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Histomorphology of chemoradiotherapy-induced oral mucositis in patients with gastrointestinal cancer.

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Abstract

Objective: To study the histomorphological changes of oral mucosa by observing the sections of lip buccal mucosae from patients with gastrointestinal cancer.

Methods: Oral mucosae from 15 patients aged 61-80 years old who died of gastrointestinal cancer were sampled. Group A (n=10) had received high-dose chemoradiotherapy. Group B (n=10) were divided two subgroups. Group B1 (n=5) had gastrointestinal cancer (before chemoradiotherapy), and Group B2 (n=5) consisted of healthy adult subjects. Oral mucosa (1 cm × 3 cm) was collected, prepared into 5-7 μm-thick paraffin sections, stained with haematoxylin-eosin, and observed under a light microscope. Meanwhile, indirect immunoperoxidase reaction was performed.

Results: No. 5 sample in Group A showed the atrophy rate of mucosal epithelial cells was 100%. No. 6 sample showed that the autologous mucosal layer was attenuated, and the hypodermal mucosa was loosened and arranged in deposited fibers. Besides, 75% of No. 3 and 9 samples showed moderate focal or diffusive inflammatory infiltrations of lymphocytes-macrophages in the basal layer of oral mucosa. The nerve fibers and muscles in 20% of No. 2 sample underwent dystrophy.

Conclusion: The oral mucosae of patients receiving chemoradiotherapy suffered from atrophy of epithelial cells and infiltration of numerous cells. In deeper layers, there was relaxation of the hypodermal mucosa as well as dystrophy of nerve fibers and muscles. The deep submucosa exhibited atrophic muscle fibers. In the meantime, the neuromodulation system was injured, especially in non-hyperplastic and hyperplastic muscle fiber bundles.

Keywords: Chemoradiotherapy, Oral mucosa, Morphology, Gastrointestinal cancer.

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Introduction

Oral mucositis is the most important acute complication of chemoradiotherapy, especially in patients with bone marrow stem cell transplantation who receive myeloablative chemotherapy and those receiving radiotherapy for malignant tumors of oral and surrounding structures [1,2]. This disease evidently affects cancer treatment outcomes [3]. Generally, oral mucositis only invades the epithelium. Chemotherapy and radiotherapy directly injure the basal cells of mucosal epithelium and affect their regeneration, so there are no new cells in the basal layer and the existing ones migrate to the surface and shed. As a result, the epithelium is gradually attenuated, giving mucosal erythemas that finally develop into ulcers. Overall, chemoradiotherapy injures the oral mucosa on the DNA level [4], which has attracted global attention [5,6]. Under the same conditions, not all cancer patients are complicated with oral mucositis, the reasons for which remain elusive [7,8]. Chemoradiotherapy regimens induce significantly high levels of acute toxicity than radiotherapy alone. Chemotherapy introduces systemic toxicity and exacerbates local tissue reactions when combined with radiotherapy. In this case, mucositis is recognized as the principal factor limiting further effective treatment [9]. Radiotherapy and multiple-drug chemotherapy that are used concurrently have apparent interactions [10]. In this study, we studied the histomorphological changes of oral mucosa by observing the sections of lip buccal mucosae from patients with gastrointestinal cancer under a light microscope, also depending on the results of immunoperoxidase reaction.

Materials and Methods

Oral mucosae from 15 patients aged 61-80 years old who died of gastrointestinal cancer were sampled. We have obtained the "Ethical Committee Permission" for patient tissue sampling

before the research (No. 2013223). Group A (n=10) had received high-dose chemoradiotherapy. Group B (n=10) was divided into two subgroups. Group B1 (n=5) had gastrointestinal cancer (before chemoradiotherapy), and Group B2 (n=5) consisted of healthy adult subjects (Figure 1). Samples of Group A were collected from of the morgue of our forensic unit. Oral mucosae (1 cm × 3 cm) were collected from lip buccal mucosae. The samples were fixed in 10% neutral formalin, paraffin-embedded, prepared into 5-7 μm-thick paraffin sections, stained with Haematoxylin-Eosin (HE), and observed under a light microscope (lens: X40 and × 90 magnifications, ocular lens: X7 magnification). Meanwhile, indirect immunoperoxidase reaction was conducted to detect leukocytes and the reaction with Leukocyte Common Antigen (LCA). Besides, CD-3, CD-20, CD-4, CD-8 and CD-56 leukocytes were also detected. All experiments were carried out by Leica Microsystems Inc. and Morphology Master video test system.

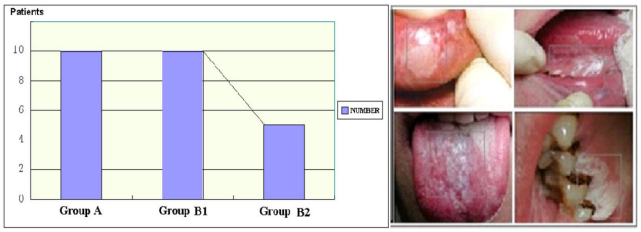

Figure 1. *Grouping and oral mucositis manifestations of some patients (before their deaths).*

The injuries of cancer to oral mucosa were first examined before determining the negative effects of chemoradiotherapy. All patients with gastrointestinal cancer received oral examinations to clarify the states of dental hard tissues, periodontal tissues as well as mouth and lip mucosae before sampling. Oral mucosal smears were prepared by scraping and smearing cells onto slides that were then oven-dried for 1 h, fixed in a 1:1 mixture of 96% ethanol and medical diethyl ether for 10 min, stained with the Romanowski-Giemsa method, and observed under the light microscope (lens: X40 and X90 magnifications, ocular lens: X7 magnification).

Results

Morphological changes

For No. 5 sample in Group A, mucosal epithelial cells had the atrophy rate of 100% (Figure 2a). As to No. 6 sample, the autologous mucosal layer was attenuated, and the hypodermal mucosa was loosely arranged in deposited fibers (Figure 2b). Moreover, 75% of No. 3 (Figure 2c) and No. 9 (Figure 2d) samples showed that lymphocytes-macrophages underwent moderate focal or diffusive inflammatory infiltrations in the basal layers of oral mucosae. Additionally, 20% of No. 2 sample was subjected to dystrophy of nerve fibers and muscles (Figure 2e). Figure 2f shows that samples from Groups B1 and B2 have similar initial states in intact oral and lip mucosae as well as injured mucosae.

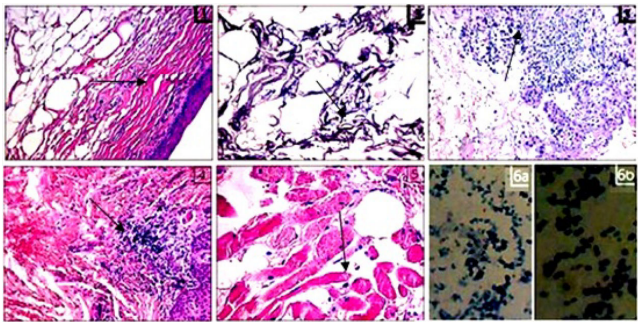

Figure 2. *Typical sections showing morphological changes. a: No. 5 sample in Group A, HE staining, X100 magnification; b: No. 6 sample in Group A, Foot's staining, X400 magnification; c: No. 9 sample in Group A, HE staining, X200 magnification; d: No. 3 sample in Group A; e: No. 2 sample in Group A, HE staining, X400 magnification; f: 1.) No. 3 sample in Group B1, 2.) No. 1 sample in Group B2.*

Results of immunoperoxidase reaction

The results of immunoperoxidase reaction are summarized in Table 1, being consistent with the morphological changes under the light microscope. Clearly, chemoradiotherapy induced the immune response of hosts and release of leukocytes.

Table 1. *Results of immunoperoxidase reaction.*

	A5	A9	A6	A3	A2	B13	B21
LCA	-	+	+	-	+	+	+
CD-3 CD-20	-	-	-	+	+	+	+
CD-4	+	-	-	-	-	-	+
CD-8	-	-	+	-	-	+	+
CD-56	+	+	+	-	+	+	-

Discussion

The pathogenesis of chemoradiotherapy-induced oral mucositis remains unclear, which has now mainly been attributed to direct or indirect injuries. Direct injury means the mucosa is directly injured by anticancer agents or radiation on the 5th~14th day on average, further inducing the apoptosis of oral mucosal epithelial cells. Indirect injury means oral mucosa is injured by the loss of saliva due to release of inflammatory mediators, radiation or anticancer agents [11-13]. In addition, methotrexate contributes to oral mucositis because of enhanced adaptability to the immune system function, manifested as increase in oral lymphocytes [14].

The pathophysiological process of chemoradiotherapy-induced oral mucositis is divided into start-up phase, inflammatory/vascular phase, epithelial phase, ulcer/bacteria phase and recovery phase [15]. In the inflammatory phase, epithelial, endothelial and connective tissues are injured, releasing free radicals, modified proteins and primitive inflammatory

cytokines such as interleukin (IL)-1β, prostaglandin and tumor necrosis factor α. Such inflammatory mediators directly or indirectly aggravate vascular permeability, increase the accumulation of cytotoxic factors, and finally exacerbate tissue injury. In contrast, release of anti-inflammatory factors such as IL-11 offsets early inflammatory response [16]. The epithelial phase starts 4-5 days after radiotherapy, during which the injury degree is directly correlated with the proliferation rate of oral epithelial tissues. Young patients are prone to radiation-induced oral mucositis and recover rapidly, whereas oral basal cells of the elderly tend to undergo mitosis [17]. Meanwhile, the patients have high levels of transforming growth factor B1 before chemoradiotherapy, which plummet after treatment while oral mucositis occurs accordingly [18].

After one week of treatment with anticancer agents, the epithelium of oral mucosa collapses, leading to oral mucositis in the ulcer phase, loss of epithelial cells and fibrin exudation. As a result, pseudomembrane and ulcer form. In this phase, gram-negative bacteria and fungi grow in to the injured mucosal surface, accompanied by decrease in leukocytes to further aggravate the disease. Moreover, release of bacterial metabolites including endotoxin results in excessive oxidation of monocytes, further enhancing the release of inflammatory mediators such as IL-1, NO and tumor necrosis factor A [5]. For patients receiving chemotherapy, the recovery phase usually lasts for 12-16 days, which is predominantly controlled by proliferation of epithelial cells, recovery of hematopoietic function, rebuilding of local microflora, and lack of factors that impede recovery [15,19].

Chemotherapy-induced oral mucositis is related with chemotherapeutic agents, route of administration, treatment duration, dosage, supplementary drugs and previous mucosal toxicity treatment [20]. The risk of oral mucositis is increased due to continuous or repeated treatment with low-dose cytotoxic drugs, increase in chemotherapy cycle and history of this disease. The degree of radiotherapy-induced oral mucositis is associated with treatment duration, radioactive source, cumulative radiation dose, dosage, volume of radiated mucosa, history of smoking or drinking, and other factors such as dry mouth and oral infections [21,22]. In immunocompetent hosts, radiotherapy-induced oral mucositis often recovers within three weeks after the treatment terminates. Commonly occurring 7-10 days after treatment, oral mucositis induced by implantation radiation becomes most severe within two weeks. Such injuries, except for large areas of mucosal ones, usually recover within several weeks. This disease can also be triggered by disrupted mechanisms of metabolic enzymes and DNA modification, deficiency of folic acid and vitamin B12, anticancer agent-induced damage of liver and kidney functions, exudative pleural and peritoneal effusions, and delayed elimination of drugs owing to the use of special drugs (e.g. leucovorin) [23,24]. Potential decrease in haemocytes [15] and previous oral lesions such as dry mouth also promote the onset of oral mucositis. This symptom limits the production of saliva, weakens its buffering effect, as well as elevates its stickiness and acidity and oral IgA level, finally affecting the onset of dental caries [25,26].

In this study, changes in the mucosal epithelial cells are typified by atrophy of epithelial cells and invasion of numerous lymphocytes and macrophages. In deeper layers, there was relaxation of the hypodermal mucosa as well as dystrophy of nerve fibers and muscles. Deeper part of the submucosal layer showed atrophy of muscle fibers, manifested as injury of the neuromodulation system. Particularly, the proliferative and nonproliferative muscle fiber bundles were more prone to neural Injury. Similarly, it has previously been reported that complications of chemoradiotherapy were induced by intramucosal matrix components but not surface structures.

After high-dose chemoradiotherapy, the percentage of viable oral epithelial cells increases. Also, cells in the buccal epithelium shift from mature to immature, possibly due to desquamation of the upper oral epithelial layer. Newer treatment modalities that can circumvent the side effects of chemotherapeutic agents should be considered. Clinical trials with larger sample sizes will give more accurate results in this regard. Decreased loco-regional control, poorer quality of life and shortened overall survival have recently been associated with unplanned treatment breaks and reduction in dose intensity [27]. Therefore, such assessment aids in mucositis may be valuable in the future. The *in vitro* assay may also be useful as an adjunct in studies focusing on oral mucositis prevention.

Conclusion

The findings herein provide pathological and physiological evidence for the prevention and treatment of radiotherapy- and chemotherapy-induced oral mucositis. However, the mucosa was collected near the cheeks or lips with the area of 1×3 cm^2, so we observed limitations of the study. We are looking forward to in-depth studies.

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