Effects of MeIQx and EGF Applications on the Hamster Tongue after Excisional Wounding

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The effects of MeIQx and EGF applications on the tongues of hamsters after excisional wounding were investigated histologically. The tongues of the control animals which received applications of distilled water after excisional wounding showed only a hyperkeratinized epithelium. In contrast, the tongues of the animals which received applications of MeIQx after excisional wounding exhibited epithelial dysplasia. The tongues of the animals which received applications of EGF after excisional wounding displayed a hyperparakeratinized epithelium without epithelial dysplasia. The tongues of the animals which received applications of both MeIQx and EGF together after excisional wounding showed a hyperkeratinized epithelium similar to those of the controls.

Key words: hamster tongue , excisional wounding , MeIQx , EGF

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Introduction

A number of the heterocyclic amines present in broiled foodstuffs have been shown to have carcinogenic potential in either mice or rats (1-8). In a previous investigation, we have reported that application of 2-amino-3,8-dimethylimidazo [4,5-f]guinoxaline (MeIQx), one of the heterocyclic amines, after excisional wounding could produce lingual epithelial dysplasia in hamsters (9). Epidermal growth factor (EGF), a single-chain polypeptide consisting of 53 amino acids, was first isolated from mouse submandibular gland (10). It is reported that EGF stimulated epidermal growth when injected into neonatal mice (11) and epithelial cellular proliferation when added to chick embryo skin fragment and mouse mammary gland in organ culture (12-14) . In the present study, the effects of both MeIQx and EGF applications on the tongues of hamsters after excisional wounding were investigated histologically.

Materials and Methods

Twenty-eight male Golden hamsters , 3 weeks old, were divided equally into 4 groups (Groups A , B , C and D). In all the groups, the tip (2 mm in longitudinal thickness) of the tongue was excised with scissors under anesthesia with ether. The animals of Group A were used as the controls. In Groups A , B , C and D, the wounds were brushed three times weekly with distilled water, 0.5% solution of MeIQx (Wako Pure Chemical Industries, Ltd.) in distilled water, 0.5% solution of EGF

(Sigma Chemical Co.,EGF E4127) in distilled water, and both the 0.5% solution of MeIQx and 0.5% solution of EGF, respectively, for 4 weeks. At the end of 4 weeks , the tips (2 mm in longitudinal thickness) of the tongues in all 4 groups were again excised with scissors. Subsequently, in Groups A , B , C and D, the wounds were painted daily with distilled water, MeIQx, EGF, and both of MeIQx and EGF, respectively, for 2 more weeks. The animals of all 4 groups were then killed with ether. The tongues of all animals were removed, fixed for 2 days in 10% neutral buffered formalin and embedded in paraffin. Serial sagittal sections at 4 μ m were prepared, stained with hematoxylin-eosin and examined histologically. The treatment of the 4 groups is summarized in Fig.1.

Result

In Group A, the wounds of all animals were completely covered by hyperkeratinized epithelium with a regular arrangement of basal, spinous-cell and granular layers (Fig.2). No epithelial projection was seen, and the underlying connective tissue showed no inflammatory cell infiltration. In Group B, the wounds of all animals showed slight to moderate epithelial dysplasia with features such as disorientation of cells, cellular pleomorphism, proliferation of basal cells, abnormal keratinization, and elongation of the epithelial projection (Fig.3). The connective tissue was similar to that in Group A. In Group C, the wounds of all animals exhibited not hyper-

Fig. 1: Treatments of the 4 groups

Fig. 2: Group A . Wound is covered by hyperkeratinized epithelium . (HE×100)

Fig. 3: Group B . Wound is covered by epithelium with moderate epithelial dysplasia . Epithelium is thicker than that in Group A . (HE×100)

Fig. 4: Group C . Wound is covered by hyperparakeratinized epithelium . Epithelium is thicker than that in Group A . (HE×100)

Fig. 5: Group D . Wound is similar to that of Group A . (HE×100)

keratinized epithelium, but hyperparakeratinized epithelium with a thickening of the spinous-cell and granular layers (Fig.4). The epithelial projection was found, but the connective tissue was similar to that in Group A. In Group D, the wounds of all animals were similar to those of Group A (Fig.5).

Discussion

The carcinogen MeIQx is one of the heterocyclic amines formed during the frying of meat or fish (7,8). It is reported that MeIQx induces hepatocellular carcinoma, lung adenocarcinoma, lymphoma and leukemia in mice, and skin squamous cell carcinoma in rats when

given continuously in the diet (7,8). The present study showed that, in Group B, MeIQx treatment in combination with two excisional woundings could produce epithelial dysplasia in the tongues of all animals, although carcinoma could not be induced in the tongue. In Group A, however, the tongues of all animals displayed only a hyperkeratinized epithelium without the features of epithelial dysplasia. These results were similar to those of a previous study (9). Therefore, the present study indicated that application of MeIQx together with excisional wounding could produce lingual epithelial dysplasia in hamsters. Furthermore, it also suggested that excisional wounding acted as a promotional stimulus in inducing

the appearance of epithelial dysplasia of the tongues initiated with MeIQx.

It is reported that topical application of EGF increases the rate of reepithelization in rabbit skin wounds and the resulting epithelium was thicker and more cellular than in untreated wounds (15). Steidler and Reade (16), investigating neonatal mice given subcutaneous injections of EGF, reported that EGF increased the thickness of the epithelium and keratin of both skin and oral mucosa. In the present study, the tongues of all animals in Group C exhibited an increase in epithelial thickness compared with those of the animals in Group A, although they did not show the epithelial dysplasia seen in the tongues of the animals in Group B. These results, as well as those of the above report (16), clearly indicated that EGF could cause the thickening of the oral epithelium in animals. This epithelial thickening was probably ascribed to the proliferation of the basal cells induced by EGF, since EGF receptors are reported to be localized on the basal cells, which alone have cell proliferative capacity in the epithelium (17-20). In Group C, the tongues of all animals displayed hyperparakeratinized epithelium instead of the hyperkeratinized epithelium which was seen in the tongues of the animals in Group A. Steidler and Reade (16) reported that mucosa which is rapidly proliferating tends to produce parakeratin rather than orthokeratin, whereas more slowly proliferating epithelium tends to be orthokeratinized. In this study, the reason for the production of hyperparakeratinized epithelium in the tongues of the animals in Group C was not clear. However, ultrastructural investigation of the effects of EGF on chick embryonic skin in organ culture revealed that EGF treatment resulted in a discontinuity and folding of the basement membrane and an inhibition of the epidermal keratinization by reducing the number of tonofilament bundles (21). In the current study, therefore, there is the possibility that EGF caused some kind of structural changes within epithelial cells, leading to the parakeratinization of these epithelial cells.

In the present study, the lingual wounds of the animals in Group D were similar to those of the control animals in Group A. This seemed to indicate that EGF prevented the production of epithelial dysplasia which might be otherwise induced by the carcinogen MeIQx, although the exact mechanism involved in the normal healing of lingual wounds receiving both MeIQx and EGF in Group D was unknown. It is reported that the topical application of EGF accelerated reepithelialization in burns and excisional injuries of the skin in pigs (22,23). The latter also showed that EGF stimulated mesenchymal cells in the healing dermis. In the human cell culture study, EGF was reported to inhibit the growth of squamous carcinoma cells of the skin, oral cavity, and esophagus (24). EGF was also demonstrated to suppress the tumor growth of human breast cancer transplanted into nude mice (25). It appeared from these

studies that EGF may have brought about the normal proliferation and differentiation of epithelial cells in the lingual wounds of hamsters in Group D.

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