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Medical Use of Bismuth: the Two Sides of the Coin

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Summary

Inorganic bismuth derivatives have good antibacterial properties and are considered to be only slightly toxic to humans because of their low uptake into human cells. Compounds containing bismuth are therefore widely used in medical applications. Bismuth-containing pharmaceuticals, partially in synergy with antibiotics, are already used or are being considered in the treatment of infections caused by certain bacteria, especially to eradicate *Helicobacter pylori*, *Pseudomonas aeruginosa*, *Burkholderia multivorans* and *B. cenocepacia*. However, careless use of bismuth containing pharmaceuticals can result in encephalopathy, renal failure and other adverse effects. Microbial methylation of bismuth by the human gut microbiota has recently been reported. As the lipophilicity and thus the membrane permeability of bismuth are increased by these methylation processes, the toxic effects on human cells and on members of the beneficial "physiological" gut microbiota must be considered in medical application of bismuth-containing drugs.

Keywords: Bismuth methylation; Gut microbiota; Colloidal bismuth subcitrate; Antibacterial; *Helicobacter pylori*; Toxicity

Introduction

Bismuth is a heavy metal and was regarded until recently to be the heaviest stable element. It was discovered around ten years ago that the only natural isotope of bismuth, ^{209}Bi, is an alpha emitter with a half-life of 1.9×10^{19} years [1]. Due to the low stability in aqueous solutions of bismuth derivatives with the oxidation number +V, bismuth with the oxidation number +III is regarded as the only relevant bismuth species in biological systems [2]. Bismuth is seen as the least toxic heavy metal for humans and is widely used in medical applications for its good antibacterial properties [3]. Bismuth-containing pharmaceuticals are most commonly used in the eradication of *Helicobacter pylori*, the causative agent for diseases like gastritis, peptic ulcer and even gastric cancer [4]. Recent evidence of bismuth methylation by human gut microbiota, resulting in more mobile and presumably more harmful derivatives, has led to new efforts to evaluate the usage of bismuth under this newfound aspect [5]. This minireview presents our current knowledge of the rare element bismuth, in particular its use in medicine, and highlights the potential health risk associated with its application.

Bismuth Application in Medicine

Bismuth has a long history in medicine on account of its antibacterial properties [4]. Salves for wound infections and pharmaceuticals for oral intake are available which contain bismuth. The main use of bismuth drug medication today is to eradicate *Helicobacter pylori*, a Gram-negative bacterium that causes peptic ulcers and other diseases of the gastrointestinal tract. The current concepts in the management of *Helicobacter pylori* infections recommend a triple therapy using a proton-pump inhibitor (PPI) or ranitidine bismuth citrate (RBC) (both 400 mg twice a day) with the antibiotics clarithromycin (500 mg twice a day) and amoxicillin (1000 mg twice a day) or metronidazole (500 mg twice a day) as first-line treatment, and a quadruple therapy consisting of PPI, bismuth subsalicylate (BSS) or subcitrate (120 mg four times a day) in combination with the antibiotics metronidazole (500 mg three times a day) and tetracycline (500 mg four times a day) for at least one week as second-line therapy [6]. Both PPI and ranitidine reduce the production of stomach acid and thus aid the healing of peptic ulcers. A recent clinical trial conducted in South Korea indicates that the first-line triple therapy without a bismuth compound has an unacceptably low eradication rate, as bacterial resistance to antibiotics and particularly to clarithromycin [7-9] is increasing globally. Bismuth is beneficial because no development of resistance to it has been observed among pathogens to date [10]. A comprehensive review of new treatment strategies to eradicate antibiotic-resistant *H. pylori* was made by Malfertheiner and Selgrad in 2010 [11].

It is also feasible that bismuth thiols can be used in the treatment of the opportunistic pathogen *Pseudomonas aeruginosa*, which causes respiratory problems among cystic fibrosis sufferers and immunocompromised patients, since such compounds show good antibacterial effects against this pathogen [12]. The thiolation of bismuth, for example by dimercaptopropanol (BAL), increases its membrane permeability. This improves its antibacterial effect against e.g. *H. pylori*, *Staphylococcus aureus* and *Clostridium difficile* at concentrations of below 17 µM Bi^{3+} [13]. An even lower, non-inhibitory (not growth impairing) concentration of 0.5 µM bismuth thiols (as bismuth-ethandithiol) reduces adherence of *P. aeruginosa* to epithelia cells by up to 28% by impairing the formation of bacterial extracellular polysaccharides (EPS) [12]. Low concentrations (3-5 µM) of bismuth-2,3-dimercaptopropanol have also been shown to inhibit capsular polysaccharide (CPS) formation of *Klebsiella pneumoniae* [14]. Good antibacterial activity against various *Staphylococcus* species, including multi-resistant *S. aureus*, by another bismuth thiol (bismuth-3,4-dimercaptotoluene), which impairs biofilm formation at 1.25 µM, has also been observed [15]. A recent study of thirteen different bismuth thiols confirmed antibacterial activity against the antibiotic-resistant *P. aeruginosa* and *S. aureus* found in chronic wounds [16]. However, the concentration of bismuth-ethandithiol required to eradicate mature *P. aeruginosa* biofilms was shown to be toxic to adenocarcinomic human

alveolar epithelial cells [17]. Application of the low concentration of bismuth-ethandithiol incorporated in liposome-loaded tobramycin, an aminoglycoside, shows good results in attenuating *P. aeruginosa* virulence factors and lower cytotoxicity for human lung cells. Another possible medical application of bismuth may be in bismuth-containing cement material for pulp capping. Recent studies report good antibacterial activity, low cytotoxicity, useful setting time and pH value, as well as good compressive strength [18].

Although bismuth drugs are not available in all countries to date, they are nevertheless promising tools for treating bacterial infections when antibiotics alone are no longer effective.

Supposed Molecular Aspects

Bi^{3+} ions generally have a high affinity to thiolate sulfur and to a lesser extent to nitrogen and oxygen ligands [19]. Interactions occur with cysteine-rich proteins, peptides including GSH, and metalloproteins [4]. In the latter, bismuth replaces catalytic or structural metals such as iron, nickel and zinc [19]. The antibacterial properties of bismuth against pathogens are thus based on a concentration-dependent inactivation of proteins that are either crucial to the pathogen in general or to its virulence. For instance, eradication of *H. pylori* by bismuth applied as colloidal bismuth subcitrate (CBS) may result from Bi^{3+} ions binding to a cysteine at the entrance to the active site of the nickel-containing enzyme urease, thus blocking the active site [20]. Urease activity is crucial for *H. pylori* in maintaining a pH value of around 6.2, as it forms ammonia and CO_2 from urea in the otherwise highly acidic environment of the stomach. The activity of the F1-ATPase, required for energy conservation, and the activity of the histidine-rich protein Hpn, which presumably controls cell nickel homeostasis in *H. pylori*, may be impaired by Bi^{3+} ions. It is also assumed that bismuth adheres to bacterial ferric ion-binding proteins (similar to human transferrin and lactotransferrin) and metallothionin, both of which are cysteine-rich and involved in iron and zinc homeostasis [4]. The binding of bismuth to these proteins or polypeptides may lead to deprivation of essential metal ions in the pathogen cell and thereby impair its growth.

Low concentrations of bismuth-ethanedithiol, i.e. concentrations that do not impair the growth of *P. aeruginosa*, were shown to reduce the virulence of this opportunistic pathogen [12]. Bismuth-ethanediitiol (BiEDT) alters the bacterial surface of *P. aeruginosa* by inhibiting formation of EPS and lipopolysaccharides (LPS) even at a low concentration (0.5 μM), thereby reducing biofilm formation and the release of endo- and exotoxins.

For their antibacterial properties to take effect, bismuth ions must be absorbed into the cell, but elemental bismuth and its ions almost without exception have low solubility. The most commonly used bismuth drugs contain bismuth subsalicylate (BSS), CBS and RBC. Of these compounds, only colloidal bismuth subcitrate (CBS) and RBC are highly soluble in water (1 g ml^{-1} pure water) [20]. Tests have shown bismuth salts to be most soluble at between pH 4 and pH 7 in gastric juice [10]. However, bismuth from these compounds precipitates in the stomach and small intestine due to the very low pH [21]. The absorption of bismuth from different tested compounds such as CBS, bismuth subnitrate (BSN) and bismuth subsalicylate (BSS) in the small intestine of rats is below 1% [22], indicating low bioavailability of bismuth from these pharmaceuticals in mammalian bodies. Relatively large amounts of bismuth, up to 480 mg per day, are therefore given in treating *H. pylori* infections. Most of the bismuth precipitates in the stomach and the small intestine as BiOCl and bismuth citrate and coats the ulcer site, building a physical barrier against colonization by the pathogen *H. pylori*.

Attempts have been made to improve the membrane permeability of bismuth by coordinating Bi^{3+} with ligands that promote its lipophilic character [13], for example, thiolate ligands, or incorporating bismuth drugs into liposomes to increase its bioavailability for pathogens and thereby decrease the amount of bismuth required to achieve an inhibitory effect [13,17, 23].

Adverse Effects of Bismuth Drugs

A comparison of bismuth-containing quadruple therapy for treatment of *H. pylori* in South Korea over one and two weeks showed an increase in the adverse effects of longer treatment with bismuth [8]. The longer quadruple therapy containing bismuth in particular caused more cases of headache and asthenia. However, it is not clear whether the longer intake of CBS or of one of the other drugs, i.e. pantoprazole, metronidazole and tetracycline, was responsible for the adverse effects observed. Nevertheless, considering the adverse effects of bismuth on human health still seems to be justified.

In France, careless use of bismuth-containing drugs (mainly CBS) led to numerous cases of encephalopathy during the 1960s and 1970s [24]. Bismuth is readily absorbed into the blood after ingestion of CBS [25]. Its transport in blood serum is thought to be mediated by human serum transferrin [4]. Some studies suggest that bismuth can enter the central nervous system by a retrograde axonal transport route, thus circumventing the blood-brain barrier, but also through blood vessels [26,27]. Autometallographical analysis of the human brain in people suffering from (suspected) bismuth intoxication after a long intake of BSN revealed an accumulation of bismuth mainly in neurons and glia cells in the cerebellum, thalamus and neocortex. This is presumably the cause of the myoclonic encephalopathy symptoms observed following (suspected) bismuth intoxication [28].

In addition to the neurotoxic effects, reversible renal failure following high-dose intake of CBS has also been reported [29,30]. The nephrotoxicity of high doses of bismuth is presumably caused by necrosis of proximal tubular epithelial cells [31]. Bismuth can destabilize the membrane of these cells and thereby causes cell death. Another study demonstrates eryptosis on exposure of erythrocytes to >500 μg l^{-1} $BiCl_3$, thus explaining the occurrence of anemia after treatment with bismuth-containing drugs [32].

Recent studies also advise caution in the extended use of bismuth. Bacterial reverse mutation tests and chromosomal aberration tests in cultured mammalian cells have indicated genotoxic effects [33]. A preliminary and as yet unpublished experiment by Bialek suggests that the inorganic bismuth derivative CBS can cause DNA single-strand breaks at concentrations of 250 μM and above in a concentration-dependent manner. The same effect was shown earlier for methylated arsenic and antimony derivatives, presumably caused by formation of reactive oxygen species [34,35].

Admittedly, all the reported toxic effects of bismuth were found after an overdose of bismuth compounds *in vivo* or usage of very high concentrations *in vitro*. Nevertheless, too little attention has been paid so far to microbial transformation of bismuth into methylated derivatives and its toxicological relevance.

Formation of Toxic Methylated Bismuth

As outlined earlier in this review, bismuth drugs have positive antibacterial properties and are beneficial because they do not appear to be met with bacterial resistance and their toxicity to human cells is considered to be low with careful use. However, some prokaryotes

are capable of transforming inorganic bismuth into highly mobile and probably very toxic methylated derivatives.

Production of volatile trimethylbismuth (TMBi) has been reported from different, mainly anaerobic environments [36]. The first report of microbial formation of TMBi was made by Michalke et al. [37]. In this study, the formation of numerous volatile metal(loid) compounds by representative members involved in the anaerobic digestion of sewage sludge was analyzed. Pure cultures of *Methanobacterium formicicum* are capable of producing TMBi from bismuth-containing pharmaceuticals (Bismofalk: bismuth subgallate and bismuth nitrate; Noemin: bismuth aluminate) [38]. Although methylation of elements such as arsenic by a variety of prokaryotes, fungi and even mammalian tissue has been documented [39,40], the capability to produce volatile TMBi does not seem to be as widespread. Methanoarchaea, which can be integral members of the human gut microbiota, are the most versatile organisms with regard to the quality and quantity of methylated derivatives of different metal(loid)s [41]. Equal capability has hitherto only been found for a near relative of the strictly anaerobic Gram-positive bacterium *Clostridium glycolicum*, strain ASI 1, isolated from an alluvial soil with only low levels of contamination by heavy metals and metalloids [42].

The capability of mammalian gut microbiota to produce TMBi was observed in human feces and different gut segments removed from mice fed with De-Nol, a CBS containing drug [43]. A follow-up study of 20 male human volunteers analyzed conversion into TMBi and subsequent distribution in the human body after intake of 215 mg of bismuth (as CBS) [25]. The highest concentrations of TMBi in human breath were observed 8-24 hours after CBS intake, with concentrations of up to 458 ng m^{-3}. TMBi was also found in blood samples. However, bismuth was mainly excreted with the feces. Trials with gut segments of conventionally raised mice and germ-free mice, both fed with chow containing CBS as the precursor for bismuth methylation, points towards involvement of the gut microbiota in metal(loid) methylation, as no TMBi was detected in the blood of germ-free mice [44]. The formation of TMBi by the gut microbiota appears to promote the dispersal of bismuth in mammalian bodies, with a significant accumulation of bismuth being detected in organ tissue.

The formation of toxic TMBi in the human colon may also affect the physiological gut microbiota, as indicated by *ex situ* experiments performed by Meyer et al. in 2008 and later by Bialek et al. [41,45]. Both studies demonstrated growth impairment of pure cultures of *Bacteroides thetaiotaomicron*, a representative of the physiological gut microbiota, by TMBi. A MIC$_{50}$ of 17-30 nM of TMBi was determined. The study by Bialek et al. [45] also showed the inhibitory effects of soluble, partly methylated mono- and dimethylbismuth with a MIC$_{50}$ also in the low nM range. In contrast, the MIC$_{50}$ of CBS is four orders of magnitude higher, demonstrating the greater antibacterial effect of methylated bismuth derivatives relative to inorganic derivatives used in medical applications.

The complex nature of the human gut microbiota and its interactions with the human host makes it difficult to attribute clinical symptoms observed after intake of bismuth to impairment of the gut microbiota by formation of TMBi. As a first step towards predicting the adverse effects of TMBi formation in the gut, investigation has already begun of the molecular consequences of *in vitro* incubation of *B. thetaiotaomicron* with TMBi, i.e. concentration-dependent modification(s) of soluble proteins, membrane-proteins, membrane-lipids and DNA. Nevertheless, many attributes of *B. thetaiotaomicron* are already known. These known attributes make some of the possible

adverse effects on growth impairment of *B. thetaiotaomicon* feasible: as shown by Backhed et al. [46], *B.thetaiotaomicon* thrives on the degradation of complex sugar molecules and releases more simple carbohydrates, which can then be utilized by the human host cells. This bacterium also stimulates angiogenesis [47]. As a consequence, impairment of *B. thetaiotaomicron* in the human gut might lead to a lower uptake of vitamins and a lower energy yield from food. *B. thetaiotaomicron* also represses transcription of proinflammatory genes and attenuates the proinflammatory response to the beneficial gut microbiota [48]. However, *B. thetaiotaomicron* induces expression of the antibacterial protein angiogenin Ang4, which is predominantly directed against pathogenic bacteria, in the Paneth cells of the small intestine [49]. *B. thetaiotaomicron* is therefore directly involved in the regulation of mammalian immune response, which could be disrupted by impairment of this gut inhabitant. While *B. thetaiotaomicron* is one of the most prominent inhabitants of the human gut, it is worth remembering that it is only one member of the very diverse human gut microbiota [46]. Other inhabitants may also be affected by TMBi. Some studies have indicated that intact gut microbiota are involved in the repair of epithelial injury caused by dextran sulfate sodium and thereby contribute to maintenance of the mucosal barrier function [48].

A promising tool for determining the specific individual composition of the gut microflora in order to monitor alterations upon exposure to TMBi. is the Simulator of the Human Intestinal Microbial Ecosystem (SHIME). This five-reactor compartment system can be inoculated with human feces samples and simulates the human intestinal tract under the physicochemical, enzymatic and microbial conditions of the stomach, small intestine and different regions of the colon [50]. This setup allows sampling of microbial communities from different simulated compartments of the human gut and therefore proves useful in studying the behavior and changes in gut microbiota on exposure to TMBi and inorganic CBS. Nevertheless, as this tool cannot simulate the interaction between the physiological gut microbiota and the human host, *in vivo* studies, e.g. with mice, are still necessary.

The methylation pathway of bismuth by Methanoarchaea seems to have been elucidated. Direct links have been shown between the methylation of bismuth and other metal(loid)s (As, Se, Sb and Te) by *Methanosarcina mazei* and a central stage of methanogenesis, the formation of the methane precursor methyl mercaptoethansulfonate (CH$_3$-S-CoM) [51]. The comparison of the methylation and hydrogenation patterns of different metal(oid)s by pure cultures of *M. mazei*, by a non induced cell-free crude extract of *M. mazei*, by recombinant methyltransferase MtaA, which catalyzes the methylcobalamin (CH$_3$-Cob(III))-dependent formation of CH$_3$-S-CoM, and by CH$_3$-Cob(III) with Cob(I)alamin as the reducing agent indicates the non enzymatic methylation and hydrogenation of numerous metal(loid)s by CH$_3$-Cob(III) in the presence of a strong reducing agent. Bismuth methylation in the human body does not necessarily require the presence of Methanoarchaea if sufficient amounts of CH$_3$-Cob(III) and a strong reducing agent are available. Other physiological groups of anaerobic microbes with a high CH$_3$-Cob(III) content, e.g. certain sulfate-reducing bacteria and homoacetogenic bacteria, may also methylate bismuth. *In vitro* analysis of different cells, such as human erythrocytes and lymphocytes, demonstrated a better uptake of monomethyl bismuth (MMBi(III)) than of inorganic CBS and bismuth glutathione (Bi-GSH) [52]. Erythrocytes absorb MMBi(III) more efficiently than highly cyto- and genotoxic monomethyl arsenic (MMAs(III)), which is around 10-times more toxic to human hepatocytes than MMBi(III) at equal molar concentrations [53,54]. However, elevated cytotoxicity of methylated bismuth derivatives relative to Bi-GSH and CBS appears to be caused

by its increased bioavailability due to higher membrane permeability. Soluble, non-volatile MMBi(III) may arise in mammalian bodies either as an intermediate of TMBi formation by the gut microbiota or through TMBi decomposition.

Toxicological studies of TMBi were performed by Sollmann and Seifter in the late 1940s and early 1950s [55]. The authors of this study described neuronal poisoning by TMBi in mammals such as dogs, cats and rats on exposure to non determined, but presumably very high, concentrations of TMBi. They also found that 3-10.5 mg of bismuth (as TMBi) per kg body weight administered intravenously caused poisoning in cats and dogs. The poisoning resulted in symptoms such as nausea, salivation, diarrhea and sometimes emesis.

Conclusion

Bismuth has various faces: it has beneficial effects for humans in that it eradicates certain pathogens, such as *H. pylori* and *P. aeruginosa*, but also adverse side effects as indicated by cases of encephalopathy, renal failure, and suspected cyto- and genotoxicity. The negative effects of TMBi on a member of the physiological gut microbiota, *B. thetaiotaomicron*, have also been demonstrated *in vitro*. This finding should motivate further research on the possible consequences for human health of the formation of TMBi by certain members of the gut microbiota. Both the beneficial and the adverse effects of bismuth are based on the same property of the metal, i.e. its strong affinity to thiols of proteins. However, it is important that bismuth is taken up by cells, a condition dependent either on the concentration or the solubility and lipophilicity of the bismuth derivative. In this context, it is essential that the concentration of bismuth applied in medication does not cause an increased accumulation of the metal in the cytoplasm of human cells. This may prove difficult in practice, as the bismuth compounds in use, i.e. CBS, RBS and BBS, only have low solubility and lipophilicity in the stomach and the small intestine on account of the low pH and are therefore given in relatively high concentrations. However, anaerobic microorganisms with an intensive methylcobalamin metabolism like Methanoarchaea are capable of converting inorganic bismuth into highly mobile, membrane-permeable and therefore toxic methylated bismuth derivatives in the human gut. These findings should be considered in the medical application of bismuth, since the formation of methylated bismuth derivatives in the human gut may damage mammalian cells as well as the physiological gut microbiota.

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