Changes in Synthetic Activity of Sulfated Mucosubstances in Healing Process of Acetic Acid Ulcer in Rats and Effect of Anti-Ulcer Agents

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It is generally believed that ulcerogenesis results from an imbalance between the aggressive and defensive factors (1). Recently, increasing interest is being shown regarding the importance of anti-ulcer therapy, presumably via the positive effects on the defensive mechanisms. Attention has been paid to the function of mucosubstances including sulfated mucosubstances (SMS) as participants in the mucosal defensive system. Previous investigations on the function of SMS have been carried out by using acute gastric ulcers such as stress or drug-induced ulcers (2-4). However, there is little information about the role of SMS in chronic gastric ulcers. The present study was undertaken to investigate the changes in the synthetic activity of SMS in the ulcerated tissue during the healing process of acetic acid ulcer and the effect of some anti-ulcer agents, using the $^{35}$S-sulfate as the index for the SMS biosynthesis.

Male Wistar strain rats weighing approx. 180 g were used. The acetic acid ulcers, 4 mm in diam., were produced by the application of glacial acetic acid to the serosal surface of the stomach as reported by Okabe et al. (5). The rats were orally given the anti-ulcer agents, suspended in 0.4% carboxymethylcellulose (CMC), for 5 days starting from 1 day or 15 days after the operation. One hour after the last dosing, approx. 100 $\mu$Ci of Na$_2$$^{35}$SO$_4$ (The Radiochemical Centre, Amer- sham) was injected intraperitoneally and the stomach removed 6 hr later. The ulcerated area was macroscopically measured in rats sacrificed 20 days after the operation. The tissues of the ulcerated portion were punched out in circular forms, 14 mm in diam., to make the tissues similar in size. The rate of incorporation of the $^{35}$S-sulfate into gastric SMS was determined according to the method of Rainsford (3). In brief, the tissues obtained were homogenized with a Physcotron in water, and three volumes of ice-cold ethanol were added to the homogenate. After allowing this to stand overnight at 4°C, the precipitate following centrifugation was defatted with 5 ml of ether-acetone (1:1, v/v). The resultant dry tissue was weighed, suspended in 1 ml of water and digested with papain (2x crystallized, Sigma) at 65°C for 24 hr. After digestion, SMS was isolated and washed free of low molecular substances using a Millipore filter (0.4 $\mu$m pore, Millipore Corp.) with 30 ml aliquots of 0.1 M Na$_2$SO$_4$ + 0.1 M H$_2$SO$_4$, 0.1 M Na$_2$SO$_4$ and water in succession. The SMS on the filter was dried and solubilized in 1 ml of 1% Soluene-350 (Packard). One half ml of the solution was neutralized with acetic acid and mixed with 10 ml of Insta-gel (Packard). The radioactivity was determined in a Packard Tricarb, Model 3255, liquid scintillation spectrophotometer.

The changes in the synthetic activity of SMS in the ulcerated portion of the glandular stomach during the healing process of the acetic acid ulcer are shown in Fig. 1. The penetrating ulcers were found until 5 days after the operation. One day after the operation, the rate of incorporation of $^{35}$S-sulfate was already twice as much as that in the normal. The incorporating activity then increased rapidly and was maintained beyond the level of three times as high as that in the normal until 20 days. The remarkable ac-
celeration of the SMS synthesis in gastric ulcers produced by clamping was also reported by Hayashi (6). These results can be interpreted as a pertinent defensive response to prevent an invasion of acid and pepsin. During a period from 20 to 30 days after the operation, the ulcer areas decreased in size and the mucosa became swollen with a slight erythema. Although the rate of incorporation tended to decrease in this period, it remained significantly above the level of the normal tissue even 30 days after the operation. This observation is of interest in the light of the results obtained by Okabe and Pfeiffer (7); the acetic acid-induced ulcer model represents an intractable chronic one, and a complete cure was not established during the long period.

As shown in Table 1, the incorporating activity at an early stage after the operation increased significantly by 40–50% compared with that in the ulcerated group, when treated with anti-ulcer drugs for 5 days. Twenty days after the operation, the ulcers were almost restored (ulcer index, 2–3 mm²) in all of the treated and non-treated groups. However, the incorporating activity in treated groups was found to be rather lower than that in the ulcerated group, suggesting that these drugs enhanced healing; and as a result, the mucosal cells practically recovered to the normal state. Therefore, it may be concluded that the anti-ulcer agents facilitate early restoration of the damaged tissues by increasing SMS synthesis, and the SMS synthetic activity does not increase in the gastric tissues having a normal function.

![Graph](image)

Table 1. Effect of anti-ulcer drugs on the incorporation of 35S-sulfate into the sulfated mucosubstances in ulcerated tissue during the healing process of acetic acid ulcer in rats

<table>
<thead>
<tr>
<th>Drug</th>
<th>Treatment (mg/kg/day x 5, p.o.)</th>
<th>5 Days after operationa)</th>
<th>20 Days after operationb)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>dpm/100 mg D.W. % Change</td>
<td>Ulcer index (mm²)</td>
<td>dpm/100 mg D.W. % Change</td>
</tr>
<tr>
<td>Normal (CMC)</td>
<td>4212 ± 358</td>
<td>2.46 ± 0.79</td>
<td>743 ± 134</td>
</tr>
<tr>
<td>Ulcer (CMC)</td>
<td>8413 ± 654*** +100c)</td>
<td>1744 ± 65*** +135c)</td>
<td></td>
</tr>
<tr>
<td>SU-88</td>
<td>200</td>
<td>2.61 ± 0.57</td>
<td>1376 ± 127g</td>
</tr>
<tr>
<td>SU-88</td>
<td>500</td>
<td>2.40 ± 0.60</td>
<td>1175 ± 34f</td>
</tr>
<tr>
<td>Gefarnate</td>
<td>200</td>
<td>3.18 ± 0.75</td>
<td>1013 ± 62ee</td>
</tr>
<tr>
<td>L-Glutamine</td>
<td>500</td>
<td>1.86 ± 0.37</td>
<td>995 ± 69gg</td>
</tr>
</tbody>
</table>

Specific activity of Na₂ 35SO₄: a) 123 mCi/mm mol. b) 38 mCi/mm mol. Significantly different from normal (***P < 0.001) and from ulcer (g) P < 0.05, f) P < 0.01, ee) P < 0.001). Each value represents the mean ± S.E. of 6 or 7 rats. c): compared to normal. d): compared to ulcer.

Fig. 1. Changes in the incorporation of 35S-sulfate into the sulfated mucosubstances in ulcerated tissue during the healing process of acetic acid ulcer in rats. Significantly different from normal (*P < 0.05, **P < 0.01, ***P < 0.001). Each point represents the mean ± S.E. of 4 or 5 rats. O–O Normal, ●–● Ulcer.

References
3 Rainsford, K.D.: The effects of aspirin and other non-steroidal anti-inflammatory/analgesic drugs on gastro-intestinal mucus glycoprotein bio-


