

The Subcellular Localisation and the Time Course of Bismuth in the Gastric Mucosa of Rats after Short-term Administration of Colloidal Bismuth Subcitrate

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Abstract

The aim of this study was to investigate if colloidal bismuth subcitrate (CBS) can penetrate the gastric mucus barrier to reach the different sites of the antral mucosa and to estimate the time course for CBS to reach and remain in the mucosa. A single dose of CBS was administered orally to rats that were sacrificed at different time intervals post treatment. The control group received gum acacia without CBS. Colloidal bismuth subcitrate, visualised as electron dense precipitate (EDP), was seen in the gastric mucus layer, intercellular spaces and intracellularly after 30 minutes and disappeared after 6 hours. Scant amounts of EDP were observed in the gastric crypts, confined only to the upper parts of these structures. We concluded that CBS can penetrate the mucus and has a wide but uneven distribution in the gastric mucosa. Colloidal bismuth subcitrate, in the concentration given only penetrated the upper two-thirds of gastric pits and not the lower one-third. We also concluded that CBS has to be given 6 hourly to ensure its continuous presence in the gastric mucosa.

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Introduction

Bismuth compounds were first introduced in medicine in the 18th century and were first advocated for the treatment of dyspepsia. They are being evaluated for their antibacterial activity against *Helicobacter pylori*, an organism with strong pathogenic implications in duodenal ulcers.

The bactericidal action of colloidal bismuth subcitrate (CBS) against *H. pylori* has previously been demonstrated.¹ Despite this, eradication rates achieved are poor. Data from different studies show that a 4-week course of CBS eradicates *H. pylori* in only 20% of patients.² A successful therapeutic agent would be one that is able to kill all the organisms present in the gastric mucosa, thus minimising relapse due to recrudescence. This would be possible if the bactericidal agent is able to reach all areas colonised by the organism. Studies on the pharmacokinetics of CBS in the gastric mucosa are limited. Although it has been shown that CBS is taken up by the antral and fundal mucosa,³ the localisation and the distribution of CBS in the different structures in the gastric mucosa has not been explored. An electron mi-

croscopic study suggests that CBS does not penetrate the mucus barrier of the stomach⁴ as opposed to the findings of other investigators.^{2,3}

The findings in our previous work indicated that CBS was ineffective in the eradication of *H. pylori* from the gastric mucosa. We postulated that one of the reasons for this is the failure of CBS in reaching some areas colonised by the organism or alternatively inadequate dosing interval. Hence, we undertook to investigate the distribution of CBS in the antral mucosa and estimate the time course for CBS to remain in the antrum.

Materials and Methods

Preparation of CBS Suspension

Colloidal bismuth subcitrate (CBS) tablets containing 120mg Bi₂O₃ were obtained from PN Gerolymatos SA, 35, Mihalakopoulou Street, 115 28 Athens, Greece by Rhone-Poulenc Malaysia. The tablets were soaked in 10% acacia gum and were left overnight to soften after which the suspension was stirred using a magnetic stirrer for 1 hour to further dissolve it. The suspension

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was vortexed each time before administration to the animals.

The dose of CBS used for an adult weighing 60 kg is 240 mg. The dose used in the rats in this study was 60 times that of the human (adult) dose based on per kg body weight.

Animals

Thirty-five male Wistar rats weighing 200 g to 250 g were divided into two groups; the treated ($n = 21$) and control groups ($n = 14$). The rats were not given any food for 48 hours prior to treatment with a known amount of CBS suspended in gum acacia while the control group received only gum acacia. Administration of the suspension was through an orogastric tube to minimised adherence of CBS particles to the oral and oesophageal mucosa.

The antral mucosa of 3 treated and 2 control rats were examined at each time point of 0, 15, 30, 60 minutes and 1, 2, 3 and 6 hours post treatment. The rats were sacrificed by cervical dislocation, the abdomen opened and the stomach removed. A complete circumference of the antrum was cut. The thickness of each circumference was 1 mm to 1.5 mm. Portions of the antrum were cut along the circumference and alternate portions were included into the electronmicroscopic study. Each cube of the antral tissue contained layers of the stomach, from the mucosal surface until the muscularis mucosa.

Preparation of Tissue for Electron Microscopy

After the excision, the antral cubes were immediately fixed in 3% glutaraldehyde and postfixed in osmium tetroxide according to previously described standard methods.⁵

After dehydration, the tissue specimens were embedded into epoxy resin. After the resin had hardened, the block of tissue was taken out using a razor blade and secured in a holder to avoid movements of the tissue while the excess resin was cut under a binocular microscope exposing the tissue. The exposed tissue was cut to the shape of a trapezium with a 50-degree surface gradient on each of its sides.

The tissue specimens, cut to a thickness of 1 μm with an ultramicrotome were collected in a small container with a few drops of water. Each tissue section was taken with a hair probe and placed on a glass slide in a drop of water and left on a heating element of 60°C for 30 minutes, after which toluidine blue was poured over the section and left heated at the same temperature for 30 seconds to 1 minute. The excess stain was washed away with tap water and the slide was dried with an absorbent paper and examined under a light microscope to ensure that tissue sectioning was properly done and that the specimens contained the whole thickness of the antral mu-

cosa. After identifying a suitable part of the specimen (i.e. that contained the whole thickness of the antral mucosa), the tissue block was further cut leaving only the portion of tissue to be examined and this was cut to the shape of a trapezium. Using an ultramicrotome, the tissue was subsequently cut to contain only one cell layer. These sections were collected in ethanol and later placed on a grid on a filter paper in a petri dish and left to dry.

The staining of the ultrathin sections with lead citrate was done according to the method described by Reynolds.⁶ The staining was to increase the ability of the tissue to reflect electron by increasing the density of electron in the specimen through the deposition of lead with a high atomic number on or inside the tissue sections. The stain was placed on the grid for 5 to 10 minutes. After that, the grid was washed with 0.02N NaOH followed by distilled water. Subsequently, the grid containing the tissue section was examined with a transmission electron microscope.

Control material was obtained from animals who had received gum acacia without CBS. The processing of these samples was identical to those above.

Results

Colloidal bismuth subcitrate was seen as electron dense particles (EDPs) in the presence of hydrochloric acid. They appeared as dark particles singularly, in clusters or as a beaded chain in the specimens. At 0 minute, EDP was seen only in the mucus layer that coated the mucosal surface and in the mucus surrounding single epithelial cells that had fragmented from the mucosa during processing. After 15 minutes, EDP was seen to have penetrated the mucus barrier and numerous particles were seen to occupy the intercellular spaces with some also seen in the epithelial cells. In the epithelial cells, EDP only congregated in the cytoplasm. None were seen in the cytoplasmic organelles or the nucleus. The particles of the bismuth complex were also seen in the coated pits, confined however, only to the upper portions of the pits. Electron dense particles were seen in the mucin containing vesicles as well as the empty ones. During this interval, EDP could also be found in the lamina propria and small particles were occasionally visualised in the endothelial cells of the artery. No EDPs were seen in the muscularis mucosa.

After 30 minutes, the quantity of the particles in the mucus layer had decreased. Electron dense particles continued to enter the epithelial cells and were frequently seen in the upper third of the gastric pit but not observed in the middle or lower third of the pit. The distribution pattern of EDPs in the animals treated for 1 and 2 hours was quite similar to that treated for 30 minutes. The bismuth particles remained readily identifiable in the cytoplasm but were occasionally seen to enter the membrane bound cytoplasmic organelles, but

none were seen in the nucleus.

The distribution of EDPs was more clustered after 3 hours, seen mainly in the deeper cells and not much in the luminal epithelial cells. The amount of particles observed in the cells had also considerably decreased compared to the section examined after 2 hours of treatment. The subsequent observations at 6 hours post treatment revealed only scant amounts of EDP, only in some superficial epithelial cells and hardly any in other areas. Controlled specimens were consistently negative for the distinctive electron dense particles. A summary of the distribution of EDPs is represented in Table I.

TABLE I: THE DISTRIBUTION OF EDPs AT DIFFERENT TIME INTERVALS

Time (min)	Mucus layer	Intercell spaces	Intracell spaces	Gastric pits	Lamina propria
	+++				
15	+++				
30					
60		+++	+++	*+	
120		+++	+++		
180		++	++	-	-
360		-	-	-	-

Heavy : +++; Moderate : ++; Scanty : +
* upper two-thirds of gastric pits

Discussion

Our study confirms that in the stomach of rodents, CBS forms electron dense particles. In this study, CBS was found in the gastric mucus layer and penetrated the gastric barrier to reach the gastric mucosa. These findings are consistent with that of Stiel et al.³ In contrast, Coghill et al⁴ observed that the accumulation of bismuth complex was only in the mucus layer but did not appear to reach the gastric epithelium even one and a half hours post treatment. Although the CBS particles reached the gastric mucosa, the distribution of these particles in the mucosa was not homogenous throughout the mucosa. Colloidal bismuth subcitrate did not penetrate to the deeper parts of the gastric pits that are colonisation sites for *H. pylori*.⁷ Organisms colonising this “sanctuary site” would be spared of the bactericidal action of CBS and, after treatment is stopped, would presumably resume propagation and colonise the gastric mucosa resulting in recrudescence. It has been shown in other studies through analysis of strains by restriction endonuclease DNA analysis that *H. pylori* subtypes isolated after relapses did not differ from that of the initial infection.⁸

Several studies have shown that, compared to treatment with only antibiotics, CBS given with or without antibiotics effectively suppressed *H. pylori* but the relapse rate is high.⁹⁻¹² Other studies have shown that the strain of the organism is the same in many cases of

reinfection.^{2,8} In our previous work,¹³ we found that after a course of CBS with ampicillin, the suppression rate for patients re-evaluated for the presence of *H. pylori* after 48 hours was twice as high as those patients re-evaluated after 2 weeks. It is possible that organisms in the sanctuary sites proliferated during the 2 weeks after cessation of therapy and recrudescence occurred as a result of incomplete eradication. The overall implication, however is that the therapy was inadequate and ineffective in killing the whole of the bacterial population.

Our study attributes that failure of CBS in the treatment of *H. pylori* infection is due to the limited time that CBS particles were present in the gastric mucus layer and the gastric mucosa for a maximum of 2 and 6 hours, respectively. This short duration of CBS presence in these two areas that are usually heavily colonised by *H. pylori* in patients with the infection indicates that CBS is not able to exert maximum bactericidal effect on the organisms due to insufficient contact time. Marshall et al¹ had previously observed rapid bacterial degradation and detachment from the epithelial surface membranes in *H. pylori* positive endoscopic biopsy specimens after a 2-hour exposure to bismuth. In contrast, *in vitro* studies have shown that most bacteria contained visible bismuth complex under the cell wall after the bacterial cultures were exposed to bismuth for at least 8 hours.¹⁴ The same study also showed that the bactericidal effect manifested only after 8 hours. Although *in vitro* conditions and the stomach environment are certainly different, and that the antibacterial action may be enhanced or modified by factors present in the gastric environment, data from clinical trials employing “a four times a day dosage regime” appear to have more favourable results compared to studies with a bi-daily dosing regime.* This suggests that a more successful treatment modality is one that is given 6 hourly as this would ensure continuous contact of CBS with the organism. This fact, in turn, is supported by our finding that CBS only stays for about 6 hours in the gastric mucosa.

Based on our findings which illustrate that CBS does not reach all areas said to be colonised by *H. pylori* coupled with the inadequate contact time of bismuth with the organism in areas where CBS is distributed, it is possible that the high relapse rates of *H. pylori* infection are probably due to submaximal antibacterial effect of CBS resulting in incomplete eradication of the organism. We suggest that CBS be given consistently four times a day and combined with other non-absorbed agents with good topical activity in an acidic environment of the stomach or with absorbed agents with good systemic activity to achieve better results.

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