Cryotherapy for oral precancers and cancers

Chuan-Hang Yu a,b, Hung-Pin Lin c,d, Shih-Jung Cheng e,f, Andy Sun e,f, Hsin-Ming Chen f,g,*

a School of Dentistry, College of Oral Medicine, Chung Shan Medical University, Taichung, Taiwan
b Department of Dentistry, Oral Medicine Center, Chung Shan Medical University Hospital, Chung Shan Medical University, Taichung, Taiwan
c Department of Dentistry, China Medical University Hospital, College of Medicine, China Medical University, Taichung, Taiwan
d School of Dentistry, College of Medicine, China Medical University, Taichung, Taiwan
e Graduate Institute of Clinical Dentistry, School of Dentistry, National Taiwan University, Taipei, Taiwan
f Department of Dentistry, National Taiwan University Hospital, College of Medicine, National Taiwan University, Taipei, Taiwan
g Graduate Institute of Oral Biology, School of Dentistry, National Taiwan University, Taipei, Taiwan

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Previous studies have used cryotherapy for the treatment of oral precancers including oral leukoplakia (OL) and oral verrucous hyperplasia (OVH) as well as oral cancers including oral verrucous carcinoma (OVC) and oral squamous cell carcinoma (OSCC). Cryotherapy is a method that locally destroys lesional tissues by freezing in situ. It can be carried out by either an "open" or a "closed" system. Lesional tissues are destroyed mainly through disruption of cell membrane, cellular dehydration, enzyme and protein damage, cell swelling and rupture, thermal shock injury to cells, damage to vasculature, and immune-mediated cytotoxicity. Cryotherapy is used frequently for the treatment of OL lesions with promising results. It can also be used to treat OVH and OVC lesions. Because OVH and OVC lesions are usually fungating and bulky, a combination therapy of shave excision and cryotherapy is needed to achieve a complete regression of the lesion. OSCCs have also been treated by cryotherapy. However, cryotherapy is not the main-stream treatment modality for OSCCs. Cryotherapy seems suitable for treatment of thin or relatively thick plaque-typed lesions such as OL lesions. By careful selection of patients, cryotherapy is a simple, safe, easy, conservative, and acceptable treatment modality for certain benign oral lesions and oral precancers.

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Introduction

Oral squamous cell carcinoma (OSCC) is the most frequent malignant tumor of the oral cavity and the sixth most common cancer in the world. OSCCs consist of more than 90% of oral malignancies. Oral verrucous carcinoma (OVC) is a low-grade variant of OSCC, which represents 1–10% of all OSCCs. In Taiwan, oral cancers rank as the sixth most prevalent cancer in both genders and account for the fourth most common cancers in males. Although great advances have been made in the diagnosis and treatment of OSCC in recent years, the 5-year survival rate for patients with advanced stages of oral cancer has remained approximately 20%. Oral precancerous lesions are even more much prevalent than oral cancers. Without further interventions, a part of these oral precancerous lesions finally transform into oral cancers. Therefore, finding a simple, safe, easy, conservative, and acceptable treatment modality to eradicate oral cancers, thereby preventing their further malignant transformation is one of the best strategies to prevent the occurrence of oral cancer.

Oral leukoplakia (OL), oral erythroleukoplakia (OEL), and oral verrucous hyperplasia (OVH) are three common oral precancerous lesions. Histologically, approximately 90% of OL lesions show hyperkeratosis and/or epithelial hyperplasia, 5% epithelial dysplasia or carcinoma in situ, and another 5% invasive carcinoma. The malignant transformation rates of OL lesions have been reported to be 1–7% for homogenous thick leukoplakia and 4–15% for granular or verruciform leukoplakia. However, approximately 50% of OEL lesions reveal some degree of epithelial dysplasia or carcinoma in situ, and another 50% superficially invasive carcinoma. OEL has a higher malignant transformation rate of 18–47%, which is higher than that of OL lesions. In Taiwan, both betel quid chewing and cigarette smoking are involved in multistate progression of oral precancers including OL and OEL lesions. A cohort study found an average dwelling period of 24 years and 7 years for OL and OEL, respectively. Furthermore, the risks of developing oral cancer after 20 years of follow up are 42.2% for OL and OEL, respectively. Furthermore, the risks of developing oral cancer after 20 years of follow up are 42.2% for OL and OEL, respectively. A retrospective clinical study showed a malignant transformation rate of 3.1% and a mean malignant transformation duration of 54.6 months for 324 OVH lesions in Taiwanese patients. Wang et al also demonstrated a 5-year malignant transformation rate of 3% for 30 plaque-typed and 17% for 30 mass-typed OVH lesions. The high malignant transformation rates of OL, OEL, and OVH lesions highlight the importance of early detection and subsequent cryotherapy on OVH and OVC lesions.

Mechanisms of tissue destruction by cryotherapy

The mechanisms for cell destruction after cryotherapy are complex involving a combination of direct and indirect effects. Direct effects consist of extracellular and intracellular formation of ice crystals, which in turn disrupt cell membranes, cellular dehydration, toxic intracellular electrolyte concentration, inhibition of enzymes, protein damage, thawing effects that cause the cell to vacuolate, swell, and rupture, and thermal shock injury to cells. Indirect effects are vascular changes that lead to ischemic necrosis of the treated tissue and immunological responses that cause cell damage through a cytotoxic immune mechanism.

Tissue changes after cryotherapy

Clinical observations

The clinical responses of oral soft tissues to cryotherapy usually include courses of tissue edema, subepithelial hemorrhage, blister formation, necrosis, sloughing, and repair. The degree of tissue destruction and the rate of tissue regeneration after cryotherapy are slightly different and depend on the size and location of the lesion and the cryotherapy system used. In general, hyperemia, edema, or erythema appears immediately or within a few hours after cryotherapy. Local swelling develops and increases for 1–2 days, followed by superficial necrosis and an ulceration covered by a layer of whitish or yellowish necrotic pseudomembrane. The whitish or yellowish slough separates from the underlying tissue within the 1st week leaving a clean, granulating surface that is partially covered by the epithelium. Epithelialization is complete after 1–4 weeks with no or very little scar formation.
Histological observations
There are very few reports with regard to the histological tissue responses after cryotherapy. Hurt and colleagues used cryotherapy for treating periodontal disease of a 33-year-old male to see the histological changes after treatment. They observed a marked edema with vesicle formation just deep to the basal cell layer of the gingival epithelium immediately after cryotherapy. They also found a striking hyperemia with many engorged vessels in the connective tissue near the vesicle. Three days after cryotherapy, the outer surface of the treated area is ulcerated and covered by a fibrinous exudate incorporating many neutrophils. The connective tissue underneath the ulcer is edematous and exhibits an infiltrate of lymphocytes, plasma cells, and neutrophils. Some eosinophilic ghost cells and multinucleated epithelial giant cells that contain 2–20 nuclei within an eosinophilic cytoplasm are also noted. Complete epithelial coverage with minimal disturbance of the deep gingival structure is seen at Day 7 after cryotherapy. The connective tissue appears slightly hyalinized with a diffuse plasma cell and lymphocyte infiltrate. Six weeks after cryotherapy, the treated site reveals an intact outer epithelium with no evidence of cryotherapy histologically.

Tal et al. used cryotherapy to treat an extensive OL in a 63-year-old white female. Four weeks after cryotherapy, they excised residual leukoplakia with a 2-mm margin of treated mucosa, and the specimen was processed for histological examination. They found that the treated epithelium that is parakeratinized or nonkeratinized shows a prominent granular cell layer. Peri-epithelial edema in the upper spinous cell layer. The treated epithelium contained a large amount of glycogen in the spinous cell layer, whereas the untreated hyperkeratotic epithelium was almost entirely lacking in glycogen. There was only a mild chronic inflammatory cell infiltrate in the superficial lamina propria of the treated mucosa, whereas the infiltrate was fairly dense in the untreated mucosa.

Closed-system cryotherapy for OL
Closed-system cryotherapy has been used for treatment of OL lesions with promising clinical outcomes. Sako et al. treated OL lesions in 60 patients using a special cryosurgical unit, and all OL lesions showed complete regression (CR) after one to five treatments. Chapin and Burke used cryotherapy with a gold cryoprobe to treat four patients with dysplastic and nondysplastic OL lesions and observed CR of all lesions after one or two treatments. Leopard used closed-system cryotherapy with two consecutive freeze–thaw cycles of up to 1.5 minutes to treat over 40 OL lesions in a 3-year period; only two extensive and long-term OL lesions failed to respond. Bekke and Baart used cryotherapy with a cryoprobe to treat 35 OL lesions in 24 patients; all lesions showed CR after one to four treatments. Al-Drouby used cryotherapy with a cryoprobe to treat 30 OL lesions in 30 patients; 23 had complete removal, six had partial removal, and there was no response in one patient. Kawczyk-Krupka et al. used cryotherapy with a contact scalpel probe to treat 37 patients with OL; a complete response was obtained in 33 patients (89.2%) and recurrence was observed in nine patients (24.3%).

Open-system cryotherapy for OL
Open-system cryotherapy is carried out by the direct application of either carbon dioxide snow or liquid nitrogen to OL lesions by the cotton swab or open spray. Yeh used cotton-swab cryotherapy to treat 102 oral lesions including 25 OL lesions. He demonstrated that small, superficial lesions show CR after one treatment, but deep, large lesions need from two to four treatments to achieve CR. Our previous study used the cotton-swab cryotherapy to treat 60 OL lesions and found CR of all OL lesions after an average of 6.3 treatments. Recently, we used the liquid nitrogen spray with a cryogun (cryogun cryotherapy) to treat 60 OL lesions that showed CR after an average of 3.1 treatments. Cryogun cryotherapy seems more efficient than cotton-swab cryotherapy to treat OL lesions, because the former needs approximately half of the treatment number to achieve CR of nearly equal-sized OL lesions compared with the latter. In fact, the cotton swab carries only a small amount of liquid nitrogen that cannot maintain a constant low temperature in the treated lesional tissues. By contrast, special spray equipment such as a cryogun can deliver a relatively large amount of liquid nitrogen onto the lesional surface thereby maintaining a more constant, lower temperature in treated lesional tissues. This can further explain why the cryogun cryotherapy is more efficient for the treatment of OL lesions than the cotton-swab cryotherapy. In addition, we also discovered that OL lesions on oral mucosal sites other than the tongue, smaller than 2 cm, with epithelial dysplasia, or with a surface keratin thickness < 55 μm require significantly fewer cryotherapy treatments to achieve CR than the corresponding OL lesions, respectively, when OL lesions are treated by either the cotton-swab or cryogun cryotherapy. Miller used a single liquid nitrogen spray for 45–60 seconds to treat OL lesions on the hard palate, soft palate, and buccal mucosa; all OL lesions were successfully eliminated after treatment. Goode and Spooner used liquid nitrogen spray to treat OL lesions in 20 patients and observed CR of all OL lesions after one to four treatments. Gongloff and Gage used liquid nitrogen spray of two consecutive freeze–thaw cycles to treat seven OL lesions; CR of all OL lesions was achieved after a single treatment. The results of the aforementioned studies indicate that either closed-system or open-system cryotherapy is very effective for the treatment of OL lesions.

Cryotherapy for OVH and OVC
Yeh treated 16 OVH lesions in 13 patients with the cotton-swab cryotherapy. All 16 OVH lesions showed CR after one to four cryotherapy treatments. However, four OVH lesions recurred after a mean follow-up period of 26 months (range: 3–46 months). The recurrent lesions were successfully treated by additional cryotherapy. Yeh also...
treated 17 OVH lesions and nine OVCs in 20 patients with a combination therapy of shave excision and subsequent simple cryotherapy. Eighteen of the 20 patients were followed up. The CR of the tumor was found in 11 lesions, and small and discrete residual tumors were noted in seven lesions, which were further removed by electrosurgery or CO₂ laser surgery. After a mean follow-up period of 23 months (range: 6–46 months), recurrence was found in three cases, wherein the lesions developed elsewhere; however, all of them were successfully treated by the same combination therapy.26

Cryotherapy for OSCC

There are relatively fewer clinical reports with regard to the use of cryotherapy for oral malignant lesions. Most of these studies were reported predominantly between 1960 and 1980. In 1965, Gage and colleagues28 used cryotherapy to treat three OSCCs and one adenoid cystic carcinoma, which all showed CR after multiple cryotherapy treatments. Three years later, Gage29 extended his study to treat 50 oral and oropharyngeal cancers. The reasons for selecting cryotherapy as the main treatment modality are resistance to radiotherapy, location in areas difficult to excise, desire to avoid the bone resection, and presence of severe cardiopulmonary diseases.30 Of the 50 patients, 39 were treated with the intent to cure the disease and 11 were treated for palliation because of the extensive cancer. Among the former 39 patients, 30 are in good condition and nine died after the treatment. Moreover, of the 24 patients treated more than a year ago, three failed to control the primary lesion, four died of heart failure or coronary artery thrombosis, five died due to unrelated diseases with no evidence of cancer; and five are still alive and eight patients died. Of the 13 survivors, nine are free from tumor after cryosurgery, two are free from tumor after supplemental therapy, and two are still under supplemental therapy. Of the eight deceased patients, five were free from tumor and three had tumor remnants at the time of death.17 Gongloff and Gage25 treated six superficially invasive OSCCs and two oral carcinoma in situ lesions with cryotherapy. All lesions show CR and no recurrence was noted after a follow-up period of 30–54 months.

Combination therapy of cryotherapy and other treatment modalities

Chang and Yu37 used PDT combined with cryotherapy to treat a large OVH and achieved CR of the lesion after six treatments of combination therapy. In that study, cryogun cryotherapy was performed immediately after topical 5-aminolevulinic acid-mediated PDT (topical ALA-PDT) at the same visit.37 Cryogun cryotherapy can also be used prior to topical ALA-PDT at the same visit to facilitate the diffusion of ALA into the precancerous and cancerous epithelial cells to improve the clinical outcome of topical ALA-PDT.12,38 By our clinical experience, hyperkeratotic OL lesions respond poorly to topical ALA-PDT, because the ALA agents are difficult to diffuse into OL lesional epithelial cells through the thick keratin layer. Therefore, if one or two treatments of cryogun cryotherapy alone are performed to remove the thick keratin layer on the surface of OL lesions, then the cryotherapy-treated residual OL lesions can have a very good response to subsequent topical ALA-PDT. Therefore, cryogun cryotherapy can be used prior to or after topical ALA-PDT at the same visit or used alone prior to topical ALA-PDT to obtain successful treatment outcomes for oral precancers and cancers treated with combination therapy.30

Cryotherapy for other potential oral lesions

In addition to oral precancers and cancers, cryotherapy has also been used for the treatment of mucocele,13,20 oral hemangioma,13,15,17,20 oral lymphangioma,17 gingival melanin pigmentation,19–21 oral melanotic macule,22 and oral lichen planus.17,20 Better clinical outcomes may be achieved, when oral lichen planus is treated by cryotherapy combined with systemic administration and local application or injection of steroid.43–45 Small oral soft-tissue tumors such as fibroma,20 pyogenic granuloma, and peripheral odontogenic fibroma46,47 may also be treated by
cryotherapy. Moreover, because the incidence of OSCC is high and the prognosis of OSCC is relatively poor in Taiwan,\cite{14,40} cryotherapy may also be used as a palliative remedy for OSCCs that fail to respond to radical surgical excision, chemotherapy, and radiotherapy, separately or in combination.

**Conclusion**

This article reviewed the studies that used cryotherapy for the treatment of oral precancers and cancers. Cryotherapy seems suitable for treating thin or relatively thick plaque-typed precancerous lesions such as OL. For OVH or OVC lesions, because these two types of verrucous lesions are usually fungating and bulky, a combination therapy of shave excision and subsequent cryotherapy is needed to achieve a CR of the lesion. OSCCs have also been treated by cryotherapy predominantly between 1960 and 1980 with comparable treatment outcomes to those treated by surgical excision. However, it is not the main-stream treatment modality for OSCCs after 1980s. Using the cryotherapy as the initial treatment for OSCCs is not recommended. By careful selection of patients, cryotherapy is a simple, safe, easy, conservative, and acceptable treatment modality for certain types of benign oral lesions and oral precancers.

**References**

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