METABOLISM OF OPIOIDS IS ALTERED IN LIVER MICROSOMES OF SICKLE CELL TRANSGENIC MICE

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ABSTRACT:

Pain in sickle cell anemia (SCA) is clinically managed with opioid analgesics. There are reports that SCA patients tolerate high doses of these drugs without adequate pain relief. The current study investigated the in vitro hepatic metabolism of opioids in mouse models of sickle cell anemia, with the hypothesis that higher dose requirements in SCA could be explained by an increased metabolism rate of opioids. Various rodent cytochrome P450 substrates, i.e., buprenorphine and codeine, and rodent uridine glucuronosyltransferase substrates, i.e., morphine, buprenorphine, and estradiol, were studied. The three groups used were: 1) control C57BL mice, 2) mice with the human α-globin and sickle β-globin transgenes (SC), and 3) mice with the human α-globin and sickle β-globin transgenes, and homozygous for the murine α-globin and hemoglobin S (SCKO). In vitro hepatic microsomal incubations were carried out for each substrate, and data were fit to the Michaelis-Menten equation. Morphine formation had a higher Vmax in SCKO microsomes (0.4 ± 0.009 nmol/min·mg; estimate ± S.E.) than controls (0.25 ± 0.007). Morphine-3-glucuronide formation had Vmax estimates of 18.9 ± 0.6, 25.1 ± 0.4, and 27.06 ± 1.1 nmol/min·mg in control, SC, and SCKO microsomes, respectively. The control Vmax for estradiol-3-glucuronide formation was 2-fold greater than in SCKO microsomes. The control Vmax for estradiol-17-glucuronide formation was 3.4- and 2.2-fold greater than in SC and SCKO microsomes. Thus, in vitro metabolism of opioids is altered in SCA mouse models, which may lead to altered clearances of these drugs.

The genetic basis of sickle cell anemia (SCA), a hemoglobin disorder, has been understood since 1949 (Pauling et al., 1949); red blood cells (RBCs) assume a sickle shape when deoxygenated due to a point mutation in the beta-globin gene of the hemoglobin molecule. In the United States alone, around 1000 babies are born every year with SCA, and it is estimated that 1 in every 600 African Americans is homozygous for the mutation (Steinberg, 1999). Sickling of RBCs affects almost all organs of the body, including the heart, lung, kidneys, and liver. Among the various complications of SCA, acute and chronic pain syndromes cause the highest number of hospital visits. The intense pain is commonly managed with opioid analgesics including morphine, buprenorphine, and nalbuphine (Embry et al., 1994). There have been various clinical reports that sickle pain is not adequately managed by typical doses of opioids, and SCA patients can tolerate very high doses of analgesics without alleviation of their symptoms (Dampier et al., 1995; Beyer, 2000; Yaster et al., 2000). Studies have been limited due to the complexity of study designs involving persons with SCA, many of whom are pediatric patients.

Studies in SCA patients have indicated that one possible reason for higher dose requirement is an alteration in drug pharmacokinetics. The elimination half-life of codeine in sickle cell patients was found to be significantly shorter than in control subjects (Mohammed et al., 1993). Dampier et al. (1995) reported that the clearance of morphine in SCA patients was higher than typical values (Glare and Walsh, 1991). SCA patients have higher than normal levels of both unconjugated and conjugated bilirubin as a result of hemolysis as well as cholestasis (Embry et al., 1994). High circulating bilirubin levels may play a role in the modulation of enzyme activity. Maddrey et al. (1978) have reported an increase in the bilirubin-conjugating activity in SCA liver biopsy samples; bilirubin is known to be glucuronidated by human uridine glucuronosyltransferase (UGT) 1A1, as well as the corresponding rodent ugt1a1 (Iyanagi et al., 1998). Interestingly, a study by Sanchez and Tephly (1973) showed that exogenous bilirubin increased rat ugt activity in vivo as well as in microsomes from treated animals.

Transgenic mouse models carrying the human sickle cell anemia gene have been developed in recent years. Sickle cell transgenic mice (SC) have a C57BL thalassemia background, and the human α-globin gene and the human sickle β-globin genes have been introduced into
the mouse genome (Fabry et al., 1992). A more severe model of the disease is in the form of the sickle cell-knockout mouse (SCKO); in addition to the introduction of the human genes, these mice are homozygous for the knockout of the murine a-globin gene and heterozygous for the knockout of the murine b-gene (Paszty et al., 1997). SCKO have a mixed genetic background. The transgenic mice have various pathological characteristics mirroring the human disease, such as extensive sickling of red blood cells on deoxygenation, retinopathy, and renal and hepatic pathology (Embry et al., 1994). The percentage of sickled erythrocytes that develop after complete deoxygenation in control mice (C57BL), SC, and SCKO is 0, 40, and 80%, respectively, and the percentage of nonalpha-globins that are comprised of the human β3-globin is 0.72, and ~100%, respectively (R. P. Hebbel, unpublished data). These animal models provide an excellent opportunity to investigate alterations in drug disposition due to SCA.

We hypothesize that enzyme induction could be one explanation for the higher observed clearance of opioid analgesics in SCA. To this end, in vitro hepatic metabolism by various rodent P450 and ugt isozymes was investigated in two different SCA mouse models. The substrates chosen were opioid analgesics commonly used in the management of SCA pain. Since buprenorphine is glucuronidated by various UGT1A and UGT2B isozymes, the glucuronidation of estradiol was also studied because estradiol is reported to be specifically glucuronidated at its 3-position by human UGT1A1 (Senafi et al., 1994). Table 1 shows the human and rodent isozymes responsible for metabolism of various substrates.

### Materials and Methods

Morphine sulfate, morphine 3-glucuronide (M3G), buprenorphine hydrochloride, estradiol, estradiol 3-glucuronide (E3G), estradiol 17-glucuronide (E17G), ethynylestradiol (EE2), codeine sulfate, alamethicin, uridine diphosphoglucuronic acid (UDPGA), glucose 6-phosphate (G6P), G6P-dehydrogenase, β-NADP, β-glucuronidase, and phenytoin were obtained from Sigma-Aldrich (St. Louis, MO). Norbuprenorphine (NB) was obtained through the National Institute on Drug Abuse, from the Research Triangle Institute (Research Triangle Park, NC). All other chemicals used were reagent or HPLC grade. All transgenic mice were bred in the laboratory of Dr. R. P. Hebbel, University of Minnesota, and wild-type C57BL/6j mice were obtained from Harlan (Indianapolis, IN). All mice were maintained under specific pathogen-free conditions.

Preparation of Mouse Liver Microsomes. Three groups of mice were used for the studies: control mice (wild-type C57BL/6j background), SC, and SCKO. For the preparation of microsomes, animals were sacrificed in a CO2 chamber, and the livers were immediately harvested. Up to five livers were pooled and homogenized for each batch of microsomes. Microsomes were prepared by differential centrifugation, by a previously published method (Nelson et al., 2001). Briefly, liver homogenates were centrifuged at 9000g for 20 min at 4°C, and the resulting supernatant was centrifuged at 100,000g for 60 min at 4°C. The microsomal pellet was suspended in 0.1 M phosphate buffer containing 2 mM EDTA and 20% glycerol and stored at −80°C until further use. The protein content was determined using the BCA protein assay kit (Pierce Chemical, Rockford, IL).

### Microsomal Incubations for Rodent P450-Catalyzed Reactions

Incubation conditions were optimized for linearity with respect to protein and incubation period for each type of microsome and for every reaction. For the conversion of buprenorphine to NB as well as codeine to morphine, preliminary experiments showed that the reactions were linear up to 60 min and 1 mg/ml of protein. The necessary cofactors were added to the incubation mixture as two solutions: solution A contained β-NADP, MgCl2, and G6P in 50 mM Tris buffer (pH 7.4 at 37°C) and yielded final concentrations of 1 mM β-NADP, 5 mM MgCl2, and 5 mM G6P. Solution B was a G6P-dehydrogenase solution in 50 mM Tris buffer and gave a final concentration of 1.0 IU/ml of G6P-dehydrogenase in the incubation. The final volume of each incubation solution was made up to 250 μl with 50 mM Tris buffer (pH 7.4 at 37°C). Mouse liver microsomes were at a final concentration of 1 mg/ml, and solutions A and B were preincubated at 37°C for 3 min. The reaction was started by the addition of varying concentrations of substrate and was terminated after 1 h by the addition of ice-cold acetonitrile (ACN) containing phenytoin as the internal standard. The solution was centrifuged to pellet the precipitated protein, and the supernatant was injected onto the HPLC column. Six replicates of each experiment were carried out with pooled mouse liver microsomes for each group.

### Microsomal Incubations for ugt-Catalyzed Reactions

Incubation conditions were optimized for linearity with respect to protein and incubation period for each type of microsome and for every reaction. The conversion of morphine to M3G was linear up to 15 min, whereas the formations of buprenorphine glucuronide (BG), E3G, and E17G were linear up to 10 min. Alamethicin, a pore-forming peptide, was used to activate the microsomal preparations before incubation. Alamethicin concentration was optimized to achieve maximal activation of the microsomes. Thus, 75 μM alamethicin was used for control and SCKO microsomes and 50 μM alamethicin was used for SC microsomes. The final incubation volume in all cases was made up to 200 μl using 0.1 M Tris buffer (pH 7.4 at 37°C). For the incubations, a mixture of 1 mg/ml of microsomal protein, alamethicin, 5 mM MgCl2, and substrate was preincubated for 3 min at 37°C. The reaction was started by adding UDPGA to give a final concentration of 3 mM. Control incubations were performed without the addition of UDPGA. For morphine and buprenorphine, the reaction was terminated by the addition of ice-cold ACN containing phenytoin as the internal standard. For estradiol, the reaction was terminated with ice-cold ACN and subsequent addition of the internal standard, EE2. In all cases, the incubation solution was centrifuged at 13,000g for 2 min and the supernatant was used for HPLC analysis. Six replicates of each experiment were performed with pooled mouse liver microsomes for each group. For incubations for the conversion of buprenorphine to BG, preliminary incubations were carried out to verify the glucuronidation reaction; β-glucuronidase was added to the reaction mixture, and the resulting disappearance of the BG peak with a corresponding increase of the buprenorphine peak confirmed that the metabolite peak was indeed BG.

### HPLC Analysis

The HPLC system used was a Beckman Gold 126 system (Beckman Coulter Inc., Fullerton, CA) with a 507e autosampler, a 166 UV detector, and a Gold Nouveau integration software (version 1.6, Beckman Coulter). Except for the formation of BG, metabolite formation was quantitated by comparing metabolite/internal standard peak area ratios in incubations.
TABLE 2

Reversed-phase HPLC conditions for the analysis of the various CYP and UGT substrates

<table>
<thead>
<tr>
<th>Compound</th>
<th>Mobile Phasea, b and ACN (64:36 v/v)</th>
<th>Column</th>
<th>Flow Rate (mL/min)</th>
<th>Detection</th>
<th>Retention Time (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Buprenorphine NB</td>
<td>Reversed-phase Luna 5-μm C18(2) 150</td>
<td>× 4.6 mm; Phenomenex®</td>
<td>1.0</td>
<td>UV 210 nm</td>
<td>26</td>
</tr>
<tr>
<td>Phenyletoin (I.S.)</td>
<td></td>
<td>Reversed-phase Luna 5-μm C18(2) 150</td>
<td>× 4.6 mm; Phenomenex®</td>
<td>7</td>
<td>11</td>
</tr>
<tr>
<td>Codeine</td>
<td></td>
<td>Reversed-phase Luna 5-μm C18(2) 150</td>
<td>× 4.6 mm; Phenomenex®</td>
<td>7</td>
<td>4.6</td>
</tr>
<tr>
<td>Morphine</td>
<td></td>
<td>Reversed-phase Luna 5-μm C18(2) 150</td>
<td>× 4.6 mm; Phenomenex®</td>
<td>7</td>
<td>12.4</td>
</tr>
<tr>
<td>Phenyletoin (I.S.)</td>
<td></td>
<td>Reversed-phase Luna 5-μm C18(2) 75</td>
<td>× 4.6 mm; Phenomenex®</td>
<td>16</td>
<td>6</td>
</tr>
<tr>
<td>Morphine M3G</td>
<td></td>
<td>Reversed-phase Luna 5-μm C18(2) 75</td>
<td>× 4.6 mm; Phenomenex®</td>
<td>16</td>
<td>22</td>
</tr>
<tr>
<td>Buprenorphine BG</td>
<td></td>
<td>Reversed-phase Luna 5-μm C18(2) 75</td>
<td>× 4.6 mm; Phenomenex®</td>
<td>16</td>
<td>22</td>
</tr>
<tr>
<td>Phenyletoin (I.S.)</td>
<td></td>
<td>Reversed-phase Luna 5-μm C18(2) 75</td>
<td>× 4.6 mm; Phenomenex®</td>
<td>16</td>
<td>22</td>
</tr>
<tr>
<td>Estradiol E3G</td>
<td>Acetate buffer and ACN, gradient elutionc</td>
<td>Reversed-phase Supelco 5-μm C18 250</td>
<td>× 4.6 mm; Supelco®</td>
<td>9.4</td>
<td>24</td>
</tr>
<tr>
<td>E17G</td>
<td></td>
<td>Reversed-phase Supelco 5-μm C18 250</td>
<td>× 4.6 mm; Supelco®</td>
<td>9.4</td>
<td>24</td>
</tr>
<tr>
<td>EE2 (I.S.)</td>
<td></td>
<td>Reversed-phase Supelco 5-μm C18 250</td>
<td>× 4.6 mm; Supelco®</td>
<td>9.4</td>
<td>24</td>
</tr>
</tbody>
</table>

a I.S., internal standard.
b 0.05 M ammonium acetate buffer, pH 4.5 (Ebner et al., 1993).
c 26% ACN for 12 min, increase to 50% ACN over 1 min; 50% ACN for 7 min, increase to 85% ACN over 0.5 min; 85% ACN for 6 min, re-equilibration at 26% ACN.
d Supelco is based in Bellefonte, PA.

test, assuming normal distribution.

TABLE 3

Rodent P450-catalyzed reactions: Michaelis-Menten parameters for the formation of NB and morphine in control mouse, SC, and SCKO liver microsomes

<table>
<thead>
<tr>
<th>Metabolite (Isozyme)</th>
<th>Vmaxa (nmol/min·mg)</th>
<th>Km (μM)</th>
<th>Vmax/Kma (μl/min·mg)</th>
<th>In Vivo Cl(int) (μl/min/g of body weight)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NB (cyp3a) Control</td>
<td>0.63 ± 0.04</td>
<td>52.5 ± 10.0</td>
<td>1.2</td>
<td>4.00</td>
</tr>
<tr>
<td>SC</td>
<td>0.65 ± 0.04</td>
<td>67.5 ± 10.5</td>
<td>0.96</td>
<td>2.69</td>
</tr>
<tr>
<td>SCKO</td>
<td>0.59 ± 0.04</td>
<td>52.1 ± 10.0</td>
<td>1.1</td>
<td>2.36</td>
</tr>
<tr>
<td>Morphine (cyp2d22)</td>
<td>0.25 ± 0.007</td>
<td>194.7 ± 16.2</td>
<td>1.3</td>
<td>4.34</td>
</tr>
<tr>
<td>Control</td>
<td>0.26 ± 0.008</td>
<td>224.0 ± 21.2</td>
<td>1.2</td>
<td>3.36</td>
</tr>
<tr>
<td>SC</td>
<td>0.40 ± 0.009*</td>
<td>167.4 ± 11.9</td>
<td>2.4</td>
<td>5.15</td>
</tr>
</tbody>
</table>

a Data expressed as parameter estimate ± S.E. (n = 6).
b Value was significantly different than corresponding control values, as determined by a two-sided t test (p < 0.01).

to a standard curve with known amounts of metabolite. Since a BG standard was unavailable, BG areas were quantitated against a buprenorphine standard curve and expressed as equivalent buprenorphine concentrations. UV spectra of both buprenorphine and BG by diode array detection in the 190- to 250-nm wavelength range showed an identical spectrum with a λmax at 212 nm. All assays were validated for inter- and intraday precision and accuracy, and the percentage of CV and bias were less than 10% throughout the concentration range studied (data not shown). The HPLC conditions for each substrate and its metabolites are shown in Table 2.

Data Analysis. Metabolite formation data were fit to the simple form of the Michaelis-Menten equation (Gibaldi and Ferrier, 1982):

$$V = \frac{V_{max} \cdot S}{K_m + S}$$

where V is the initial rate of metabolite formation, Vmax is the maximum rate of metabolite formation, S is the substrate concentration, and Km is the Michaelis-Menten constant. Eadie-Hofstee plots were evaluated for linearity for each reaction.

The nonlinear fit was carried out with KaleidaGraph 3.5 (Synergy Software, Reading, PA). Statistical comparisons for the Michaelis-Menten parameter estimates were carried out using a two-sided t test, assuming normal distribution of parameter estimates. A p value less than 0.01 was considered significant.

Liver weights were compared for statistical significance with StatView 5.0 (SAS Institute Inc., Cary, NC).

The ratio of Vmax to Km is the in vitro intrinsic clearance of the unbound drug. A theoretical in vivo intrinsic clearance of the unbound drug was calculated by multiplying the Vmax/Km value by the microsomal protein yield for each type of liver. The microsomal protein yield was calculated from the liver weights, with a scaling factor of 45 mg/g of liver (Houston, 1994).

Results

The mean body weights of age-matched male control mice, SC, and SCKO were 21.0 ± 2.1 (mean ± S.D.), 33.1 ± 4.1, and 33.8 ± 7.0 g, respectively, and the mean liver weights were 1.56 ± 0.2, 2.06 ± 0.22, and 1.62 ± 0.3 g, respectively. The mean body weight normalized liver weights (gram per gram of body weight) in control mice, SC, and SCKO were 0.074, 0.062, and 0.048, respectively. The microsomal protein yield was calculated using a scaling factor of 45 mg/g of liver (Houston, 1994). These values were 70.2, 92.7, and 72.5 mg for control mice, SC, and SCKO microsomes, respectively. The microsomal protein yield values were used to calculate theoretical in vivo Cl(int) values in Tables 3 and 4.

Figure 1, a and b, shows the formation of NB from buprenorphine and morphine formation from codeine, with the corresponding Michaelis-Menten parameters of these rodent P450-catalyzed reactions reported in Table 3. All three groups showed similar NB formation kinetics. The formation of morphine from codeine showed a significantly higher Vmax in SCKO microsomes than in controls. The Eadie-Hofsteet plots were linear for both NB and morphine formation (data not shown).

Figure 2 shows the ugt-catalyzed formation curves for M3G (Fig.
TABLE 4

<table>
<thead>
<tr>
<th>Metabolite (Isozyme)</th>
<th>$V_{\text{max}}$&lt;sup&gt;a&lt;/sup&gt; (nmol/min·mg)</th>
<th>$K_{\text{m}}$&lt;sup&gt;a&lt;/sup&gt; (μM)</th>
<th>$V_{\text{max}}/K_{\text{m}}$ (μl/min·mg)</th>
<th>In Vivo Cl&lt;sub&gt;int&lt;/sub&gt; (ml/min/g of body weight)</th>
</tr>
</thead>
<tbody>
<tr>
<td>M3G (ugt2b)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>18.90 ± 0.6</td>
<td>276.6 ± 39.4</td>
<td>67.5</td>
<td>0.23</td>
</tr>
<tr>
<td>SC</td>
<td>26.51 ± 0.33*</td>
<td>269.9 ± 20.3</td>
<td>93.0</td>
<td>0.26</td>
</tr>
<tr>
<td>SCKO</td>
<td>27.06 ± 1.12*</td>
<td>193.7 ± 36.0</td>
<td>140.2</td>
<td>0.30</td>
</tr>
<tr>
<td>E3G (ugt1a1)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>7.01 ± 0.73</td>
<td>190.64 ± 40.9</td>
<td>36.8</td>
<td>0.12</td>
</tr>
<tr>
<td>SC</td>
<td>5.02 ± 0.57</td>
<td>159.13 ± 32.5</td>
<td>31.5</td>
<td>0.09</td>
</tr>
<tr>
<td>SCKO</td>
<td>3.46 ± 0.28*</td>
<td>136.8 ± 21.3</td>
<td>25.3</td>
<td>0.05</td>
</tr>
<tr>
<td>E17G (ugt1a, 2b)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>0.71 ± 0.09</td>
<td>60.4 ± 19.2</td>
<td>11.75</td>
<td>0.04</td>
</tr>
<tr>
<td>SC</td>
<td>0.21 ± 0.01*</td>
<td>26.2 ± 2.3</td>
<td>8.0</td>
<td>0.02</td>
</tr>
<tr>
<td>SCKO</td>
<td>0.32 ± 0.03*</td>
<td>29.6 ± 8.6</td>
<td>10.8</td>
<td>0.02</td>
</tr>
</tbody>
</table>

<sup>a</sup> Data expressed as parameter estimate ± S.E. ($n = 6$).

<sup>*</sup> Values were significantly different than corresponding control values, as determined by a two-sided $t$ test ($p < 0.01$).

The transgenic mouse models closely approximate the human disease, especially with phenotypic similarities such as enlarged liver, retinopathy, and renal pathology. A significant alteration in the metabolism of drugs by both rodent P450 and ugt isozymes in the transgenic mice was observed. Also, each isozyme shows a unique change in its drug-metabolizing capacity in these mouse models.
Specifically, our results indicate the following: 1) the mouse cyp3a isoform responsible for the formation of NB from buprenorphine shows no change in activity among the three groups; 2) the mouse cyp2d22 isoform responsible for the conversion of codeine to morphine is induced in SCKO microsomes, showing a significantly higher $V_{\text{max}}$ than in control microsomes; 3) the mouse ugt1b isoform responsible for morphine glucuronidation is induced in both SC and SCKO microsomes and shows a significantly higher $V_{\text{max}}$ than controls; and 4) the mouse ugt1a isoform responsible for estradiol glucuronidation shows decreased $V_{\text{max}}$ values for E3G formation in SC microsomes and decreased $V_{\text{max}}$ values for E3G and E17G formation in SCKO microsomes. The $V_{\text{max}}/K_{\text{m}}$ ratios in either case, however, are not very different from control values. In all cases, the observed significant differences were more pronounced in the SCKO, a more severe model of SCA.

The increased conversion of codeine to morphine does not support the hypothesis that the decreased analgesic effect in SCA is due to enhanced metabolism, since morphine is a potent analgesic. However, the sequential conversion of morphine to the inactive M3G is also increased, and the relative rates of the two reactions in vivo would determine the overall analgesic effect. It is of interest to note that in preliminary studies, there were significant differences in baseline response to tail-flick tests among the control mice, SC, and SCKO, indicating differences in nociceptive response (Nagar, 2003).

All the rodent P450- and ugt-catalyzed reactions studied except BG formation showed atypical kinetics (Figs. 1b and 3) with nonlinear Eadie-Hofstee plots. This may be due to the involvement of more than one ugt isoform. Atypical Eadie-Hofstee curves such as hook-shaped plots are usually obtained due to allosteric sites, multiple isozymes, or activation kinetics (Fisher et al., 2000; Kenworthy et al., 2001; Oda and Kharasch, 2001).

The $V_{\text{max}}/K_{\text{m}}$ ratio is the maximum in vitro Cl$_{\text{int}}$ value for a given substrate. A theoretical in vivo Cl$_{\text{int}}$ value was calculated from mean values of $V_{\text{max}}$ and $K_{\text{m}}$ with the use of the microsomal protein yield as a scaling factor (Houston, 1994). The average liver weight of SC was significantly higher than controls, leading to a higher value of microsomal protein yield. Differences in the theoretical in vivo Cl$_{\text{int}}$ values followed the same trend as those in the corresponding $V_{\text{max}}/K_{\text{m}}$ ratios (Tables 3 and 4). Liver enlargement is also very common in SCA patients (Embury et al., 1994). Thus, if human P450 and UGT levels were induced in SCA, a significant increase in the intrinsic clearance of a drug per gram of liver tissue would translate to an even greater increase in the total hepatic intrinsic clearance per liver weight.

The mechanisms for differential alterations in enzyme activity in SCA need to be determined. SCA patients are known to have increased levels of both unconjugated and conjugated bilirubin as a result of hemolysis and biliary obstruction (Schubert, 1986). Sickle cell transgenic mice are hyperbilirubinemic, with higher conjugated and unconjugated serum bilirubin levels than control mice (Nagar, 2003). An increase in rat ugt activity has been reported after bilirubin treatment both in vitro and in vivo (Sanchez and Tephly, 1973; Munoz et al., 1987; Li et al., 2000). Bilirubin has been implicated in the aryl
hydrocarbon receptor-mediated induction of rodent cyp1a1 (Sinal and Bend, 1997; Phelan et al., 1998). In addition, it appears that increased bilirubin levels in mice activate the nuclear hormone receptor, constitutive androstane receptor, resulting in an increase in bilirubin clearance (Huang et al., 2003). On the other hand, recent studies of human UGT1A1 activity have shown that UGT1A1-catalyzed reactions like E3G formation can be slightly inhibited by bilirubin, which acts as a competitive inhibitor (Williams et al., 2002). These reports support the hypothesis that high bilirubin levels may play an important role in the activation and inhibition of various human P450 and UGT isozymes in SCA. The effect of bilirubin on morphine disposition is currently being studied in mouse and rat models.

In addition to altered pharmacokinetics, the pharmacodynamics of analgesics also need to be evaluated in SCA. It is also important to note that morphine is converted only to M3G in rodents, whereas in humans it is also glucuronidated at the 6-position, with the resulting morphine 6-glucuronide being much more potent than even the parent compound as an analgesic (Christrup, 1997). How the increased glucuronidation of morphine would thus affect the analgesic activity of morphine in human SCA patients needs to be investigated. However, previous clinical reports indicate that patients require significantly larger doses of morphine for adequate pain control.

In conclusion, there is a marked alteration in the in vitro microsomal enzyme kinetics of various ugt and rodent P450 isozymes in the sickle cell transgenic mouse models studied. There is evidence for the induction of the rodent P450 isozyme responsible for converting codeine to morphine and the ugt isozyme responsible for glucuronidating morphine. Further studies in human patients are needed to validate the animal models as well as to clarify the role of altered pharmacokinetics and pharmacodynamics in sickle cell anemia pain.

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