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Distribution of Bismuth in the Rat after Oral Dosing with Ranitidine Bismuth Citrate and Bismuth Subcitrate

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Abstract

Bismuth preparations are used world-wide for the management of peptic ulcer disease, for eradication of *Helicobacter pylori*, and in the prevention and treatment of diarrhoea. However neurological toxicity of bismuth has always been a major concern and evidence has been found of the absorption of bismuth. Recent studies have suggested that the absorption of bismuth increases when bismuth salts are used with ranitidine hydrochloride. The absorption and deposition of bismuth as a result of the use of the new drug ranitidine bismuth citrate have not been yet clarified.

After 15 days of twice daily oral gavage with bismuth subcitrate, 13·7 mg kg^{-1} day^{-1} to eight rats, deposition of bismuth was found in all the tissues studied, especially the kidney ($30·81 \pm 8·59$ $\mu g\ g^{-1}$ dry weight). A similar pattern of distribution and tissue concentrations was found when bismuth subcitrate was given with ranitidine hydrochloride 8·6 mg kg^{-1} day^{-1} to another eight rats, although this combination resulted in lower brain levels ($3·12 + 1·31$ $\mu g\ g^{-1}$ dry weight) than after administration of bismuth subcitrate alone ($4·77 \pm 0·97$ $\mu g\ g^{-1}$ dry weight). When six rats were given ranitidine bismuth citrate by gavage at 22·8 mg kg^{-1} day^{-1} for 15 days, kidney levels were lower ($4·24 \pm 1·75$ $\mu g\ g^{-1}$ dry weight) and brain levels were below detection limits; the bismuth concentrations in the faeces from this group were also significantly lower ($1603 \pm 104·0$ $\mu g\ g^{-1}$ dry weight) than for the two other groups. After dosing with bismuth alone or in association with ranitidine hydrochloride, bismuth was detected in several organs and deposition was not influenced by gastric pH. Blood levels correlate poorly with organ deposition and brain deposition was not always associated with encephalopathy. After administration of ranitidine bismuth citrate, significantly lower concentrations of bismuth were found in the kidney and bismuth was not detectable in the brain, suggesting lower bismuth absorption. This was confirmed by higher levels in the faeces after dosing with ranitidine bismuth citrate. Thirty days after dosing with ranitidine bismuth citrate or bismuth subcitrate, bismuth could not be detected in any of the organs examined but could be found in the urine.

In conclusion, bismuth was deposited in the kidney, brain, lung and liver of rats after oral dosing with bismuth subcitrate. After oral dosing with an equivalent amount of bismuth in the form of ranitidine bismuth citrate, significantly lower concentrations of bismuth were deposited in the kidney; in the brain bismuth was not detectable.

Bismuth salts have been used for more than two centuries for the management of a variety of gastrointestinal complaints. Over the past few decades they have been used most commonly for the prevention and treatment of diarrhoea and dyspepsia, and for the management of peptic ulcer disease. Their use for treatment of peptic ulcer disease has recently received renewed interest because in combination with antibiotics they effectively eradicate *Helicobacter pylori* (Van der Hulst et al 1996; Penston & McColl 1997), the bacterium now generally agreed to play a major role in the

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development of peptic ulceration (Misiewicz 1996).

The subject of bismuth toxicity was raised in the mid-1970s when neurological disorders were found to be associated with oral intake of bismuth salts in Australia and in France and other European countries. Most cases of bismuth neurotoxicity have been associated with bismuth subgallate and subnitrate; only three recorded cases of encephalopathy have been related to therapeutic doses of the two preparations most widely used in modern times, bismuth subsalicylate and colloidal bismuth subcitrate (Gorbach 1990). Recent studies have suggested that concomitant use with H_2-receptor antagonists enhance bismuth absorption from colloidal bismuth subcitrate, increasing the risk of bismuth deposition and, therefore, brain neurotoxicity (Nwokolo et al 1991).

Ranitidine bismuth citrate (pylorid) is a new drug developed specifically for use with one or two antibiotics in *H. pylori* eradication regimens. It is a novel salt of ranitidine with a complex of bismuth and citrate which has the anti-*Helicobacter* and mucosal protective properties of bismuth and the gastric anti-secretory effects of ranitidine (Stables et al 1993; McColm et al 1996).

The purpose of this study was to examine bismuth levels in selected organs (brain, liver, kidney, lung), blood, urine and faeces of the rat after oral dosing with ranitidine bismuth citrate. In addition, to determine any influence of an increase in gastric pH the study has compared these results with bismuth deposition results obtained after dosing with bismuth subcitrate alone and with bismuth subcitrate plus ranitidine hydrochloride. As it is known that bismuth accumulates and is retained in some tissues, the bismuth content of organs was measured 30 days after 15 days oral dosing with ranitidine bismuth citrate or bismuth subcitrate.

Materials and Methods

Drugs used

Ranitidine bismuth citrate and ranitidine hydrochloride were from Glaxo Wellcome (Lisbon, Portugal) and bismuth subcitrate was from Yamanouchi (Lisbon, Portugal). The drugs were dissolved in 0·9% NaCl and given by gavage in a volume of 10 mL kg^{-1}.

Animals

Male Wistar rats (Fundação Calouste Gulbenkian, Lisbon, Portugal), approximately 220 g, were used in all experiments. Animals were caged individually with free access to food (standard rat chow, CRM Autoclavel, Interfauna Iberica, Barcelona) and water. The colony room was maintained at 20–22°C, humidity 60–70%, and was illuminated on a 12 h light–dark cycle (light on 0900–2100 h).

Study 1

Rats were randomly allocated to four groups and were given one of four treatments by oral gavage twice daily at 0900 h and 2100 h for 15 days: 0·9% NaCl (n = 8); bismuth subcitrate 13·7 mg kg^{-1} day^{-1} (n = 8); bismuth subcitrate 13·7 mg kg^{-1} day^{-1} plus ranitidine hydrochloride 8·6 mg kg^{-1} day^{-1} (n = 8); or ranitidine bismuth citrate 22·8 mg kg^{-1} day^{-1} (n = 6).

Food was withheld on day 9 after oral gavage at 2100 h; 6 h after the morning gavage on the tenth day of dosing, rats were anaesthetized by intraperitoneal injection of pentobarbitone sodium (Sanofi Winthrop, Produtos Farmacêuticos, Lisbon, Portugal; 25% solution, 0·25 mL). After laparotomy a glass electrode (Ingold, Stockholm) was introduced into the stomach through an incision in the lesser curvature and three measurements of gastric pH were made at 2-min intervals in the lumen of the stomach (Digitrapper, Mark II Gold, Synetics Medical, Stockholm).

After measurement of gastric pH both gastrostomy and laparotomy were sutured and the animals left in their cages to recover. On day 15 of the study the rats were killed by pentobarbitone sodium overdose (20% solution, 0·5 mL, i.p.) and samples taken of liver, brain, blood, kidney, lung, faeces and urine.

Study 2

Rats were randomly allotted to three treatment groups to receive 0·9% NaCl (n = 6) or bismuth subcitrate 13·7 mg kg^{-1} day^{-1} (n = 6) or ranitidine bismuth citrate 22·8 mg kg^{-1} day^{-1} (n = 6). Drugs were given twice daily by oral gavage at 0900 h and 2100 h for 15 days. After 30 days the rats were killed by pentobarbitone sodium overdose and samples of liver, brain, blood, kidney, lung, faeces and urine were collected.

Measurements and analytical methods

During both studies animals were tested twice daily for signs of encephalopathy: rats that did not move outside a 30 cm^2 area were considered to show loss of activity; animals unable to right themselves after being placed on their back were considered to have lost righting ability, and immobile rats with loss of corneal reflex were considered comatose.

Assessment of bismuth in tissue samples was by particle-induced X-ray emission (PIXE) (Araújo et al 1993). Each sample of liver, brain, blood, kidney, lung, faeces and urine was weighed to deter-

mine wet weight, freeze-dried and then stored in a polyethylene or Teflon vial which had been cleaned with analytical-grade nitric acid. The samples were then dried in an oven at 75°C for 12 h before digestion in 9 M suprapure nitric acid (Merck, Darmstadt, Germany) containing yttrium (Alfa Products, Johnson Matthey, Karlsruhe, Germany) as internal standard. Digestion, in an oven at 130°C for 12 h, was performed in a closed Teflon vessel ('Teflon bomb'; Parr Instruments, Moline, IL), sealed in a stainless steel casing. After cooling, PIXE targets were prepared by pipetting the resulting solution (10 μL) on to a 7·5-μm Kapton film (Cambridge, UK) mounted on a Teflon frame. The film had previously been treated with high-purity 14 M nitric acid (Darmstadt, Germany) and washed with 18 MΩ cm water (Millipore GmbH, Eschborn, Germany). The targets were dried in a vacuum desiccator. Typically, four targets were prepared from each tissue sample for determination of bismuth content.

The PIXE analysis methodology and equipment used, installed at the Van de Graaf accelerator (3 MV-High Voltage, The Netherlands) of the ITN (Nuclear and Technological Institute, Lisbon, Portugal), has been described in detail by Araújo et al (1993). Bombardment was performed in-vacuo with a 2·4 MeV proton beam of 5 mm diameter. For determination of bismuth an absorber of 350-μm thick Mylar was placed in front of the Si (Li) detector to absorb the low-energy X-rays originating from the major and minor elements in the sample.

The PIXE spectra were analysed using the non-linear least-squares fitting program, AXIL (Van Espen et al 1986), and quantitative analysis was performed by use of the computer package DATTPIXE as described by Reis & Alves (1992). The detection limits for bismuth were 2 μg g^{-1} in liver, brain, kidney, lung and faeces, 1·5 μg g^{-1} in blood, and 0·005 μg mL^{-1} in urine.

The reproducibility of the analytical method was determined by oral dosing of three rats with bismuth subcitrate for 15 days under the conditions described for study 1. The animals were killed and the same tissues and other samples prepared as in studies 1 and 2. For each the bismuth content was assessed by three analyses performed on different days. A variation of less than 5% was obtained between individual tissues and other samples.

Statistics

Data are presented as means ± s.e.m. of concentrations of bismuth in those tissues for which bismuth content was above the limit of detection. Statistical analysis was performed by Student's *t*-test and the level of significance was set at $P < 0.05$.

Results

Study 1

Mean intragastric pH measured on day 10 of dosing was 1·7 ± 0·2 for the vehicle (0·9% NaCl) and 2·3 ± 0·1 for bismuth subcitrate. Intragastric acidity was suppressed to a similar extent in rats given ranitidine bismuth citrate and ranitidine hydrochloride with bismuth subcitrate (mean pH 3·5 ± 0·1 and 3·6 ± 0·2, respectively).

Bismuth was not detectable in all samples from rats treated orally with 0·9% NaCl. Table 1 lists mean ± s.e.m. bismuth concentrations in samples

Table 1. Bismuth concentrations (μg g^{-1} dry weight except for urine, μg mL^{-1}) in tissue and other samples from rats treated for 15 days.

Sample	Treatment		
	Ranitidine bismuth citrate 22·8 mg kg^{-1} day^{-1} (n = 6)	Bismuth subcitrate 13·7 mg kg^{-1} day^{-1} (n = 8)	Ranitidine hydrochloride 8·6 mg kg^{-1} day^{-1} with bismuth subcitrate 13·7 mg kg^{-1} day^{-1} (n = 8)
Brain	< Detection limit (100%)	4·77 ± 0·97 (50%)	3·12 ± 1·31 (100%)
Lung	3·20 ± 0·39 (33%)	4·07 ± 1·92 (100%)	2·95 ± 0·66 (100%)
Liver	2·17 ± 0·63 (33%)	2·36 ± 0·29 (100%)	2·38 ± 0·37 (100%)
Kidney	4·24 ± 1·75 (100%)*	30·81 ± 8·59 (100%)	32·44 ± 13·1 (100%)
Blood	1·82 ± 0·23 (87·5%)	6·58 ± 3·74 (75%)	5·91 ± 3·20 (37·5%)
Faeces	1603·0 ± 104·0 (100%)	287·0 ± 84·40 (100%)†	497·66 ± 355·42 (100%)
Urine	0·016 ± 0·006 (50%)	0·095 ± 0·006 (100%)	0·085 ± 0·005 (37·5%)

The percentage in parentheses indicates in how many rats bismuth was detectable and from which the mean ± s.e.m. was determined. *$P < 0.001$, significant difference between results from ranitidine bismuth citrate and those from ranitidine hydrochloride with bismuth subcitrate, and between results from ranitidine bismuth citrate and those from bismuth subcitrate. †$P < 0.01$, significant difference between results from bismuth subcitrate and those from ranitidine hydrochloride with bismuth subcitrate.

from rats treated twice daily for 15 days with ranitidine bismuth citrate, with bismuth subcitrate, or with bismuth subcitrate plus ranitidine hydrochloride.

Study 2

In all rats given 0·9% NaCl by gavage, bismuth was below the limit of detection in all organs and in faeces and urine. Among the group of six rats dosed with bismuth subcitrate, bismuth was detectable in the urine of five; the mean ± s.e.m. concentration was $0{\cdot}115 \pm 0{\cdot}018$ $\mu g\ mL^{-1}$. Bismuth was not detectable in organs or faeces. Similarly, bismuth was detected in the urine only of rats ($n = 3/6$) which had received ranitidine bismuth citrate (mean ± s.e.m. $0{\cdot}0525 \pm 0{\cdot}017$ $\mu g\ mL^{-1}$) and was below the limit of detection in all other organs and in faeces.

Signs of encephalopathy

During both series of experiments there was no evidence of encephalopathy in any of the 48 rats studied.

Discussion

This study showed that immediately after 15 days of oral dosing with either bismuth subcitrate 13·7 mg kg^{-1} day^{-1} or with bismuth subcitrate 13·7 mg kg^{-1} day^{-1} plus ranitidine hydrochloride 8·6 mg kg^{-1} day^{-1}, bismuth was present in the kidney, blood, brain, lung and liver (in descending order of abundance). There were no differences between organ concentrations after the two treatments, suggesting that bismuth deposition after oral bismuth subcitrate is not influenced by a concurrent elevation of gastric pH. A differing pattern of bismuth deposition was observed after the same duration of dosing with ranitidine bismuth citrate 22·8 mg kg^{-1} day^{-1}. Significantly lower concentrations of bismuth were found in the kidney and bismuth was not detectable in the brain. A second study, performed 30 days after acute oral dosing with either bismuth subcitrate or ranitidine bismuth citrate, showed that although bismuth could be found in the urine, none could be detected in the kidney, blood, brain, lung, liver or faeces.

The bismuth content of sample tissues in these studies was measured by particle-induced X-ray emission, a procedure with high sensitivity and specificity and capable of very accurate measurements with low-mass samples. The method, which is based on X-ray spectrometry, has been described by several authors (Duflow et al 1987; Pinheiro et al 1990; Araújo et al 1993). Its usefulness in detecting trace elements in rat tissues is discussed elsewhere (Pinheiro et al 1996).

The distribution of bismuth in the tissue of rats has previously been reported. For example, Dresow et al (1991) measured distribution of retained ^{205}Bi activity among organs 20 days after a single oral dose of ^{205}Bi-labelled colloidal bismuth citrate. The organ containing the greatest amount of ^{205}Bi was the kidney, followed by bone, red blood cells and lung. The organ which contained the least amount of bismuth was the brain. Bioavailability of ^{205}Bi from a number of labelled pharmaceutical oral bismuth preparations in rats was also assessed. Intestinal absorption of bismuth was small and for all compounds studied more than 99% of orally administered ^{205}Bi was excreted in the faeces. The higher amount (statistically significant) of bismuth detected in the faeces after oral dosing with ranitidine bismuth citrate when compared with other groups suggests lower absorption of bismuth from ranitidine bismuth citrate.

Rao et al (1997) have measured bismuth levels in the rat after single oral doses of bismuth salts. The kidney contained the greatest amount of bismuth. Brain levels of bismuth were below quantifiable limits in all animals dosed with bismuth subcitrate whereas trace amounts at the lower limit of quantitation (by graphite-furnace atomic-absorption spectrophotometry) were seen in one rat of each of two groups of six rats which had received bismuth subsalicylate or bismuth sucrose octasulphate.

The pattern of distribution of bismuth after chronic dosing in the rat has also been reported (Lee et al 1980). Bismuth subcitrate (172 mg kg^{-1} per 6 or 7 days)$^{-1}$ was given by oral feeding tube to 10 rats for 14 months. Measurement of bismuth in tissues was by atomic-absorption spectrometry. The highest concentrations of bismuth were measured in the kidney, followed by the lung, spleen, liver, brain, heart and skeletal muscles.

Oral bismuth compounds are associated with a low incidence of adverse events—a review of data from clinical studies over an eight-year period found just five reports of adverse events attributable to colloidal bismuth subcitrate (Bader 1987). Nevertheless, concern is inevitably expressed about the possibility of neurotoxicity as a consequence of the still unexplained French and Australian 'epidemics' of neurological symptoms after prolonged use of excessively high oral doses (up to 20 g day^{-1}) of bismuth subnitrate or bismuth subgallate (Winship 1983; Bader 1987; Slikkerveer & de Wolff 1989). The clinical picture of reported cases of bismuth encephalopathy has been consistent—symptoms are abnormal gait, myoclonus and memory loss with dementia, depression and confusion.

In both series of studies reported here there was no evidence of encephalopathy among any of the

experimental animals. Lee et al (1980) also have reported that after 14 months chronic dosing of rats with bismuth subcitrate (172 mg kg^{-1} per 6 or 7 days), all animals appeared normal and showed no unusual gait or behaviour. No evidence of bismuth toxicity was observed during clinical development of ranitidine bismuth citrate (Pipkin et al 1996). This probably reflects the low systemic bioavailability of bismuth from ranitidine bismuth citrate; during twice daily dosing with 800 mg for 28 days < 0·5% of the amount of the bismuth administered is absorbed (Koch et al 1996).

In conclusion, bismuth was deposited in the kidney, brain, lung and liver of the rat after oral dosing with bismuth subcitrate. When rats were dosed orally with an equivalent amount of bismuth in the form of ranitidine bismuth citrate, significantly lower concentrations of bismuth were deposited in the kidney and bismuth was not detectable in the brain.

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