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The Effect of Age on P-Glycoprotein Expression and Function in the Fischer-344 Rat

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ABSTRACT

We investigated the effect of age on P-glycoprotein (P-gp) expression and function in rat liver, intestine, kidney, and endothelial cells of the blood-brain barrier (BBB) and lymphocytes. Flow cytometric analysis was used to examine P-gp expression in lymphocytes from male Fischer-344 rats from three age groups (young at 3–4 months, intermediate at 13–14 months, and old at 25–26 months). In addition, P-gp function in lymphocytes was assessed by measuring the ability of the P-gp inhibitor verapamil to limit the efflux of the fluorescent P-gp substrate rhodamine 123. P-gp expression was evaluated in the remaining four tissues by Western blot analysis. The effect of age on P-gp expression was tissue-specific. Although lymphocytic and hepatic P-gp expression increased with age, renal P-gp content was lower in the old kidneys. No statistical difference was observed in P-gp expression in intestinal microsomes or in BBB cell lysates among the three age groups. P-gp function was also increased by 6- to 8-fold in lymphocytes from the old rats. When P-gp expression was compared with CYP3A expression in these rats (reported elsewhere in this journal), we found that P-gp expression increased with age, whereas CYP3A expression and activity declined in the old livers. The converse pattern was observed in the kidney. Thus, age-related changes in P-gp expression and function are likely to be tissue-specific, and these changes may be inversely related to differences in CYP3A expression.

The importance of P-glycoprotein (P-gp), the gene product of the multidrug resistance gene 1 (MDR1), in limiting the entry of chemotherapeutics into cancer cells has been recognized for more than two decades (Juliano and Ling, 1976). P-gp has been found to transport a variety of structurally unrelated compounds from numerous therapeutic classes and is expressed in a variety of normal tissues such as the liver, gastrointestinal tract, kidney, blood-brain barrier (BBB), and hemopoietic cells (Thiebaut et al., 1987; Schinkel, 1999; Ayrton and Morgan, 2001). The expression of P-gp in sites of secretory or barrier function (Lo and Burckart, 1999) and broad substrate specificity suggests that it may play a critical role in the absorption, distribution, metabolism, and elimination of a variety of agents (Wandel et al., 1999).

Significant overlap in function and expression exists between P-gp and the drug-metabolizing enzyme, cytochrome P450 3A (CYP3A) (Schuetz et al., 1995, 2000; Seree et al., 1998). CYP3A is the most abundantly expressed cytochrome P450 in the human liver and intestine and contributes to the metabolism of a wide spectrum of pharmaceutical agents. Although differences have been noted between these proteins, CYP3A and P-gp have been found to display overlapping tissue expression patterns (Wacher et al., 1995) such as common expression in the liver, intestine, kidney, adrenal gland, and, possibly, lymphocytes (Thiebaut et al., 1987; Wacher et al., 1995; Witkowski and Miller, 1999). These two proteins also share many substrates, inducers, and inhibitors (Salphati and Benet, 1998). Moreover, genes encoding P-gp and CYP3A map to adjacent regions on chromosome 7 (loci 7q21.1 and 7q22.1, respectively; Wacher et al., 1995). Recent studies have found that the transcription of these two proteins may be regulated, in part, by the pregnane X receptor (Gibson et al., 2002).

A number of studies have demonstrated a reduced clearance of CYP3A substrates in the elderly in vivo (Greenblatt et al., 1982; Cotreau et al., 2004). Although these age-related differences may reflect a reduction in CYP3A expression or function (Warrington et al., 2000), a variety of other factors may contribute to these in vivo findings such as changes in liver size and hepatic blood flow (Greenblatt et al., 1982). Since CYP3A and P-gp exhibit overlap in tissue expression and substrate specificity, it is possible that some of the age-related changes observed in vivo may reflect an age-related change in P-gp expression or function.

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ABBREVIATIONS: P-gp, P-glycoprotein; MDR1, multidrug resistance gene 1; P450, cytochrome P450; BBB, blood-brain barrier; PBMC, peripheral blood mononuclear cell; FITC, fluorescein isothiocyanate; PE, phycoerythrin; ANOVA, analysis of variance.

Previous studies on the effect of age on P-glycoprotein have been largely limited to lymphocytes and cancer cells. Increased P-gp mRNA expression, protein expression, and/or activity have been identified in T lymphocytes in the elderly (Gupta, 1995; Aggarwal et al., 1997) and in aging mice (Witkowski and Miller, 1999).

Other studies have identified an increased resistance to chemotherapeutics in elderly leukemia patients. Leith et al. (1999) found that P-gp expression and drug efflux increased in older acute myeloid leukemia patients and suggested that differences in survival between the young and old may be due to differences in MDR1 expression.

Studies of age-related differences in P-gp expression or function in other tissues have been limited. Lown et al. (1997) found no change in age in intestinal P-gp expression in 25 renal patients between 21 and 67 years of age, whereas Batetta et al. (1999) found that MDR1 mRNA expression was increased in the atherosclerotic plaques of elderly subjects.

Age-related differences have been identified in the pharmacokinetics of P-gp substrates that are not metabolized by CYP3A such as digoxin (Hanratty et al., 2000) and fexofenadine (Simons and Simons, 1999). Modulation of P-gp function with age may contribute to the different pharmacokinetic profile observed in the old for these drugs.

To our knowledge, the effect of age on P-gp expression and activity has not been examined directly in nonhemopoietic cells known to express this protein such as the bile canaliculi of the liver, the kidney proximal tubules, intestinal epithelial cells, or the endothelial cells of the BBB in either human or animal models. Nonetheless, age-related differences in P-gp expression or function in these tissues could alter drug distribution and bioavailability.

Thus, we examined the effect of age on P-glycoprotein expression and function in five tissues or cell types of the Fischer-344 male rat: the liver, intestine, kidney, lymphocytes, and endothelial cells of the blood-brain barrier. We also compared these P-gp expression studies with changes in CYP3A expression and activity in these rats (Warrington et al., 2004).

Materials and Methods

Materials

Rats. Three age groups of male Fischer-344 rats were acquired from the National Institute on Aging (young, 2–3 months, $n = 10$; intermediate, 12–13 months, $n = 10$; old, 24–25 months, $n = 12$) (Warrington et al., 2003, 2004). Animals were aged at the Tufts University School of Medicine Animal Facilities for an additional 1 to 2 months and sacrificed by decapitation. Animals were examined for gross pathology, trunk blood was collected (~7 ml), and livers and kidneys were harvested and stored at −80°C until microsomal preparation. Intestinal microsomal preparations were performed directly after decapitation. Isolation of endothelial cells from the blood-brain barrier and peripheral blood mononuclear cells (PBMCs) were performed within 3 to 6 h of sacrifice. These storage times and conditions have been routinely used in previous studies (Jackson and Warner, 1986; Jette et al., 1993). Four old rats were excluded from the study due to the development of disease (infection, $n = 1$; superficial gross tumors, $n = 2$; and hepatomegaly, $n = 1$). The remaining rats appeared healthy and displayed no evidence of gross pathological processes.

Reagents. Antibodies, verapamil, rhodamine 123, and other reagents were purchased from commercial sources. Verapamil and rhodamine 123 were dissolved in methanol and stored at −20°C until use. For studies of P-gp function, appropriate volumes of verapamil were aliquotted and evaporated to dryness in a vacuum oven at 37°C.

Tissue Preparations

Microsomal Preparations. Hepatic, intestinal, and renal microsomes were prepared and suspended in 0.1 M potassium phosphate buffer containing 20% glycerol and stored at −80°C until use (Warrington et al., 2003).

Isolation of Endothelial Cells of the BBB. Endothelial cells were isolated by differential centrifugation (Jette et al., 1997). Briefly, cerebral cortices were dissected from the rest of the brain and cleared of meninges and superficial vessels. Electron microscopic analysis of this cellular preparation was used to confirm the presence of intact endothelial cells (data not shown). Red blood cells were also found, but at concentrations less than 1%. Myelin and organelles likely to have been derived from neuronal cells were also found at low concentrations. Cells were lysed with a lysis buffer containing 1% Triton X-100 and 0.5% deoxycholate and homogenized with a Dounce homogenizer. Samples were allowed to shake for 30 min to solubilize the capillaries and were then centrifuged to remove cellular debris. Cell lysates were stored at −80°C until use.

Quantification of Protein Content. The protein content of microsomes and cell lysates was quantified using a bicinchoninic acid protein assay kit, as described by the product's manufacturer (Pierce Chemical Co., Rockford, IL). Known quantities of albumin, subjected to the same conditions, were used as reference standards.

Peripheral Blood Mononuclear Cell Isolation. Trunk blood was collected into heparinized tubes and stored at 25°C for 4 to 6 h during which the relative proportions of lymphocyte subsets have been shown to remain unchanged (Jackson and Warner, 1986). PBMCs were isolated by Ficoll-Hypaque density gradient centrifugation. Briefly, anticoagulated trunk blood (~6–7 ml) was diluted to 15 ml with 0.9% saline. Samples were layered onto Histopaque-1077 (Sigma-Aldrich Laboratories, St. Louis, MO) and centrifuged at 2000 rpm.

After washing with saline, cell number and viability were determined by a trypan blue exclusion assay. Cells (1/5th vol) were incubated with 0.9% saline and 0.4% trypan blue (MP Biomedicals, Irvine, CA) for 15 min to allow the stain to penetrate the cells. Cells were mixed thoroughly and transferred to a hemocytometer. Cells were counted and concentrations were determined according to the following equation:

$$\text{Cells/ml} = \frac{\sum \text{Count/square}}{n} \cdot \text{DF} \cdot 10^4 \quad (1)$$

in which DF represents the dilution factor of cells and n represents the number of squares counted. Cells that actively removed trypan blue were counted as viable, whereas those filled with dye were counted as dead.

Western Blot Analysis of P-gp Expression

Western blot analysis was conducted as described previously (Perloff et al., 2001). Briefly, samples containing 25% sampling buffer (40% glycerol, 8% β-mercaptoethanol, 8% SDS, 0.4 M Tris, and pyronin Y) were loaded onto a 4 to 15% polyacrylamide gel at varying protein concentrations for hepatic (3–25 μg), intestinal (18–25 μg), renal (15–25 μg), and BBB (5–60 μg) samples. Using a Mini Protean II Western analysis unit (Bio-Rad Laboratories, Hercules, CA), gels were submerged in running buffer (0.19 M glycine, 250 mM Tris-base, and 0.1% SDS), and samples were separated electrophoretically at 100 V for 1.5 h on ice. Using transfer buffer (0.19 M glycine, 250 mM Tris-base, and 20% methanol), protein was transferred onto a polyvinylidene fluoride membrane at 100 V for 1.5 h on ice. Membranes were exposed to gel fixation buffer (50% methanol with 10% glacial acetic acid) and washed with 1× Tris-buffered saline/Tween

20 (0.15 M NaCl, 40 mM Tris-HCl, 40 mM Tris-base, and 0.06% Tween 20).

After a 2-h preincubation with 6% Blotto (dry nonfat milk in 1× Tris-buffered saline/Tween 20) at 25°C, membranes were probed with C219 (1:1000; Signet Laboratories, Dedham, MA) for 22 h at 4°C, followed by 3 to 5 h at 25°C. Membranes were washed and reprobed with a sheep anti-mouse IgG-horseradish peroxidase antibody (1:3000; Amersham Biosciences Inc., Piscataway, NJ) for 2 h at 25°C. A chemiluminescent substrate was added, and samples were analyzed using a Kodak imager and Kodak 1D image analysis software (Eastman Kodak, Rochester, NY). Since C219 is known to cross-react with the gene product of mdr2 and this protein is highly expressed in the rat liver, a hepatic P-gp Western blot was stripped with stripping buffer (0.2 M glycine, 0.1% SDS, and 1% Tween 20, pH 2.2) for 1 h and reprobed with another P-gp antibody, C494 (1:1667; Signet Laboratories), which is not known to cross-react with this protein. Findings were similar using this antibody (data not shown).

Flow Cytometric Analysis

P-gp functional assays were adapted from previous studies using Caco-2 cells (Perloff et al., 2001; Störmer et al., 2001). Isolated PBMCs (1.5×10^6 cells) were incubated in 1× minimal essential media-α (containing 1% bovine serum albumin) in the presence or absence of verapamil (100 μM) for 1.25 h at 37°C. Cells were incubated with rhodamine 123 (11 μM), a fluorescent P-gp substrate, for 1 h at 37°C. After five washing steps, cells were resuspended in media and divided into thirds. Samples were centrifuged at 2000 rpm for 5 min, resuspended in 0.9% NaCl (1 ml), and centrifuged for another 5 min. Cells were incubated with either 0.9% saline, phycoerythrin (PE)-labeled IgG, or CD45 antibodies (50 μl) for 30 min on ice in the dark. Cells were washed, resuspended in saline, and analyzed on a FACScan flow cytometer.

P-gp expression studies were adapted from that described by Maillefert et al. (1996). Isolated PBMCs (5×10^5) were incubated with either 0.9% saline or MRK16 (diluted 1:4; Kamiya Biomedical Co., Seattle, WA) on ice for 45 min. After washing, samples were incubated with a fluorescein isothiocyanate (FITC)-labeled anti-mouse secondary antibody (Calbiochem, La Jolla, CA) for 30 min at 4°C in the dark. An additional 1 ml of NaCl was added. Samples were centrifuged and resuspended in 0.9% saline for analysis on a FACScan flow cytometer.

Cells (5000 or 10,000) were separated using a Becton Dickinson FACScan flow cytometer and analyzed using Cell Quest software (BD Biosciences, San Jose CA). Both cell counts are considered sufficient for analysis of peripheral blood lymphocytes (Lewis, 1993).

Using Summit software (version 3.1, DakoCytomation California Inc., Carpenteria CA) a two-parameter histogram, in which the integrated forward-angle light scatter was plotted relative to the integrated right-angle light scatter (side-scatter, Fig. 1), was used to identify the lymphocyte population within the collection of PBMCs as previously described (Lewis, 1993). In the conditions used in these assays, forward-angle light scatter reflects the cell size, whereas right-angle light scatter reflects the cell granularity (Lewis, 1993).

Two cell populations were observed (Fig. 1A). Based upon visual inspection and comparison to previously published analyses in humans (Schwartz and Fernandez-Repollet, 1994) and mice (Vacchio and Shores, 2000), these individual populations are likely to represent small and large lymphocytes (Jackson and Warner, 1986).

Cells were further characterized using PE-labeled CD45 antibodies and a PE-labeled isotype control. CD45-positive cells were identified in both cell populations (Fig. 1B, $80.0 \pm 1.9\%$, $n = 8$), and these populations were considered collectively throughout these studies. No differences were apparent across age groups or treatment groups in the percentage of $CD45^+$ cells in these lymphocyte populations.

MRK16 positive cells were determined by comparison to a phosphate buffer plus FITC-labeled antibody control. In pilot studies, samples were incubated with an IgG isotype control or 50 mM potassium phosphate buffer in parallel with samples probed with MRK16. After further incubation with the FITC-labeled secondary antibody, no differences were identified between samples containing either the isotype control or phosphate buffer. Accordingly, buffer was incubated with the secondary FITC-labeled antibody in these assays.

After gating on lymphocytes (Fig. 1A) or on lymphocytes that also expressed CD45 (Fig. 1, A and B), samples were analyzed for the content of rhodamine 123, as measured by the green fluorescent intensity. Samples incubated in the presence or absence of verapamil (100 μM) were compared, and values were expressed relative to the negative control.

Statistical Analysis

One-way ANOVA or one-way Kruskal-Wallis ANOVA on ranks tests was used to evaluate age-related differences. To determine differences between individual groups, either Student-Newman-Keuls or Dunn's multiple comparisons tests were used for parametric and nonparametric analyses, respectively. For all statistical tests, α was set at 0.05.

Results

P-gp Expression and Function in Rat Lymphocytes. P-gp function, as measured by the ability of verapamil (100 μM) to inhibit rhodamine (11 μM) efflux, was increased in the old rats in $CD45^+$ cells and in lymphocytes, gated as shown in Fig. 1. In gated lymphocytes, age-related differences reached statistical significance (Fig. 2; ANOVA, $p < 0.05$). Findings were similar in $CD45^+$ cells (Kruskal-Wallis ANOVA on ranks, $p = 0.09$). Although only a limited number of rats could be analyzed for both P-gp functional studies ($n = 8$), the percentage of rhodamine 123-positive cells relative to controls were similar in both assays. The old animals exhibited mean values of 6- to 8-fold greater than the young in both experiments.

P-gp expression was measured by the ability of MRK16, an antibody that targets the extracellular epitope of membrane-

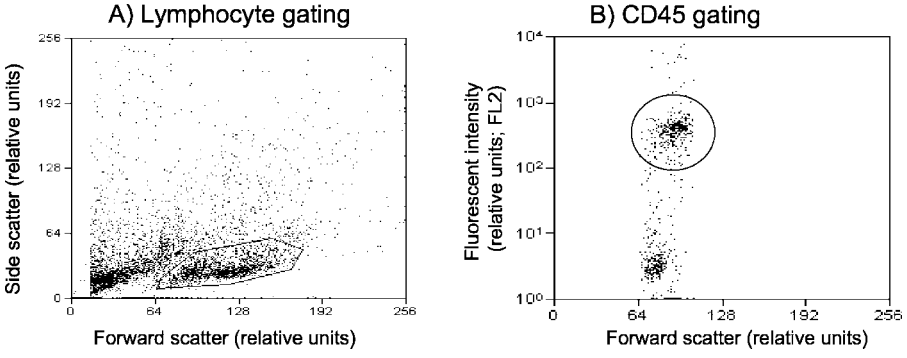

Fig. 1. Representative samples of gating on cell populations. A, two-parameter histograms expressing forward and side-scatter values (relative units) were used to identify lymphocyte populations, which are circumscribed by a polygon. B, after applying the gate described in panel A, $CD45^+$ populations (circumscribed by a circle) were identified in comparison to the isotype control (data not shown).

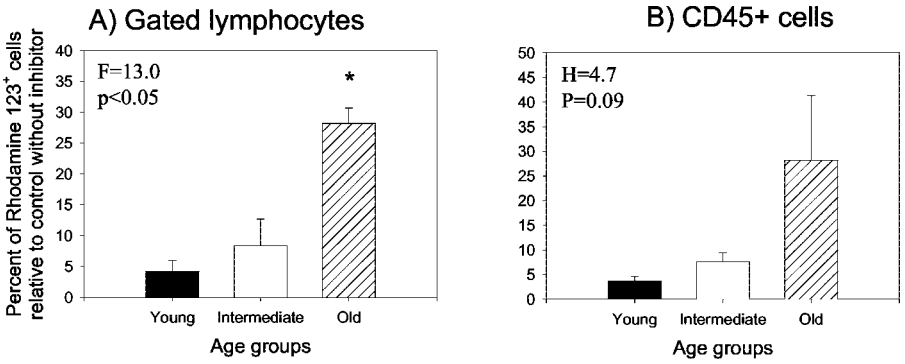

Fig. 2. Rhodamine 123 efflux studies in rat lymphocytes. Gated lymphocytes (A) and $CD45^+$ cells (B) from young ($n = 3$), intermediate ($n = 3$), and old ($n = 2$) were compared. Values are expressed as the mean ± S.E. and represent the percentage of rhodamine 123-positive cells in verapamil-containing samples minus that in samples without inhibitor. ∗, a statistical difference of the old from the intermediate and young groups (ANOVA, $p < 0.05$; Student-Newman-Keuls, $p < 0.05$). B, the statistical analysis was based on a Kruskal-Wallis ANOVA on ranks.

bound P-gp, to bind cells. Approximately 20% of gated lymphocytes were $MRK16^+$ in young rats (19.9 ± 3.4%, $n = 5$). The relative percentage of $MRK16^+$ cells increased with age in these cells to an average of 46.0 ± 7.1% in the old ($n = 4$, Fig. 3); however, while both P-gp expression and rhodamine 123 efflux assays demonstrated an increase with age, the magnitude of these changes differed. Although P-gp function was ~7-fold higher in the old animals, the expression studies displayed a ~2- to 3-fold increase with age.

P-gp Expression by Western Blot Analysis. Although P-gp expression in PBMC lysates could not be detected by immunoblotting (data not shown), the P-gp antibody C219 (Signet Laboratories) detected one band (~170 kDa) in the remaining four tissues (Figs. 4–7). A second, fainter band, was also detected at ~150 kDa in intestinal samples. Previous studies have also detected bands at this molecular weight using C219, although the origin of this second band is not known (Jette et al., 1996).

Age-related differences were observed in hepatic P-gp expression (Fig. 4; Kruskal-Wallis ANOVA, $p < 0.001$). The old animals expressed 4.4-fold higher protein content than the young. The variation was greatest among the old livers, which was largely attributable to the inclusion of one old outlier. This outlier expressed P-gp levels that were 3.4-fold greater than the average P-gp levels of the remaining seven old livers. Removal of this outlier resulted in the detection of a statistical difference between all groups (ANOVA, $p < 0.001$; Student-Newman-Keuls, $p < 0.05$). A trend toward an

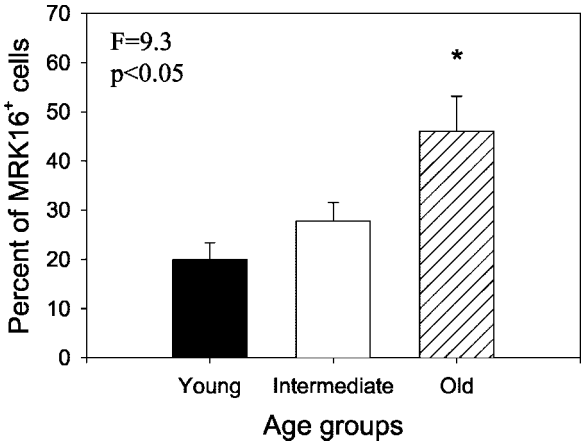

Fig. 3. P-gp expression in PBMCs gated for lymphocyte populations. Cells from young ($n = 5$), intermediate ($n = 5$), and old ($n = 4$) rats were compared for MRK16 positivity. ∗, a statistical difference of old from young and intermediate (ANOVA, $p < 0.005$; Student-Newman-Keuls, $p < 0.05$).

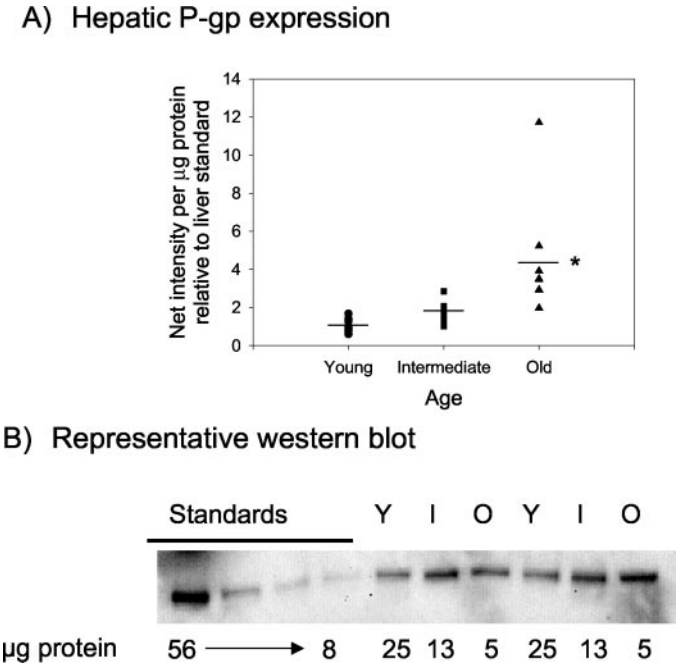

Fig. 4. The effect of age on hepatic P-gp expression. A, P-gp expression in young (circles, $n = 10$), intermediate (squares, $n = 10$), and old (triangles, $n = 8$) hepatic microsomes were evaluated. Values are expressed as the net intensity relative to a liver microsomal standard. Lines represent mean values. ∗, the old from the young and intermediate groups (Kruskal-Wallis ANOVA on ranks, $p < 0.001$; Dunn's test, $p < 0.05$). B, a representative Western blot of hepatic P-gp expression is provided.

increased intestinal P-gp expression was observed in the old; however, differences were not statistically significant (Fig. 5; $p = 0.15$). Age-related changes in renal P-gp expression were evident (Fig. 6; ANOVA, $p < 0.001$). An increase in renal P-gp levels was identified between the young and intermediate age groups, whereas a decline was observed in the old rats (Student-Newman-Keuls, $p < 0.05$). No age-related differences were detected in P-gp expression in the BBB (Fig. 7), although a high degree of variation was observed within each age group. Thus, age-related changes in P-gp expression varied depending upon the tissue under study. A comparison of tissue-specific changes with age is provided in Fig. 8.

There were weak or no correlations in most pairwise comparisons of P-gp expression across tissues. The strongest correlation for P-gp expression between tissues was observed in pairwise comparisons between lymphocytes and the liver ($r^2 = 0.49$) or the intestine ($r^2 = 0.39$).

Age-related differences in P-gp expression were compared with changes in CYP3A expression in these tissues using the

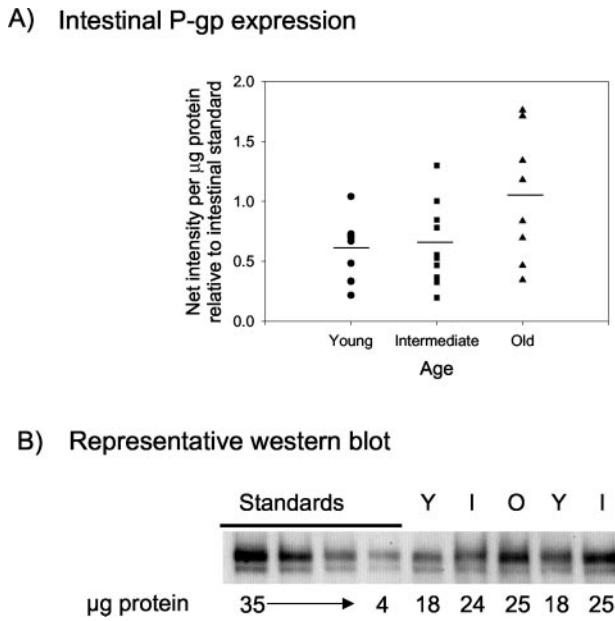

Fig. 5. Age-related differences in intestinal P-gp expression. A, intestinal microsomes from young (circles, $n = 10$), intermediate (squares, $n = 10$), and old (triangles, $n = 8$) rats were examined for P-gp expression. Values are expressed as the net intensity relative to an intestinal microsomal standard, and lines represent mean values. No statistical differences were observed among the age groups. B, a representative Western blot of intestinal P-gp expression is provided.

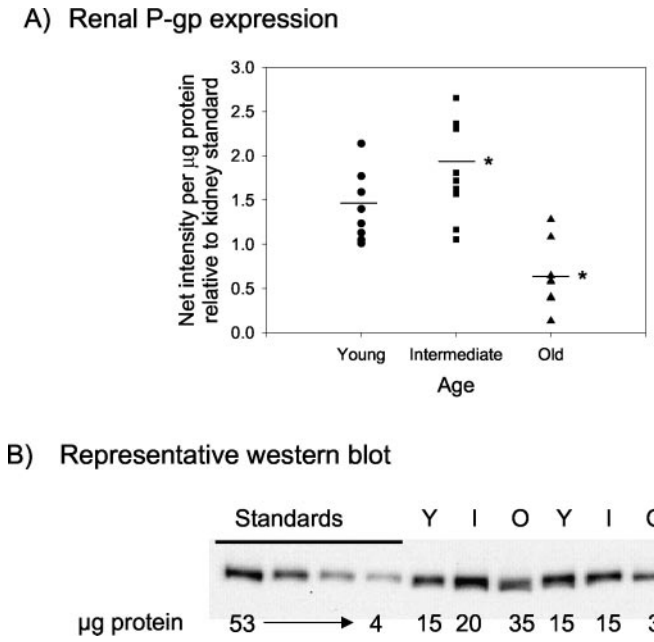

Fig. 6. P-gp expression in aging rat kidneys. A, microsomes from young (circles, $n = 9$), intermediate (squares, $n = 9$), and old (triangles, $n = 8$) rat kidneys were evaluated for P-gp expression. Values are expressed as the net intensity relative to a renal microsomal standard, and lines represent mean values. A statistical difference was found between all age groups (ANOVA, $p < 0.001$). *, a difference from the young (Student-Newman-Keuls, $p < 0.05$). B, a representative Western blot of renal P-gp expression is provided.

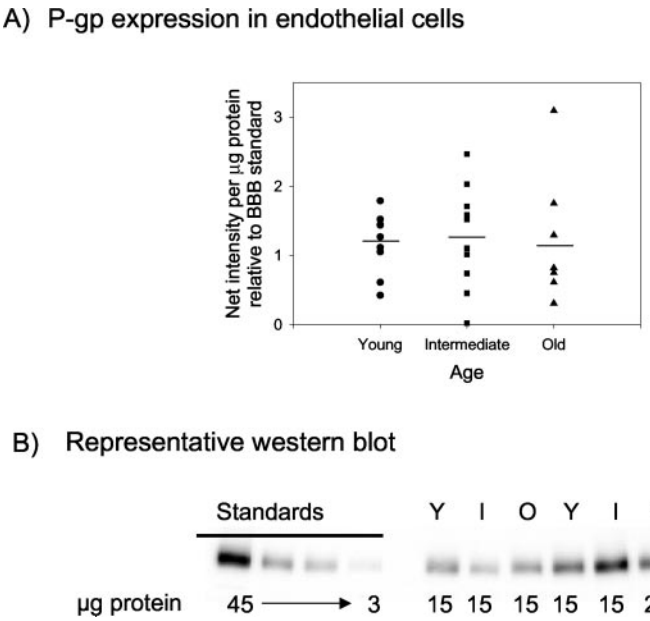

Fig. 7. The effect of age on P-gp expression in aging rat BBB cell lysates. A, endothelial cell lysates from the BBB of young (circles, $n = 9$), intermediate (squares, $n = 9$), and old (triangles, $n = 8$) rats were evaluated for P-gp expression. Values are expressed as the net intensity relative to a BBB lysate standard, and lines represent mean values. No statistical difference was identified by ANOVA. No statistical differences were observed among the age groups. B, a representative Western blot of P-gp expression in the BBB is provided.

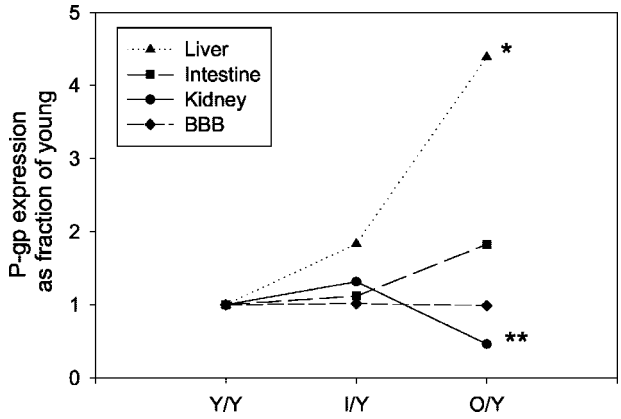

Fig. 8. Tissue-specific changes in P-gp expression with age. The effect of age on P-gp expression in four tissues (liver, dotted line; intestine, long dashed line; kidney, solid line; BBB, short dashed line) was evaluated by Western blot analysis. Values are expressed as a fraction of the young and represent the mean values for each age group relative to the mean value for the young. *, a difference of the old from the young and intermediate groups (Dunn's test, $p < 0.05$). **, reflects that a Student-Newman-Keuls test detected a difference between all groups ($p < 0.05$).

same rats (Warrington et al., 2004; Fig. 9). The correlation between net CYP3A and P-gp expression was tissue-dependent. In the liver, there was a correlation between CYP3A and P-gp expression ($r^2 = 0.46$). However, in the intestine and the kidney, there was no correlation between CYP3A and P-gp expression (r^2 values of 0.04 and 0.02, respectively). Using the individual CYP3A isoforms (CYP3A1 and -3A2) in place of net CYP3A expression resulted in little change in correlation values in any of the three tissues. When old values were expressed as a percentage of values from the young, CYP3A and P-gp were inversely related in the liver and kidney. Although P-gp expression was increased in the old livers (by 339%), hepatic CYP3A expression was reduced in the old (by 67%). On the other hand, in the kidney, P-gp expression declined with age (by 54%), whereas there was a small trend toward an increase in renal CYP3A expression (by 11%) that was not statistically significant. In both tis-

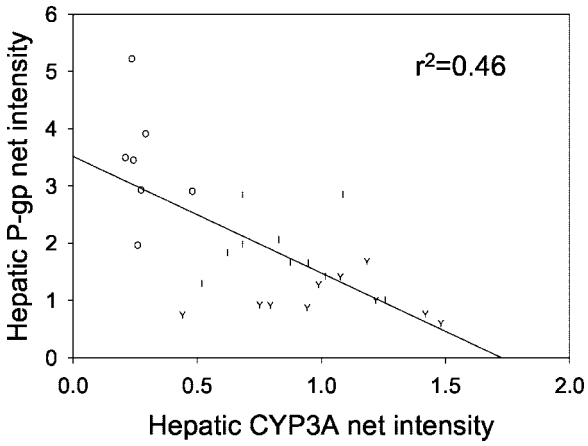

Fig. 9. A correlation analysis between hepatic P-gp expression and reported hepatic CYP3A expression in these rats (Warrington et al., 2004). Using linear regression, young (Y, $n = 10$), intermediate (I, $n = 10$), and old (O, $n = 8$) rats were compared.

sues, the magnitude of change in P-gp expression was greater than that observed for CYP3A expression. No statistically significant age-related differences were found in either intestinal P-gp or CYP3A expression.

Hepatic P-gp expression was also compared with triazolam hydroxylation activity, a CYP3A-mediated reaction, in rat liver microsomes (Warrington et al., 2004). Correlations determined between P-gp expression and CYP3A-mediated activity were similar to that observed for CYP3A expression ($r^2 = 0.45$). Interestingly, the extent of age-related changes in hepatic P-gp expression and CYP3A activity was similar. A ratio of the age-related increase in hepatic P-gp expression and the age-related decline in CYP3A activity approached unity (0.90).

Discussion

In lymphocytes, an age-related increase in P-gp expression and function was observed, although the age-related changes in P-gp function were more marked. These findings were consistent with that observed previously in humans (Gupta, 1995; Aggarwal et al., 1997). Although an age-associated change in P-gp function was observed in mice, a concomitant increase in P-gp expression was not found (Witkowski et al., 1999). Although a limited number of data points was included in our lymphocyte studies ($n = 8$), lymphocytic P-gp expression and function were well correlated ($r^2 = 0.70$). Ivy et al. (1996) found a similar correlation between P-gp expression and function in human leukemia patients.

The effect of age on P-gp expression differed markedly across the five tissues examined. An age-related increase in P-gp expression was evident in the liver and lymphocytes, whereas a reduction was observed in the kidney. In intestinal cells and endothelial cells of the BBB, there was no apparent change with age. Of all tissues examined, P-gp expression in the intestine and the BBB displayed the greatest variability.

Although correlations of P-gp expression across tissues were weak for many pairwise comparisons, no correlations in P-gp expression were found between the BBB and other tissues. Previous studies have suggested that the regulation of P-gp expression in brain capillaries may be different from hepatic, intestinal, and renal P-gp expression (Jette et al., 1996, 1997).

Our studies suggest an inverse relationship between P-gp and CYP3A with age. In the liver, P-gp expression increased with age, whereas CYP3A expression and activity declined in the old animals. The converse pattern was observed in the kidney. Although no statistical differences were found with age in the intestine, a possible inverse relationship may have been observed between P-gp expression and CYP3A activity.

Previous studies have reported an inverse relationship between these two proteins (Schuetz et al., 1995, 2000; Seree et al., 1998), and several observations support this hypothesis. First, although both enzymes are expressed in the mature enterocytes at the tip of the villus, maximal expression along the length of the intestine appears to be inversely related. CYP3A is most highly expressed in the proximal small intestine (Kolars et al., 1992; Paine et al., 1997; Zhang et al., 1999), but P-gp expression is higher distally (Ayrton and Morgan, 2001). Second, studies using knockout mice indicate a possible inverse relationship. For example, in mdr1a and mdr1a/1b knockout mice, Perloff et al. (1999) found a trend toward an increase in the V_{max} values for midazolam hydroxylation, which is predominantly mediated by CYP3A in the mouse. Schuetz et al. (2000) also found a significant increase in CYP3A expression in males and females in knockout mice housed in Amsterdam; however, in the same study, mice housed in the United States showed variable expression patterns across sexes and knockout genotypes. Third, Western blot analysis of CYP3A and P-gp expression in human liver microsomes displayed a possible inverse relationship; however, this finding did not reach statistical significance (Schuetz et al., 1995). Fourth, induction by dexamethasone in the adrenal gland resulted in an up-regulation of CYP3A and a corresponding down-regulation of P-gp in the rat (Seree et al., 1998).

On the other hand, several studies have suggested that CYP3A and P-gp are instead modulated in a similar direction or are governed by independent regulatory mechanisms (Perloff et al., 1999; Wandel et al., 1999). For example, although many CYP3A inhibitors also inhibit P-gp, these agents inhibit with different relative potencies for each protein (Wandel et al., 1999). Also, although many substrates are shared between P-gp and CYP3A, there are P-gp substrates that are not metabolized by CYP3A (such as fexofenadine) as well as CYP3A substrates that are not substrates for transport by P-gp (such as trazodone) (Störmer et al., 2001).

Although the extent of the interrelationship between P-gp and CYP3A remains unclear (Wandel et al., 1999; Schuetz et al., 2000), it is becoming increasingly apparent that the effect of P-gp on the pharmacokinetics of many CYP3A-mediated reactions may need to be considered (Lan et al., 2000).

Our data suggest that P-gp expression may change with age in a tissue-specific manner. These changes may be inversely related to changes in CYP3A expression. Nonetheless, further analysis of age-related changes in P-gp function in other tissues as well as age-related changes in P-gp expression and function in females would be of significant value. In addition, to our knowledge, the effect of age on other drug transporters has not been studied.

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