Influence of Omeprazole on Multidrug Resistance Protein 3 Expression in Human Liver

MONIKA HITZL, KATHRIN KLEIN, ULRICH M. ZANGER, PETER FRITZ, ANDREAS K. NÜSSLER, PETER NEUHAUS, and MARTIN F. FROMM

Dr. Margarete Fischer-Bosch-Institute of Clinical Pharmacology, Stuttgart, Germany (M.H., K.K., U.M.Z., M.F.F.); Department of Pathology, Robert-Bosch-Hospital, Stuttgart, Germany (P.F.); Department of Surgery, Charité, Campus Virchow-Clinic, Humboldt University, Berlin, Germany (A.K.N., P.N.); and Institute of Experimental an Clinical Pharmacology and Toxicology, University of Erlangen-Nuremberg, Erlangen, Germany (M.F.F.)

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ABSTRACT

Multidrug resistance protein (MRP) 3 transports bile salts and conjugated xenobiotics from cells (hepatocytes and enterocytes) into the blood. Hepatic MRP3 expression is low under normal conditions but is markedly up-regulated during cholestasis. Since little is known about additional factors increasing human hepatic MRP3 expression, we investigated the variability of MRP3 expression in a large collection of human livers and factors contributing to variable MRP3 expression in liver and HepG2 cells. MRP3 was measured in 62 human livers from patients with and without omeprazole treatment and in HepG2 cells with and without omeprazole or β-naphthoflavone treatment. Livers of patients treated with omeprazole showed 4.8-fold (P < 0.0001) higher MRP3 protein expression compared with the remainder of the population. Accordingly, MRP3 mRNA and protein were induced 2.4- and 1.8-fold, respectively (P < 0.01 and P < 0.05), in HepG2 cells treated with omeprazole. Finally, MRP3 was induced in HepG2 cells by β-naphthoflavone. In summary, treatment with omeprazole and β-naphthoflavone is a determinant of variable human hepatic MRP3 expression.

It is increasingly recognized that active uptake and efflux processes are important determinants for disposition of endogenous compounds (e.g., bile salts) and drugs (e.g., cyclosporine and HIV protease inhibitors) (Kusuhara et al., 1998; Trauner et al., 1998; Borst et al., 2000; Fromm, 2000; Keppler and König, 2000). MRP3 is a member of the multidrug resistance protein (MRP) family (for review, see Borst et al., 2000; Keppler and König, 2000). These membrane proteins mediate ATP-dependent efflux of lipophilic substances conjugated to glucuronate, glutathione, and sulfate. Substrates of the basolateral export pump MRP3 are sulfated and nonsulfated bile salts, 17β-glucuronosyl estradiol, leukotriene C4, and several anticancer drugs (König et al., 1999a; Zeng et al., 1999; Hirohashi et al., 2000; Zelcer et al., 2001). MRP3 appears to be involved in bile salt homeostasis in several ways. First, expression of MRP3 in the basolateral membrane of enterocytes indicates that MRP3 could be involved in intestinal transport of organic anions from the cells into portal blood (Rost et al., 2002). Second, MRP3 expression was found in the basolateral membrane of human hepatocytes, with exceptionally high expression in livers from patients with Dubin-Johnson syndrome and a patient with primary biliary cirrhosis, in the lateral membrane of intrahepatic bile duct epithelial cells (cholangiocytes), and in the basolateral membrane of human gallbladder epithelium (König et al., 1999b; Kool et al., 1999; Gotoh et al., 2000; Rost et al., 2001). Since in animal models of cholestasis, MRP2 is down-regulated whereas MRP3 expression is induced (Hirohashi et al., 1998; Ogawa et al., 2000; Donner and Keppler, 2001), it appears that MRP3 compensates for impaired MRP2 function and responds to bile salts at the transcriptional level (Schuetz et al., 2001). Accordingly, recent data indicate that intestinal MRP3 is also induced by bile acids (Inokuchi et al., 2001).

In addition to increased MRP3 expression in humans during cholestasis, there is little information on the variability of MRP3 expression in human livers and on factors contributing to MRP3 expression in liver, such as comedication (e.g., by the proton pump inhibitor omeprazole), age, gender, and smoking. We therefore investigated various aspects of MRP3

ABBREVIATIONS: HIV, human immunodeficiency virus; MRP, multidrug resistance protein; PCR, polymerase chain reaction; TBS, Tris-buffered saline; TBS-T, Tris-buffered saline/Tween 20; DMSO, dimethyl sulfoxide; RT-PCR, reverse transcription polymerase chain reaction; a.u., arbitrary units.
expression and regulation in 62 human livers and HepG2 cells.

Materials and Methods

Tissue Collection. Human liver tissue (n = 62) was obtained as nontumorous tissue surrounding surgically removed liver tumors or metastases or material that was surgically resected for other reasons. Extensive documentation was obtained for each of the samples, including demographic data of the patient (age: mean ± S.D., 56.1 ± 16.5 years; range: 4–85 years; and sex: 31 male and 31 female), diagnosis, smoking status (11 smokers, 49 nonsmokers, and 2 unknown), and drug intake. The tissues were stored at −80°C until nuclear/membrane pellets were prepared according to the following standard procedure. One gram of tissue was homogenized in buffer containing 1 mM EDTA, 1 mM dithiothreitol, 10 mM HEPES pH 7.4, 0.2 mM Pefabloc (Roche, Karlsruhe, Germany) according to the manufacturer's instructions in 1% nonfat dry milk in TBS-T (Thermo Scientific, Rockford, IL). Total RNA was isolated using RNeasy columns (QIAGEN, Hilden, Germany) according to the manufacturer's instructions. High-quality RNA was obtained by homogenation of RNase by the reagent and then stored at −80°C.

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statistical significance by analysis of variance with subsequent Student-Newman-Keuls tests.

Results

Histological Analysis of Human Livers. Histological analysis revealed that the samples were noncirrhotic and nontumorous. Since it has been reported that cholestasis results in a pronounced up-regulation of MRP3 in liver, we particularly examined the samples for any signs of local cholestasis. However, none of the livers investigated revealed signs of local cholestasis.

MRP3 mRNA in Human Liver. We analyzed MRP3 mRNA in 62 human livers by TaqMan real-time RT-PCR and found a MRP3 mRNA distribution as indicated in Fig. 1. The expression of MRP3 mRNA in human liver was highly variable and varied by a factor of 86. MRP3 mRNA was not significantly affected by age, gender, smoking, or drug treatment (e.g., omeprazole).

MRP3 Protein in Human Liver. A representative Western blot for MRP3 in human livers is shown in Fig. 2. We observed the previously described pattern of two bands, a possible result of different levels of glycosylation (König et al., 1999b). Western blot analysis of MRP3 protein in 62 human livers showed a highly variable protein expression as indicated in Fig. 3. All samples had constant β-actin protein levels (Fig. 2). No MRP3 protein was detectable in two livers (with log MRP3 mRNA/β-actin being −5.7 and −5.2; see Fig. 1). In the remainder of the population, MRP3 expression varied 193-fold. No correlation was found between expression of MRP3 protein and MRP3 mRNA levels in human liver. In addition, we analyzed whether medication has an influence on MRP3 protein levels. We found significant differences in MRP3 protein levels of subjects treated with omeprazole compared with untreated subjects (Fig. 3). Mean MRP3 protein expression of 10 subjects treated with omeprazole is 152.1 ± 117.0 (range, 0–413.1) compared with 31.8 ± 35.1 a.u./μg of total protein (P < 0.0001; range, 0–175.4) without omeprazole. Moreover, smokers had a (nonsignificant) trend toward higher MRP3 protein expression in comparison with nonsmokers [n = 7 versus 43 (omeprazole-treated patients were excluded) and 43.1 ± 61.5 versus 30.1 ± 30.4 a.u./μg of total protein, not significant]. Gender, age, and any other comedication (e.g., benzodiazepines) did not significantly affect MRP3 protein in human livers.
Fig. 4. Induction of MRP3 mRNA by omeprazole in in vitro studies. HepG2 cells were incubated with vehicle (0.1% DMSO), 10, and 100 μM omeprazole for 48 h. MRP3 mRNA was analyzed by real-time TaqMan RT-PCR. Results obtained for MRP3 mRNA were normalized for -actin mRNA (, P < 0.05; **, P < 0.01).

Fig. 3. MRP3 protein expression in 62 human livers. Fifty micrograms of nucleus/membrane fractions of each human liver were analyzed by Western blot using a dilution series of a standard liver sample for quantification. Each arrow (in the insert, *) represents one subject treated with omeprazole. Two additional subjects are not shown in the log-transformed part of the figure since they did not have detectable MRP3 protein expression. One of these two individuals was also treated with omeprazole. The insert displays the distribution of the linear expression data.
Effect of Omeprazole on MRP3 Expression in HepG2 Cells. To investigate the potential molecular basis for the increase in hepatic MRP3 in omeprazole-treated patients, we incubated HepG2 cells with omeprazole. MRP3 mRNA levels were $1.04 \pm 0.36, 1.14 \pm 0.61$, and $2.46 \pm 0.54$ a.u. in control, 10, and 100 $\mu$M omeprazole-treated cells, respectively (control versus 100 $\mu$M omeprazole, $P < 0.01$; Fig. 4). Moreover, MRP3 protein levels in control, 10, and 100 $\mu$M omeprazole-treated cells were $4.5 \pm 0.7, 6.3 \pm 2.3$, and $8.2 \pm 2.7$ a.u./$\mu$g of total protein (Fig. 5; control versus 100 $\mu$M omeprazole, $P < 0.05$), respectively.

To investigate the potential involvement of the Ah receptor pathway in MRP3 induction, we incubated HepG2 cells with 5 and 10 $\mu$M $\beta$-naphthoflavone, which is known to be a ligand for this receptor. MRP3 mRNA levels were $1.78 \pm 0.86, 5.47 \pm 0.94$, and $5.81 \pm 0.29$ a.u. in control, 5, and 10 $\mu$M $\beta$-naphthoflavone-treated cells, respectively. Cells, which were treated with 5 and 10 $\mu$M $\beta$-naphthoflavone, showed on average a 10.5- and 8.5-fold higher resorufine formation in comparison to control cells, respectively (Fig. 6).

Discussion

Our study in a large panel of human liver tissues revealed a considerable variability of MRP3 expression. Therapy with the proton pump inhibitor omeprazole was identified as an environmental factor causing increased hepatic MRP3 expression. Additional in vitro studies indicate activation of the Ah receptor pathway as a putative mechanism of hepatic MRP3 induction by omeprazole. The potential clinical rele-

Fig. 5. Induction of MRP3 protein by omeprazole in in vitro studies. After 48 h of incubation of HepG2 cells with vehicle (0.1% DMSO), 10, and 100 $\mu$M omeprazole, cells were homogenized and analyzed for MRP3 protein by Western blot. Fifty micrograms of cell homogenate were analyzed for MRP3 and quantified using a dilution series of a standard liver sample. A, MRP3 Western blot (7.5 $\mu$g of protein were loaded from the standard liver); B, mean MRP3 protein expression from duplicate measurements of three independent experiments (*, $P < 0.05$).
MDRI single nucleotide polymorphisms are associated with altered P-glycoprotein expression and function (Hoffmeyer et al., 2000; Hitzl et al., 2001; Kim et al., 2001) and determine the response of an individual to drug treatment (e.g., HIV) (Felley et al., 2002). It is currently unknown whether genetic mutations contribute to variable MRP3 expression in humans, but for example, DNA from the only two patients with no detectable MRP3 might be very useful for mutation screening.

Using a large collection of human liver tissues, we identified omeprazole as an inducer of MRP3 expression. This effect could be reproduced with HepG2 hepatoma cells, which showed a concentration-dependent induction of MRP3 expression by omeprazole. Interestingly, we observed increased MRP3 mRNA and protein levels in HepG2 cells but only increased protein levels (without increase of MRP3 mRNA) in our collection of human livers. The underlying mechanism of these differences is unclear at the moment, but similar observations have been made during MRP3 induction studies in rats (Ogawa et al., 2000). It should be noted that we collected only nontumorous and nonpathologic human liver tissue for this study. We cannot, however, completely rule out that the vicinity of the collected tissue to a tumor or local inflammation affected MRP3 expression to some extent.

Although it was not the goal of this work to investigate the molecular mechanism of MRP3 induction, the following data indicate that activation of the Ah receptor pathway could be one mechanism involved in MRP3 induction by omeprazole. First, activation of the Ah receptor pathway mediates CYP1A1 induction by omeprazole (Daujat et al., 1992; Backlund et al., 1997; Dzeletovic et al., 1997). Second, dioxin-responsive elements to which the Ah receptor/Arnt heterodimer may bind have been identified in the 5′-flanking region of human MRP3 (Takada et al., 2000). Third, in vitro data indicate that MRP3 expression is also induced by β-naphthoflavone (unpublished data) and 2-acetylaminofluorene (Stöckel et al., 2000; Schrenk et al., 2001), which are known activators of the Ah receptor pathway. Finally, our own data indicate that smokers have on average 1.4-fold higher MRP3 protein levels in comparison to nonsmokers. Despite the low MRP3 expression in human liver, we identified a commonly used drug as inducer of hepatic MRP3 expression and provide evidence for involvement of the Ah receptor pathway in omeprazole-mediated MRP3 induction.

Taken together, using in vitro studies in combination with clinical samples, induction of an ABC transporter involved in homeostasis of endogenous compounds as well as in drug disposition was found. Moreover, evidence is provided for the putative mechanism of MRP3 induction. Further studies are required to determine the impact of highly variable MRP3 expression on disposition of endogenous compounds and drugs and the contribution of genetic polymorphisms to variable MRP3 expression.

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References


Address correspondence to: Dr. Martin F. Fromm, Dr. Margarete Fischer-Bosch-Institute of Clinical Pharmacology, Auerbachstr. 112, 70376 Stuttgart, Germany. E-mail: martin.fromm@ikp-stuttgart.de