Effects of Dose and Route on the Disposition and Kinetics of 1-Butyl-1-methylpyrrolidinium Chloride in Male F-344 Rats

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Received June 18, 2009; accepted August 17, 2009

ABSTRACT:

Studies were conducted to characterize the effects of dose and route of administration on the disposition of 1-butyl-1-methylpyrrolidinium (BmPy-Cl) in male Fischer-344 rats. After a single oral administration of [^{14}C]BmPy-Cl (50 mg/kg), BmPy-Cl in the blood decreased rapidly after C_{max} of 89.1 min with a distribution half-life ($t_{1/2\alpha}$) of 21 min, an elimination half-life ($t_{1/2\beta}$) of 5.6 h, and a total body clearance of 7.6 ml/min. After oral administration (50, 5, and 0.5 mg/kg), 50 to 70% of the administered radioactivity was recovered in the feces, with the remainder recovered in the urine. Serial daily oral administrations of [^{14}C]BmPy-Cl (50 mg/kg/day for 5 days) did not result in a notable alteration in disposition or elimination. After each administration, 88 to 94% of the dose was eliminated in a 24-h period, with 63 to 76% of dose recovered in the feces. Intravenous administration of [^{14}C]BmPy-Cl (5 mg/kg) re-

sulted in biphasic elimination. Oral systemic bioavailability was 43.4%, approximately equal to the dose recovered in urine after oral administration (29–38%). Total dermal absorption of [^{14}C]BmPy-Cl (5 mg/kg) was moderate when it was applied in dimethylformamide-water ($34 \pm 13\%$), variable in water ($22 \pm 8\%$), or minimal in ethanol-water ($13 \pm 1\%$) vehicles. Urine was the predominant route of elimination regardless of vehicle. Only parent [^{14}C]BmPy-Cl was detected in the urine after all doses and routes of administration. BmPy-Cl was found to be a substrate for ($K_t = 37 \mu\text{M}$) and inhibitor of ($\text{IC}_{50/\text{tetraethylammonium}} = 0.5 \mu\text{M}$) human organic cation transporter 2. In summary, BmPy-Cl is moderately absorbed, extracted by the kidney, and eliminated in the urine as parent compound, independent of dose, number, or route of administration.

1-Butyl-1-methylpyrrolidinium chloride (BmPy-Cl) is a cyclic quaternary amine that belongs to a growing class of industrial compounds known as “ionic liquids.” A wide variety of applications for ionic liquids (ILs) have been proposed, the principal one being use as application-specific alternatives to conventional solvents (Welton, 1999). Many classes of cations have been explored for potential commercial application including 1-alkyl-3-methylimidazolium, *N*-alkylpyridinium, *N*-methyl-*N*-alkylpyrrolidinium, and tetraalkylammonium compounds. New pyrrolidinium-based ionic liquids have been synthesized by neutralization reactions between pyrrolidine and Brønsted acids to produce protonated pyrrolidinium salts called “protic ionic liquids” (Anouti et al., 2008). Based on their resulting chemical and physical properties, pyrrolidinium protic ILs have promising widespread application as electrolytes in aqueous batteries and

fuel cell devices, as thermal transfer fluids, and as replacements for conventional acid catalysts (Anouti et al., 2008).

As a class of compounds, ILs are not likely to contribute to air pollution, because of their intrinsically low vapor pressure (Huddleston et al., 1998). However, their excellent solubility in aqueous media increases the likelihood of environmental exposure to a variety of IL species via water pollution. A growing body of evidence suggests that ILs may be ecologically hazardous (Bernot et al., 2005a,b). The majority of toxicity studies have focused on the imidazolium- or pyridinium-based ionic liquids using a variety of aquatic microorganisms including algae, marine bacteria, and freshwater snails to assess the potential ecotoxicological effects of these compounds. These studies have demonstrated that toxicity is determined mainly by the cation and that toxicity increased with increasing length of the alkyl chains (Bernot et al., 2005a).

There are few reports of the potential systemic toxicity of the various cations in mammalian systems. An EC_{50} value of approximately 20 mM was reported for BmPy-Cl in IPC-8 rat promyelocytic leukemia cells, based on results of tetrazolium dye formation assays (Ranke et al., 2007). Kumar et al. (2009) recently compared the in vitro cytotoxicity of ILs containing various cations and anions using the MCF7 human breast cancer cell line. Consistent with previous

This work was supported in part by the National Institutes of Health National Institute of Environmental Health Sciences [Grants N01-ES45529, ES06694]; the National Institutes of Health National Institute of Environmental Health Sciences, Intramural Research Program, Research Project Number 1 [Grant Z01-ES045004-11 BB]; and the National Institutes of Health National Institute of Diabetes and Digestive and Kidney Diseases [Grant DK58251].

Article, publication date, and citation information can be found at <http://dmd.aspetjournals.org>.

doi:10.1124/dmd.109.029082.

ABBREVIATIONS: BmPy-Cl, 1-butyl-1-methylpyrrolidinium chloride; IL, ionic liquid; Bmim-Cl, 1-butyl-3-methylimidazolium chloride; NBuPy-Cl, *N*-butylpyridinium chloride; TEA, tetraethylammonium trifluoroacetate; DMF, dimethylformamide; HPLC, high-pressure liquid chromatography; JVC, jugular vein cannula; OCT, organic cation transporter; h, human; CHO, Chinese hamster ovary; K_t , concentration of substrate that results in half maximal transport; EtOH, ethanol; C_{max} , maximum concentration of ^{14}C radioactivity in blood.

FIG. 1. Chemical structure of 1-butyl-1-methylpyrrolidinium chloride (177.72 g/mol). *, ^{14}C radiolabel.

reports for other cations, the IC_{50} values of the water-soluble pyrrolidinium compounds decreased with increasing alkyl chain length (Kumar et al., 2009). It is interesting to note that the propyl- and butyl-substituted cationic pyrrolidiniums were less toxic than their nonionic precursor, methyl pyrrolidine, whereas the octyl-substituted pyrrolidinium was more toxic. Three ILs, 1-butyl-3-methylimidazolium chloride (Bmim-Cl), 1-*N*-butylpyridinium chloride (NBuPy-Cl), and BmPy-Cl, were nominated to the National Toxicology Program for toxicological testing because they are representative of the most common cation classes of ILs and are the starting materials for many other ILs. Subchronic toxicity studies of these three ILs in rats and mice are in progress. Previous data from our laboratory have shown that Bmim-Cl and NBuPy-Cl are readily absorbed from the gastrointestinal tract (60–70% of dose), and both are eliminated rapidly in the urine by glomerular filtration and renal secretion (Sipes et al., 2008; Cheng et al., 2009). Renal secretion of these ILs was attributed to activity of renal organic cation transporter 2. Because no information is available on the dispositional fate of BmPy-Cl in mammals, these studies were designed to characterize the toxicokinetics of BmPy-Cl after intravenous and oral dosing, to determine the extent of its systemic bioavailability after oral or dermal dosing, and to determine whether escalated or repeated dosing alters its elimination profile. In addition, the renal secretion process(es) involved in BmPy-Cl elimination were investigated.

Materials and Methods

Chemicals. ^{14}C -radiolabeled BmPy-Cl (lot 9719-75) was received from RTI International (Research Triangle Park, NC). The location of the ^{14}C label is indicated by an asterisk on the chemical structure (Fig. 1). The radiochemical purity of BmPy-Cl was 97.5% and the specific activity was 27.5 mCi/mmol. [^3H]Tetraethylammonium trifluoroacetate (TEA; 54 Ci/mmol) was synthesized by GE Healthcare (Little Chalfont, Buckinghamshire, UK). The radiolabeled chemicals were stored at 4°C. BmPy-Cl (142.2 g/mol) was purchased from EMD Chemicals, Inc. (Gibbstown, NJ) and the purity was 98%. Soluene-350 tissue solubilizer and Pico-Flour 40 scintillation cocktail solution were received from PerkinElmer Life and Analytical Sciences (Torrance, CA). Dimethylformamide (DMF) was obtained from Mallinckrodt Baker, Inc. (Phillipsburg, NJ). Hydrogen peroxide (30%) was obtained from VWR (West Chester, PA). All reagents used in animal experiments were HPLC or analytical grade. For *in vitro* studies, Ham's F12 medium, fetal bovine serum, hygromycin B, penicillin, and streptomycin were obtained from Invitrogen (Carlsbad, CA).

Animals. Male Fischer-344 (F-344) rats of 8 to 9 weeks of age (161–190 g) without surgical alteration or with an in-dwelling jugular vein cannula (JVC) were obtained from Harlan Inc. (Indianapolis, IN). Animals were maintained and housed in the University of Arizona's Association for Assessment and Accreditation of Laboratory Animal Care-approved University Animal Care facility, as described by Kuester et al. (2007).

Dose Selection. Because no toxicological data were available for BmPy-Cl, the dose selection was based on data for a related IL, Bmim-Cl (Sipes et al., 2008). A toxicological screen of the highest dose tested of Bmim-Cl or BmPy-Cl (50 mg/kg) resulted in no notable symptoms of toxicity (matted hair, weight loss, decreased water consumption, ocular bleeding, nasal discharge, or

difficulty breathing) or histological abnormalities in liver or kidney tissues (Sipes et al., 2008). Therefore, doses of 50, 5, and 0.5 mg/kg were chosen for metabolism and disposition studies after oral administration of BmPy-Cl. A 5 mg/kg dose was used in intravenous and dermal studies to minimize the likelihood of adverse reactions while maintaining sufficient systemic levels of compound for quantification at later time points.

Dose Administration. To assess the effect of dose and route on excretion, BmPy-Cl was administered as single doses orally (0.5, 5, or 50 mg/kg, 50 $\mu\text{Ci/kg}$) or intravenously (5 mg/kg, 50 $\mu\text{Ci/kg}$) to male F-344 rats. In the repeat dose study, BmPy-Cl was administered orally to male F-344 rats at 50 mg/kg/day (50 $\mu\text{Ci/kg/day}$) for 5 days. For the dermal absorption studies, dose applications were similar to those described by Winter and Sipes (1993). In brief, the dorsal surface of the rat was shaved and an aluminum "skin depot" was affixed to the skin, followed by application of the dose (5 mg/kg, 100 $\mu\text{Ci/kg}$, 125 $\mu\text{g/cm}^2$) within the defined area (approximately 10 cm^2) in vehicles of DMF-water (63:37, v/v), ethanol-water (63:37, v/v), or water. Because of the low volatility of BmPy-Cl, the application site was not occluded (i.e., activated carbon and mesh coverings were not used). For the BmPy-Cl toxicokinetic studies, JVC rats were dosed once with BmPy-Cl by intravenous (5 mg/kg, 100 $\mu\text{Ci/kg}$) or by oral gavage (50 mg/kg, 100 $\mu\text{Ci/kg}$). Normal saline (0.9%) was used as the dosing vehicle in all cases except dermal application.

Sample Collection. Procedures for the collection of excreta and tissues after intravenous, oral, or dermal administration of BmPy-Cl were performed as described by Sipes et al. (2008) and Cheng et al. (2009). In brief, animals were placed into Nalgene metabolism cages after dosing and urine and feces were collected over time. Cages were rinsed with approximately 15 ml of water after collections, and radioactivity recovered in cage rinses was added to that determined for urine. Blood samples (300 μl /time point) were collected via the JVC into heparinized syringes. Aliquots of blood were replaced with an equal volume of saline. Animals were euthanized by CO_2 inhalation at the end of each study, and tissues were collected. Excreta collections were performed at times noted in figures and tables. In topical application studies, the application area was washed (five times) and tape-stripped (five times) to remove radioactivity remaining on the surface of the skin before excision. After collections, feces and tissues were chemically solubilized before liquid scintillation counting (Thompson and Burns, 1996). Systemic exposure to the compound was estimated based on disposition of compound in tissues and compound eliminated in urine and feces at 96 h after dosing.

Analytical Methods. Samples of blood and urine collected during the course of the experiments were analyzed using HPLC-radiometric detection or HPLC-mass spectrometry using acidified water and acidified acetonitrile mobile phases (Agilent Technologies, Palo Alto, CA). Nonradiolabeled samples were analyzed using HPLC-mass spectrometry with mass spectrometer conditions set as described by Hoehle et al. (2009). Samples containing ^{14}C radioactivity were analyzed using liquid scintillation counting and by HPLC with an in-line flow-through radiometric detector (IN/US Systems, Tampa, FL). Gradient parameters and runtimes were as described previously by Sipes et al. (2008).

Samples were prepared for HPLC analyses as described by Sipes et al. (2008). In brief, urine samples were diluted with water (1:1, v/v), vortexed, and filtered using 0.45- μm Whatman nylon syringe filters. Blood samples were extracted into acetonitrile, evaporated to dryness, and reconstituted in water-acetonitrile (93:7%, v/v). Calibration standards and quality control samples were prepared from concentrated stock solution. The limit of detection for ^{14}C by liquid scintillation counting was 32 dpm (0.16 $\mu\text{g/ml}$), and the limit of quantification was 124 dpm (0.62 $\mu\text{g/ml}$), as determined using the methods described by Zhu et al. (2005).

Kinetic Analyses. Blood data obtained at various times points after oral or intravenous administration of BmPy-Cl were analyzed as described by Knudsen et al. (2007). The blood concentration-time data were analyzed with the WinNonlin pharmacokinetic modeling program (Pharsight, Rainbow Technologies, Inc., 1998–2008, Mountain View, CA) using nonlinear regression analysis and assuming first-order kinetics for all processes.

In Vitro Transport Studies. Transport of BmPy-Cl and the inhibitory effect of BmPy-Cl on TEA transport by human organic cation transporter 2 (hOCT2) were characterized in Chinese hamster ovary cells (CHO) with stably expressed hOCT2 transporters (CHO_hOCT2). The expression of hOCT2 in

TABLE 1

Dose recovered after a single intravenous,^a single oral,^a or repeated oral^b administration of [¹⁴C]BmPy-Cl to male F-344 rats

n = 4/dose group.

	Dose Recovered									
	1 Dose (5 mg/kg i.v.)		1 Dose (0.5 mg/kg p.o.)		1 Dose (5 mg/kg p.o.)		1 Dose (50 mg/kg p.o.)		5 Doses (5 mg/kg p.o.)	
	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.
	%									
Feces	8.4	1.3	64.8	6.8	68.6	4.3	51.0	6.2	69.4	3.4
Urine	86	12.5	38.0	8.6	28.6	1.6	37.2	10.0	22.3	1.4
Tissues	0.1	0.1	N.D.	N.D.	N.D.	N.D.	0.3	0.6	0.9	0.3
Total recovery	92.2	11.4	102.8	7.6	97.2	5.2	88.5	10.8	92.7	4.4

N.D., not determined.

^a Studies were terminated 72 h after administration.^b Studies were terminated 24 h after final administration.

CHO cells was confirmed by using Western blot analysis (Suhre et al., 2005) and TEA transport kinetic studies (Cheng et al., 2009). CHO_hOCT2 cells were maintained in Ham's F12 medium with FBS (10%, v/v), penicillin (100 U/ml), streptomycin (100 µg/ml), and hygromycin B (100 µg/ml). The detailed procedures for the transport assay were described by Cheng et al. (2009). In brief, the intracellular uptake of [¹⁴C]BmPy-Cl (22 µM) or [³H]TEA (15 nM) was measured in CHO_hOCT2 in the presence of increasing concentrations of BmPy-Cl after a 30-s incubation. The apparent Michaelis-Menten constant (concentration for half-maximal transport rate, K_t) and the BmPy-Cl concentration necessary to inhibit [³H]TEA transport (IC_{50}) were determined using the modified Michaelis-Menten equation (Malo and Berteloot, 1991) as described by Cheng et al. (2009).

Statistical Analyses. Statistical analyses were performed by using Sigma-Stat 3.0 (SPSS Inc., Chicago, IL). Results with $p < 0.05$ were considered statistically significant.

Results

Disposition and Elimination after Oral Administration. The disposition, metabolism, and elimination of BmPy-Cl after oral administrations were assessed over a range of doses. After oral administration of BmPy-Cl (0.5, 5, or 50 mg/kg) as a single bolus dose, 28 to 38% of the total administered radioactivity was eliminated in the urine over the 72-h collection period (Table 1). Peak urinary excretion was observed between 6 and 12 h for all doses. The elimination of ¹⁴C radioactivity in feces was greater than 50% of the dose for all doses administered orally. The time course of elimination of radioactivity after a single oral administration of BmPy-Cl (50 mg/kg) is shown in Fig. 2A. Repeated daily administration (50 mg/kg/day) did not result in changes in apparent rate or route of elimination over the 5-day period (Fig. 2B). Statistical analysis of recoveries at each time point showed no significant differences across dose groups at any time point. Figure 3A shows the HPLC profile of urine samples collected at 6 or 12 h after a single oral dose of 50 mg/kg [¹⁴C]BmPy-Cl. One peak was detected, which coeluted with the BmPy-Cl standard ($R_t = 14.6$ min). Its structure was confirmed to be BmPy-Cl by mass spectrometry (6 h) (Fig. 3B). Examination of the total ion chromatogram detected a $M + 1$ ion at $R_t = 14.6$ min with a mass/charge ratio of 142.2 Da, consistent with the mass of the BmPy⁺ cation. Collision-induced fragmentation of this ion resulted in the loss of 56 Da and formation of a single daughter ion ($m/z = 86.3$), consistent with the mass of protonated methyl pyrrolidinium. It is noteworthy that spectra of the compound detected in urine were identical to those of the BmPy-Cl standard.

Disposition and Elimination after Intravenous Administration. After intravenous administration of BmPy-Cl, blood and excreta were collected and analyzed as described above. Elimination of [¹⁴C]BmPy-Cl was rapid, with greater than 84% of the dose being

FIG. 2. Cumulative elimination of ¹⁴C radioactivity after oral administration of BmPy-Cl (50 mg/kg). A, total radioactivity recovered in urine and feces after a single oral administration ($n = 4$, mean $\pm$ S.D.; $\square$, total; $\diamond$, urine; $\circ$, feces). B, total radioactivity recovered in urine and feces after five daily oral administrations ($n = 4$ per dosing group, mean $\pm$ S.D., open symbols, one administration; closed symbols, five administrations; $\square/\blacksquare$, total; $\diamond/\blacklozenge$, urine; $\circ/\bullet$, feces).

eliminated in the urine within 12 h of administration; a total of $86 \pm 12.5\%$ was recovered by 72 h (Fig. 4A). HPLC-radiometric analysis of urine and blood extracts showed a single peak that coeluted with the [¹⁴C]BmPy-Cl standard ($R_t = 14.6$ min) (Fig. 4B). The majority of the ¹⁴C radioactivity was recovered in urine (Table 1). Recovery of radioactivity in feces was low.

Disposition and Elimination after Dermal Administration. Dermal application of BmPy-Cl using vehicles of DMF-water, EtOH-water, or water resulted in a recovery of approximately 19.1, 2.5, or 9.6% of the dose in the urine, respectively. Cumulative recoveries in urine and feces for each vehicle are shown Fig. 5A. Total recoveries of applied doses are listed in Table 2. Depending on the vehicle, approximately 13 to 34% of the applied dose was determined to be absorbed (sum of dose eliminated in urine, feces, and nonrecoverable from site of application). ¹⁴C radioactivity detected in urine after dermal administrations coeluted with the [¹⁴C]BmPy-Cl authentic standard, independent of dosing vehicle (Fig. 5B).

BmPy-Cl Toxicokinetics. HPLC-radiometric analyses of blood extracts obtained after intravenous or oral dosing revealed a single peak that coeluted with the [¹⁴C]BmPy-Cl standard (Fig. 6C). Be-

FIG. 3. The characterization of urinary excretion products after oral administration of BmPy-Cl (50 mg/kg; $n = 4$, mean $\pm$ S.D.). A, representative HPLC-radiochromatogram of urine collected after administration (solid line: $[^{14}\text{C}]$ BmPy-Cl standard; dashed line: 6 h; dotted line: 12 h postdose). B, mass spectral analysis of urine collected at 6 h. Representative positive ionization electrospray mass spectrum of urine after administration (top panel: $m/z = 142.2$). Collision-induced fragmentation of $m/z = 142.2$ gives a loss of 56 (bottom panel: $m/z = 86.3$ approximates the mass of methyl pyrrolidinium). Mass spectrometry analyses of samples collected at all other times yielded similar spectra.

cause no metabolites were detected in blood extracts or urine, blood toxicokinetics were determined using ^{14}C radioactivity in whole blood. Figure 6, A and B, show the time-blood concentration curves after intravenous (5 mg/kg, 100 $\mu\text{Ci/kg}$) or oral (50 mg/kg, 100 $\mu\text{Ci/kg}$) administration of BmPy-Cl. After oral dosing C_{max} was reached at approximately 1.5 h. The slope of the terminal phase yielded a half-life of 5.6 h that was similar to the elimination half-life calculated for intravenous administration of BmPy-Cl (6.8 h). Based on the area under the curve after intravenous administration (185 $\mu\text{g} \cdot \text{min/ml}$) and the area under the curve after oral administration (1193 $\mu\text{g} \cdot \text{min/ml}$) and adjustment for dose (dose_{oral}, 8756 $\pm$ 70 μg ; dose_{intravenous}, 1047 $\pm$ 14 μg), the systemic bioavailability was estimated to be 43.4% after oral dosing (Table 3).

Transport and Inhibition of BmPy-Cl in Vitro. The transport of BmPy-Cl was characterized in CHO cells expressing hOCT2. As

show in Fig. 7A, the intracellular accumulation of $[^{14}\text{C}]$ BmPy-Cl was decreased by increasing concentrations of extracellular unlabeled BmPy-Cl. From these data, the K_i was determined to be 36.5 ± 3.6 μM . To determine whether BmPy-Cl could act as an inhibitor of hOCT2 transport processes, increasing concentrations of BmPy-Cl (0–100 μM) were coincubated with $[^3\text{H}]$ TEA (15 nM) for 30 s. The presence of BmPy-Cl decreased the intracellular uptake of $[^3\text{H}]$ TEA in a concentration-dependent manner (Fig. 7B), resulting in an IC_{50} value of 0.5 ± 0.1 μM .

Discussion

Recent initiatives such as the Montreal Protocol and its subsequent versions have been initiated to curb the effects of solvents on human and environmental health. These have led to the development of a variety of alternative solvents and conditions for the use of chemical

FIG. 4. Elimination of BmPy-Cl after a single intravenous administration to male F-344 rats (5 mg/kg; $n = 4$, mean $\pm$ S.D.). A, cumulative elimination of radioactivity ($\square$, total; $\diamond$, urine; $\circ$, feces). B, representative HPLC-radiochromatograms of urine collected after intravenous administration of BmPy-Cl (solid line, $[^{14}\text{C}]$ BmPy-Cl standard; dotted line, 6 h; dashed line, 12 h).

FIG. 5. Cumulative elimination of ^{14}C radioactivity after dermal application of BmPy-Cl in various vehicles (5 mg/kg; $n = 3$ –5 per vehicle group, mean $\pm$ S.D.). A, total radioactivity recovered in urine and feces ($\blacksquare$, DMF-water; $\bullet$, water; $\blacktriangledown$, EtOH-water). B, representative HPLC-radiochromatogram of urine collected at 6 h after application (solid line, $[^{14}\text{C}]$ BmPy-Cl standard; dash-dot-dot line, DMF-water; dash-dot line, water; dotted line, EtOH-water).

solvents (Velders et al., 2007). More environmentally friendly alternatives have been proposed for a wide variety of applications, from cleaning and coatings to separations and synthesis (Sherman et al., 1998). The ionic liquid discussed in this article, BmPy-Cl, has structural and physical properties similar to those of other “green” ionic liquids that have been proposed for use in a variety of organic synthesis reactions (Seddon, 1997).

This article represents the third study in a series exploring the disposition and metabolism of ionic liquids in rats (Bmim-Cl, NBuPy-Cl, and BmPy-Cl). All three compounds contain a butyl substitution on a different nitrogenous base. The butyl substitution provided opportunities for cytochrome P450-mediated formation of hydroxyl derivatives. The results of the in vivo disposition studies (Sipes et al.,

TABLE 2

Dose recovered in 96 h after dermal application of $[^{14}\text{C}]$ BmPy-Cl to male F-344 rats (5 mg/kg)

DMF-H₂O: 63/37, v/v; EtOH-H₂O: 63/37, v/v.

	DMF-H ₂ O ($n = 3$)		EtOH-H ₂ O ($n = 3$)		H ₂ O ($n = 5$)	
	Mean	S.D.	Mean	S.D.	Mean	S.D.
Feces	4.1	1.8	3.4	1.9	5.1	4.3
Urine	19.1	3.4	2.5	0.7	9.6	3.6
Tissues	0.2	0.2	0.4	0.3	0.2	0.2
Skin						
Absorbed ^a	11.0	7.5	7.1	1.9	7.4	4.3
Unabsorbed ^b	55.5	15.7	80.7	2.4	72.5	8.3
Total recovery in tissues	89.9	5.3	94.2	1.7	91.8	11.0

^a Nonrecoverable ^{14}C radioactivity present in skin at site of application.

^b ^{14}C radioactivity recovered by washing and tape-stripping.

FIG. 6. Time course of BmPy-Cl in blood after administration in male F-344 rats ($n = 4$, mean $\pm$ S.D.). Predicted values were calculated based on best-fit analyses of a two- or one-compartment model fit, respectively. Samples containing analyte concentrations below the limit of quantitation were not used in the best-fit analyses. A, intravenous dose (5 mg/kg). B, oral dose (50 mg/kg). C, representative radiochromatograms of $[^{14}\text{C}]$ BmPy-Cl equivalents in whole blood [solid line, $[^{14}\text{C}]$ BmPy-Cl standard; dashed line, 1.5 h after oral administration (50 mg/kg); dotted line, 7.5 min after intravenous dose (5 mg/kg)].

2008; Cheng et al., 2009; and data herein) provided no evidence for metabolism of any of these three compounds by mammalian enzymes. After intravenous administration to rats, each of the compounds was readily excreted in the urine as parent compound as determined by HPLC-radiometric analyses. In addition, no circulating metabolites of Bmim-Cl, NBuPy-Cl, or BmPy-Cl were detected in the blood. Thus,

TABLE 3

Toxicokinetic parameters for BmPy-Cl after intravenous (5 mg/kg) or oral (50 mg/kg) administration to male F-344 rats

n = 4/dose route.

	Dose	AUC _{0-∞}	<i>t</i> _{1/2α}	<i>t</i> _{1/2β}	CL _r	V _{ss}	C _{max}	T _{max}	F
	μg	min · μg/ml	min	h	ml/min	ml	μg/ml	min	%
Intravenous	907	274	33.3	6.8	3.3	1495	— ^a	—	—
Oral	8157	1069	20.9	5.6	7.6	—	1.8	89.1	43.4

AUC_{0-∞}, area under the blood concentration-time curve from 0 to infinity; *t*_{1/2α}, distribution half-life; *t*_{1/2β}, terminal elimination half-life; CL_r, total final systemic clearance from blood; V_{ss}, volume of distribution at steady-state; C_{max}, maximum concentration of analyte in blood; T_{max}, time to maximal concentration; F, systemic bioavailability of BmPy-Cl.

^a —, data not predicted by pharmacokinetic models.

FIG. 7. Kinetics of [¹⁴C]BmPy-Cl (A) or [³H]TEA (B) uptake in the presence of increasing concentration of unlabeled BmPy-Cl in CHO cells expressing hOCT2 (*n* = 3, mean ± S.D.). These results yielded a *K_t* of 37 μM and IC₅₀ of 0.5 μM.

it can be concluded that metabolism of these agents by rats is minimal, if it occurs at all. In addition, studies with Bmim-Cl (Sipes et al., 2008) and NBuPy-Cl (Cheng et al., 2009) in mice have failed to detect any metabolites in blood or urine. Microbial metabolism of the unabsorbed portions of these compounds may occur. Indeed, chromatographic profiles suggest the presence of some metabolites in fecal extracts. However, no attempts were made to confirm or identify these additional peaks as metabolites (data not shown).

We recently reported on the effects of dose and route of administration of two structurally similar ILs, Bmim-Cl and NBuPy-Cl. Systemically available compound was rapidly eliminated in the urine as parent compound (Sipes et al., 2008; Cheng et al., 2009). Both of these compounds were found to be highly available to the systemic circulation (62–65%) after oral administration. The mechanism of absorption of these compounds from the intestine is unknown but may

be facilitated by OCTs, which are known to be expressed in the intestine (Zaïr et al., 2008). In the studies reported here, it was found that the BmPy-Cl that is systemically available was eliminated in the urine as parent compound as well, but bioavailability was lower and clearance rate and elimination half-life were as much as 2-fold lower than those reported for Bmim-Cl and NBuPy-Cl. As with Bmim-Cl and NBuPy-Cl, up to 35% of topically applied BmPy-Cl was absorbed. The extent of absorption was increased when applied in a DMF-H₂O, a more hydrophobic vehicle.

The blood clearance for Bmim-Cl, NBuPy-Cl, and BmPy-Cl exceeds published values for the glomerular filtration rate (0.5–1.2 ml/min/100-g rat) (Corley et al., 2005), suggesting a secretory mechanism for these ILs. Secretion is mediated by a variety of transporters, many of which are known to be critical in the elimination of a variety of clinically used drugs (Jonker and Schinkel, 2004) as well as occupationally and environmentally relevant compounds (Prasad et al., 2007). Indeed, NBuPy-Cl has been shown to be a substrate for hOCT2 and its renal elimination in rats appears to be mediated, in part, by OCT transport (Cheng et al., 2009). Renal transport was also suggested to occur for Bmim-Cl (Sipes et al., 2008) and was subsequently shown to occur (data not presented).

The rapid clearance by the kidney suggests that BmPy-Cl is also transported by OCTs present on the basolateral membrane of proximal tubule of the nephron. The hOCT2-mediated transport of BmPy-Cl (*K_t* = 37 μM) resulted in transport kinetics similar to that of the OCT2 model substrate, TEA (*K_t* = 27–48 μM) (Suhre et al., 2005; Cheng et al., 2009). Additional data indicate that BmPy-Cl strongly inhibited hOCT2-mediated transport of TEA (IC₅₀ = 0.5 μM). Previous reports by Suhre et al. (2005) showed that only ethylacridinium inhibited hOCT2-mediated transport of TEA at a lower concentration than BmPy-Cl (IC₅₀ = 0.09 ± 0.03 μM).

Data indicating that only OCT2 is expressed in the kidney in humans (Gründemann et al., 1998), in association with the *in vivo* toxicokinetic data presented here, indicate that exposure to BmPy-Cl and other ILs secreted by OCT2 may affect the pharmacokinetic profiles of a variety of therapeutic, occupational, and environmental compounds that exist as organic cationic compounds. These data indicate that ILs as a chemical class may function as substrates and/or inhibitors of human organic cation transporters. Future studies are required to determine the potential of the ILs that may modify the *in vivo* pharmacokinetics of such compounds.

Acknowledgments. We thank Dr. M. L. Cunningham [National Toxicology Program and National Center for Toxicogenomics, National Institute of Environmental Health Sciences (NIEHS)] for advice and support as well as Leigh Jacobs, Aniko Solyom, and Veronica Rodriguez for assistance in this project. We also thank Drs. Mike Sanders and Suramya Waidyanatha (National Toxicology Program, NIEHS) for critical review of this manuscript. We give special thanks to Dr. Steven Wright for providing the CHO_hOCT2 cells.

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