Effect of Sofalcone on the Gastric Cell Proliferation in the Experimental Gastritis of Rats

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Abstract—Effects of sofalcone on the morphometrical glandular structure and the generative cell proliferation in the gastric mucosa of gastritis, induced in rats by the treatment of sodium taurocholate (TCA) for 3 and 6 months, were examined by means of $^3$H-thymidine autoradiography. Morphometrical observation revealed that the ratios of the length of the glandular portion/the total length of the gastric gland were generally decreased in both fundic and pyloric glands with TCA treatments, which may indicate mucosal atrophy. These atrophic changes were improved to the normal levels by the administration of sofalcone for 3 weeks after the treatment of TCA. On the other hand, cell proliferative activity, indicated by the labeling indexes in the generative cell zone of gastric mucosa, was increased in TCA induced gastritis rat, which seems to be the response to replace the increased cell loss. The administration of sofalcone to the gastritis rats further increased the labeling indexes, especially in pyloric gland with statistical significance in both TCA treated rats for 3 and 6 months. From these results, it is suggested that sofalcone stimulates the compensatory increase of proliferative activity of generative cells, which would be available to repair the gastric mucosa with gastritis.

Sofalcone, 2'-carboxymethoxy-4,4'-bis(3-methyl-2-butenyloxy)chalcone, has an anti-ulcer effect on experimental ulcers (1-3) and shows a cytoprotective potency against necrotizing agents in rat stomach (4, 5). Many possibilities have been reported concerning the mechanisms of these protective effects: e.g., increase of gastric mucosal blood flow (6), maintenance of mucosal macromolecular glycoprotein (4, 7, 8) and increase of endogenous prostaglandin (PG) (9).

Recently, Kishimoto et al. (10, 11) have been successful in inducing erosive and atrophic gastritis by the long term administration of taurocholic acid (TCA), a component of bile acids, in rats. They also have previously demonstrated that sofalcone has a therapeutic effect on this experimental gastritis (12). The involvement of endogenous PG was postulated as one of the possible mechanisms of this effect (13).

On the other hand, changes in the cell proliferation kinetics in gastric mucosa play a significant role in the pathogenesis of gastric disease, which also appear to be important in the development of gastric atrophic lesions (14-16). However, little was known about the cell dynamics of the damaged gastric mucosa such as gastritis, especially in the healing process. In the present study, $^3$H-thymidine autoradiography was employed to evaluate the effect of sofalcone on the generative cell proliferation of gastric mucosa with reference to the morphometrical changes of the glandular structure in TCA induced gastritis.

Materials and Methods

Male Wistar rats weighing about 100 g at the beginning of the experiments were used in this study. Experimental atrophic gastritis was induced according to the method of Kishimoto et al. (10). The rats were fed with standard meal (Orient Yeast) and water containing 5 mM sodium taurocholate (TCA,
Ditco) for 3 months in one experiment and for 6 months in another experiment. In both experiments, one group of TCA treated rats was fed with standard meal but containing 1% of sofalcone (Taisho Pharmaceutical Co., Ltd.) and plain water for 3 weeks after withdrawal of TCA (TCA+sofalcone group), and the other group of TCA treated rats was fed with standard meal and plain water for 3 weeks after withdrawal of TCA (TCA group). Control rats were fed with a standard meal and plain water throughout the experiments.

Autoradiography: Each group of rats received a single subcutaneous injection of $^3$H-thymidine (1 μCi/g of body weight; specific activity of 22 Ci/m mole, Amersham). Ninety min after the injection, the rats were killed with ether anesthesia and the stomach was removed. The stomach was fixed with 10% buffered formalin, embedded in paraffin and sectioned longitudinally along the axis of the glandular tubule. Sections of 5 microns in thickness were mounted on a glass slide, dipped in Kodak NTB-2 nuclear emulsion and developed after 2 weeks exposure. They were stained with hematoxylin and eosin.

Measurement of glandular structure and labeling index: In the autoradiograph, cell nuclei covered with more than 20 grains were identified as labeled. To measure the morphometrical changes of the glandular structure, the total length of the gastric gland (l) and that of the glandular portion (g), regarded as the portion below the lowermost labeled cell in the isthmus, of more than 500 gastric glands were measured and estimated by the parameter of g/l (Fig. 1). The labeling index of the proliferating cell zone was counted and expressed as the percentage of labeled cells in a total count of more than 1000 cells confined to the isthmus region of fundic and pyloric glands.

Results

Morphometrical findings: By gross observation, no gastric lesions were detected in any group of rats. On the other hand, in the autoradiograph, mucosal surface injury (erosion) and interstitial fibrosis were evident in experimental gastritis rats induced by TCA treatments for 3 and 6 months, while these histological changes were slight in the mucosa of sofalcone administered rats (Figs. 2 and 3). As shown in Table 1, the ratio of g/l showed a slight increase in the fundic gland, whereas there was a slight decrease in the pyloric gland by the treatment of TCA for 3 months, although this was not statistically significant. However, these ratios reached almost the same values as that of control rats after the administration of sofalcone. In the rats treated with TCA for 6 months, g/l ratios were significantly decreased in both fundic and pyloric glands compared with that of control rats (P<0.05), but it was increased significantly (P<0.01) in both glands by the administration of sofalcone.

Cell proliferative activity: For the evaluation of cell proliferative activity, the labeling index was counted in the generative cell zone in gastric mucosa. As indicated in Table 2, in the rats treated with TCA for 3 months, higher labeling indexes were shown in fundic and pyloric glands compared with that of
Fig. 2. Autoradiographs of the gastric mucosa, showing the effect of sofalcone on the generative cell labeling 90 min after ³H-thymidine injection to the gastritis rats with 3 months TCA treatment (H.E. x 100). Labeled cells are identified by accumulation of black grains which overlie cell nuclei. The rats were fed with standard meal and water containing 5 mM sodium taurocholate (TCA) for the indicated period. The TCA group was fed with standard meal and plain water for 3 weeks after withdrawal of TCA. The TCA+sofalcone group was fed with standard meal but containing 1% sofalcone and plain water for 3 weeks after withdrawal of TCA. (a) TCA, fundic glands, (b) TCA, pyloric glands, (c) TCA+sofalcone, fundic glands, (d) TCA+sofalcone, pyloric glands.

Discussion

In the present study, the experimental atrophic gastritis induced by 6 months TCA treatment was significantly improved by the administration of sofalcone for 3 weeks. As to the changes of glandular structure estimated by the parameter of g/l, the g/l ratio tended to be reduced in pyloric gland. In the rats treated with TCA for 6 months, higher labeling indexes were also shown in both fundic and pyloric glands than that of the control, but the difference was not statistically significant. In the rats treated with TCA for 3 months, the labeling indexes were further elevated with significant difference from the TCA treated rats in the pyloric gland (P<0.01), and different from the control rats in both glands (P<0.05, P<0.01).
Fig. 3. Autoradiographs of the gastric mucosa, showing the effect of sofalcone on the generative cell labeling 90 min after $^3$H-thymidine injection to the gastritis rats with 6 months TCA treatment (H.E. x100). Labeled cells are identified by accumulation of black grains which overlie cell nuclei. (a) TCA, fundic glands, (b) TCA, pyloric glands, (c) TCA+sofalcone, fundic glands, (d) TCA+sofalcone, pyloric glands. See Fig. 2 for other details of the methods.

Table 1. Effect of sofalcone on the morphometrical glandular structure of the gastric glands in TCA induced gastritis rats

<table>
<thead>
<tr>
<th>Group</th>
<th>g/l ratio</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Fundic gland</td>
</tr>
<tr>
<td>3 months TCA treatment</td>
<td></td>
</tr>
<tr>
<td>Cont</td>
<td>0.650±0.004</td>
</tr>
<tr>
<td>TCA</td>
<td>0.672±0.020</td>
</tr>
<tr>
<td>TCA+Sofalcone</td>
<td>0.655±0.003</td>
</tr>
<tr>
<td>6 months TCA treatment</td>
<td></td>
</tr>
<tr>
<td>Cont</td>
<td>0.698±0.008</td>
</tr>
<tr>
<td>TCA</td>
<td>0.661±0.007</td>
</tr>
<tr>
<td>TCA+Sofalcone</td>
<td>0.721±0.008</td>
</tr>
</tbody>
</table>

*Ratio of the length of the glandular portion (g) to the total length of the gland (l) of more than 500 gastric glands were measured in the autoradiograph. *P<0.05, **P<0.01. Each value represents the mean ±S.E. (N=3 or 4). See Fig. 2 for other details of the methods.

mucosal injury, which would have resulted in shortening of the tubular portion relative to the glandular portion. As for the degree of these changes, 6 months TCA treatment provided significant atrophic changes and also improvement by the administration of
Table 2. Effect of sofalcone on the labeling index of the gastric glands in TCA induced gastritis rats

<table>
<thead>
<tr>
<th>Group</th>
<th>Labeling index (%)</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Fundic gland</td>
</tr>
<tr>
<td>3 months TCA treatment</td>
<td></td>
</tr>
<tr>
<td>Cont</td>
<td>9.1±0.3</td>
</tr>
<tr>
<td>TCA</td>
<td>11.9±1.1</td>
</tr>
<tr>
<td>TCA+Sofalcone</td>
<td>14.8±2.7</td>
</tr>
<tr>
<td>6 months TCA treatment</td>
<td></td>
</tr>
<tr>
<td>Cont</td>
<td>8.1±0.5</td>
</tr>
<tr>
<td>TCA</td>
<td>8.7±0.8</td>
</tr>
<tr>
<td>TCA+Sofalcone</td>
<td>10.2±0.8</td>
</tr>
</tbody>
</table>

*Cell nuclei covered with more than 20 grains were identified as labeled in the autoradiograph. Labeled cells were counted and expressed as the percentage in a total count of more than 1000 cells confined to the isthmus region of the gastric glands. *P<0.05, **P<0.01. Each value represents the mean±S.E. (N=3 or 4). See Fig. 2 for other details of the methods.

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sofalcone in comparison with 3 months TCA treatment. The present results are in good accordance with the histopathological study of Kishimoto et al. (12), who observed shortened mucosal thickness with reduction of parietal cells by the TCA treatment for 6 months and the curative effect of sofalcone on these atrophic changes of gastric mucosa.

With respect to the cell kinetics of the gastric mucosa, it is generally accepted that the gastric epithelium is in a kind of dynamic equilibrium, balanced on one hand by cell loss from mucosal epithelial surfaces and on the other by continuous cell production at the site of cell proliferation (14). Several investigators have reported the higher rate of cell proliferation and the increased rates of epithelial cell exfoliation in patients with atrophic gastritis, suggesting a more rapid turnover of the gastric mucosa (17-19). The present study has shown the similar tendency of cell proliferation in gastritis rats induced by TCA treatment, as indicated by the increased labeling indexes of generative cells in both fundic and pyloric glands. From these results, it could be assumed that with advancing degree of gastritis, the rate of cell renewal increases in order to replace the increased cell loss by TCA treatment. In contrast to the case of 3 months TCA treatment, 6 months treatment which caused a significant atrophic feature showed a relatively low proliferation rate. A possible explanation for this difference is that it may result from an inability of the generative cells to maintain the high proliferative activity, as observed in the case of severe gastritis in man (20).

On the other hand, it is well known that nutritional, hormonal, neural and also pharmacologic factors can alter the rate of gastric cell proliferation and/or migration in the mucosa (21-23). Among these factors, PGE2 has been shown to have a stimulating effect on the cell proliferation in pyloric gland (23). Sofalcone itself has also a similar accelerating effect on the cell proliferation in pyloric gland of normal mouse (H. Inoguchi et al., unpublished data). In the present study, when sofalcone was administered to the TCA treated rats, cell proliferative activity was further elevated with statistical significance in the pyloric gland of both rats treated with TCA for 6 months and those treated with TCA for 3 months. Besides, it may be worthwhile to mention that sofalcone has been shown to elevate PGE2 contents in gastric mucosa via the inhibition of PGs metabolizing enzyme activity (9). These evidences may be interpreted to mean that a high cell proliferation induced by sofalcone, especially in pyloric gland, is due to the increase of mucosal endogeneous PG contents. Considering these findings, it is suggested that sofalcone promotes the compensatory increase of proliferative activity of generative cells by the
possible increase of PG, which would be available to repair the gastric mucosa with erosive and atrophic gastritis.

However, there are unresolved questions concerning the mode of cell differentiation and migration, which could explain a decreased number of parietal cells in atrophic gastritis as well as its healing process initiated by the administration of sofalcone. In the recent studies, many authors have confirmed the importance of mesenchymal influences on normal epithelial cell differentiation (24, 25). Further, Hattori (26) demonstrated that the tight stromal sheath in fundic mucosa, which enclosing the generative cell zone tightly, may play an important role in maintaining the proliferative and also differentiative capacity of the cells. Actually, the mucosal structure of the gastric glands was loose, irregular, and occasionally tortuous in the TCA induced gastritis rats (12). In order to resolve these problems, a detailed study on the dynamic aspects of cellular differentiation and migration, in relation to the possible action of sofalcone on the stromal tissue, seems to be necessary.

References
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