

THE EFFECT OF SNAIL MUCIN ON THE ULCER HEALING RATE OF CLARITHROMYCIN

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ABSTRACT

The effect of mucin on the ulcer-healing rate of clarithromycin has been investigated. The snail mucin extract was obtained from the giant African snail, *Achatina achatina* (Family Arionidae). The mucin was precipitated with acetone, collected and air-dried. Ulcers were induced with indomethacin in rats. The ulcer-healing rate was determined for clarithromycin alone, mucin alone and the combination of both. The control group did not receive any of the drugs.

The results showed that the healing rate of clarithromycin alone was less than the healing rate achieved with clarithromycin and mucin combinations. The healing rate increased with increasing concentration of mucin. It was also observed that the ulcer-healing rate of mucin alone was higher than the healing rate of clarithromycin alone.

Key words: *Snail mucin, effect, ulcer healing, clarithromycin.*

INTRODUCTION

Peptic ulcers are erosions in the stomach or duodenum (the first part of the small intestine) ¹. The term "peptic" distinguishes peptic ulcer from ulcerations that affect other parts of the body (e.g. diabetic leg ulcers). Peptic ulcer may be caused by infection from *Helicobacter pylori* (*H. pylori*) ². Thus a combination of antibiotics with other anti-ulcer drugs is often used in the treatment of people with peptic ulcer diseases. The cause of many peptic ulcers is not completely understood. One common factor found in almost all peptic ulcer patients is increased gastric acid (and pepsin) secretion. Hydrochloric acid activates pepsin secretion. Pepsin, in turn, begins digestion of protein over excessive secretion of hydrochloric acid, which, however, may result in erosion or ulceration and possible perforation of the gastric walls leading to ulcer disease. In addition, factors relating to mucosal resistance to acid and pepsin may also be important in gastric ulcer disease. Cholinergic (parasympathetic) division of the autonomic nervous system increases gastrointestinal tract motility and gastric acid secretion. Thus cholinergic nerve stimulation may initiate or aggravate peptic ulcer disease. Peptic ulcer therapy has undergone many strides over the past few years and a number of drugs are now available for treatment. These drugs are broadly classified into two, those that decrease or counter acid pepsin secretion and those that affect cytoprotection by virtue of their effects on mucosal defensive factors.

Peptic ulcers are typically treated with antibiotics such as amoxicillin, clarithromycin, and tetracycline, to

eliminate infection with *H. pylori*. In addition, other medications such as famotidine, nizatidine, ranitidine and antacids may be prescribed to control stomach acidity ³.

Snail mucin has been shown to have some wound healing effects ⁴. The mucin from this source has been recently evaluated and found to be physiologically safe especially when employed orally ⁵.

Clarithromycin is a semi-synthetic derivative of erythromycin. In this study, a combination of clarithromycin and snail mucin is evaluated in the treatment of peptic ulcer disease.

MATERIALS AND METHODS

Collection and preparation of animal extract

The snails were purchased from local markets in Nsukka in Nsukka Local Government Area of Enugu State, Nigeria. The snails were washed and the shells were broken and removed and the fleshy part of the snail soaked inside purified water for the slimy liquid to flow inside a bowl that contains little quantity of water. Fifty snails were treated in this manner and the total volume of the slime was collected in 100 ml of water. The pooled slime was precipitated by adding twice the volume of acetone and further washed several times with 100 ml volumes of acetone. The wooly precipitate was collected on a Buchner funnel by means of suction from a vacuum pump and air-dried for 48h. The precipitate was pulverized by means of an end runner mill, weighed and stored in a refrigerator until used.

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Experimental animals

Adult and healthy animals were used for these experiments. The animals used were albino rats weighing between 150–220 g. The rats were obtained from the Department of Pharmacology and Toxicology animal house, University of Nigeria, Nsukka. The rats were fed on standard pellets. All the rats had free access to water.

Induction of ulcer using indomethacin

The method of Suzuki et al⁶ was employed. A Group of 7 albino rats each weighing 150 – 220 g were fasted for 24 h but allowed access to water *ad libitum*. Indomethacin was dissolved in 10 ml of water to obtain a concentration of 25 mg/ml and administered to the rats orally with the help of an intragastric intubation (sonde) at a dose level of 30 mg/kg body weight. At the end of one hour the animals were sacrificed and the stomach excised along the greater curvature and observed with a hand lens for ulcer formation. The numbers as well as the diameters of the ulcers formed were determined using a magnifying glass (x 10). The ulcer index was expressed as the sum of the scores described below⁶.

Effect of the drugs on ulcer healing

The animals were treated as stated under induction of ulcers and the drugs administered at the end of 24h. The method used for determining the level of ulcer induction was then repeated. One group of the rats received mucin alone; another received clarithromycin alone while the other three groups received varying amounts of the mucin in combination with the antibiotic. The rat stomach was invasively isolated after anaesthetizing the rats with chloroform and dissected to open the abdomen. The stomach was then incised with a dissecting blade and placed in a dish containing tyrode solution^{6,7}. The stomach was transferred onto a watch glass and viewed with a hand lens, and the diameters of the ulcers recorded at given time intervals to determine the healing rate of the ulcers.

Ulcer index

The ulcers noted were scored as follows depending on their diameters.

Score 1 = ulcer spot less than 1 mm in diameter

Score 2 = maximal diameter 2 - 4 mm

Score 10 = an ulcer of over 5 mm in diameter

Score 25 = a perforated ulcer.

The percentage of ulcer healing was also calculated.

The process was repeated with same dose of the drug with mucin but the results were checked after 48h. Both the ulcer index and the percentage of ulcer healing were also determined.

Statistical analysis

The results were tested for significance using the Students t-test at the 95 % level of significance ($P=0.05$).

RESULTS

Tables 1a and b show the ulcer formation when the animals were given indomethacin. The results indicate rapid formation of the ulcer, as the ulcer radii were very high. Similarly the ulcer indices were also very high. After treatment with Clarithromycin alone, mucin alone and with the combinations there was rapid decrease in the ulcer parameters such as the ulcer indices and the radii of the ulcers. The use of Clarithromycin alone was not very beneficial as the reduction of the ulcer radii was minimal. In fact there were no significant changes in the ulcer radii when tested statistically using the Student's *t*-test ($P=0.05$). The results obtained with mucin alone and mucin combined with the antibiotics was more significant when tested on the ulcerated animals for 48 h. and the total remissions of the ulcers were noted.

Table 1. Effect of clarithromycin combinations with mucin on the rate of ulcer healing

1a. Twenty four h after drug administration

Drug Combinations (Mg/kg b.w.)	Initial ulcer radius (mm)	*U. I.	Radius after 24 h (mm)	*U. I.	% Ulcer healing
Snail mucin alone 100 mg	15.0	10	3.5	5	76.66
Clarithromycin alone 100 mg	24.2	10	20.2	10	42.14
100 mg of mucin + 100 mg of clarithromycin	19.0	10	1.5	2	92.10
200 mg of mucin + 100 mg of clarithromycin	19.5	10	0	-	100.00
300 mg of mucin + 100mg of clarithromycin	25.0	10	4.0	2	84.00

Note : In table 1b ulcer index after 48 hours is 25 with last 2 groups (i.e more than base line UI = 10) , but % of ulcer healing is 100 – Explain . The results are contra dicator

1b. Forty eight h after drug administration

Drug Combinations (Mg/kg b.w.)	Initial ulcer radius (mm)	after 48 h (mm) *U.I.	*U. I.	% Ulcer healing	
Snail mucin alone 100 mg	19.0	10	5	2	97.36
Clarithromycin alone 100 mg	22.5	10	19.5	10	86.66
100 mg of mucin + 100 mg of clarithromycin	21.0	10	1	1	95.23
200 mg of mucin + 100 mg of clarithromycin	21.0	10	0	25	100.00
300 mg of mucin + 100mg of clarithromycin	20.0	10	0	25	100.00

DISCUSSION

The results obtained with clarithromycin alone may be due to the aetiology of this ulcer. It is drug (indomethacin)-induced ulcer. Antibiotics are more beneficial in ulcers infected with *Helicobacter pylori*⁷. For drug-induced ulcers the prostaglandin system is often implicated⁸. The mechanism of development of ulcers induced by indomethacin and other non-steroidal anti inflammatory drugs has been claimed to be primarily due to their ability to inhibit prostaglandins⁸. Prostaglandins especially have anti secretory and cytoprotective properties and they may have a physiological role in peptic ulcer disease. The

mechanism of cytoprotection by prostaglandins may include: Increased mucus synthesis and secretion, increased epithelial cell half life; strengthening of the gastric mucosal barrier, and increased secretion by the non-acid producing cells of the gastrointestinal tract. Increase in mucosal blood flow, stimulation of bicarbonate secretion, stimulation of sodium ion and chloride ion transport and stimulation of cyclic AMP formation are other proposed mechanisms of cytoprotection⁸.

The effectiveness of mucin as an antiulcer agent needs further investigations. It may be due to a physical coating of the gastric mucosa thereby leading to an increased integrity of the mucous layer building a layer that is resistant to the gastric acid. A similar compound, known as mastic gum has been shown to have some antiulcer effects^{9, 10}. Similarly, other materials with mucoadhesive properties have been shown to enhance the wound healing properties of snail mucin^{11, 12}. Its effect needs to be tested in ulcers. It is worthy of noting that mucin is known to bind many microorganisms¹³⁻¹⁶ but this has not been evaluated for *Helicobacter pylori* induced ulcers.

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