ANNALS OF GASTROENTEROLOGY 2000, 13(2):118-121

*Original article*

# The influence of ranitidine bismuth citrate on rat gastric mucosal microcirculation and adherent mucus gel layer

Katerina Kotzampassi, D. Paramythiotis, A. Voudouris, K. Milias, E. Eleftheriadis


## SUMMARY

**Ranitidine Bismuth Citrate (RBC), recently developed to fight H. pylori, exhibits a triple action: anti-bacterial, anti-secretive and mucosal-protective. Since less research has focused upon its mucosal protective action, we decided to study two parameters concerning that topic, i.e. the gastric mucosal blood flow and the adherent mucus gel thickness. Twenty rats were treated by gavage with either 25mg/kg RBC or equal volume (1ml) of drinking water; 60 min later, laparotomy was performed, the stomach opened along the greater curvature and gastric mucosal blood flow assessed by laser-Doppler flowmetry. After this measurement, five - 20mm long- fullthickness strips were sectioned from the stomach, mounted transversely on glass plates and viewed under a light microscopy using an eyepiece graticule (4 measurements/strip). Rats treated with RBC were found to exhibit a statistically significant increase in gastric mucosal blood flow (36.29±16.64 vs 88.99±12.23 relative units of flow, p=0.001) as well as in adherent mucus gel thickness (69.30±21.7 vs 222.39±47.71μm, p=0.0001). It is thus concluded that the ability of RBC to increase gastric mucosal blood flow and adherent mucus gel thickness may contribute to the protective action of this compound.**


**Key words:** gastric mucosal blood flow, adherent mucus, ranitidine bismuth citrate, H. pylori

## INTRODUCTION

Ranitidine bismuth citrate (RBC) is a relative new compound that seems to prossess the therapeutic properties of both ranitidine and bismuth, i.e. anti-secretory, mucosal protective and anti-bacterial against H. pylori.[1-4] Since the major goals of this therapy are ulcer healing and H. pylori eradication, pharmacological research has focused mainly on this area, leaving the mucosal protective properties of this compound not fully elucidated.

The three studies, two experimental[5,6] and one clinical,[7] performed to date, and dealing with its cytoprotective effects refer essentially to its properties in preventing ethanol or indomethacin-induced mucosal injuries; inhibition of acid secretion, pepsin isoenzymes, gastric mucosal blood flow and mucus secretion were tested against the extent of gastric mucosal damage. However, the intact gastric mucosa, i.e. the gastric mucosa not challenged by damaging agents, is considered as the optimum condition for testing the cytoprotective properties of RBC, since no prostaglandins or other compensative mechanisms interfere.

Thus, the present experimental study was designed to investigate in the intact rat gastric mucosa whether RBC works towards the strengthening of mucosal defence factors, namely the gastric mucosal blood flow and the adherent mucus gel thickness.

## MATERIAL AND METHODS

### *Study Design*

Twenty adult male Wistar rats (250-300gr) were included in this study. All rats were housed together for a 1-week adaptation period, in room temperature of 24˚C and with an alternating 12h light-dark cycle and had free access to tap water and stadard rat food (Biozokat, Plati, Greece).

Eighteen hours prior to the experiment the rats were deprived of food but allowed free access to water, while

*Department of Surgery, University of Thessaloniki, Greece*

*Author for correspondence:*

E. Eleftheriadis, 33, Them. Sofouli str, Thessaloniki, GR 546 55 Greece, fax: (3031) 993496, e-mail: elemakis@med.auth.gr

60 min before they were treated by gavage with either 1ml of drinking water (control group) or 25mg/kg of body weight of RBC (Pylorid, GlaxoWellcome, Greece), diluted in equal volume of drinking water.

Sixty minutes after treatment, all rats were subjected to a midline laparotomy, under 50mg/Kg BW ketamine plus 10mg/Kg BW xylazine anesthesia. The stomach was dissected along the greater curvature, from the forestomach to the first duodenal portion, and the posterior mucosa wall was gently wetted with 1ml of 0.15M NaCl, to prevent dehydration.

### *Gastric mucosal microcirculation measurements*

A laser-Doppler self-adhesive single fibre probe (p318, Perimed, Sweden) was attached to the mucosa of the anterior stomach wall, at a standard area of the gastric corpus, for mucosal blood flow measurements. This area, considered to be the highest perfused one, according to our previous studies,[8] is defined as the cross-section of two hypothetical axis, a horizontal and a vertical, dividing the gastric wall into quarters. Four consecutive measurements were blindly performed per animal, each one lasting for 1 min.

The Periflux PF2B (Perimed, Sweden) flowmeter was used, working at a frequency of 4kHz with a time constant of 0.2 sec, and flow values, stored in a PC, through an A/D convector, were expressed in units of relative flux.

### *Measurement of adherent mucus gel thickness*

After compltion of the blood flow measurement, the stomach was removed and four adacent strips, 20mm long, were cut from the posterior wall, using a homemade cutting instrument, consisting of five parallel surgical blades fixed 1,0mm apart. Sections were mounted transversely on glass plates and examined under light microscope (magnification x125). Surface gel thickness was determined (in μm) using an eyepiece graticule. Measurements were performed at 5mm intervals along each strip and means of mucus thickness of each stomach were computed from 20 measurements (5 readings x4 strips) per animal. The whole procedure - blindly performed by a method established by Sandzen et al[9] and used by us in a previous study[10] - lasted, from the time of stomach excision until measurement of gel thickness, less than 10min.

### *Statistical analysis*

All data were expressed as mean + SD. Student's t-test for unpaired variables was used for statistical evaluation of measurements and a p value less than 0.05 was considered as significant.

## RESULTS

Rats treated with RBC were found to exhibit a statistically significant increase in gastric mucosal blood flow (88.99±12.23 relative units of flow) in relation to controls (36.29±16.64, p=0.001). Similarly, the adherent mucus gel thickness was found significantly increased (222.39±47.71μm) in RBC treated rats in relation to controls (69.30±21.7μm, p=0.0001) (Figure 1).

## DISCUSSION

The few experimental studies that have been performed to date concerning the gastro-protective properties of RBC, deal with its effects on ethanos (fundic)[5] or indomethacin-induced (antral) damage,[5,6] after administration as pretreatment, but no study has been conducted mainly for the evaluation of its action on the intact gastric mucosa.

The present experimental study was designed to evaluate the gastroprotective properties of the anti-ulcer agent RBC, which is considered to combine in one molecule the gastric antisecretory activity of ranitidine with the mucosal protective, antipepsin and anti H. pylori properties of bismuth containing compounds.[1,5,11] In respect to gastric mucosal blood flow, the data obtained from our study revealed a significant improvement in gastric mucosal perfusion just an hour after a single oral dose of 25mg/Kg BW of RBC. This is an interesting finding, as it is widely accepted that blood flow plays a fundamental role in the maintenance of gastric mucosal integrity, since it regulates the transport of oxygen and nutritional substances and the fast removal of toxic metabolites and back-diffused hydrogen ions and other irritants.[12,13]

Additionally, considering peptic ulcers as low perfused areas, it has long been accepted that increased mucosal blood flow to the ulcer margin at the healing stage has a significant effect on the healing process.[12,14-16] Similar results of increased gastric mucosal blood flow have been reported by us previously, concerning the influence of long-term colloidal bismuth subcitrate treatment on the rat's gastric mucosal microcirculation.[17]

On the contrary, Tanaka et al,[6] working in an indomethacin-induced gastric corpus injury rat model, reported no alteration in gastric mucosal blood flow, despite the fourfold dose of GG311 compound (RBC) used (100mg/Kg BW in relation to 25mg/Kg) by us. This dis-

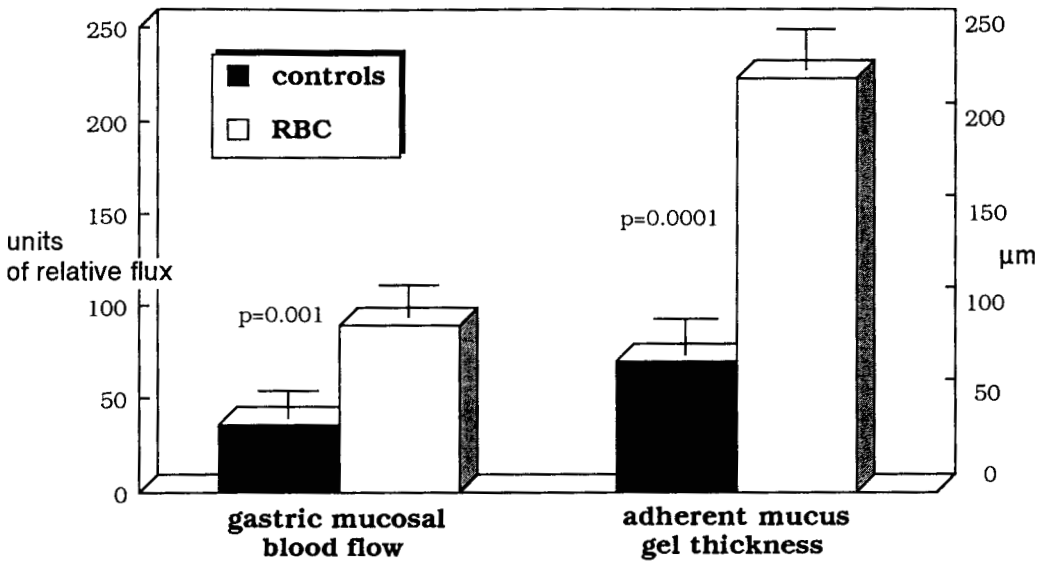


***Figure 1.*** A significantly increase in gastric mucosal blood flow and adherent mucus gel thickness after RBC treatment.

crepancy supports different hypothesis about the way by which bismuth containing compounds work, but the exact mechanism of their action is, as yet, undefined. Thus, it is accepted that they increase neutral glycoproteins biosynthesis, inactivate pepsin, increase bicarbonate secretion, enhance endogenous prostanoids, and act as a physical protective coating of the gastric mucosa.[11,18,19]

However, Konturek et al,[18] based on the fact that colloid bismuth subcitrate was able to prevent aspirin-induced gastric lesions, when the generation of prostaglandin was suppressed by indomethacin, suggested that other factors, not related to prostaglandin, may also be involved in the protection mechanism. Thus, it is not unreasonable to suggest that such a factor may be the improvement of mucosal hemodynamics, which is associated with the increased mucus and bicarbonate secretion.[12]

This argument led us to the other part of our study, concerning the adherent mucus gel thickness as a protective mechanism. Mucus is the other important defense factor of the gastric mucosal barrier. its main active role consists in causing a delay of backdiffused $H^+$ and enabling their neutralization by $HCO_3^-$ contained in its mesh. The adherent mucus gel layer acts as a physical barrier against luminal pepsin, its function depended on its thickness and the gel structure, which is linearly depended on the polymeric mucin content.[20] The findings of the present study support the gastroprotective properties of RBC, by means of increasing the adherent mucus gel thickness, to a highly significant degre. Our findings are in contrast with those of Tanaka et al,[6] who reported no significant effect of RBC on the adherent mucus gel thickness of the normal gastric mucosa used as control, but significant differences exist between the studies: the dose of RBC, the time of measurement and the technique used. The dose administered to our experiment was the same used by Stable et al,[5] who found it to be sufficient enough to prevent ethanol-or indomethacin-induced gastric mucosal damage. This dose, being four times less than that used by Tanaka et al,[6] resulted in a significant increase of adherent mucus gel thickness. Additionally, the time interval between RBC administration and microscopy was as long as 150 min in Tanaka's study, but 60min in our own. Finally, difference exists in the technique used: Tanaka assessed by means of in vivo microscopy one optical field per rat/stomach, while we measured the adherent mucus gel thickness at 5 points X 4 specimens per rat/stomach by means of direct light microscopy.

Although bismuth-containing-compounds are implicated with the enhancement of mucus production, through prostaglandin's pathway, many experiments deal with increased mucus contents by $H_2$ antagonists, too. From the time of cimetidine, an increased biosynthesis of mucoproteins has been demonstrated in the mucus-membrane of the corpus-antrum region in cimetidine treated rats.[21] A previously performed study in our laboratory revealed a highly significant increase of prostaglandin $E_2$, as well as both intracellular mucus accumulation and adherent mucus gel thickness, after famotidine

treatment[9] in the rat. Similar findings were reported for roxatidine, which caused increases in the secretion and synthsis of mucus in a dose-related manner,[22] while Ichikawa et al,[23] using an organ culture system of the rat stomach, reported that roxatidine-induced activation of mucin biosynthesis is mediated by nitric oxide. Additionally, it is reported that bismuth accumulation in the gastric mucus during the evolution of mucosal injury may play an important role in the gastroprotective effect against indomethacin-induced injury.[24] Thus, we may suggest that both bismuth and ranitidine are implicated in the process of strengthening the mucus barrier.

In conclusion, the present study performed in fasted rats let us to suggest that the ability of RBC to increase gastric mucosal microcirculation and adherent mucus gel thickness may contribute to the protective action of this compound.

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