

PHARMACOKINETICS OF A MODEL ORGANIC NITRITE INHALANT AND ITS ALCOHOL METABOLITE IN RATS

WILLIAM KIELBASA AND HO-LEUNG FUNG

Department of Pharmaceutics, School of Pharmacy, University at Buffalo, State University of New York, Buffalo, New York

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ABSTRACT:

Volatile organic nitrites were originally used to relieve the chest pain that is associated with angina pectoris. Today, these inhalants are predominantly used as drugs of abuse. Little is known regarding the bioavailability and disposition of volatile nitrites. In this study, the pharmacokinetics of a major organic nitrite inhalant, isobutyl nitrite (ISBN), and its primary metabolite, isobutyl alcohol (ISBA), were investigated after inhalation and i.v. administration. ISBN blood concentrations in the rat declined mono-exponentially with a half-life of 1.4 min and a blood clearance of 2.9 l/min/kg that vastly exceeded cardiac output (0.3 l/min/kg). Approximately 98% of ISBN was metabolized to ISBA, which declined monoexponen-

tially with a half-life of 5.3 min when the infusion of ISBN was terminated. The bioavailability of inhaled ISBN, over a range of 300 to 900 ppm, was estimated to be 43%. After inhaled ISBN, the half-life of ISBA decreased approximately 4-fold ($t_{1/2}$ inh = 1.5 min versus $t_{1/2}$ i.v. = 5.3 min; $P < .001$), whereas no pharmacokinetic difference was observed for ISBN. Inhalation of another nitrite, isoamyl nitrite, accelerated the apparent clearance of ISBA, suggesting that nitrite inhalation could change the disposition of another compound. A pharmacokinetic model was developed to describe the concentration-time profile of ISBA and ISBN after inhalation and i.v. administration.

More than a century ago, Thomas Lauder Brunton suggested using vasodilators as a remedy for treating painful and distressing heart symptoms associated with angina pectoris. Isoamyl nitrite (ISAN)¹ was used because it had a powerful vapor that, when inhaled, reduced arterial tension, and "might be repeated as often as necessary without detriment to the patients health" (Brunton, 1867). Today, ISAN is available by prescription in the United States, but is seldom used for the treatment of angina. ISAN has been used in the diagnostic evaluation of mitral regurgitation and ventricular septal defect (Lembo et al., 1988), but its most common application is in emergency situations as an antidote for cyanide poisoning (Nicholson et al., 1994). ISAN is dispensed in single dose cloth-covered glass ampoules, and when used, is crushed between the fingers allowing the vapors of the clear yellow liquid to be inhaled. When the ampoule is broken, it makes a snapping sound, thus giving them the nickname "snappers" or "pop-pers".

Abuse of nitrites has been documented in the teenage and homosexual populations. Deep inhalations of nitrites can cause a decrease in blood pressure, flushed face, dizziness, and euphoric feelings that usually last from a few seconds to minutes. Nitrites, in particular isobutyl nitrite (ISBN), have been marketed as room odorizers in an attempt to avoid regulatory control that restricts the sale of these inhalants. Nitrites were used just as much as crack, cocaine, heroin,

barbiturates, and steroids in the high school graduating class of 1996 in the United States (National Institute on Drug Abuse, 1996). Epidemiological studies have suggested nitrite abuse as an independent risk factor for seropositivity for Human Immunodeficiency Virus (Seague et al., 1992) and Kaposi's Sarcoma (Archibald et al., 1993), the most common cancer reported in AIDS patients. However, it has not been established that chronic nitrite use contributes to these disease states.

Despite their use in medicine over the last century and their recent popularity as inhalant drugs of abuse, the pharmacokinetics of organic nitrite inhalants is largely unknown. We recently developed a gas chromatographic assay for the analysis of ISBN in blood and have demonstrated that this assay can be applied to gain useful pharmacokinetic information (Kielbasa et al., 1999). In preliminary studies, we showed that the clearance of ISBN is rapid, implying almost instantaneous biotransformation to isobutyl alcohol (ISBA), its denitrated metabolite. ISBA has been shown to cause hepatotoxicity when injected into the peritoneum of rats. The appearance and rate of development of this toxicity was similar to the toxicity caused by ISBN, suggesting that ISBN metabolism to ISBA may play a role in causing damage to the liver (Maickel and McFadden, 1979). Soderberg et al. (1998) recently suggested that nitric oxide (NO) is not responsible for the immunotoxicity associated with ISBN, but it is unknown whether the alcohol metabolite plays any role in mediating this immunomodulating effect.

Detailed pharmacokinetic analysis of organic nitrites and their metabolites have not been carried out. Here, we conducted studies using ISBN/ISBA as a model nitrite inhalant/metabolite pair to determine the effect of the inhalation route on the pharmacokinetics of these compounds. This study represents the first detailed pharmacokinetic characterization of a nitrite inhalant.

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¹ Abbreviations used are: ISAN, isoamyl nitrite; ISBN, isobutyl nitrite; ISBA, isobutyl alcohol; NO, nitric oxide.

Send reprint requests to: Ho-Leung Fung, Ph.D., Dept. of Pharmaceutics, School of Pharmacy, University of Buffalo, State University of New York, Buffalo, NY 14260. E-mail: HLFung@acsu.buffalo.edu

Materials and Methods

Chemicals and Reagents. ISBN, ISBA, ISAN, *n*-propyl nitrate, and 1-butanol were purchased from Aldrich Chemical Co., Inc. (Milwaukee, WI). Pentane and dichloromethane were of capillary gas chromatography-gas chromatography/mass spectrometry solvent grade (Burdick and Jackson, Muskegon, MI). Sulfatase and glucuronidase enzymes were purchased from Sigma Chemical Co. (St. Louis, MO).

Animal Preparation. Male Sprague-Dawley rats in the range of 300 to 325 g were used for the pharmacokinetic studies. For implantation of cannulas, rats were anesthetized with a mixture of ketamine (Dodge Laboratories, Fort Dodge, IA) and xylazine (Bayer Corporation, Shawnee Mission, KS) at a dose of 90 and 9 mg/kg, respectively. For the i.v. studies, cannulas were implanted via the left femoral artery and vein for blood withdrawal and drug administration, respectively. For the inhalation studies, cannulas were implanted via the left femoral artery for blood withdrawal. In both cases, the rats were allowed to recover for 24 h after surgery.

ISBN Sample Preparation and Instrumentation. The details of the assay procedure have been reported elsewhere (Kielbasa et al., 1999). Briefly, blood samples containing ISBN (0.4 ml) were taken through a heparinized cannula with a 0.5-ml gas-tight glass syringe stored at 0°C just before sampling. The blood was immediately processed under ice-cold gas-tight conditions by vortexing with an equal volume of pentane (0°C) containing *n*-propyl nitrate (78.3 ng/ml). An aliquot of pentane was subsequently removed and placed in glass conical chromatography vials for injection. Samples were injected (3 μ l) on the gas chromatograph and the analysis of ISBN was carried out using a 5890A gas chromatograph (Hewlett-Packard, Avondale, PA) equipped with an electron capture detector and a J & W Scientific (Folsom, CA) DB-1 capillary column (30-m length; 0.32-mm i.d.; 1- μ m film thickness). Samples were analyzed under the following conditions: an initial temperature of 30°C was maintained for 9.5 min, followed by a linear increase in temperature to 45°C at 60°C/min. The total run time was 18.3 min. Extra dry grade nitrogen was used as the carrier gas and the detector gas. The injector and detector temperatures were set at 45°C and 195°C, respectively.

We assembled an apparatus for nitrite inhalation in the rat using a fluothane vaporizer (Cyprane LTD, Keighley, England) calibrated for ISBN and ISAN, medical grade air (Strate Welding, Buffalo, NY), and a gas anesthetizing box (Braintree Scientific, Braintree, MA). The exposure concentration of nitrite was determined experimentally by analyzing air containing ISBN or ISAN in the inhalation chamber using a gas chromatograph with flame ionization detection. Briefly, 1.0 ml was withdrawn from the chamber using a gas-tight syringe followed by injection of a 30- μ l aliquot on the gas chromatograph. Calibration standards ranging from 0 to 1500 ppm were prepared by injecting microliter volumes of ISBN or ISAN into gas-tight vials that were allowed to vaporize before analysis. Exposure concentrations of nitrite in the inhalation chamber were determined three times during the dosing interval and are reported as the mean \pm S.D. Inter- and intraday variabilities were each less than 13% for ISBN and ISAN.

ISBA Sample Preparation and Instrumentation. Blood samples containing ISBA (0.2 ml) were taken through a heparinized cannula with a 0.5-ml gas-tight glass syringe stored at 0°C just before sampling. The blood was immediately processed under ice-cold gas-tight conditions by extraction with an equal volume of dichloromethane (0°C) containing 1-butanol (2.0 μ g/ml). The sample was rapidly vortexed and centrifuged, and an aliquot of dichloromethane was placed in glass conical chromatography vials for injection. Samples were injected (5 μ l) on the gas chromatograph and the analysis of ISBA was carried out using a 5890A gas chromatograph (Hewlett-Packard) equipped with a flame ionization detector and a J & W Scientific DB-225 capillary column (30-m length; 0.25- μ m i.d.; 0.25- μ m film thickness). The samples were chromatographed using gradient temperature elution: 40°C for the initial 2.0 min, 70°C for the next 1.5 min, followed by 150°C for the remaining 6.0 min. The oven temperature was increased at a rate of 60°C/min. The total run time was 11.3 min. Extra dry grade nitrogen was used as the carrier gas (2.7 ml/min) and the make-up gas (24.6 ml/min). Hydrogen and air (Strate Welding) flowed at 28.3 and 258.4 ml/min, respectively. The injector and detector temperatures were 125°C and 175°C, respectively. The assay was determined to have a sensitivity limit of 1.2 μ M in blood, with inter- and intraday variabilities less than 5%.

Pharmacokinetic Analysis. The blood clearance of ISBN (CL_{ISBN}) after infusion to steady state was obtained by $CL_{ISBN} = k_{0(i.v.)}/C_{SS_{ISBN}}$, where $k_{0(i.v.)}$ is the infusion rate of ISBN and $C_{SS_{ISBN}}$ is the steady-state blood concentration of ISBN. The volume of distribution and half-life of ISBN were obtained as $V_{ISBN} = CL_{ISBN}/k_{ISBN}$ and $t_{1/2} = 0.693/k_{ISBN}$, where k_{ISBN} is the elimination rate constant for ISBN.

The apparent bioavailability of inhaled ISBN (*F*) was estimated using eq. 1:

$$F = \frac{k_{0(obs, inh)}}{k_{0(theor, inh)}} = \frac{C_{SS(obs, inh)} \times CL_{(inh)}}{RR \times TV \times IC} \quad (1)$$

where $k_{0(obs, inh)}$ and $k_{0(theor, inh)}$ are the observed and theoretical input rates of ISBN, respectively. $C_{SS(obs, inh)}$ is the apparent steady-state concentration of ISBN observed after inhalation and $CL_{(inh)}$ is the clearance of ISBN after inhalation. *RR* and *TV* are the estimated average respiration rate (85 breaths/min) and tidal volume (1.7 ml/breath) for a normal 300-g rat, respectively (Davies and Morris, 1993). *IC* is the average exposure concentration of ISBN during the dosing interval. The rate constant $k_{0(obs, inh)}$ represents the apparent zero order input of ISBN into the systemic circulation, whereas $k_{0(theor, inh)}$ is the theoretical dosing rate based on experimentally determined exposure concentrations of ISBN and estimated physiological parameters.

The fraction of ISBN converted to ISBA (*f_m*) was obtained using eq. 2:

$$f_m = \frac{C_{SS(ISBA)} \times CL_{(ISBA)}}{k_{0(iv)}} \quad (2)$$

where $C_{SS(ISBA)}$ is the steady-state concentration of ISBA after ISBN infusion, $CL_{(ISBA)}$ is the clearance of ISBA after i.v. bolus administration of ISBA, and $k_{0(i.v.)}$ is the infusion rate of ISBN.

Intravenous Administration of ISBA. One day after surgery, conscious rats were injected with 25, 50, or 100 mg/kg (equivalent to 0.34, 0.68, or 1.35 mmol/kg) ISBA dissolved in 0.25 to 1.0 ml of saline. Blood samples (0.2 ml) were taken at 2, 5, 8, 12, 15, 20, 25, 35, 40, and 45 min. After the 50 mg/kg dose, rats were placed in metabolism cages for 4 h and urine was collected and analyzed for unchanged, sulfated, and glucuronidated ISBA.

Intravenous Administration of ISBN. One day after surgery, ISBN was infused to conscious rats at a rate of 3 or 6 μ l/h (equivalent to 24.6 or 49.2 μ mol/h) for 90 min using a gas-tight syringe. Blood samples (0.4 ml) were taken at 30, 60, and 90 min after onset of infusion for analysis of ISBN. After 90 min, the infusion was either increased to 6 μ l/h or decreased to 3 μ l/h, and blood samples were taken 30, 60, and 90 min later. In similar studies, rats were infused ISBN at a rate of 6 μ l/h for 90 min. Blood samples were taken at 30, 60, 90, 92, 94, and 96 min.

In other rats, blood ISBA concentrations were determined when ISBN was infused at a rate of 3 or 6 μ l/h for 90 min. Blood samples (0.2 ml) were taken 30, 60, and 90 min after onset of infusion. After 90 min, the infusion was either increased to 6 μ l/h or decreased to 3 μ l/h, and blood samples were taken at 30, 60, and 90 min later. In similar studies, rats were infused ISBN at a rate of 6 μ l/h for 90 min. Blood samples were taken at 30, 60, 90, 95, 100, 105, and 110 min.

Inhalation of ISBN. One day after surgery, rats were subjected to an average dose of 309 or 907 ppm inhaled ISBN for 45 min (equivalent to 13.8 or 40.5 μ M ISBN for 45 min). Blood samples (0.4 ml) were taken at 15, 30, 45, 46, 48, 50, and 52 min. In other rats, blood ISBA levels were determined under similar dosing conditions. Blood samples (0.2 ml) were taken at 2, 5, 25, 45, 46, 48, and 50 min.

Intravenous Administration of ISBA-Inhalation of ISAN. One day after surgery, conscious rats were injected with 25 mg/kg (equivalent to 0.34 mmol/kg) ISBA dissolved in 0.25 ml of saline. Blood samples (0.2 ml) were taken at 2, 5, 10, 15, 20, 25, and 35 min after ISBA administration and replaced with an equal volume of saline. After a 60-min washout period, a second 25-mg/kg bolus of ISBA was given to rats concomitantly exposed to an average dose of 296 ppm ISAN. Blood samples (0.2 ml) were taken at 2, 4, 6, 8, 10, and 12 min after ISBA administration. Blood samples were analyzed for ISBA as described previously.

Statistical Analysis. For the ISBA dose-ranging studies, statistical analyses were performed using ANOVA followed by Newman-Keuls post hoc test. Student's *t* test was used to compare the half-life and clearance of ISBA when given with or without inhaled ISAN and to evaluate the elimination half-lives

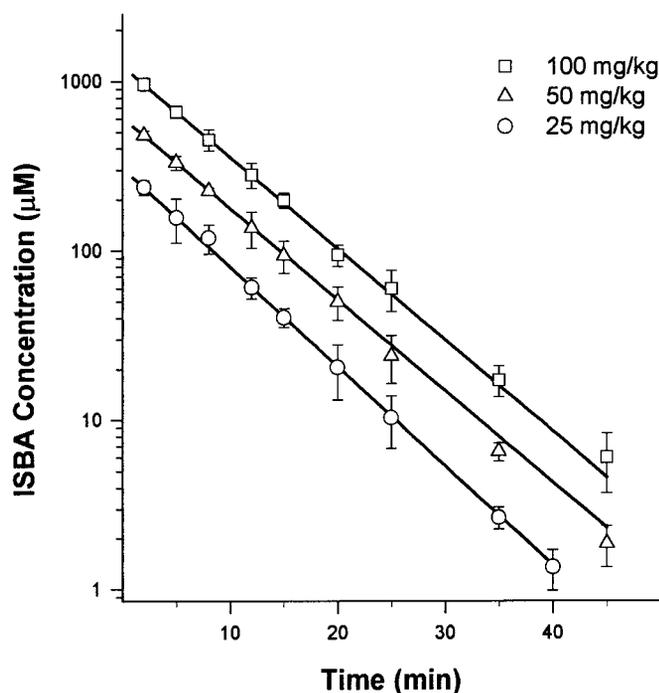


FIG. 1. Mean blood concentration-time profile of ISBA after i.v. injection of ISBA.

The lines represent the computer fit of the data to a one compartment pharmacokinetic model ($n = 3$ or 4 each dose).

of ISBA after i.v. and inhalation of ISBN. Significance was concluded when $P < .05$. The data were expressed as mean \pm S.D.

The data obtained were fitted to the proposed pharmacokinetic model using ADAPT II software (Biomedical Simulations Resource, Los Angeles, CA).

Results

Intravenous Bolus Doses of ISBA. The concentration-time profiles of ISBA obtained after doses of 25, 50, and 100 mg/kg are shown in Fig. 1. Blood ISBA concentrations declined monoexponentially after all doses. There were no statistical differences ($P > .05$) in blood clearance, volume of distribution, or half-life between doses (see Table 1). The urinary excretion of unchanged ISBA was determined to be less than 1% of the dose, and there was no evidence of sulfate or glucuronide conjugates in the urine.

Intravenous Infusion of ISBN—ISBN Pharmacokinetics. The individual blood concentration-time profiles of ISBN during an infusion of 3 and 6 $\mu\text{l/h}$ are shown in Fig. 2A. There appears to be a dose-dependent change in the steady-state concentration of ISBN based on the infusion rate of ISBN. When ISBN was administered at 3 or 6 $\mu\text{l/h}$, the steady-state blood ISBN concentrations were 0.50 ± 0.12 and 0.96 ± 0.14 μM , respectively. The clearance of ISBN was calculated to be 2.8 ± 0.2 and 3.0 ± 0.3 l/min/kg for the 3- and 6- $\mu\text{l/h}$ infusions, respectively. The pharmacokinetics of ISBN appeared to be stationary (i.e., they did not change over time), because the blood ISBN concentrations were similar regardless of the order of the infusion.

In a separate study, the wash-out kinetics of ISBN were determined after a 6- $\mu\text{l/h}$ infusion for 90 min (Fig. 3). The half-life and volume of distribution of ISBN were determined to be 1.3 ± 0.2 min and 5.8 ± 0.4 l/kg, respectively.

Intravenous Infusion of ISBN—ISBA Pharmacokinetics. The individual blood concentrations versus time profiles of ISBA during an infusion of ISBN at 3 and 6 $\mu\text{l/h}$ are shown in Fig. 2B. There appeared to be a dose-proportional change in the steady-state concen-

TABLE 1

Pharmacokinetic parameters of ISBA after i.v. bolus administration ($n = 3$ or 4 each dose)

Parameter	25 mg/kg	50 mg/kg	100 mg/kg
CL (liters/min/kg)	0.15 ± 0.01	0.13 ± 0.02	0.15 ± 0.01
V (liters/kg)	1.1 ± 0.1	1.1 ± 0.01	1.2 ± 0.1
$t_{1/2}$ (min)	5.1 ± 0.2	5.6 ± 0.4	5.6 ± 0.3
Mean Residence Time (min)	7.4 ± 0.3	8.0 ± 0.6	8.1 ± 0.5
k_{el} (min^{-1})	0.14 ± 0.01	0.13 ± 0.01	0.12 ± 0.01
Urinary excretion (% dose)	^a	0.49 ± 0.1	^a

^a not determined.

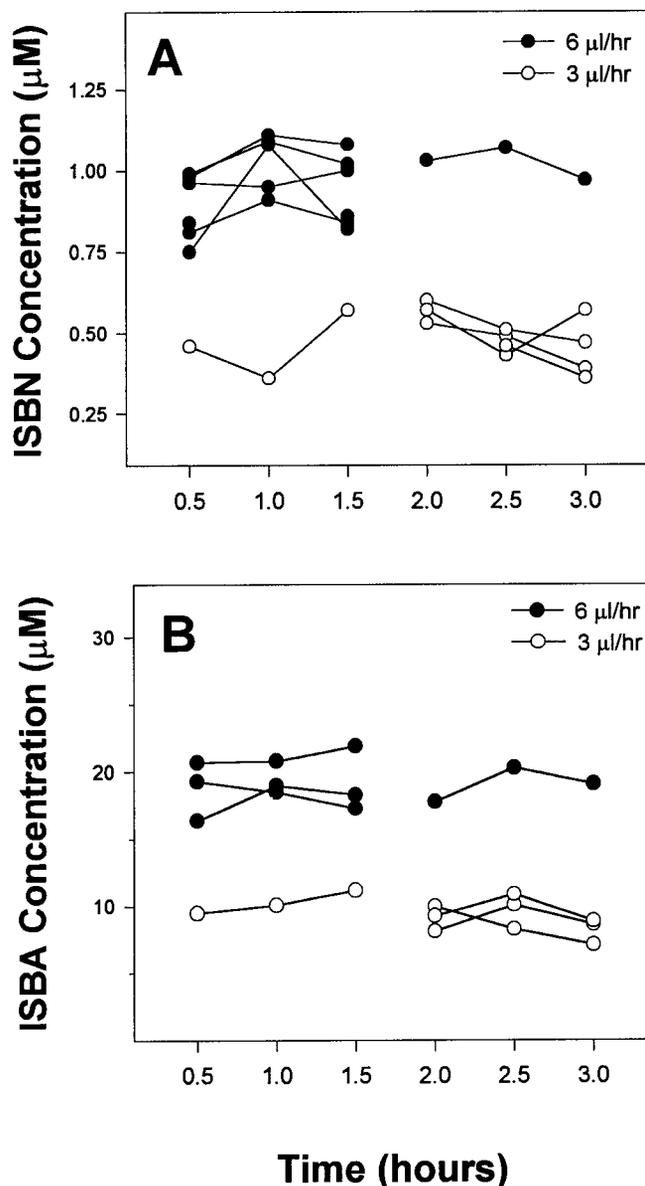


FIG. 2. Individual steady-state blood concentrations of ISBN and ISBA after infusions of 3 or 6 $\mu\text{l/h}$ ISBN (A: $n = 7$ for blood ISBN determination; B: $n = 4$ for blood ISBA determination).

tration of ISBA based on the infusion rate of ISBN. When ISBN was administered at 3 or 6 $\mu\text{l/h}$, the blood ISBA concentrations were 9.4 ± 2.0 and 19.1 ± 2.2 μM , respectively. Via eq. 2, we determined that 98% of ISBN was metabolized to ISBA. The data showed that ISBA pharmacokinetics also appeared to be stationary, because the blood

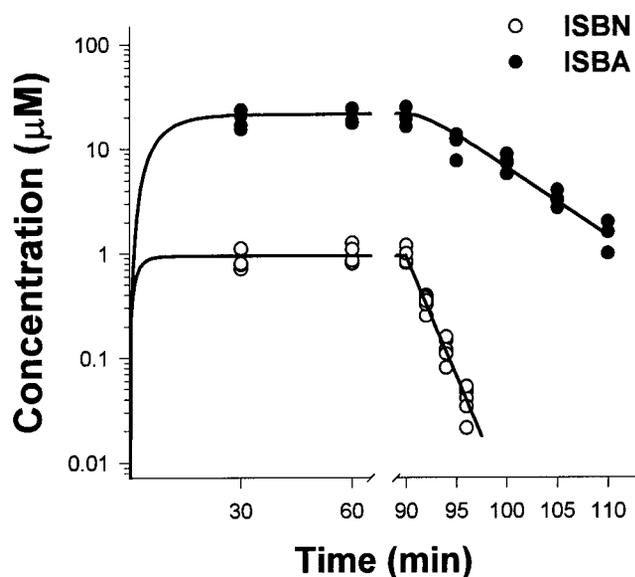


FIG. 3. Concentration-time profile of ISBN and ISBA after a 90-min infusion of ISBN (6 μ l/h).

The lines were obtained from computer fitting of the data to the pharmacokinetic model shown in Fig. 6A ($n = 4$ for ISBA and ISBN).

ISBA concentrations were similar regardless of the order of the infusion of ISBN.

In a separate study, the wash-out kinetics of ISBA were determined after a 6- μ l/h infusion of ISBN for 90 min (Fig. 3). The average half-life of ISBA observed was 5.4 min, which was similar to that obtained after ISBA bolus administration (Table 2).

Inhalation of ISBN—ISBN Pharmacokinetics. The pharmacokinetic profile of inhaled ISBN after doses of 309 ± 19 and 907 ± 12 ppm for 45 min are shown in Fig. 4, A and B, respectively. The apparent steady-state blood ISBN concentrations were 0.8 ± 0.1 and 2.7 ± 0.5 μ M for the average doses of 309 and 907 ppm ISBN, respectively. The apparent steady-state concentrations of ISBN seemed to increase proportionally with dose. The half-life of ISBN was 1.4 ± 0.2 and 1.5 ± 0.2 min, after the two doses (Table 2), and was similar to the elimination half-life after infusion ($P > .05$), suggesting that the clearance of ISBN did not change based on route of administration. The bioavailability of the average doses of 309 and 907 ppm inhaled ISBN was estimated to be 41 ± 6 and $45 \pm 3\%$, respectively, using eq. 1.

Inhalation of ISBN—ISBA Pharmacokinetics. The concentration-time profile of ISBA after average doses of 309 and 907 ppm inhaled ISBN for 45 min are shown in Fig. 4, A and B, respectively. The apparent steady-state blood ISBA concentrations after average doses of 309 and 907 ppm inhaled ISBN were 4.6 ± 0.7 and 11.2 ± 2.2 μ M, respectively, and appeared to increase proportionally with dose. The half-life of ISBA after average doses of 309 and 907 ppm ISBN was 1.4 ± 0.3 and 1.5 ± 0.2 min, respectively. The half-life of ISBA after inhaled ISBN was significantly decreased compared with its half-life after infusion of ISBN (Table 2).

Inhalation of ISAN—ISBA Pharmacokinetics. The concentration-time profiles of an i.v. bolus of 25 mg/kg ISBA given alone or in conjunction with 296 ± 16 ppm inhaled ISAN are shown in Fig. 5. As expected, when given alone, ISBA produced similar pharmacokinetics as shown previously. However, concurrent administration of inhaled ISAN significantly changed the pharmacokinetic profile of ISBA. The clearance was increased from 0.13 ± 0.03 to 0.28 ± 0.04 l/min/kg,

although there was no significant difference in the volume of distribution ($P > .05$).

The pharmacokinetic parameters of ISBN and ISBA after different doses and routes of administration are shown in Table 2.

Discussion

To our knowledge, only one pharmacokinetic study has been reported so far regarding nitrite inhalants, with this study describing the time course of the transformation products in blood and plasma of rats acutely exposed to *n*-butyl nitrite (Osterloh and Goldfield, 1984). The authors were unable to detect *n*-butyl nitrite in plasma, whereas *n*-butyl alcohol was detectable only at early time points after termination of the dose. Although these results suggested biotransformation of *n*-butyl nitrite to *n*-butyl alcohol, the study was not of sufficient detail to fully describe the pharmacokinetics of this inhalant or its metabolite. Until now, the pharmacokinetics of nitrite inhalants and their denitrated metabolites, in general, were largely unknown.

In this study, we conducted systematic experiments to determine the pharmacokinetics of ISBN and ISBA after different doses and routes of administration. After i.v. infusion, the systemic clearance of ISBN was estimated to be 2.9 liters/min/kg, greatly exceeding liver blood flow (0.06 liters/min/kg) and cardiac output (0.3 liters/min/kg) in the rat (Davies and Morris, 1993). This high clearance value could be artifactual if there was loss of ISBN due to either blood degradation or volatility before analysis. Therefore, in an *in vitro* experiment, we simulated our blood-sampling procedure from rats by withdrawing ISBN-spiked blood from a gas-tight vial stored at 37°C. Samples were withdrawn using a gas-tight syringe stored at 0°C before use via a cannula of similar length and diameter of those implanted in the femoral artery of rats used for the pharmacokinetic studies. The results showed that our procedure did not lead to significant loss of ISBN in the blood samples (<5% loss before analysis), indicating that the large clearance of ISBN was not artifactual from our methodology.

A clearance value of ISBN greater than the reported cardiac output in the rat suggests that the metabolism of ISBN could be due to a number of drug-eliminating processes *in vivo*. We have determined the degradation rates of ISBN in plasma and rat whole blood, allowing us to estimate the *in vivo* plasma and blood clearance of ISBN by multiplying the *in vitro* degradation rate constant of ISBN by the physiologic fluid volume. Using volume estimates of 38 and 65 ml/kg for plasma and blood volume, respectively (Farris and Griffith, 1949), we determined that the clearance from plasma and blood, as such, was 3.4 ± 0.6 and 27.0 ± 3.3 ml/min/kg, respectively (Kielbasa et al., 1999). Thus, although plasma and blood can degrade ISBN rather quickly, these processes do not appear to contribute significantly to the *in vivo* systemic clearance of ISBN (2.9 liters/min/kg).

Because the value of cardiac output equals blood flow to the lung, clearance values greater than cardiac output can suggest that extensive lung clearance of ISBN may be occurring. It has been shown that lung tissue contains GST activity and has a high selectivity for alkyl nitrites, indicating that GST may be of major importance in nitrite metabolism (Akerboom et al., 1997). Contribution from other metabolizing organs, such as the liver and kidneys, would provide an additive effect, thus increasing the clearance value. Also, the systemic vasculature and muscle may be contributing to the clearance of ISBN, because it has been shown that bovine vascular smooth muscle cells contain enzymes capable of metabolizing organic nitrites (Kowaluk and Fung, 1991). However, it is not known whether the same enzymes in bovine smooth muscle cells exist in the rat. At this time it is unclear as to what processes contribute to the high systemic clearance of ISBN.

Based on eq. 2, our results indicate that ISBN is almost completely metabolized to ISBA. This equation is valid as long as the clearance

TABLE 2
Pharmacokinetic parameters of ISBN and ISBA after different doses and routes of administration

Route	i.v.		Inhalation		
	ISBA	ISBN	ISBN		ISAN + ISBA
Compound administered					
Dose	25 mg/kg	6 μ l/h	309 \pm 19 ppm	907 \pm 12 ppm	296 \pm 16 ppm + 25 mg/kg
ISBA Pharmacokinetics					
C_{ss} (μ M)		19.1 \pm 2.4	4.6 \pm 0.7	11.2 \pm 2.2	
$t_{1/2}$ (min)	5.6 \pm 1.1	5.3 \pm 0.4	1.4 \pm 0.3**	1.5 \pm 0.2**	1.9 \pm 0.5*
V (liters/kg)	1.1 \pm 0.1				0.8 \pm 0.2
CL (liters/min/kg)	0.13 \pm 0.03				0.28 \pm 0.04*
ISBN Pharmacokinetics					
C_{ss} (μ M)		0.96 \pm 0.1	0.83 \pm 0.1	2.7 \pm 0.5	
$t_{1/2}$ (min)		1.3 \pm 0.2	1.4 \pm 0.2	1.4 \pm 0.2	
V (liters/kg)		5.8 \pm 0.4			
CL (liters/min/kg)		3.0 \pm 0.3			
F (%)			41 \pm 6	45 \pm 3	

* $P < .01$ compared with 25 mg/kg ISBA.

** $P < .001$ compared with 6 μ l/h ISBN.

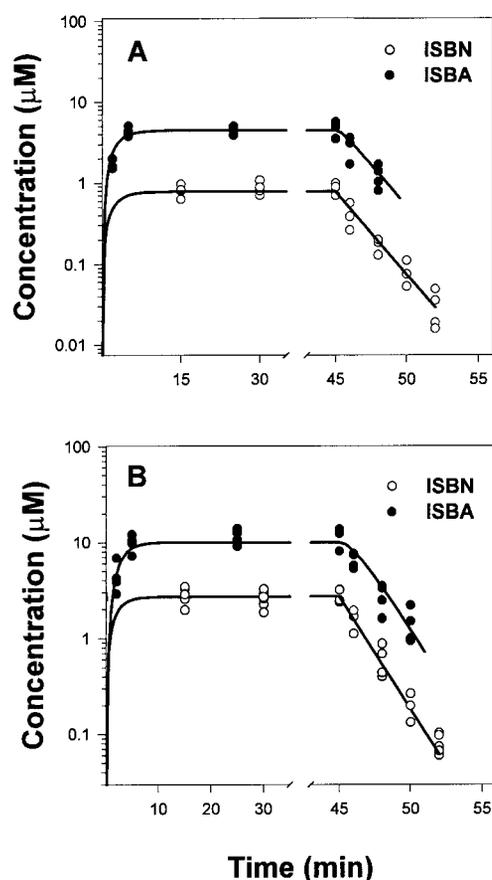


FIG. 4. Concentration-time profile of ISBN and ISBA after 45 min of inhaled ISBN.

The lines were obtained from computer fitting of the data to the pharmacokinetic model shown in Fig. 6B (A: 309 \pm 19 ppm, $n = 4$ each; B: 907 \pm 12 ppm, $n = 4$ and 6 for ISBA and ISBN, respectively).

of ISBA after ISBN administration remains the same. This assumption appears to be applicable because the half-life of ISBA after direct i.v. injection and after termination of infused ISBN was identical (Table 2). Therefore, after i.v. administration, the metabolite pharmacokinetics can be described as elimination-rate limited. The clearance of ISBA (0.15 liters/min/kg) was large, and exceeded liver blood flow in the rat, implying extrahepatic metabolism. Moreover, studies exam-

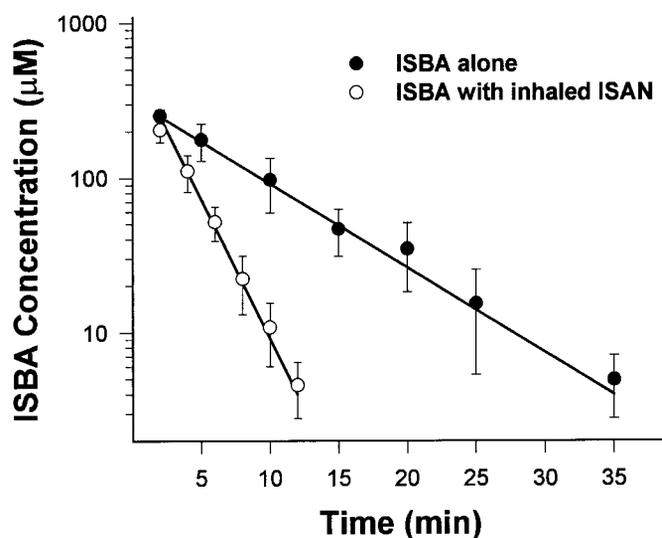


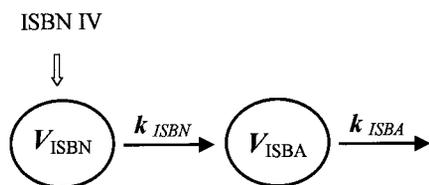
FIG. 5. Mean blood concentrations of ISBA after i.v. injection of ISBA alone or with concomitant administration of 296 \pm 16 ppm inhaled ISAN ($n = 4$ each).

The lines represent the computer fit of the data to a one-compartment pharmacokinetic model.

ining the urinary kinetics of ISBA showed that there was no substantial excretion of unchanged ISBA (<1% of the administered dose) or evidence of glucuronidated or sulfated conjugates of ISBA.

Historically, the clinical route of administration of nitrites has been via inhalation. Using our animal model, we were able to examine the pharmacokinetics of ISBN and ISBA after the administration of an average dose of 309 and 907 ppm inhaled ISBN over 45 min. The half-life of ISBN after termination of breathing of the vapors declined similarly to what was observed after infused ISBN, suggesting that there was no change in the clearance of ISBN based on the route of administration (Table 2). This assumption was used in estimating the bioavailability of inhaled ISBN, using literature values of average respiratory rate and tidal volume. Because we estimated that ISBN is primarily converted in vivo to ISBA, it follows that ISBN is unlikely to be eliminated unchanged by lung expiration, as might be possible with such a volatile compound. Based on the large clearance value of ISBN, it seems likely that there would be a first-pass effect by the lung. Our studies estimated that 43% of inhaled ISBN reached the systemic circulation.

A



B

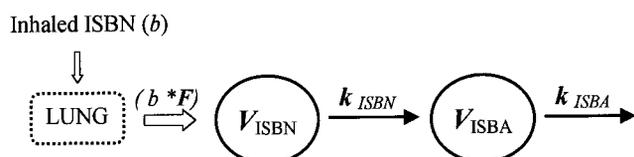


FIG. 6. Schematic representation of the pharmacokinetic models used to describe the concentration-time profile of ISBN and ISBA after different routes of administration (b = dose of ISBN via inhalation; F = bioavailability).

The pharmacokinetics of ISBA formed from ISBN appeared to depend on the route of administration of the nitrite. The rate of elimination of ISBA from inhaled ISBN approached that of the parent nitrite (Fig. 4), whereas the half-life of ISBA elimination was longer than that of infused ISBN (Fig. 3). Because the clearance of ISBA after direct injection was larger than liver blood flow in the rat, other organs such as the lung may be contributing to its excretion. For isopropyl alcohol, it was estimated that 81 to 89% of the administered dose to rats was exhaled as unmetabolized isopropyl alcohol, acetone, or carbon dioxide (Slaughter et al., 1994). Because ISBA is structurally similar, it is possible that the lungs may play a role in its elimination. ISBN is a NO prodrug and acts as a potent vasodilator. The change in the disposition of ISBA may well be due to the liberation of NO from inhaled ISBN, because nitrites have been reported to cause vasorelaxation via NO formation in vivo (Bauer and Fung, 1995). Relaxation of smooth muscle caused by inhalation of ISBN may affect the hemodynamics of the lung by altering blood flow to this organ. If the lung contributes to the clearance of ISBA, then inhalation of any volatile nitrite may have an effect on its elimination. We examined this hypothesis by administering ISAN, a pharmacologically similar but analytically distinct compound that has been shown to increase lung blood flow by 66% in humans (de Leon and Perloff, 1965), while simultaneously administering an i.v. bolus of ISBA. Our results showed that concomitant inhalation of ISAN decreased the clearance of ISBA without changing the volume of distribution (Table 2). Because no studies examining expired ISBA were carried out, it is yet unknown if ISBA is eliminated via the lung as unchanged or metabolized form.

We then constructed pharmacokinetic models to describe the concentration-time profiles of ISBN and ISBA in rats after inhalation and i.v. administration (Fig. 6). ISBN and ISBA were described by one-compartment systems with complete biotransformation of ISBN to ISBA. Although we hypothesized that the decreased half-life of ISBA after inhalation of ISBN may be due to alterations in blood flow to the lung, a physiologically based pharmacokinetic model using organ

TABLE 3

Model parameter estimation and 95% confidence interval after i.v. and inhaled ISBN (907 ± 12 ppm) using ADAPT II software

Data are presented as parameter estimate (confidence interval).

Route of ISBN	i.v.	Inhalation
ISBA Pharmacokinetics		
V (liters/kg)	0.94 (0.80–1.08)	1.1 (1.0–1.2)
k_{el} (min^{-1})	0.14 (0.13–0.15)	0.50 (0.47–0.53)
ISBN Pharmacokinetics		
V (liters/kg)	5.7 (4.9–6.5)	5.1 (4.8–5.4)
k_{el} (min^{-1})	0.54 (0.48–0.59)	0.56 (0.51–0.61)
F (%)		42 (38–46)

flow rates was not developed because our data set was not comprehensive enough to justify the added parameters for such a model. Also, separate pharmacokinetic models for i.v. (Fig. 6A) and inhalation (Fig. 6B) were developed because a single model did not allow for the change in elimination of ISBA based on the route of administration. Nevertheless, our models appear to reasonably predict the observed disposition data of ISBN and ISBA (Figs. 3 and 4). The model parameter estimates for ISBN and ISBA (Table 3) compared well to compartmental calculations (Table 2), indicating that the models adequately describe their apparent in vivo behavior.

In summary, we have carried out a detailed examination of the pharmacokinetics of ISBN and ISBA and have constructed models to describe their in vivo pharmacokinetic behavior. We have determined that the large clearance of ISBN is not due primarily to blood metabolism, because this process accounts for only a small portion of its systemic clearance. ISBN appears to be completely metabolized to ISBA. The clearance of ISBN is not dependent on the route of administration, but nitrite inhalation appears to affect the disposition of its alcohol metabolite.

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