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Multidrug Resistance Gene Expression in Rodents and Rodent Hepatocytes Treated with Mitoxantrone

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ABSTRACT. Overexpression of P-glycoprotein in tumor cells can represent a severe drawback for cancer chemotherapy. P-glycoprotein acts as an efflux transporter for a variety of chemotherapeutic agents. It is encoded by multidrug resistance (*mdr*) genes of the subfamily 1 in humans (*MDR1*) and rodents (*mdr1a* and *1b*). Because *mdr1* gene expression is inducible in cultured rat hepatocytes and in rat liver with chemical carcinogens such as 2-acetylaminofluorene or aflatoxin B_1, which form DNA-binding electrophiles during their metabolism, we investigated whether the DNA-damaging chemotherapeutic drug mitoxantrone may induce multidrug resistance in rodents and in hepatocytes in primary culture. In H4IIE rat hepatoma cells stably transfected with a luciferase construct containing the rat *mdr1b* promoter, mitoxantrone caused a concentration-dependent increase in promoter activity. *Mdr1* gene expression in cultured rat hepatocytes was enhanced at mitoxantrone concentrations greater than or equal to 0.1 μM and in mouse hepatocytes at 5 μM. In hepatocytes from both species, a correlation was found between *mdr1* induction and the inhibition of protein synthesis. *In vivo*, mitoxantrone was a very powerful inducer of *mdr1* gene expression in rat liver and small intestine. In rat kidney, induction of mRNA was lower, and a marginal effect was seen in lung. In contrast with rats, no significant induction of *mdr1* gene expression was obtained in mouse liver. Probably as a consequence of inhibition of protein synthesis, mitoxantrone did not lead to a pronounced elevation of P-glycoprotein levels in rat liver and kidney. BIOCHEM PHARMACOL 52;9:1453–1460, 1996. *Copyright © 1996 Elsevier Science Inc.*

KEY WORDS. extrahepatic organs; hepatocytes; induction; mitoxantrone; multidrug resistance; rodent liver

Resistance toward a variety of structurally distinct chemotherapeutic drugs, referred to as multidrug resistance, frequently hampers effective chemotherapy of tumours [1]. Overexpression of P-glycoprotein, a multidrug excretion transporter, represents a major mechanism of multidrug resistance [2, 3]. In recent years, *mdr* genes# encoding P-glycoprotein have been identified in human, hamster, mouse and rat [3–7]. Within the *mdr* gene family, comprised of two genes in man and three in rodents, expression of *mdr1a* and *mdr1b* (referred to as *mdr1*) in rodents or *MDR1* in humans is related to multidrug resistance. The *mdr1* gene product P-glycoprotein is also found in normal tissues such as liver, kidney, adrenal gland, bone marrow and the lower gastrointestinal tract [8–10]. Its localization at the biliary domain of the hepatocyte [9–12] suggests a role for P-glycoprotein in the biliary excretion of xenobiotics and other substrates [13].

Mdr1 gene expression in rat liver [14, 15] and in rat hepatocytes in primary culture [16] is inducible with xenobiotics such as 2-AAF, leading e.g. to enhanced biliary excretion of the P-glycoprotein substrate vinblastine [12]. From experiments with inhibitors and intermediates of the metabolic activation of 2-AAF, it was concluded that the ultimate electrophilic metabolite of 2-AAF acts as inducer of *mdr1* gene expression in rat hepatocytes [17]. Interaction of electrophiles with DNA or a receptor protein may result in transcriptional activation of *mdr1* genes. Mitoxantrone, a widely used chemotherapeutic agent, interacts directly with the DNA-topoisomerase II complex and is activated in humans and in rat liver microsomes to electrophilic metabolites [18, 19]. Both mechanisms may contribute to the DNA-damaging potency of mitoxantrone. In rodent cell lines, mitoxantrone induced *mdr1* gene expression [20], and indirect evidence showed that mitoxantrone may be a sub-

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Abbreviations: 2-AAF, 2-acetylaminofluorene; DMEM, Dulbecco's modified Eagle's medium; EDTA, ethylenediamine tetraacetic acid; EGTA, ethylene glycol-bis (β-aminoethyl ether)N,N,N′,N′-tetraacetic acid; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; HEPES, N-[2-hydroxy-ethyl]piperazine-N′-[2-ethanesulfonic acid]; LDH, lactate dehydrogenase; *mdr* genes, multidrug resistance genes; PBS, phosphate buffered saline; SDS, sodium dodecyl sulfate; TCA, trichloroacetic acid.

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strate of P-glycoprotein [21, 22]. Therefore, a possible role of mitoxantrone as inducer of *mdr1* gene expression in rat and mouse liver and in hepatocytes in primary culture was investigated. Furthermore, the question was addressed if expression of *mdr1* genes is also inducible in rodents in extrahepatic organs such as small intestine, kidney and lung.

MATERIALS AND METHODS

Materials

Mitoxantrone (NOVANTRON^R) was obtained from Lederle (Wolfratshausen, Germany). [^{3}H]-vinblastine sulphate was purchased from Amersham (Braunschweig, Germany) at a specific radioactivity of 777 GBq/mmol. By dilution with unlabeled vinblastine sulphate (Sigma, St. Louis, MO, USA), a final specific radioactivity of 37 MBq/mmol was achieved. L-[4,5-^{3}H]-leucine (Amersham) was purchased at a specific radioactivity of 4.4 TBq/mmol.

Animal Treatment

All animals were obtained from Savo (Kisslegg, Germany). Male Wistar rats were kept on a standard diet and tap water *ad libitum*. At a body weight of 190–250 g, the animals were treated intraperitoneally with saline or 0.01, 0.1 or 0.5 mg mitoxantrone/kg in aqueous solution. After 3 days, the animals were decapitated, organs were removed and washed with ice-cold PBS, frozen in liquid nitrogen and kept at −80°C until further analysis. Similarly, male NMRI mice (weighing 21–23 g) were treated with mitoxantrone and organs were isolated 3 days after treatment.

Cell Culture

Hepatocytes were isolated from male Wistar rats (150–200 g body weight) as described elsewhere [16]. Isolation of hepatocytes from male NMRI mice (21–23 g body weight) was performed under essentially identical conditions, i.e. by sequential perfusion with EGTA-containing and collagenase/calcium-containing buffers via the portal vein. Hepatocyte preparations showing a trypan blue exclusion rate of more than 90% were seeded on 90-mm Petri dishes in a 1:1 mixture of DMEM and Waymouth's MD 705/1 medium (Life Technologies, Gaithersburg, MD, USA) containing 10 mM HEPES, 50 nM dexamethasone, 5 μg each of insulin, transferrin and selenous acid per milliliter (ITS premix; Becton Dickinson, Bedford, MA, USA), 10% fetal bovine serum and 50 μg/mL gentamicin. For preparation of RNA, cells were plated on 150-mm dishes (Falcon, Becton Dickinson, Bedford, MA, USA) at a density of 100,000 cells/cm^2. After 3 hr, medium was replaced by a 1:1 mixture of DMEM and Waymouth's MD 705/1 supplemented with 10 mM HEPES, 50 nM dexamethasone, 6.25 μg each of insulin, transferrin and selenous acid per milliliter, 1.25 mg/mL bovine serum albumin, 5.35 μg/mL linoleic acid (ITS$^+$ premix; Collaborative Research) and 50 μg/mL gentamicin.

Northern Analysis

For analysis of gene expression in rodent organs, total RNA was isolated by cesium chloride gradient centrifugation, and mRNA was prepared by polyA-selection [23]. For preparation of total RNA from hepatocyte cultures, cells were lysed on the plate by using 0.5 M sodium chloride, 10 mM Tris, pH 7.5, 2 mM EDTA, 1% Nonidet P-40, 0.5% sodium deoxycholate and 1% β-mercaptoethanol. Nuclei were removed by centrifugation, and total RNA was prepared by the sequential phenol/chloroform extraction method as previously described [16]. After electrophoresis in a 1% agarose gel containing 2.2 M formaldehyde [24], RNA was blotted to nylon membranes (Magnagraph, MSI, Westboro, MA, USA) by using capillary transfer with 10× SSC. As molecular weight standard, an RNA ladder of fragment size 0.24–9.5 kB (Gibco) was used. Analysis of *mdr1* mRNA expression was achieved by hybridization to a 353-bp fragment of the rat *mdr1b* cDNA [7] or to a 3′-most 850-bp fragment (PstI/EcoRI) of the murine full length pC1.5 clone described by Hsu *et al.* [6]. Loading of the gels was controlled by using a cDNA probe for the rat GAPDH gene [25]. The probes were labeled by the random primer method using [α-^{32}P] dCTP [26]. Membranes were prehybridized and hybridized in 50% deionized formamide, 6× SSC, 5× Denhardt's solution and 1% SDS. Hybridized mRNA was visualized by autoradiography at −80°C using intensifying screens for 1–4 days. Densitometric analysis was performed by using a Bio Image densitometry unit (Milipore, Bedford, MA, USA).

Reporter Gene Assay

The derivation of H4IIE cells stably transfected with a luciferase reporter construct containing *mdr1b* DNA corresponding to bases −941 to +608 relative to the transcription start point has been previously described [27]. The cells were grown in 12-well dishes and treated with 0, 0.01, 0.1, 0.5, 1 and 5 μM mitoxantrone for 24 hr, washed with PBS, and lysed with 50 μL 0.1 mM potassium phosphate buffer, pH 7.8, 1 mM dithiothreitol, 2 mM EDTA, and 1% Triton X-100. One hundred microliters of 1 mM luciferin and 350 μL of 25 mM glycylglycine (pH 7.8), 5 mM ATP, 15 mM $MgSO_4$ were mixed with a 5-μL aliquot of lysate, and luminescence was measured for 30 sec in a Berthold Lumat LB9501 (Berthold, Wildbad, Germany) luminometer. Variability in cell number from sample to sample was corrected by dividing luciferase activity by protein concentration. Protein concentration was determined by a modified Bradford method following the supplier's instructions (Bio-Rad, Hercules, CA, USA).

Western Analysis

P-glycoprotein levels in rodent organs and in hepatocytes in primary culture were analyzed as described elsewhere [12, 16]. Proteins were electroblotted on Immobilon P mem-

branes (Milipore, Dreieich, Germany), and P-glycoprotein was detected with the C-219 monoclonal antibody (Signet, Dedham, MA, USA). The immunoreaction was visualized by using a horseradish peroxidase-conjugated secondary antibody (Sigma), and the Amersham ECL technique (Amersham).

Cytotoxicity

Leakage of LDH into the medium was used as a parameter for cytotoxic damage. After 9 hr of incubation in the presence of different concentrations of mitoxantrone, LDH activity in the medium was determined according to the method of Benford and Hubbard [28]. Leakage was expressed as percentage of total leakage after addition of a solubilizing agent (0.9% NaCl, 0.1% Triton X-100, 0.1% bovine serum albumin). At all concentrations used, LDH activity in the medium was at maximum 8–12 hr after addition of mitoxantrone (not shown).

Protein Synthesis

For determination of protein synthesis, 3.7×10^4 Bq L-[4,5-^{3}H]-leucine/mL were added to hepatocyte cultures. After 12 hr, cultures were washed three times with ice-cold PBS and homogenized in a Dounce homogenizer. After adding PBS to a final volume of approximately 3 mL, protein was precipitated by addition of 1 mL 5% TCA. After 1 hr on ice, the precipitate was sedimented by centrifugation, washed with 3 mL 5% TCA and dissolved in 3 mL 1 N NaOH at 60°C over 30 min. After centrifugation at 10,000*g*, 100 µL of the clear supernatant were analyzed by liquid scintillation spectrometry. Another 100-µL aliquot was used for protein measurement [29]. Protein synthesis was expressed as incorporated radioactivity per milligram of protein.

RESULTS

In mRNA preparations from rat organs and rat hepatocytes in primary culture, two transcripts hybridizing to a 353-bb fragment of the rat *mdr1b* gene were detectable at 4.4 and 5.2 kb as previously reported [16]. From the use of gene-specific probes, the upper band probably represents the rat *mdr1a* and the lower band the *mdr1b* transcript, respectively [30]. Because both transcripts were increased in induction experiments, the term "*mdr1* induction" is used.

Treatment of rats with the chemotherapeutic drug mitoxantrone led to a dose-dependent induction of *mdr1* gene expression in liver and intestine 3 days after treatment (Fig. 1). Based on ethidium bromide staining of Northern blots (not shown) and on the amount of the GAPDH transcript, equal loading was confirmed. At doses of 0.01 and 0.1 mg/kg, a marked increase in hepatic and intestinal *mdr1* expression was observed. In kidney, maximal induction was obtained with 0.01 mg/kg, whereas 0.1 mg/kg caused a less pronounced effect. Induction in rat lung was marginal compared with other organs. After treatment with 0.5 mg/kg,

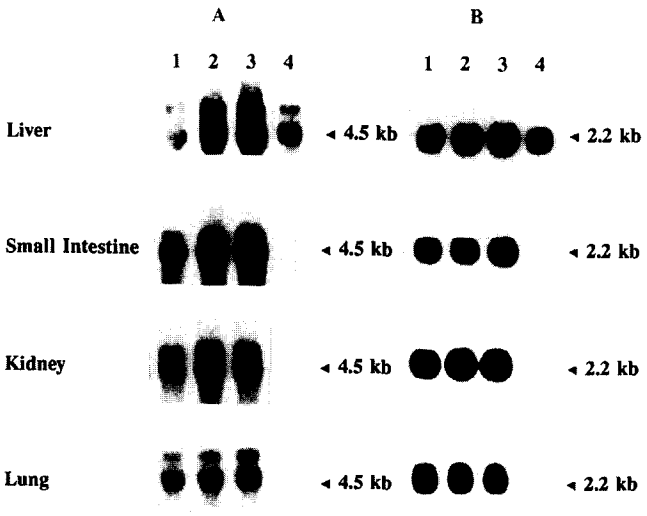

FIG. 1. Northern blot analysis showing the effect of mitoxantrone on *mdr1* (A) mRNA expression in rat organs. PolyA-selected RNA (5 µg) was obtained 3 days after treatment of male Wistar rats with saline (lane 1) or mitoxantrone: 0.01 (lane 2), 0.1 (lane 3) or 0.5 (lane 4) mg/kg, i.p. GAPDH (B) mRNA expression was detected in the same blots (gel loading control). Blots are representative for series of four blots.

RNA isolated from lung, kidney and small intestine was partially degraded and not used for hybridization, whereas typical hybridization signals of *mdr1* transcripts were found with hepatic RNA, showing a lower extent of induction than with 0.01 and 0.1 mg/kg. Moreover, the amount of RNA and polyA-selected RNA isolated from the same amount of liver tissue was markedly lower, indicating a pertubation of RNA synthesis and/or turnover.

Two days after treatment, a higher degree of interindividual variability of induction was obtained, whereas induction was less pronounced 4 days after treatment (not shown).

In Northern blots of RNA from mouse liver (and mouse hepatocytes), the 850-bp fragment of the murine *mdr1b* clone hybridized to a 4.6-kb transcript (Fig. 2), which therefore was addressed as *mdr1b* transcript according to Hsu *et al.* [6]. In contrast with the rat, no significant increase in *mdr1* gene expression was obtained in mouse liver after mitoxantrone treatment, which was confirmed by densitometric analysis of the Northern blot (Fig. 2) and of three additional Northern blots obtained from independent experiments (not shown).

Western analysis of P-glycoprotein in rat liver and kidney homogenates using the C219 antibody did not reveal an increase after mitoxantrone treatment. In mouse liver, a small increase at 0.1 mg/kg was observed (Fig. 3). Likewise, mitoxantrone treatment did not lead to a significant increase in P-glycoprotein levels in rodent hepatocytes in primary culture (not shown).

In rat hepatocytes in primary culture, 0.1 µM and higher concentrations of mitoxantrone resulted in a significant induction of *mdr1* expression 36 hr after addition to the medium (Fig. 4). Twenty-four hours after treatment, induc-

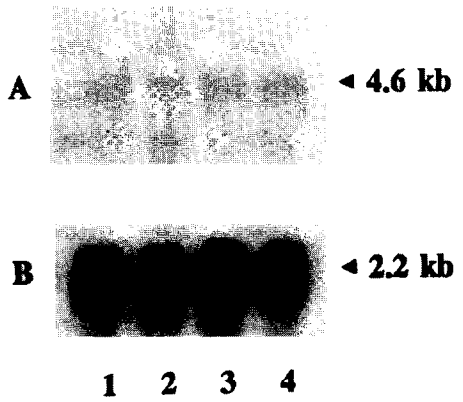

FIG. 2. Northern blot analysis showing the effect of mitoxantrone on *mdr1b* (A) mRNA expression in mouse liver. PolyA-selected RNA (5 μg) was obtained 3 days after treatment of male NMRI mice with saline (lane 1) or mitoxantrone: 0.01 (lane 2), 0.1 (lane 3) or 0.5 (lane 4) mg/kg body weight, i.p. GAPDH (B) mRNA expression was detected in the same blots (gel loading control). Blots are representative for series of four blots.

tion was lower, whereas incubation over more than 36 hr resulted in a considerable "spontaneous" increase in *mdr1* gene expression (not shown). At 5 μM, considerable cell loss was observed, and both total yield of RNA and expression of *mdr1* were decreased. Experiments with the cytochrome P-450 inhibitor metyrapone did not reveal a major role of metabolic activation of mitoxantrone in *mdr1* induction in rat hepatocytes (data not shown). In mouse hepatocytes in primary culture, very low basal expression of the *mdr1b* gene was detectable (Fig. 5). Concentrations of

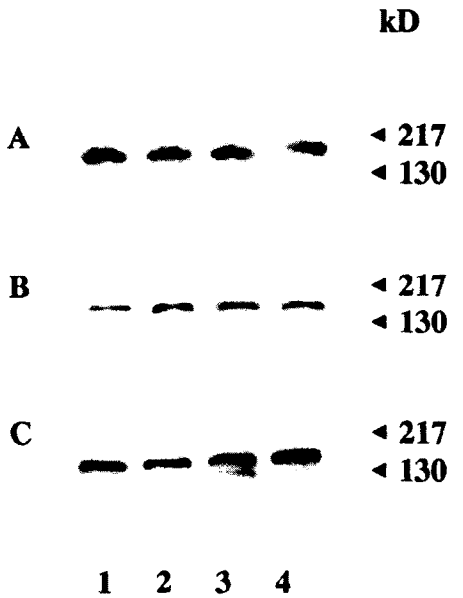

FIG. 3. Western analysis of P-glycoprotein in rat liver (A), rat kidney (B) and mouse liver (C) 3 days after treatment with saline (lane 1) or mitoxantrone: 0.01 (lane 2), 0.05 (lane 3) or 0.1 (lane 4) mg/kg body weight, i.p. Blots are representative for series of four blots.

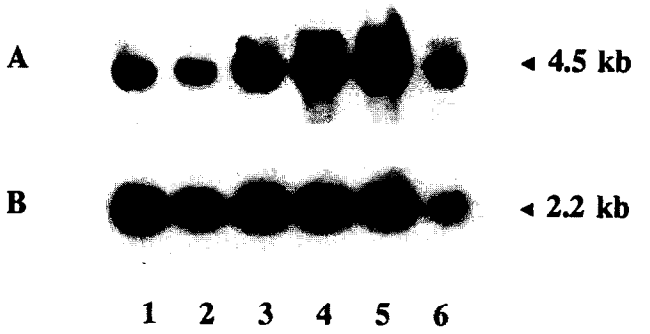

FIG. 4. Northern blot analysis of *mdr1* (A) mRNA expression in rat hepatocytes in primary culture treated with mitoxantrone. Total RNA (20 μg) from saline-treated cultures (lane 1) or from cultures treated for 36 hr with 0.01 (lane 2), 0.1 (lane 3), 0.5 (lane 4), 1.0 (lane 5) or 5.0 (lane 6) μM mitoxantrone (initial concentration). GAPDH (B) mRNA expression detected in the same blot as shown in A (gel loading control). Blots are representative for series of four blots.

less than 5 μM did not lead to a significant induction of *mdr1b* gene expression, whereas *mdr1b* mRNA was strongly increased after treatment with 5 μM, a concentration that, however, led to a considerable decline in cell number.

To determine whether rat *mdr1b* gene transcription was elevated in response to mitoxantrone, we measured the activity of luciferase in H4IIE rat hepatoma cells stably transfected with a luciferase construct of the rat *mdr1b* promoter. This construct contained the promoter elements necessary for basal and drug-regulation of *mdr1b* expression [27]. Mitoxantrone caused a concentration-dependent increase in *mdr1b* promoter activity with a maximum of 27-fold induction over control levels (Fig. 6). Expression of the endogenous gene was also increased by mitoxantrone treatment.

Because mitoxantrone has been reported to be highly cytotoxic in Hep G2 hepatoma cells [31], cytotoxicity in rat

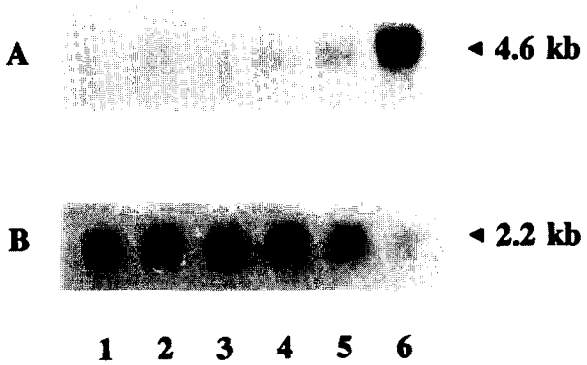

FIG. 5. Northern blot analysis of *mdr1* (A) mRNA expression in mouse hepatocytes in primary culture treated with mitoxantrone. Total RNA (20 mg) from saline-treated cultures (lane 1) or from cultures treated for 36 hr with 0.01 (lane 2), 0.1 (lane 3), 0.5 (lane 4), 1.0 (lane 5) or 5.0 (lane 6) μM mitoxantrone (initial concentration). GAPDH (B) mRNA expression detected in the same blot as shown in A (gel loading control). Blots are representative for series of four blots.

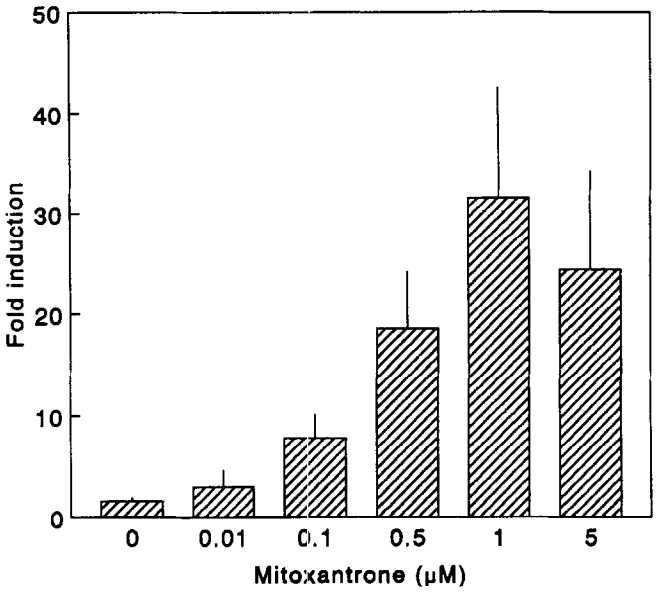

FIG. 6. Activation of *mdr1b* transcription by mitoxantrone. H4IIE cells were stably transfected with a luciferase reporter construct containing *mdr1b* DNA corresponding to bases -941 to +608 relative to the transcription start point. Cells were exposed to the indicated concentrations of mitoxantrone for 24 hr. Luciferase activity is expressed as fold induction over control. Protein concentration was used to correct for interwell variability. Data represent the means ± SEM (n = 8).

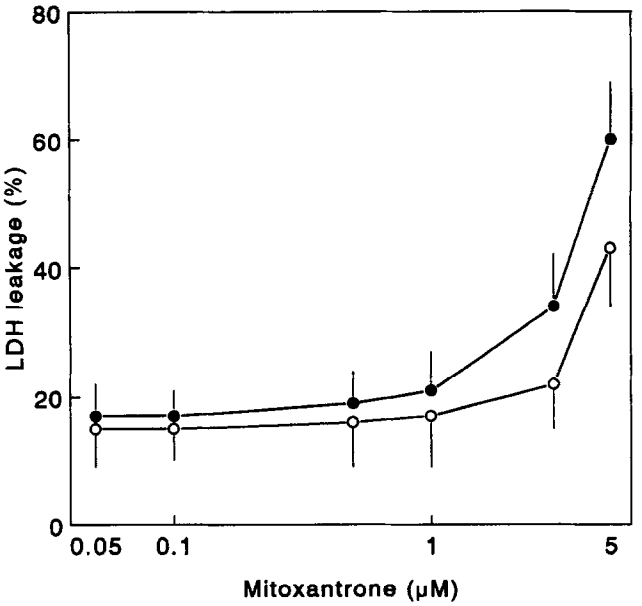

FIG. 7. Cytotoxicity of mitoxantrone in primary cultures of rat (solid circle) and mouse (open circle) hepatocytes. Cells were incubated for 9 hr in the presence of different concentrations of mitoxantrone, and LDH activity was determined in the medium as described in Materials and Methods. Data represent the means ± SD (n = 5).

hepatocyte culture was assessed by measuring LDH leakage into the medium. Concentrations that strongly induced *mdr1* gene expression (0.5 and 1 μM) did not increase LDH leakage (Fig. 7) significantly. Cytotoxicity was obvious at higher concentrations, which is similar to a report by Mewes *et al.* [32]. Analysis of LDH leakage in mitoxantrone-treated mouse hepatocytes did not reveal significant cytotoxicity at mitoxantrone concentrations of less than 5 μM.

Because inhibitors of protein synthesis have been reported to induce *mdr1* gene expression in hepatocytes from human and rodents [30, 33], the influence of mitoxantrone on protein synthesis in hepatocyte cultures was also investigated. A correlation was found between the relative amount of gene expression and the inhibition of protein synthesis (Fig. 8) in rat and mouse hepatocytes in primary culture. Mouse hepatocytes were more resistant to the inhibitory action of mitoxantrone on protein synthesis than were rat hepatocytes.

DISCUSSION

An increase in *mdr1* gene expression can represent a critical event in the acquisition of drug resistance by malignant tumors. Although permanent *mdr1* overexpression is found in many multidrug-resistant tumors, adaptive induction of *mdr1* gene expression may also occur during cancer chemotherapy. Furthermore, *mdr1* induction in nonmalignant cells may be of clinical relevance because of its possible beneficial role as a protective mechanism in nontarget tissues. Likewise, the occurrence of hepatotoxic side effects of chemotherapeutic drugs, frequently a limiting factor during therapy, may be prevented.

In this report, we show that mitoxantrone, a chemotherapeutic drug widely used in the treatment of mammary cancer, non-Hodgkin's lymphoma, and acute myeloid leukemia, is a potent inducer of *mdr1* gene expression, both *in vivo* in rat liver and *in vitro* in rodent hepatocytes in primary culture. Induction of *mdr1* gene expression could also be demonstrated in extrahepatic rat organs. Interestingly, mitoxantrone caused a strong induction in small intestine, which already shows a relatively high basal *mdr1* expression. Pharmacokinetic factors such as biliary excretion and enterohepatic circulation of mitoxantrone and its conjugates may contribute to the marked effects in the small intestine. After treatment with 0.5 mg/kg, the findings of partially degraded RNA from extrahepatic tissues, of a lower yield of RNA isolated from liver samples and of a smaller extent of induction of *mdr1* gene expression indicate that this dose of mitoxantrone inhibits RNA synthesis and/or leads to tissue damage.

In contrast with the rat, no significant induction of *mdr1* mRNA was observable in mouse liver in the dose range applied. Western analysis in rat liver and kidney showed that, in contrast with strong increases in mRNA, no comparable increases in P-glycoprotein were detectable, probably as a consequence of inhibition of protein synthesis by mitoxantrone. In mouse liver, a marginal increase in hepatic P-glycoprotein was observed.

Induction of rat hepatic *mdr1* gene expression could also

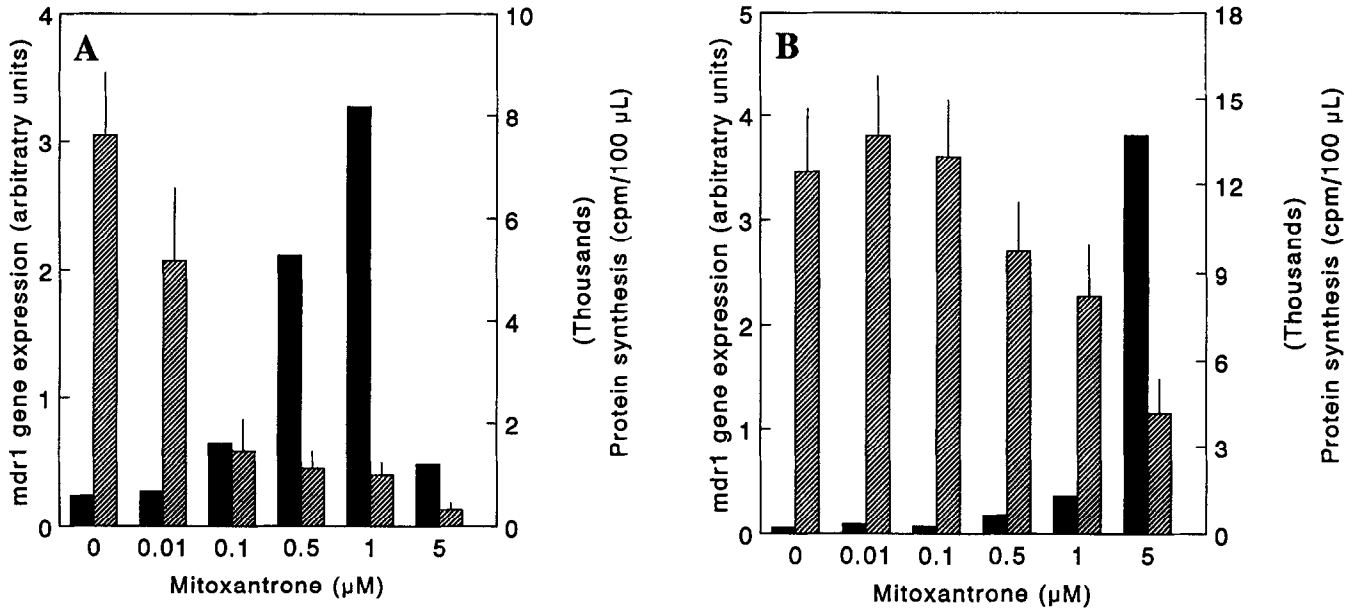

FIG. 8. Induction of *mdr1* gene expression and inhibition of protein synthesis in rat (A) and mouse (B) hepatocytes treated with mitoxantrone. *Mdr1* mRNA levels (solid bars) were assessed by densitometric analysis of autoradiographs shown in Figs. 4 and 5. *Mdr1* gene expression was corrected for RNA loading reflected by GAPDH signal intensity. Hatched bars show means ± SD (n = 3) of protein synthesis, analyzed as ^{3}H-leucine incorporated into cellular protein.

be observed in mitoxantrone-treated rat hepatocytes in culture. Compared with 2-AAF, which shows an EC_{50} of approximately 10 µM as inducer of *mdr1* gene expression in rat hepatocytes [16], mitoxantrone was more potent by at least one order of magnitude. Mouse hepatocytes were less susceptible to *mdr1* induction by mitoxantrone than were rat hepatocytes, i.e. a 10-fold higher mitoxantrone concentration was required for induction. Interestingly, 2-AAF was reported to be ineffective as an inducer of P-glycoprotein in mouse liver [34]. These findings suggest that fundamental differences in the regulation and inducibility of *mdr1* gene expression prevail between these species.

Mitoxantrone is an anthraquinone derivative that is known for its pronounced cytotoxicity and genotoxicity. Both effects may be responsible for its antitumor properties. The drug intercalates with DNA and binds to DNA by electrostatic interaction of the alkylamino side chains [35–39]. Interference with the DNA-topoisomerase II complex may be a major mode of action of mitoxantrone, finally leading to DNA damage [40]. In addition, enzymatic formation of reactive metabolites of the drug is involved in cell damage [33, 41] and covalent modification of DNA [42]. Blanz *et al.* [19] identified a glutathione conjugate from human urine, indicating the formation of an electrophilic naphthoquinoxaline derivative in patients treated with mitoxantrone. The monotherapeutic initial dose recommended by the manufacturer for the treatment of mammary carcinoma, non-Hodgkin's lymphoma and hepatocellular carcinoma is 14 mg/m^2. In patients, continuous intravenous infusion at the highest tolerated dose has been reported [43] to lead to a steady-state plasma concentration in the range of 0.005 µM.

The precise mechanism of induction of *mdr1* gene expression by mitoxantrone remains to be clarified. Enhanced transcription of an *mdr1b* reporter gene construct stably transfected in rat H4IIE hepatoma cells suggests that transcriptional activation of the *mdr1b* gene contributes to induction. In a recent paper [27], the role of a broad sequence of the promoter in xenobiotic-induced *mdr1b* induction could be demonstrated. However, no particular sequence motif responsible for this effect could be identified. Chin *et al.* [20] suggested that both transcriptional and posttranscriptional mechanisms are involved in the induction of *mdr1* gene expression by chemotherapeutic agents such as mitoxantrone or adriamycin. A possible role for the cytoskeleton in posttranscriptional stabilization of *mdr1* mRNA in rat hepatocytes treated with certain inducing agents was suggested by Lee *et al.* [44].

In our experiments, induction of *mdr1* gene expression in rat hepatocytes was seen at mitoxantrone concentrations that did not lead to a significant leakage of LDH. Leakage of LDH is one of the most widely used tests for gross cell damage, indicating irreversible damage to the plasma membrane and/or cell death [45]. Our findings demonstrate that *mdr1* induction with mitoxantrone, at least in rat hepatocytes, is not related to effects of this type but represent a more specific cellular response. Furthermore, induction was correlated to inhibition of protein synthesis, which is in agreement with the hypothesis that "cycloheximide-type" inducers may inhibit the synthesis of a repressor of *mdr1* transcription [29]. Alternatively, inhibition of protein synthesis may be a side effect of mitoxantrone, which coincides with *mdr1* induction. Schuetz *et al.* [46] provided evidence that cycloheximide increases *mdr1* mRNA in rat and human liver cells through posttranscriptional mechanisms such as polysomal stabilization. It remains to be clarified if

mitoxantrone, in addition to stimulating transcription of the rat *mdr1b* gene, also regulates *mdr1* gene expression posttranscriptionally.

In conclusion, our findings demonstrate that the chemotherapeutic drug mitoxantrone acts as a potent inducer of *mdr1* gene expression in rat liver, small intestine and kidney but not in mouse liver. Mitoxantrone was also highly effective as an inducer in rat hepatocytes *in vitro* but much less potent in cultured mouse hepatocytes. Results from experiments with stable transfectants indicate that enhanced transcription of the rat *mdr1b* gene contributes to *mdr1* induction with mitoxantrone. Probably as a consequence of inhibition of protein synthesis, mitoxantrone treatment did not result in a pronounced elevation of P-glycoprotein levels in rat liver and kidney. The lower susceptibility of mouse hepatocytes was related to their relative resistance toward mitoxantrone-mediated inhibition of protein synthesis but may also reflect fundamental differences in *mdr1* regulation between the species. The marked species differences in inducibility may provide a promising tool for further studies on the mechanisms of regulation of *mdr1* gene expression. To evaluate the possible implications of our findings for the use of mitoxantrone in cancer therapy, comparative studies in human cells are required.

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