A new method of separation and quantitation of mucus glycoprotein in rat gastric mucus gel layer and its application to mucus secretion induced by 16,16-dimethyl PGE\(_2\)

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Summary: A method was established for recovering the mucus gel layer of rat gastric mucosa without damage to underlying surface epithelium. The mucus gel was solubilized by stirring the gastric mucosa in a solution of N-acetylcysteine (NAC), a mucolytic agent. Optimal mucus gel solubilization was possible by treatment with 2% NAC for 5 minutes at room temperature. Mucus glycoprotein was quantitatively extracted and measured from the mucus gel sample obtained by the NAC treatment. This treatment caused no damage to surface epithelial cells, as observed by a light microscope. Besides NAC, pronase solution was also adequate for solubilizing the mucus gel layer without any damage to the surface epithelium. However, extraction and measurement of mucus glycoprotein from the pronase-treated mucus gel sample was not possible due to contamination by high molecular hexose-containing substances which were eluted along with the mucus glycoprotein from the column of Bio-Gel A-1.5m. This NAC method was used to examine changes in mucus glycoprotein content in the mucus gel at one hour following the oral administration of 16,16-dimethyl prostaglandin E\(_2\). A significant increase in mucus glycoprotein of the gel was brought about by the prostaglandin treatment. Thus, the present method was suitable for estimating the amount of mucus secreted in to the mucus gel layer. *Gastroenterol Jpn* 1991;26:582-587

Key words: 16,16-dimethyl prostaglandin E\(_2\); mucus gel; mucus glycoprotein; N-acetylcysteine

Introduction
The mucus gel layer, which covers the mucosal surface of the stomach, has lubricant and viscoelastic features which serve to protect the surface epithelium from mechanical irritation by food or chemical stimulation by ethanol, hydrochloric acid and various drugs. The mucus gel may possible hold bicarbonate ions that are secreted from the mucosa to neutralize hydrogen ions diffused from the gastric lumen. In this manner, it may protect the gastric mucosa against endogenous stimulants and maintain the homeostasis of the gastric mucosa. A number of morphological and physiological investigations have been undertaken to clarify the functions of the mucus gel layer by examining mucus gel thickness with pathological changes in the gastric mucosa\(^{1,2}\), permeability of hydrogen ions in the isolated mucus gel\(^3\), and the neutralization of back-diffused hydrogen ions by bicarbonate ions in the mucus gel\(^4,5\). In regard to the gastric mucus glycoprotein which is a major and important component of the gastric mucus gel, the preservation of its polymeric structure may be essential for maintaining its gel forming and viscoelastic features\(^6,7\). Until recently, no appropriate method has been available for recovering the mucus gel without damage to surface epithelial cells. The present study was thus conducted to devise
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a method for recovering the mucus gel layer from the rat gastric mucosa without the possibility of such damage and to determine mucus glycoprotein content of the recovered specimen. Using this method, changes in mucus glycoprotein content in the mucus gel layer were determined following the oral administration of 16,16-dimethyl prostaglandin E₂, which has been found to affect mucus secretion in the rat gastric mucosa.

Materials and Methods

1. Experimental animals

Ninety-six animals of 7-week-old male Wistar rats each weighing approximately 160g were used. All were made to fast for 24 hours before the experiments.

2. Methods

Recovery of the mucus gel layer

The excised stomach from each rat was cut along the greater curvature and the stomach contents and gastric juice were gently rinsed out from the gastric mucosa with Ca²⁺, Mg²⁺-free phosphate buffered saline (PBS(−)). The duodenum connected to the stomach was clipped to the inner wall of a plastic vessel containing 20 ml of PBS(−) in which there were 0.5% pronase and 0.5-5% w/v N-acetylcysteine, a proteolytic and mucolytic agent, respectively. Three stomachs in a vessel were immersed in a solution of each of these agents and gently stirred about with a magnetic stirrer for 2, 5 or 10 minutes at room temperature. Then the stomach were gently washed with 10 ml of PBS(−). The solutions obtained from these two steps of stomach rinsing were pooled and lyophilized. The powdered sample thus obtained was dissolved in 4 ml of 50 mM Tris-HCl, pH7.2, containing 2% Triton X-100 (Triton-Tris buffer), after centrifugation at 4°C for 20 min, the supernatant was collected.

Isolation and measurement of mucus glycoprotein in the mucus gel layer

An aliquot of the supernatant was applied onto a Bio-Gel A-1.5 m column (φ1.5 × 50 cm) and eluted with 2% Triton-Tris buffer. The void volume fraction (Fr. 1) was collected as mucus glycoprotein. Glucose content in this fraction was measured by the phenol-sulfuric acid method using galactose as the standard. Mucus glycoprotein content was expressed as μg glucose per stomach.

Light microscopical observation of gastric mucosal surface epithelium

Examination was made to determine whether the gastric mucosal surface epithelium had been damaged by treatment with NAC or pronase, or by conventional mechanical treatment of mucus gel removal such as scraping by a rubber spatula or wiping with surgical gauze. For this purpose, gastric tissues of six groups of two animals each were fixed in cold phosphate-buffered 10% formalin, embedded in paraffin blocks and serially cut into sections. These sections were stained with the hematoxylin-eosin (H-E) or alcian blue (pH 2.5)-periodic acid Schiff (AB-PAS) sequence, and observed by light microscopy.

Changes in mucus glycoprotein content in the mucus gel layer and mucosa following prostaglandin treatment

i) Drug administration

16,16-dimethyl prostaglandin E₂ (PGE₂) was dissolved in 0.5% ethanol at an appropriate concentration so that its injection volume per 200g body weight would be 1 ml. The rats were orally given PGE₂ at a dose of 10 μg/kg or 100 μg/kg body weight by esophageal intubation. Control rats were orally given 1 ml of 0.5% ethanol. At 1 hour following drug administration, the animals were sacrificed by decapitation and their stomachs excised.

ii) Measurement of mucus glycoprotein content in the mucus gel layer and mucosa

The mucus gel was solubilized by 2% NAC solution for 5 minutes. Mucus glycoprotein in the gel was isolated and quantified as described above. Following NAC treatment, the glandular portion of the stomach was taken, lyophilized and powdered. Mucus glycoprotein in this powder was isolated and quantified by a previously de-