Synergistic Effects of Cisplatin-epigel and Interstitial KTP Laser Treatment on a Xenografted Squamous Cell Carcinoma

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Background and Objectives

Cisplatin is an important chemotherapy drug for the treatment of head and neck cancer. Interstitial laser treatment (ILT) has cosmetic utility and is very important for maintaining the function of the head and neck after cancer treatment. This study examined the synergistic effects of locally injected cisplatin-epigel and high fever induced by an interstitial potassium titanyl phosphate (KTP) laser treatment on a xenografted human Heinz squamous cell carcinoma.

Materials and Methods

SNU-1041 (107 cells/0.1 ml) cells were xenografted into the back of nude mice by subcutaneous injection. The ILT group (n = 10) was treated with a KTP laser (1 J/mm³) through a cylindrical diffuser tip inserted into the tumor, monitoring the temperature at 43-45°C. In the combined treatment group (n = 10), local hyperthermia was induced by intratumoral injection of 100-200 µg of cisplatin into a collagen-based gel carrier (cisplatin-epigel), which was released slowly four hours before ILT. After four weeks of follow-up, the treated tumors were evaluated for tumor remission and volume change.

Results

Eight (80%) of the combined group showed complete tumor remission at the four-week follow-up, whereas only three (30%) of the ILT group showed remission (30%) ($p < 0.01$).

Conclusion

The current study has shown the synergistic effects of a local cisplatin injection and high fever from ILT on a xenografted human Heinz squamous cell carcinoma.

Key words

Interstitial laser therapy; Cisplatin-epigel; Squamous cell carcinoma

INTRODUCTION

The head and neck region has many restrictions for the treatment of cancers that develop in this region because of its functional and cosmetic importance. The speech disorders associated with surgery for laryngeal cancer and the functional cosmetic deficits associated with surgery for oral and maxillary cancer have placed many restrictions on the postoperative social adaptation for patients. The ultimate aim is to preserve the normal tissue and selectively remove the lesion site. Interstitial laser therapy can improve the quality of life for post-treatment patients with head and neck cancer.^{1,2}

Until recently, there have been some reports that combined treatment with cisplatin and hyperthermia can enhance the therapeutic effect of cancer in the head and neck region.³⁻⁷ On the other hand, high fever increases vascular blood flow, the permeability of the cell membranes, facilitates the intratumoral absorption of cisplatin, and increases the sensitivity of cisplatin to living cells.^{3,8} Davidson et al. reported that after surgical removal of recurrent cancer, cisplatin gel could be administered locally to prevent recurrence if the arm remains on a frozen section examination.⁹

Cisplatin-epigel can be administered topically and remain concentrated in the local tissue for four hours or more to reduce the systemic side effects and improve the local therapeutic effect.^{9,10} Combination therapy with hyperthermia has been studied because the local administration of cisplatin alone cannot completely cure cancer.^{10,11}

This study examined the anticancer effects of interstitial laser treatment merge treatment using cisplatin-epigel and potassium titanyl phosphate (KTP), which have excellent tissue persistence, after local administration in xenografted head and neck squamous epithelial cell tumors.

MATERIALS AND METHODS

Head and neck cancer cell line

The SNU-1041 squamous cell carcinoma cell line established from hypopharynx cancer patients was cultured in a culture flask (Nunc Inc., Naperville, IL, USA) to RPMI-1041 (Gibco BRL, Grand Island, NY, USA) media 500 ml, fetal bovine serum (Gibco BRL) 50 ml, and antibiotics. The cell culture medium mixed with 5 ml of antimycotic (Gibco BRL) was cultured in a CO₂ incubator.¹²

Experimental animals and breeding conditions

In this study, six-week-old BALB/C/nu/nu male nude

mice were bred in a filter-top cage while being supplied with sterilized water and radioactivity survey sterilized feed. The mice were kept aseptically in an animal laboratory with the appropriate temperature and humidity control. This study was approved by the Institutional Review Board (IRB) of Dankook University of Medicine, Cheonan, Korea. All of the experiment protocols on animals were conducted in accordance with the Institutional Animal Care guidelines of Dankook University of Medicine, Cheonan, Korea.

Xenotransplantation of cell lines

To xenograft the cultured cell lines, such as nude mice, the 107 SNU-1041 cell lines were made into 107 cells 0.1 ml and 0.1 ml each was injected subcutaneously into a nude mice through a 30G insulin syringe.

Cisplatin-epigel

Cisplatin-epigel (Matrix pharmaceutical, Fremont, CA, USA) was made by mixing a cisplatin solution (3.3 mg/ml), epi-nephin solution (1 mg/ml), and bovine collagen (65 mg/ml) using two syringes, 30 gauge. The gel was injected into the tumor through a syringe.

Treatment of xenografted head and neck tumors

Treatment was performed when the size of the xenografted tumor grew to approximately 200 to 1000 mm³. Tumor size was monitored twice weekly, and its volume calculated as $V = (a \times b)/2$, where a is the length and b is width.

The experimental groups were divided into two groups and treated as follows.

Group 1 (n = 10): Serious laser treatment

A KTP 532 laser (Laserscope, San Jose, CA, USA) was examined intratumorally at 0.5 W at 1 J/mm³ using a cylindrical diffuser tip (600 μm diameter; Laserscope). A temperature probe during treatment was used to maintain the temperature of the tumor margin at 43-45°C.

Group 2 (n = 10): Cisplatin-epigel plus KTP laser therapy

Cisplatin-epigel was administered topically. Four hours later, the tumors were treated with a KTP laser at 0.5 W at 1 J/mm³ using a diffuser tip while maintaining the temperature of the tumor margin at 43-45°C.

Analysis of experimental results

The degree of the initial response of the tumors for treatment in groups 1 and 2 was classified as complete

remission, partial remission, and non-responsive. Complete remission groups are defined as the tumor volume decreased less than 1 mm^3 . In addition, the clinical course, recurrence rate, and changes in tumor volume were observed through the follow-up for four weeks or longer (Fig. 1). Therapeutic effects of each experimental group were compared and analyzed. A chi-square test was used for statistical processing.

RESULTS

In this experiment, no side effects, such as vomiting and loss of appetite, due to cisplatin-epigel, were observed. In the first group, which received only interstitial laser treatment, partial tumor remission was observed in seven patients (70%) and complete remission was observed in three patients (30%). In the second group, which merged cisplatin-epigel with an interstitial laser treatment, minimal scar tissue was found in the second week, when complete remission of the tumor was observed 3-5 days after treatment because of the previous case (Fig. 2).

Looking at the average volume change of the tumor, in the first group, the tumor suppression effect was observed only by the interstitial laser treatment using the KTP laser at the average of 575.4 mm^3 before the treatment and 178.9 mm^3 at the 4th week after the treatment. In the second group, the 530.9 mm^3 size tumor before treatment decreased to 27.2 mm^3 at four weeks after treatment, and a greater anticancer effect was observed compared to the laser alone group (Fig. 3). The follow-up

results four weeks after treatment showed complete disappearance of the tumor in three patients (30%) in group 1 and eight patients (80%) in group 2, showing a statistically significant difference.

DISCUSSION

Interstitial laser treatment can reduce the postoperative exchange rate compared to palliative surgical treatment and can be used repeatedly to enhance the therapeutic effects.^{1,2} In addition, squamous cell carcinoma of the head and neck can be treated accurately by interstitial laser treatment under the supervision of magnetic resonance imaging and Doppler.^{1,13-16} Castro et al. performed repetitive interstitial laser treatment in patients with recurrent squamous cell carcinoma of the head and neck region to reduce pain and improve their respiratory and swallowing functions. In most patients, complete disappearance of the tumor was observed, but in most cases,

Fig. 1. (A) SNU-1041 cells xenografted nude mice. (B) Xenografted mice after cisplatin and potassium titanyl phosphate (KTP) laser treatment.

Fig. 2. Comparison of the tumor remission rate between two groups.

Fig. 3. Comparison of the tumor volume change between two groups.

recurrence occurred, and it was not possible to improve survival.^{1,2} The KTP laser used in this study is the most frequently used laser in interstitial laser treatment because it penetrates approximately 5 to 6 mm in the tissue and is in the visible light region.¹⁷⁻¹⁹

Cisplatin induces tissue necrosis when administered topically. Most drugs, particularly when administered directly to tumors with developed blood vessels, spread to the periphery of the tumor and throughout the body in a short period, increasing the concentration of anticancer drugs at the tumor site. The necrosis of normal tissue around the tumor can be rehabilitated without maintaining the cancer.^{11,20} The purpose of topical chemotherapy is to increase the concentration of anticancer drugs in the vicinity of the cells of the tumor tissue.²¹ When cisplatin is administered locally, the drug escapes from the tumor in a short time. Therefore, studies have been conducted using drug carriers, such as liposomes and magnetic albumin spheres.²² The cisplatin-epigel used in this study is an inexpensive cisplatin for collagen, and epinephrine was added to keep the drug in the tumor at a high concentration for 24 hours or more. Yu et al. performed the topical administration and reported temporary remission of some tumors, but all recurred during the follow-up.²¹ Paiva et al. administered cisplatin topically to achieve partial tumor remission, and in recent clinical trials, cisplatin-epigel was administered locally to head and neck cancer and gradually administered intratumorally. A method of releasing cisplatin components to enhance the effects of tumor treatment and reduce systemic side effects was reported.^{1,11,20}

Hyperthermia promotes the intracellular absorption of cisplatin and increases the sensitivity of the tumor cells to drugs.⁴⁻⁶ Moreover, even tumors resistant to cisplatin can be treated in combination with hyperthermia at 43°C.⁷ When cisplatin is administered systemically, hyperthermia appears to have a synergistic effect before and after administration. On the other hand, when cisplatin is administered locally the maximum effect can be obtained if administered before hyperthermia is performed.³⁻⁵ Therefore, in this study, cisplatin-epigel was administered intratumorally, and four hours later, the thermal effect of the interstitial laser treatment was induced to maximize the synergistic effects. When the KTP laser is used at 0.5 watts through the diffuser tip, the temperature can be maintained at 43-45°C at 5 mm from irradiated area, thus activating cisplatin. The tumor tissue is removed, and normal tissue is maintained. Hence, this laser is considered the most suitable laser.

Graeber et al. reported that mice xenografted with a

squamous cell carcinoma originating from the lung were treated with cisplatin and an Nd:YAG laser. The treatment results were superior to those of the laser or cisplatin-alone administration groups.¹⁰ In this experiment, a five-watt Nd:YAG laser was inserted into the tumor via a bare fiber tip at an interval of 3 mm. The follow-up test showed that the tissue temperature was instantaneously over 100°C. Elevated Nd:YAG laser tissue permeability caused severe damage not only to the tumor but also to the normal tissue around the tumor.¹⁰ In this study, the author transmitted a 0.5-watt KTP laser into the tumor using a diffuser fiber tip to prevent coagulation and charring in the center of the tumor and improve the tumor tissue penetration of the laser. In addition, the temperature of the tumor margin was maintained at 43 to 45°C. to prevent protein destruction due to the laser alone effect, and the synergistic effects of the cisplatin laser hyperthermia could be observed.^{23,24}

Castro et al. reported that intratumoral treatment with a laser could reduce the prevalence compared to conventional treatment, but would recur at the margin of the tumor if a significant portion of normal tissue was not treated concomitantly.^{1,2} Paiva et al. performed conjugation therapy using a bare fiber after the administration of cisplatin-Epigel in xenografted lung tumors to compensate for these shortcomings, but maintained the laser at 1 watt or more. By keeping the temperature in the tumor tissue too high, the benefits of the combined therapy could not be exploited.¹¹ In addition, many researchers have reported the results of combination therapy of anticancer drugs and lasers, particularly combination therapy using anticancer drugs, such as cisplatin and daunomycin, and the heat and light of the laser. Although it was presented using multiple studies, and there are no reports of its effects on squamous cell carcinoma of the head and neck, in this study, cancer cells cultured in hypopharynx cancer patients were transplanted heterologously into nude mice. The results confirmed the therapeutic effect of this treatment on the resulting cancer.

CONCLUSION

The intratumoral administration of cisplatin for xenografted head and neck squamous cell tumors and interstitial laser treatment with minimal laser power and a diffuser tip to maintain an intratumoral temperature above 43°C, produced excellent therapeutic effects. In the future, it is expected that it can be developed as an effective treatment method that can minimize the functional cosmetic damage to head and neck cancer and reduce

the systemic side effects through animal experiments and clinical trials.

CONFLICT OF INTEREST

Seung Hoon Woo is the Editor-in-Chief of the journal but was not involved in the review process of this manuscript. Phil-Sang Chung is an editorial board members of the journal but was not involved in the review process of this manuscript. Otherwise, there is no conflict of interest to declare.

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