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Original Article

Combination of cryotherapy and mitomycin C in excision for treatment of ocular surface squamous neoplasia

Qing Chen, Hua Wang, Ying Zhao, Pingbao Wang

Institute of Ophthalmology, Xiangya Hospital, Central South University, Changsha, Hunan Province, China

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Abstract: The aim of this study is to retrospectively analyze the treatment outcome of cryotherapy combined with mitomycin C (MMC) after ocular surface squamous neoplasia (OSSN) excision. Nineteen patients with OSSN treated between August 2010 and August 2017 were included in this study. All patients underwent excision of the tumor, followed by cryotherapy of the excision margin and incubation of the bare sclera with 0.04% MMC-soaked sponge. Amniotic membrane transplantation (AMT) was performed after tumor excision in all patients. All eyes were completely re-epithelialized. No serious complications such as limbal stem cell deficiency, scleral dissolution, or symblepharon occurred. Postoperative histopathological diagnosis was dysplasia in 10 (52.6%) patients, carcinoma in situ in 4 (21.1%) patients, and invasive squamous cell carcinoma (SCC) in 5 (26.3%) patients. Two patients had recurrence (recurrence rate 9%) within 6 months of operation; both required orbital exenteration. The recurrence rate remained at 9% at the end of 5 years. The combination of cryotherapy and 0.04% MMC after excision appears to be an effective treatment for OSSN.

Keywords: Cryotherapy, mitomycin C (MMC), ocular surface squamous neoplasia (OSSN), amniotic membrane transplantation (AMT)

Introduction

Ocular surface squamous neoplasia (OSSN) is the most common non-pigmented malignancy of the ocular surface and refers to a wide spectrum of conjunctival and corneal disease ranging from dysplasia, carcinoma in situ, to invasive squamous cell carcinoma (SCC) [1-4]. Treatment options include surgical excision following the Shields' "no-touch" technique with or without cryotherapy, radiation therapy, topical chemotherapy, immunotherapy, and even enucleation or orbital exenteration for advanced cases [5-7]. For topical chemotherapy, the commonly used drugs include urea, retinoic acid, cidofovir, dinitrochlorobenzene, 5-fluorouracil, interferon, and mitomycin C (MMC) [8]. MMC is a potent alkylating agent isolated from Streptomyces caespitosus that inhibits DNA synthesis and causes cell death [9]. It is widely used for treatment of recurrent pterygia, conjunctival-corneal intraepithelial neoplasia, and recurrent conjunctival-corneal squamous cell carcinoma [10-12].

OSSN patients may present with severe symptoms, and many fail to attend regular follow-up due to time and geographic constraints or other reasons. It is therefore very important to ensure the success of initial therapy. Different combinations of the treatment options mentioned above have been reported to be successful in various studies in the past [13-15]. In the present study, we aim to retrospectively analyze the treatment outcome of cryotherapy combined with MMC after surgical excision for both primary and recurrent OSSN.

Material and methods

Patients

This study was approved by the Institutional Review Board and Clinical Research Ethics Committee of Xiangya Hospital Central South University and was performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments. Written informed consents were obtained from all patients before surgery.

A total of 19 patients (19 eyes) with OSSN were treated with the combination of cryotherapy and MMC after surgical excision at Xiangya Hospital, Central South University, China, between August 2010 and August 2017. All surgeries were performed by the same experienced surgeon (PBW). All patients underwent ultrasound examination of head, neck and the abdomen preoperatively and were followed up for a mean length of 55.8 ± 25.4 months (range: 18-102 months) after surgery.

Procedure

The surgery was performed under topical anesthesia with 0.4% obuvacaine, or retrobulbar anesthesia with 1.0% lidocaine and 0.25% bupivacaine, or general anesthesia. The "no-touch" technique was used to remove the mass region of lesion, including a 2-mm tumor-free margin of normal conjunctival tissue and a 1-mm tumor-free margin of corneal epithelium. The removed tissue was then sent for histopathological diagnosis. Cryotherapy was applied to the conjunctival and limbal edges using a double freeze-thaw method, followed by 0.04% MMC incubation. Briefly, the cryoprobe was placed at a point on the lifted conjunctival margin for 15 seconds and then removed, allowing the frozen tissue to thaw naturally. The freeze-thaw cycle was then applied to the adjacent area, overlapping the previous area slightly to ensure no area left untreated. The procedure was then repeated over the entire perimeter. After the double freeze-thaw cryotherapy was completed, a sponge soaked with 0.04% MMC was placed on the lesion area and subconjunctival tissue for 3 minutes. The sponge was then removed, and the MMC was washed off with sterile saline. Finally amniotic membrane transplantation (AMT) was performed. Amniotic membrane was placed with the epithelial side up over the area of excision and fixed in place with 10-0 nylon sutures. A new set of sterile instruments was used for this procedure to avoid contamination of tissue with tumor cells.

Data collection

Data were collected by reviewing clinical records and the presurgical anterior segment photographs. Information was collected regarding demographic characteristics (age, sex), previous history of OSSN, previous treatment if any, characteristics of the ocular surface tumor, tumor stage according to American Joint Committee on Cancer (AJCC) [16], ocular structures involved and appearance, histopathological diagnosis, any additional treatment, complications, and recurrence. For analysis, tumors were categorized into three groups as follows: small (less than 3-clock-hour involvement or less than 5 mm in greatest dimension); medium (3-to 6-clock-hour involvement or greatest dimension 5-10 mm); or large (greater than 6-clock-hour involvement or greatest dimension more than 10 mm) [17]. Recurrence was defined as the reappearance of pathologically confirmed OSSN at the same site or at an adjacent site. Follow-up was calculated from the first day after the surgery to the last visit. Complications include redness, irritation, itching, pain, limbal stem cell deficiency, hyphema, infection, scleral dissolution, symblepharon, and diplopia.

Statistical analysis

The main outcome measure was the recurrence rate. For recurrence analyses, we used the Kaplan-Meier method to analyze the correlation between variables and disease-free survival. The measurement data are represented by average ± standard deviation. The data were analyzed using SPSS 22.0 (IBM Corp, Armonk, NY, USA). $P \leq 0.05$ was regarded as statistically significant.

Results

A total of 19 patients (13 males and 6 females) were included in this retrospective review, with a mean age of 59.9 ± 11.4 years (range: 34-78 years) and a mean follow-up of 55.8 ± 25.4 months (range: 18-102 months). **Table 1** presents the demographic data and tumor characteristics. Two patients in our sample had recurrent tumor; both had been treated with cryotherapy and AMT in excision at another hospital earlier.

16 patients had tumor involving both the conjunctiva and the cornea, whereas 3 patients had tumor involving only the conjunctiva. Only 7 patients had lesion less than 6-clock-hour involvement or smaller than 10 mm in greatest dimension, whereas the other 12 patients had larger lesions. Histopathological diagnosis included dysplasia in 10 (52.6%) patients, carci-

Table 1. Demographic data and characteristics of ocular surface squamous neoplasia

Characteristics	
Age, yrs, mean (range)	59.9 ± 11.4 (34-78)
Sex n (%)	
Male	13 (68.4)
Female	6 (31.6)
Involved eye, n (%)	
Right	10 (52.6)
Left	9 (47.4)
Previous OSSN, n (%)	2 (10.5)
Location, n (%)	
Nasal	6 (31.6)
Temporal	7 (36.8)
Superior	2 (10.5)
Inferior	2 (10.5)
Whole	2 (10.5)
Size, n (%)	
Small	4 (21.1)
Medium	3 (15.8)
Large	12 (63.2)
Tissue involved, n (%)	
Conjunctiva	3 (15.8)
Conjunctiva and cornea	16 (84.2)
Appearance, n (%)	
Leukoplakia	1 (5.3)
Papillomatous	9 (47.4)
Nodular	4 (21.1)
Gelatinous	5 (26.3)
Clinical AJCC stage, n (%)	
T_1	2 (10.5)
T_2	5 (26.3)
T_3	12 (63.2)
Pathology, n (%)	
Dysplasia, mild	2 (10.5)
Dysplasia, moderate	2 (10.5)
Dysplasia, severe	6 (31.6)
Carcinoma *in situ*	4 (21.1)
Squamous cell carcinoma invasive	5 (26.3)

OSSN = Ocular surface squamous neoplasia; AJCC = American Joint Committee on Cancer. Small means that the tumor's diameter is less than 5 mm or 3 clock hours in range; Medium means that tumor's diameter is in the range of 5-10 mm, or the range between 3 and 6 clock hours; Large means that the size or range of the tumor is larger than the medium. Whole refers to all surrounding conjunctival and/or corneal tissue involved.

noma in situ in 4 (21.1%) patients, and SCC in 5 (26.3%) patients. No patient had metastasis. The AJCC clinical stage was T1 in 2 (10.5%) patients, T2 in 5 (26.3%) patients and T3 (63.2%) in 12 patients.

All patients got complete epithelialization of ocular surfaces after surgery. Full cure, with no signs of recurrence, was achieved in 17 (89.5%) patients. However, 2 patients developed recurrences (at 3 months and 6 months after operation). One recurrent patient had initially presented with a recurrent tumor after treatment at another hospital. Both patients had progressive diminution of vision and did not receive any further radiation therapy, topical chemotherapy, or immunotherapy, but ultimately required orbital exenteration due to tumor spread to the orbit. Postoperative pathological examination revealed that the tumor had changed from carcinoma in situ to invasive SCC in one patient. Neither patient had any further recurrence during the follow-up period. Kaplan-Meier analysis showed a 91.0% recurrence-free survival rate (9.0% recurrence rate) at 6-month, 1-year, and 5-years (**Figure 1**).

For complications, redness of the eye occurred in all (100%), while irritation, itching, and pain in the eyes occurred in 15 (78.9%), 17 (89.5%), and 4 (21.1%) patients, respectively (**Table 2**). No severe complications such as limbal stem cell deficiency, infection, scleral dissolution, or diplopia occurred in any patient during the follow-up period.

Discussion

We retrospectively analyzed the outcomes of OSSN treated with a combination of cryotherapy and MMC at our hospital. In this sample of 19 patients, full cure was achieved in 17 patients, while 2 patients had recurrence and ultimately required exenteration.

In the current study, almost two-third of our samples were from men, with a mean age of 59.9 years, which is consistent with past studies showing OSSN primarily occurred in elderly men [4, 18, 19]. Although multiple treatments are available for OSSN, surgical excision is the most commonly used option, especially for patients with large tumors and poor likelihood of follow-up compliance. However, failure to completely excise the mass can result in recurrence [20-22], which usually presents with more aggressive behavior and a tendency for diffuse lateral

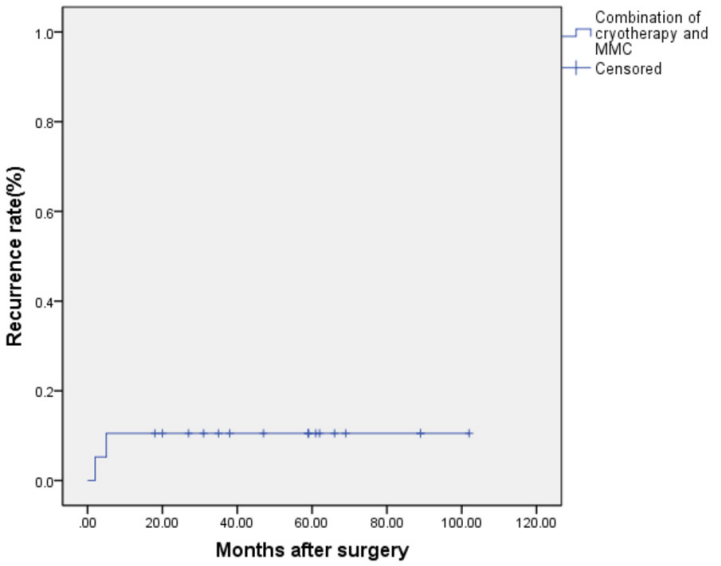

Figure 1. Kaplan-Meier plot depicting time to recurrence in ocular surface squamous neoplasia patients treated with cryotherapy and mitomycin C (MMC) after excision.

Table 2. Complications following treatment of ocular surface squamous neoplasia with the combination of cryotherapy and mitomycin C

Complications	n (%)
Itching	17 (89.5)
Irritation	15 (78.9)
Pain	4 (21.1)
Redness	19 (100)
Limbal stem cell deficiency	0
Hyphema	0
Infection	0
Scleral dissolution	0
Symblepharon	0
Diplopia	0

growth and spread along the basal layer [19]. In our sample, one patient with recurrence developed SCC from carcinoma in situ. Therefore initial therapy should aim at eradication of the tumor and, to that end, combination therapies or additional treatment such as radiation therapy, topical chemotherapy, and immunotherapy should be considered.

We used a combination of cryotherapy and topical MMC after surgical excision for treatment of OSSN. Cryotherapy can help prevent tumor recurrence by destroying residual tumor cells in the excision margin [17, 23-26]. Moreover, cryotherapy has been recommended to be done on the conjunctival margin rather than the sclera or cornea after excision [5, 20]. MMC has also been proved to be effective in killing residual tumor cells and preventing recurrence [11, 15, 27, 28]. However, MMC may cause limbal cell deficiency when used continuously in the form of eye drops [29].

No patient in our cohort had any serious complication, indicating that the combination of surgical excision, cryotherapy and MMC after surgical excision may be a safe and effective treatment option. Our results indicate that short-term MMC application during surgery is less likely to cause adverse effects than prolonged use of low-concentration MMC drops after operation. Although cure was achieved in 17 of our patients, 2 patients had recurrence and finally needed orbital exenteration, showing that the combination of cryotherapy and MMC may have its own limitations. Recurrence occurred at 3 months and 6 months after the operation, implying the importance of long-term follow-up. Accord-ing to a previous report [30], close follow-up is essential, especially for the first 2 years.

Some authors [30, 31] have pointed out that local chemotherapeutic drugs alone can be effective for treatment of OSSN. However, the high risk of complications and high cost of treatment cannot be ignored, especially in patients with large tumors and in need of close follow-up. Furthermore, without an excisional biopsy the nature of lesion will be unknown, which increases the risk of misdiagnosis or overtreatment.

In conclusion, the combination of cryotherapy and MMC after excision appears to be an effective and safe treatment for OSSN. It may be especially useful for patients with large tumors and low likelihood of compliance with follow-up. Further studies are needed to clarify the optimum timing (before or after surgery) and duration of local chemotherapy.

Disclosure of conflict of interest

None.

Address correspondence to: Dr. Pingbao Wang, Institute of Ophthalmology, Xiangya Hospital, Central South University, 87 Xiangya Road, Kaifu District, Changsha, Hunan Province, China. Tel: +86-13787-285023; E-mail: pingbao_wang@hotmail.com

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