutic efficacy following repeated dosing. Moreover, parasitic resistance, based on the plasma and tissue levels of the drug falling below the threshold for effective antiparasitic concentration, will be facilitated. In addition, mebendazole may accelerate the metabolism of other simultaneously or successively administered drugs that are metabolised by P4501A enzymes. The induction of P4501A by mebendazole may also increase the risk of mutagenesis and cocarcinogenesis, as P4501A is the major enzyme involved in the biological activation of various environmental pollutants (Parke et al 1990). Co-treatment with tiabendazole and mebendazole may result in increasing toxicity of tiabendazole as tiabendazole metabolites (products of P4501A2) were reported to be responsible for the nephrotoxicity and teratogenity of tiabendazole (Mizutani et al 1992; Rey-Grobellet et al 1996a; Coulet et al 2000). Simultaneous or successive therapy with the benzimidazole anthelmintic mebendazole and the antiulcer drug omeprazole, or with tiabendazole, may lead to significant increase of P4501A induction. This could lead to enhancement of the toxicological consequences of P4501A induction.

Conclusions

Based on our results, we assume that mebendazole, even though it is not a sulfur-containing benzimidazole derivative, possesses significant induction potency in rat hepatocytes and HepG2 cells. Thus, the pharmacological and toxicological consequences of P4501A induction should be taken into account when mebendazole is used for long-term therapy in man. One of these consequences is a risk of parasitic resistance, which is presently a worldwide problem in anthelmintic therapy.

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