STUDIES ON ACID SOLUBLE THIOLS IN THE HUMAN GASTRIC JUICE

S. Nalini and K.A. Balasubramanian

The Wellcome Trust Research Laboratory, Department of Gastrointestinal Sciences, Christian Medical College & Hospital Vellore 632 004, INDIA.

Received October 27, 1993
Received after revision, January 19, 1994

SUMMARY: Human gastric juice was found to contain high levels of thiols as detected by reaction with dithionitrobenzoic acid (Ellman's reagent). Analysis of gastric juice for glutathione and cysteine, the known acid soluble thiols in biological system, by HPLC and calorimetric methods, indicated that they are absent in the gastric juice. Further studies on the nature of thiols in the gastric juice, by Sephadex G-25 gel filtration indicated the presence of both high and low molecular weight protein and peptide associated thiols. Dialysis of the gastric juice retained nearly 70% thiols in non-dialysable fraction. These studies suggest that biologically important low molecular weight thiols, glutathione and cysteine are absent in the human gastric juice and the thiols detected are mainly acid soluble proteins and possibly peptide associated thiols.

INTRODUCTION

Thiols play an important role in cellular function which include modulation of activity of thiol sensitive receptors and enzymes and as free radical scavengers [1]. The important cellular nonprotein thiols are glutathione (GSH) and cysteine and earlier studies have stressed the importance of GSH in gastric cytoprotection [2]. It was shown that GSH is involved in protection against gastric ulceration [3] and it inhibits stress induced gastric injury [4] but later studies have shown that depletion of GSH by diethyl maleate offers protection to gastric mucosa [5]. High levels of thiol as detected by Ellman's reagent (DTNB) was found in the acid soluble fraction of stomach mucosa and these thiols were assumed to be glutathione (6-9). We have earlier shown that human gastric juice contains high content of thiols and there was no significant difference in the gastric juice thiols between normals and patients with duodenal ulcer [10]. In all these studies thiols were estimated with Ellman's
reagent which can give positive colour with both protein and peptide thiols. Since gastric juice contains acid soluble proteins, the present study looks at the nature of acid soluble thiols in the human gastric juice.

**MATERIALS AND METHODS**

Bovine serum albumin (BSA), 5,5′ dithiobis 2-nitro benzoic acid (DTNB), 1-fluoro-2,4 dinitro benzene (FDNB), reduced and oxidised glutathione, cysteine, cystine and Sephadex G25 were all purchased from Sigma Chemical Co., U.S.A. All other reagents used were of analytical grade.

**Gastric juice collection:** Gastric juice was collected from subjects undergoing upper GI endoscopy as part of the routine investigation after an overnight fast. A sterile teflon cannula was passed through the biopsy channel of the endoscope and the residual gastric juice in the stomach was aspirated into a syringe and discarded. Gastric juice was then aspirated for the next 15 min. taking care to avoid trauma to the mucosa. Gastric juice was centrifuged at 10,000g for 20 min in a refrigerated centrifuge to remove mucus and other debris. Juice contaminated with bile or blood by visual detection was not used. The pH was measured and the juice was then frozen at -20°C and analysed within 4 weeks.

**Thiol group estimation:** Thiols in the gastric juice was measured using Gillman's reagent as described [11]. To an aliquot of the gastric juice (made upto 1ml with water), 2ml of 200mm Tris/ACl buffer pH 8.6 containing 2mm EDTA and 30μl of 10mm DTNB were added, the tubes mixed well and kept in the dark for 15min at room temperature. The intense yellow colour of the nitromercapto benzoate anion formed from DTNB reaction with thiol was read at 412nm. Glutathione was used as standard and the values are expressed as nmol/ml juice.

**Glutathione estimation:** Reduced (GSH) and oxidised glutathione (GSSG) in the gastric juice were quantitated using HPLC after derivatisation as described [12]. The derivatisation procedure included reaction of iodoacetic acid with thiols to form s-carboxymethyl derivatives which is followed by chromophore derivatisation (dinitrophenyl) of primary amines with 1-fluoro-2,4 dinitro benzene. Dinitrophenyl derivatives were separated on Ultrasil NH2 column using a gradient of methanol and sodium acetate and detected at 365 nm.

**Estimation of cysteine and cystine:** Cysteine and cystine were estimated colorimetrically using acid ninhydrin reagent [13]. The reaction mixture contained 1ml of the gastric juice, 1ml of glacial acetic acid and 1ml of ninhydrin reagent (250mg of ninhydrin in a mixture of 6ml glacial acetic acid and 4ml of 0.6M phosphoric acid). The tubes were heated in a boiling water bath for 10 min, rapidly cooled and diluted with 5ml of 95% ethanol and the absorbance was read at 560nm. A standard curve for cysteine was run simultaneously.
For cystine estimation the pH of the gastric juice was adjusted to pH 8.0-8.5 with NaOH using phenolphthalein as indicator. To this, 5mmole of dithiothreitol was added to reduce cystine to cysteine. After incubating this mixture for 30min at room temperature, total cysteine was determined using the above ninhydrin method. The amount of cystine was obtained by the difference between the two values.

Gel Filtration: Approximately 50-60 ml of the clear gastric juice was lyophilised and this was dissolved in 4ml of glass distilled water. 2ml of this concentrated gastric juice was subjected to gel filtration on a Sephadex G-25 column (50cm x 1cm) previously equilibrated with 5M ammonium acetate pH 6.0. The flow rate was kept at 20ml/hour and 2ml fractions were collected. The fractions were checked for 280nm absorption and thiol groups (412 nm) measured using Ellman's reagent.

RESULTS AND DISCUSSION

Gastric juice obtained from different individuals were measured for thiols which showed high levels of DTNB reactive thiols ranging from 7 to 50 nmole/ml juice. The content of DTNB reactive thiols varied from individual to individual. Since the gastric juice proteins were not precipitable by acids, the DTNB reactive thiols represent protein as well as non-protein thiols.

In order to further characterise this acid-soluble thiols, gastric juice was dialysed for 4 hours against glass-distilled water pH 6.0 at 4°C and the thiols were measured in the contents of the dialysis bag and in the concentrated dialysate. It was observed that approximately 30% of the total thiols were dialysable and there was variation in the dialysable thiol content in different juices. Gastric juice from 6 individuals were separately dialysed and analysed for thiols. Gastric juice thiols were found to be heat stable. No reduction in the thiol content was observed when the gastric juice was subjected to heating in a boiling water bath for 10 minutes. Further, when concentrated gastric juice was subjected to gel filtration on Sephadex G-25, both high and low molecular weight thiols were observed (Figure 1). Four individual juice were subjected to gel filtration on Sephadex G-25 after concentration and very little variation in the pattern of gastric juice thiols were observed among different gastric juice. Molecular weight fractionation range for Sephadex G-25 is 1000-5000 and appearance of a significant portion of thiol in the void volume suggest the
presence of high molecular weight protein associated thiols in the juice. This also suggested that gastric juice contain number of different molecular weight thiol containing compounds.

Glutathione and cysteine are the major physiologically important acid-soluble thiols and they also react with DTNB. Quantitation of glutathione by a specific HPLC method after derivatisation showed the absence of this thiol in the gastric juice (Figure 2A). This again was carried out in 6 individual gastric juices. Glutathione was not detected by HPLC even in the concentrated gastric juice. When recovery experiment was performed by adding exogenous GSH to the gastric juice and then subjected to HPLC separation, it was observed that approximately 80% was recovered as GSH and the rest 20% appeared as the oxidation product, GSSG (Figure 2B). This suggested that gastric juice contents do not interfere in the estimation of glutathione by HPLC and further confirms the absence of this compound in the gastric juice. Absence of cysteine and cystine in the juice was confirmed by specific calorimetric method.

These results suggest that human gastric juice contains DTNB reactive acid soluble thiols which is probably
Figure 2: Quantitation of gastric juice glutathione by HPLC. (A) Native gastric juice. (B) Exogenous glutathione added gastric juice. The detailed methodology is given in text.

contributed by both high molecular weight acid soluble protein thiols and low molecular weight peptide thiols, probably derived by protease action on proteins [14]. Earlier work by others on the gastric mucosal thiols have shown that more than 80% of the thiols are contributed by glutathione and this was shown by comparison of the Ellman's reaction and specific HPLC method [15,16]. However this study has shown by specific methods, the absence of glutathione and cysteine in the gastric juice. Although gastric mucosa is known to contain considerable amount of glutathione, to our knowledge this is the first report to show the absence of glutathione and cysteine in the human gastric juice. This may prove to be more significant in the light of the recent search for SH-related gastroprotective compounds as drugs in clinical gastroenterology.
ACKNOWLEDGEMENTS: The Wellcome Research Unit is supported by the Wellcome Trust, London. Financial assistance from the Council of Scientific and Industrial Research and Indian Council of Medical Research, Government of India is acknowledged. S.N. is a Senior Research Fellow of the Council of Scientific and Industrial Research, Government of India. The authors thank Dr. B.S. Ramakrishna for supplying gastric juice samples and Professor V.I. Mathan for his keen interest in this work.

REFERENCES


454