Iodine penetration and glycogen distribution in vital staining of oral mucosa with iodine solution

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Objective. To assess and compare iodine penetration and glycogen distribution in a vital staining of oral mucosa with iodine solution.
Study Design. Twenty samples were obtained including both iodine-stained and -unstained mucosa. Intraepithelial iodine was examined using frozen sections. Glycogen distribution was assessed by periodic acid–Schiff staining and transmission electron microscopy.
Results. Iodine accumulation was observed mainly superficially and in the upper and middle thirds of prickle cell layers, with glycogen in almost the whole epithelium except for the para- and basal cell layers. The pattern of iodine and glycogen distribution was classified into 3 types (full-, surround-, and scatter-type). The iodine color was mainly derived from the cells with full- and surround-type distributed glycogen in the upper half of the oral epithelium.
Conclusions. The results of this study suggested that iodine penetrated into nonkeratinized oral epithelium and reacted mainly with intraepithelial glycogen homogeneously distributed in the cytoplasm. (Oral Surg Oral Med Oral Pathol Oral Radiol 2014;117: 754-759)

Iodine atoms react with starch/glycogen by fitting into the helical coils of amylose to form the iodine-starch/glycogen complex, giving a visible sharp blue-black or brown-black color.[1] In medical practice, iodine solution can delineate precancerous or malignant mucosa from normal mucosa in the upper part of the digestive tract, including the oral cavity. Iodine is able to infiltrate into normal epithelial cells and combine with intraepithelial glycogen to present a brown-black background, whereas poor intracellular glycogen contents in the mucosa of premalignant or malignant lesions lead to a weak or absent color reaction.[2-11] However, little is known about the details of iodine penetration within the epithelium, because conventional histopathologic methods are not able to retain iodine in the tissue sections to allow examination and comparison of the intraepithelial expression of iodine staining.[4] Our recent study achieved fixation of the iodine by freezing sections for light microscopic examination,[12] and we noted an interesting observation: the infiltrated iodine distribution was not always consistent with the glycogen distribution, especially in the deep layers of normal oral epithelium. In some cases, only weak iodine staining was observed in the cytoplasm that still retained some amount of glycogen. These findings caused us to consider whether the iodine really reacted with the glycogen or with some other substance in the cells. The purpose of our study, therefore, was to assess and compare iodine penetration and glycogen distribution within the oral mucosa stained by iodine solution.

MATERIALS AND METHODS

Tissue samples

Tissue samples were obtained from 20 patients (14 men and 6 women; median age, 65 years; range, 30-85) who underwent tumor surgery for primary oral squamous cell carcinoma (OSCC). Before the tumor surgery, iodine vital staining was carried out as described by Kurita and Kurashina.[5] All cases exhibited clear brown-black staining in nonkeratinized normal oral epithelium, with no staining reaction in the dysplastic/cancerous epithelium surrounding OSCC (Figure 1). Tissue samples containing both iodine-stained and -unstained

Statement of Clinical Relevance

Iodine solution can often produce clear mucosal margin between high-grade dysplasia to oral squamous cell carcinoma and normal- to low-grade dysplasia. Our findings suggest that not only the glycogen but also the epithelial permeability may play a role in this vital staining method.

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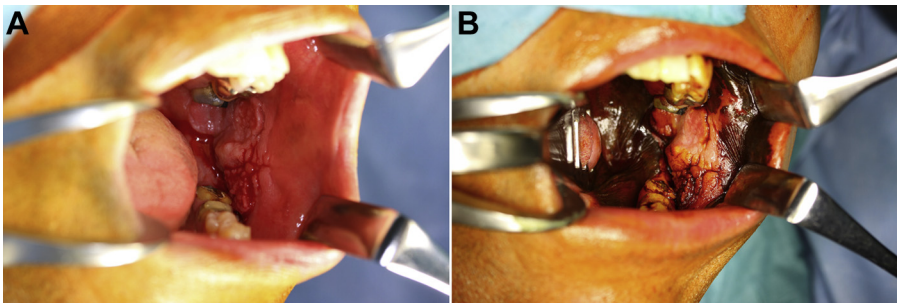

Fig. 1. Iodine vital staining in left buccal oral squamous cell carcinoma. **A**, Before iodine staining. **B**, After iodine staining.

epithelium (across the color boundary line) were cut out: 10 samples were obtained from the tongue, 6 from the buccal mucosa, 2 from the floor of the mouth, and 1 from the retromolar trigone and soft palate.

Each tissue sample was cut in half; one half was snap-frozen in dry ice−cooled acetone and stored at −80°C until needed, and the other half was processed for transmission electron microscopy. As normal controls, 8 nonkeratinized mucosal samples (located in the retromolar trigone without any signs of inflammation) were obtained from volunteers who underwent extraction of third molars. Written informed consent was obtained from each patient. The study was approved by the Ethics Committee of Shinshu University School of Medicine.

Light microscopy

Serial 5-μm sections were cut and examined by light microscopy for intraepithelial iodine analysis, hematoxylin-eosin staining, and periodic acid−Schiff (PAS) staining. Iodine examination in the first section was performed as previously described.[12] Briefly, this freshly cut section was immersed into xylene for 30 seconds after 30 minutes of air-drying at room temperature. Using a nonaqueous mounting medium prevented the intracellular iodine from being removed by water. A brown-black color in the cytoplasm was defined as iodine stain. PAS stain was used to detect glycogen on tissue sections. The glycogen showed as magenta staining, which disappeared when treated with the α-amylase digestion test before PAS staining.

Transmission electron microscopy

The fresh specimen was fixed in 2.5% glutaraldehyde for 1 hour at room temperature, cut into 1-mm^3 cubes, and left at 4°C for 3 hours. After post fixation with 1% osmium tetroxide for 1 hour, hydration through graded alcohol, and embedding in epoxy resin, ultrathin sections were prepared and double-stained with uranyl acetate and lead citrate. The glycogen features in the cytoplasm were examined by JEM-1400 electron microscopy (JEOL Ltd, Tokyo, Japan).

RESULTS

Iodine vital staining showed a clear boundary line between iodine-stained and -unstained areas. All 20 sections included both iodine-stained and -unstained mucosa. Histopathologic diagnoses of iodine-unstained areas included moderate dysplasia in 7 specimens, severe dysplasia in 4, carcinoma in situ in 4, and squamous cell carcinoma in 5, according to the criteria proposed by the World Health Organization.[13] In the iodine stained areas, the greater part of the epithelium was normal, but basal cell hyperplasia (in 2 samples) and mild dysplasia (in 6 samples) were occasionally observed in limited areas. These sections were obtained from iodine-defined margin areas rather than from the central regions of tumors. Diagnoses of hyperplasia and dysplasia for some iodine stained mucosa were based on a few abnormal epithelial cells neighboring the iodine-defined margin, whereas the remainder of the epithelial cells distal to the color margin appeared completely normal.

Iodine penetration was assessed by examining the raw frozen sections. There were no obvious differences between iodine-stained normal control and iodine-positive mucosa at the tumor periphery. Iodine was present mainly in the upper epithelium of iodine-stained areas. The depth of iodine infiltration depended on the epithelial thickness. Only 2 to 4 surface cell layers had a brown or brown-black color in the thin oral mucosal epithelium (often seen in the floor of the mouth, retromolar trigone, and soft palate) (Figure 2, A, B). In contrast, in the thick oral mucosal epithelium (mainly on the buccal mucosa and tongue), iodine often extended much deeper but rarely reached the lower third of the prickle cell layers (Figure 2, D, E).

The intracellular iodine expression in the oral epithelium showed diverse patterns according to the differentiation and shape of epithelial cells (Figure 3). In the superficial layer, the flat epithelial cells showed homogeneous iodine staining without an obvious cell-cell border and distinguishable nucleus. Beneath these layers (in the upper third of prickle cell layers), some cells were completely filled with iodine, resembling the superficial layer cells, whereas others had clear