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Report

Disposition of Bismuth in the Rat. II. Pharmacokinetics and Biliary Excretion

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The pharmacokinetics and biliary excretion profile of intravenously administered bismuth ions were investigated in male Sprague Dawley rats. The data indicated that in the dose range studied, the percentage of dose excreted in urine ranged from 58 to 63%. The mean residence time for bismuth ions was 3.93, 4.07, and 5.45 hr for the 0.5, 0.75, and 1.0 mg/kg dose, respectively, while the volume of distribution at steady state was 0.75, 1.24, and 1.38 L/kg for the three doses. Blood clearance values ranged from 0.2 to 0.32 L/hr/kg. Blood bismuth ion concentrations toward the latter part of the sampling schedule indicated significant variability. The bile-to-blood concentration ratio of intravenously administered bismuth exceeded 1.0 for the three doses studied, suggesting that transport of bismuth from blood to bile may be carrier mediated.

KEY WORDS: bismuth; pharmacokinetics; biliary excretion.

INTRODUCTION

The use of bismuth compounds in the treatment of gastrointestinal disorders and peptic ulcers is common (1,2). The chronic use of a number of these compounds has led to encephalopathies with myoclonic seizures, motor incoordination and even death (3). The pharmacokinetic profile of this metal is poorly defined. Pharmacokinetic studies conducted by Sollman and Seifter (4) suffered from a lack of assay sensitivity. In a paper published by Russ *et al.* (5), the whole-body half-life of radiolabeled bismuth following intraperitoneal administration of bismuth citrate was reported to be 122 hr in mature female rats. The short radioactive half-life of the isotope used, in relation to the determined biological half-life, could have led to erroneous conclusions about this parameter.

Studies on the biliary excretion of bismuth ions conducted by Schafer and Forth (6) indicate that bismuth ion is excreted from blood into the intestines of adult Wistar rats. The authors found a dose-dependent decrease in the fraction of the bismuth dose excreted into the jejunum. Another paper reported a dose dependent increase in the fraction of bismuth dose excreted into bile (7). The greater fraction excreted in bile reported in this study, in contrast to the values reported by Schafer and Forth (6), may be due to the infusion technique of drug administration in this study, necessitated by the low aqueous solubility of bismuth nitrate.

The objective of the present study was to examine the pharmacokinetics of intravenously administered bismuth in the rat. In another series of studies, the biliary excretion profile of bismuth was investigated following intravenous dosing of three doses of bismuth nitrate.

MATERIALS AND METHODS

Analytical

Bismuth was analyzed using atomic absorption spectrophotometry utilizing a flameless technique. The analytical procedure employed is outlined elsewhere (8). The extraction of bismuth ions from blood, bile, and urine was carried out using the technique of chelation followed by extraction into methyl isobutyl ketone. The detection limit of the assay was 5 ng/ml, with a coefficient of variation 7.03%. Since it is not possible to differentiate between the various species of bismuth using atomic absorption spectroscopy, references to bismuth in the manuscript indicate trivalent bismuth ions present in bismuth nitrate.

Animal Model

Male Sprague Dawley rats were used as the animal model. All animals were subjected to an overnight fast prior to the day of the commencement of the experiment. They were allowed free access to drinking water. After the fasting period, these rats lost an average of 6–8% of their total body weight.

Biliary Excretion

Male Sprague Dawley rats weighing 350–400 g were anesthetized with 1.0 mg/kg body weight urethane. Following a midline abdominal incision, the common bile duct was exposed and the distal end ligated with a silk suture. The bile duct was cannulated with a small length of PE-10 tubing.

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Bismuth was administered via the right jugular vein, which was cannulated with a PE-50 tubing. Bismuth was injected at doses of 0.5, 0.75, and 1.0 mg/kg. Bile was collected at 0, 0.5, 1.0, 1.5, 2.0, 2.5, and 3.0 hr postdosing. Blood samples were collected at the midpoint of the bile collection interval. After each blood sample withdrawal, 0.3 ml heparinized saline (50 units/ml) was introduced into the cannula.

Pharmacokinetics

The pharmacokinetic properties of bismuth ions were studied as a function of three doses, 0.5, 0.75, and 1.0 mg/kg body weight. Male Sprague Dawley rats weighing 250–300 g were anesthetized with 40 mg/kg body weight intraperitoneal dose of pentobarbital. The right jugular vein was cannulated with a PE-50 tubing (Clay Adams) and secured with surgical thread (Deknatel Surgical Suture, Size 4-0). The cannula was kept patent by flushing with 50 units/ml herparinized saline. The tubing was exteriorized at the nape of the neck and passed through a spring harness attachment, the end of which was attached to a 1-ml plastic syringe containing heparinized saline. The syringe was taped to the spring to allow free movement to the animal. The animals were housed in Nalgene metabolism cages and allowed free access to water and food (Purina Rat Chow). The animals were allowed to recover for 48 hr after surgery. A 0-hr blood sample was drawn and a blank urine sample collected. Bismuth was administered through the cannula and flushed with 0.5 ml of heparinized saline (50 units/ml). Blood samples (0.3 ml) were drawn at 0.083, 0.33, 0.67, 1.0, 2.0, 3.0, 6.0, 9.0, 12.0, and 24.0 hr following bismuth ion administration and then once every 24 hr up to 240 hr. The cannula was flushed twice a day with 0.5 ml of 50 units/ml heparinized saline. Total urine samples were collected at 0–6, 6–12, and 12–24 hr following drug administration and then once every 24 hr for 240 hr. Total urine volume was noted and 4-ml aliquots were placed in plastic culture tubes. All samples were frozen at −70°C. Blood and urine collection tubes were color and symbol coded for identification purposes and the date and time of sampling noted. Blood samples, instead of plasma, were collected because bismuth is sequestered in the red blood cell fraction of whole blood (8).

Data Analysis

Biliary excretion of bismuth was expressed as the biliary clearance Cl_b, using the following equation:

$$Cl_b = \frac{dX_{bile}/dt}{C_{mid}} \quad (1)$$

where dX_{bi}/dt is the excretion rate of bismuth into bile and C_{mid} is the concentration of bismuth ions in blood at the midpoint of the bile collection interval. The bile to blood concentration ratio was calculated as

$$R_{bi:b} = \frac{C_{bile}}{C_{mid}} \quad (2)$$

where C_{bile} is the concentration of bismuth ions in bile and C_{mid} is the concentration of bismuth ions in blood at the midpoint of the bile collection interval.

The pharmacokinetic analysis of bismuth was conducted using parameters based upon statistical moment theory (9). The parameters that were calculated were the area under the curve (AUC), area under the moment curve (AUMC), mean residence time (MRT), clearance (CL), and volume of distribution at steady state (V_{ss}). Urinary recovery of bismuth was determined experimentally, and the amount excreted at the end of 240 hr expressed as a fraction of the administered dose. Statistical tests for significance were carried out on a HP-41CX programmable calculator equipped with a Math/Stat application module. Differences were considerd to be significant at the $P < 0.05$ level for all experiments.

All chemicals were used as received except methyl isobutyl ketone, which was saturated with deionized water prior to its use for extraction of bismuth ions from the various matrices.

RESULTS

The amount of bismuth ions excreted in bile as a function of time following an intravenous dose of 0.75 mg/kg is shown in Table I. The data in Table I suggests the presence of an active transport mechanism. The concentration of bismuth ions in blood and the biliary excretion profile as a function of time for the 1.0 mg/kg dose is illustrated in Fig. 1. Table II shows that the fraction excreted in bile decreased from approximately 0.014 for the lowest dose to about 0.008 for the highest dose. Biliary clearance was significantly higher for the 0.5 and 0.75 mg/kg doses compared to the 1.0 mg/kg dose ($P < 0.05$).

The whole-blood concentration profile of bismuth ions following intravenous administration of the 1.0 mg/kg dose is illustrated in Fig. 2. Bismuth concentrations declined triphasically. The initial loss of bismuth ions from blood was extremely rapid and was followed by a slower phase which extended to the 9-hr sampling interval. The concentrations beyond the 12-hr blood sample tended to be variable; in some rats there were peaks and valleys in the blood concentration as one went further out in time. In most animals blood concentrations were below detectable levels after the 48-hr blood sample. While the blood concentrations of bismuth ions were below detectable levels 48 hr after drug administration, urinary levels were high. This suggests that bismuth ions may be highly bound to kidneys. Moreover, urinary levels of bismuth ions tended to be less prone to the

Table I. Biliary Excretion Profile Following Intravenous Administration of 0.75 mg/kg Bismuth[a]

Time (min)	Amount in Bile (μg)	Bile-to-Blood Concentration Ratio
30	1.280 ± 0.780	0.936 ± 0.313
60	1.840 ± 0.620	3.944 ± 0.866
90	1.280 ± 0.360	4.582 ± 1.292
120	0.620 ± 0.220	3.172 ± 1.383
150	0.310 ± 0.140	1.864 ± 0.768
180	0.180 ± 0.030	1.225 ± 0.078

[a] Mean ± SD of five rats.

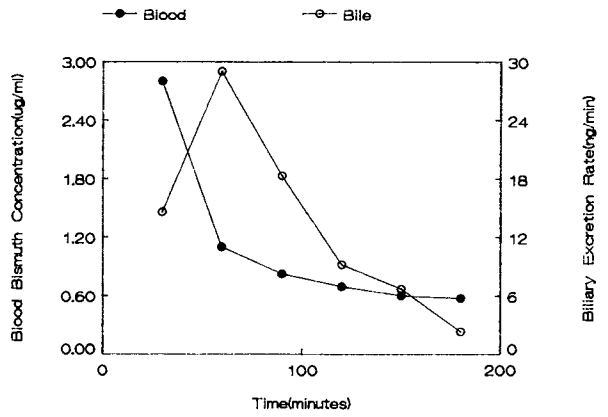

Fig. 1. Biliary excretion and blood concentration profile of bismuth ions, following 1.0 mg/kg intravenous dose.

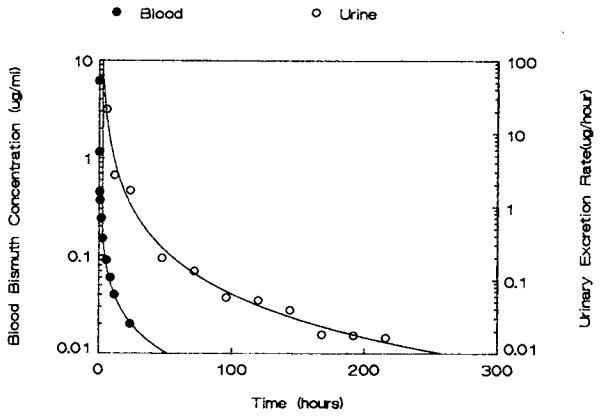

Fig. 2. Typical blood concentration and urinary excretion rate profile of bismuth ions following 1.0 mg/kg intravenous dose.

variation observed in the blood concentrations (Fig. 2). Urinary elimination of bismuth was multiphasic.

The pharmacokinetic parameters of bismuth ions were derived using statistical moment theory and are shown in Table III. Because of variability in the data beyond the 12-hr time point, differences in the parameters between the three doses were masked. The fraction of dose eliminated in urine was approximately 0.60 for the three doses studied, suggesting that urinary elimination is the primary route of bismuth ions elimination following intravenous administration. The volume of distribution at steady state, mean residence time, and clearance were not significantly different at the three doses studied. The data indicated that elimination of bismuth ions occurs for a longer time than can be predicted by blood data alone. Biliary clearance of bismuth ions accounted for about 1% of the overall clearance of bismuth. The data also indicated that when biliary clearance was expressed as a fraction of urinary clearance, the ratio decreased from 0.14 for the lowest dose to 0.02 for the highest dose, providing evidence that biliary excretion of bismuth ions in the rat may be a saturable process.

DISCUSSION

The biliary clearance values and the fraction of dose excreted in bile for bismuth ions following the 0.5, 0.75, and 1.0 mg/kg intravenous doses suggested saturation of bismuth ions excretion into bile. An examination of the bile/blood concentration ratio of bismuth ions following the 0.75 mg/kg dose indicates that it was greater than 1.0. This demonstrates that bismuth ions may be transported by a carrier mediated mechanism. The maximum concentration ratio of bismuth ions occurred approximately 90 min after administration, indicating that there is a rate-limiting step in the transport of bismuth ions from blood to bile, either from plasma to liver or from liver to bile. These data are consistent with the results reported by Schafer and Forth (6).

Table II. Effect of Intravenous Dose on Biliary Clearance of Bismuth

Dose (mg/kg)	Fraction of Dose in Bile	Biliary Clearance (ml/hr)
0.50	0.014 ± 0.009	6.080 ± 1.050*
0.75	0.020 ± 0.006	7.102 ± 3.010*
1.00	0.008 ± 0.004	2.460 ± 1.140

* $P < 0.05$.

An average of about 1.5% of the administered dose of 0.5 mg/kg dose of bismuth ions was excreted in bile at the end of 3 hr. This is in contrast to the value of 4.75% of a dose of 0.3 mg/kg excreted in 2 hr reported by Klassen and Gregus (7). One of the reasons for this difference could be due to the low aqueous solubility of bismuth nitrate. This salt is extremely unstable and converts to the insoluble subnitrate form on contact with water (10). Because of this experimental difficulty, they may have administered the salt as an i.v. infusion. This would lead to differences in blood level of the metal and thereby alter the interpretation of the results. In our experiments, bismuth was administered intravenously as a bolus. Bismuth nitrate was solubilized using glycerin as a cosolvent. The presence of trace amounts of glycerin in blood may have affected bismuth ion elimination in bile. This was not investigated. According to the classification developed by Brauer (11), bismuth ions would be categorized as a class B substance since it has a bile-to-blood concentration ratio greater than 1.0. If plasma levels of bismuth ions were to have been measured, this ratio would have been greater since bismuth is present to a greater extent in red blood cells than in plasma (8). The present study indicated that bismuth ion elimination from bile represents a minor pathway for its elimination from blood and biliary clearance is a small fraction of the overall clearance. It is possible that red blood cell binding of bismuth ions may affect its elimination in bile. A low concentration in plasma would lead to a small fraction available for biliary elimination. For biliary elimination to be significant, bismuth ions would have to diffuse rapidly out of red blood cells into plasma. However, the implications of this are significant. The presence of competing ions could lead to changes in its biliary excretion profile. The rapid elimination of bismuth ions by kidneys further decrease the amount of bismuth ions available for biliary elimination. The nature of bismuth ions in bile is not clearly known. Although there have been suggestions regarding the existence of more than one ionic form, no evidence is forthcoming (10). It is possible that bismuth may be complexed by glutathione and eliminated in bile.

Table III. Pharmacokinetic Parameters of Bismuth as a Function of Dose[a]

Dose (mg/kg)	AUC (μg · hr/ml)	AUMC (μg · hr²/ml)	MRT (hr)	CL (L/hr/kg)	V_{ss} (L/kg)	f_e
0.5	2.77 (0.89)[b]	11.52 (8.36)	3.93 (2.07)	0.20 (0.09)	0.75 (0.38)	0.58 (0.12)
0.75	2.51 (0.69)	10.63 (7.02)	4.07 (1.95)	0.32 (0.09)	1.24 (0.47)	0.62 (0.12)
1.0	3.82 (1.26)	23.87 (25.26)	5.45 (3.47)	0.28 (0.07)	1.38 (0.54)	0.63 (0.12)

[a] AUC, area under the curve; AUMC, area under the moment curve; MRT, mean residence time; CL, clearance; V_{ss}, volume of distribution; f_e, fraction excreted in urine in 240 hr.
[b] Figures in parentheses indicate standard deviation.

The kinetic profile of bismuth has been described using a conventional compartmental model (5). There is difficulty in using a conventional pharmacokinetic model to describe bismuth ion pharmacokinetics due to the apparent complexity of the disposition process. In most cases, 48 hr after administration, bismuth ion concentrations in blood were below detectable levels. However, urinary levels of bismuth ions were extremely high and could be detected up to 240 hr postadministration. Half-life data generated from blood concentration measurements would grossly underestimate whole-body burden of bismuth ions. Selective binding of bismuth ions to renal tubules and subsequent release may have led to prolonged levels in urine.

Russ *et al.* (5) found variable concentrations of bismuth ions in blood and determined its half-life in various organs. In these studies most of the administered bismuth dose was eliminated renally, a finding similar to the results obtained in the present study. The fraction of dose that was eliminated in urine at the end of 240 hr was similar for the three doses, with most of the dose excreted in the first 6 hr. The average percentage of the dose excreted in urine at the end of 6 hr was approximately 47, 40, and 40% for the 1.0, 0.75, and 0.5 mg/kg doses, respectively. Thus, kidneys seem to be responsible for the rapidly clearing initial phase following i.v. administration of bismuth nitrate in the rat. The data in Fig. 2 suggest that bismuth ions elimination in urine is triphasic. After the initial rapid decline, urinary excretion rate continued to decline up to 48 hr. During the terminal phase it was excreted in submicrogram quantities. Bismuth binds to kidneys selectively (12), and this could account for the slow phase of elimination. Half-life values derived from urine data may reflect bismuth ion elimination from kidneys.

The variability of blood concentrations of bismuth ions reflect an unusual feature of bismuth pharmacokinetics in the rat. The reasons for this variability are not clear. Bismuth and other heavy metal ions show a marked affinity for thiol-containing compounds (13) such as l-cysteine. These are widespread in the body and represent binding sites for bismuth ions in liver, kidneys, and other organs. Other compounds which complex heavy metal ions are metallothionine and glutathione. Fluctuations in the levels of these enzymes could lead to variability in the concentration of those metals that interact with them. This offers a possible explanation for the intraindividual variability noticed with bismuth ion concentrations in blood as a function of time. The release of bismuth ions from its binding sites would also affect its blood levels and add to the variability. Another possible source of variability is in the assay methodology. The assay method that was used had a coefficient of variation value of 7.03% at the lower end of the concentration range. While this value represents good precision in flameless atomic absorption spectroscopy, the small fluctuations noticed could have been artifacts of the analytical technique. However, in certain instances, bismuth ion concentrations increased from below detectable levels to about 25 ng/ml for the same rat between two successive samples and may represent release from storage sites such as liver. The role of red blood cell uptake of bismuth ions in modulating their disposition is not clear. In view of the significant *in vitro* uptake by red blood cells, it would appear that elimination would be extremely slow. The rapid initial elimination into urine suggests that this is not so. However, it is possible that a certain fraction may be associated with red blood cells and may account for part of the slower phase of elimination from blood. This aspect requires further investigation.

In conclusion, it appears that bismuth is excreted primarily in urine when bismuth nitrate is administered intravenously in the rat. The mean residence time values calculated using blood concentration data would seriously underestimate the whole-body burden since bismuth appears to bind to tissues and is released in urine slowly. Biliary excretion of bismuth appears to be by an active transport mechanism and represents a minor pathway for bismuth ion elimination from blood.

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