Original Article
Impact of preoperative chemotherapy combined with surgery on cellular and humoral immunity in patients with oral cancer

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Abstract: Most oral cancers belong to squamous epithelium, therefore, they are named oral squamous cell carcinoma (OSCC). Incidence of OSCC has increased year by year. This study aimed to explore the effects of preoperative chemotherapy combined with surgery on cellular and humoral immune function in OSCC patients. A total of 60 OSCC patients were randomly and equally divided into a surgery group and preoperative chemotherapy group. Preoperative oral cancer patients were used as controls. Flow cytometry was used to detect expression of CD4+ T-cells, CD8+ T lymphocytes, and Treg cells. Cytokines interleukin-2 (IL-2), IL-4, IL-5, and IL-17 levels were detected by enzyme-linked immunosorbent assay (ELISA). Expression of immunoglobulin G (IgG), IgA, IgM, and IgE were detected by an automated protein analyzer. Compared to the control group, CD4+ T-cells increased, while CD8+ T-cells and Treg cells decreased. IL-2 secretion was elevated in surgical and preoperative chemotherapy groups. IL-17, IL-4, and IL-5 secretion decreased, while IgA and IgM secretion decreased in the treatment group (P < 0.05). IgG and IgE showed no statistical differences (P > 0.05). Compared to the surgical group, the preoperative chemotherapy group exhibited more significant changes (P < 0.05). Preoperative chemotherapy combined with surgery can promote the increase of CD4+ T-cells, decrease of CD8+ T-cells and Treg cells, increase of IL-2 secretion, reduction of IL-17, IL-4, and IL-5 secretion, elevation of IgA, and IgM secretion in OSCC to regulate cellular and humoral immune function.

Keywords: Oral squamous cell carcinoma, cellular immunity, humoral immunity, antibody, T-cells

Introduction

Oral squamous cell carcinoma (OSCC) is a general term for malignant tumors that occur in the oral cavity. Most belong to squamous cell carcinoma, the so-called mucosal mutation. OSCC is one of the most common types of malignant tumors of the head and neck. Its incidence accounts for 5% of all malignant tumors, occupying the top six [1, 2]. Due to the influence of life and eating habits and changes in the living environment, incidence of OSCC in China is about 5-6/100,000, with a younger and increasing trend [3, 4]. OSCC can metastasize or infiltrate in the early stages of the disease, accompanied by distant metastasis. It has the characteristics of rapid progression and invasiveness, leading to poor prognosis [5, 6]. Following the advancement of medical technology, diagnosis and treatment methods for oral cancer continue to improve. However, the prognosis of oral cancer remains unsatisfactory. Its 5-year survival rate is only 50% to 60%, with about 1/3 of patients relapsing. Furthermore, surgical treatment can seriously affect swallowing, language function, and facial features, threatening health and quality of life [7, 8]. The pathogenesis of oral cancer is complex, but is related to foreign body irritation and oral HPV infections [9]. At present, surgery is still the main method for treatment of oral cancer. Recently, preoperative adjuvant chemotherapy has reportedly obtained good efficacy in oral cancer patients [10].

In the development of oral cancer, participation of the immune system is one of the most important factors. The immune system includes immune organs, tissues, immune cells, and molecules. These are involved in innate immune
response and adaptive immune response, respectively. Of these, adaptive immune response plays an important role in anti-tumor immunity [11]. T-cells and B-cells are crucial cells involved in anti-tumor immunity, in which T-cells mainly participate in cellular immune response in the adaptive immune response, while B-cells are activated by antigen stimulation and differentiate into plasma cells, thus secreting antibodies to participate in humoral immune response [12]. T-cells can be divided into CD4+ T lymphocytes, CD8+ T lymphocytes, and CD4+CD25+FoxP3 Treg cells, based on their markers. These three types of T-cell subsets have an important influence on occurrence and development of tumors [13, 14]. Cellular immune response and humoral immune response play key regulatory roles in the development of tumors [15, 16]. This study aimed to investigate the effects of preoperative chemotherapy combined with surgery on cellular and humoral immunity in OSCC patients.

Materials and methods

General information

This study selected 60 OSCC patients admitted to the First Affiliated Hospital of Henan University of Science and Technology, from March 2017 to February 2018, diagnosed by surgery. There were 33 males and 27 females with a mean age of 41 d age of surgery. There were patients received no anti-tumor treatment before admission. Systemic diseases, severe cardiovascular and cerebrovascular diseases, and abnormalities in the blood and endocrine system were excluded. Sites of the disease included the tongue, cheeks, gums, and mouth floor. Clinical stages were III and IV. Patients were randomly divided into an operation group and preoperative chemotherapy group after providing informed consent. Patients in the operation group were directly operated after admission. Patients in the preoperative chemotherapy group received paclitaxel 150 mg/m² iv plus cisplatin 100 mg/m² iv chemotherapy before surgery. The study was approved by the Medical Ethics Committee of the First Affiliated Hospital of Henan University of Science and Technology. All selected subjects agreed to the study and provided informed consent.

Main reagents and instruments

Mouse anti-human CD4-FITC monoclonal antibody, mouse anti-human CD8-PE monoclonal antibody, mouse anti-human CD4-PE monoclonal antibody, mouse anti-human CD8-PE monoclonal antibody, fixing solution, and the membrane disrupter were purchased from Miltenyi Biotec (Germany). RPMI1640 cell culture medium, fetal bovine serum (FBS), and Hankd Hankk antibody, mouse anti-human FOXP3-PE monoclonal antibody, mouse anti-human CD8-PE monoclonal antibody, fixing soluHuman lymphocyte stratified liquid was purchased from Tianjin Haoyang Biological Co., Ltd. Bechtop was purchased from Suzhou Antai Instrument Co., Ltd. The flow cytometer-EPICS XL was purchased from BECKMAN-COULTER (USA). BN II full-automatic protein analyzer was purchased from Dade Behring (USA).

Methods

Specimen collection: A total of 10 mL of fasting blood was extracted from the elbow superficial vein at 24 hours within admission and after surgery. A total of 5 mL were used to isolate human peripheral blood mononuclear cells (PBMCs). The remaining 5 mL was centrifuged at 3000 rpm for 15 minutes. Serum was placed in Eppendorf (Ep) tubes and stored at -20 Eppendorf (Ep) tubenbl.

Flow cytometry: PBMCs were centrifuged at 1000 rpm for 5 minutes and resuspended in 1.5 mL flow-washing solution. Next, the cells were blocked by serum at 4r 5 min mononutes. After centrifugation at 1000 rpm for 5 minutes, the cells were incubated in 10 m of CD4-FITC, CD8-PE, CD25-APC, and FOXP3-PE at 4on, avoiding light, for 30 minutes. After centrifugation, the cells were treated with 750 μ fixing solution, avoid lighting, for 20 minutes and 1.2 mL membrane-destroying agent. Finally, the cells were mixed in 300 llflow-washing solution and detected on flow cytometry.

ELISA: Serum levels of IL-2, IL-17, IL-4, and IL-5 in each group were detected by ELISA. Collected peripheral blood was centrifuged and the supernatant was taken. This experimental procedure was performed according to ELISA kit instructions. The 50 μL diluted standard substance and samples were added to 96-well plate at 37 ThLISA. t 44 lutes. After washing 5 times, the plate was added with 50 added with 50 s wereμl reagent B at 370 e was added. Finally, the plate was added with 50 μw stop solution and tested on the microplate reader.
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The standard curve was prepared based on the OD value to calculate sample concentrations.

**Immunoglobulin detection:** IgG, IgA, IgM, and IgE protein concentrations were tested on a fully-automatic protein analyzer.

**Statistical analysis**

All data analyses were performed using SPSS 22.0 software. Measurement data are expressed as mean Measurement data onion SD) and were compared by one-way ANOVA or t-test. The test level was taken as value to calculate indicates statistical significance.

**Results**

**CD4⁺ T-cell distribution in OSCC patients receiving preoperative chemotherapy combined with surgery**

Blood was isolated before treatment and preoperative chemotherapy combined surgery to analyze CD4⁺ T-cell distribution changes, using flow cytometry. Results showed that, compared with the control group, CD4⁺ T expression significantly increased in patients treated with preoperative chemotherapy combined with surgery (P < 0.05). The distribution of CD4⁺ T-cells in patients undergoing chemotherapy and surgery was significantly different from that in the surgical group (P < 0.05) (Figure 1). This suggests that surgery or preoperative chemotherapy combined with surgery obviously upregulated CD4⁺ T-cell distribution more significantly in the combination group.

**CD8⁺ T-cell distribution in OSCC patients receiving preoperative chemotherapy combined with surgery**

PBMCs were isolated before treatment and preoperative chemotherapy combined surgery to analyze CD8⁺ T cell distribution changes, using flow cytometry. Results showed that, compared with the control group, CD8⁺ T expression was markedly reduced in patients treated with preoperative chemotherapy combined with sur-
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The distribution of CD8$^+$ T-cells distribution more significantly in the combination group.

CD4$^+$CD25$^+$Foxp3 Treg cell distribution in OSCC patients receiving preoperative chemotherapy combined with surgery

PBMCs were isolated before treatment and preoperative chemotherapy combined surgery to analyze CD4$^+$CD25$^+$Foxp3 Treg cell distribution changes, using flow cytometry. Results showed that, compared with the control group, CD4$^+$CD25$^+$Foxp3 Treg expression obviously declined in patients treated with preoperative chemotherapy combined with surgery ($P < 0.05$). The distribution of CD4$^+$CD25$^+$Foxp3 Treg cells in patients undergoing chemotherapy and surgery was significantly different from that in the surgical group ($P < 0.05$) (Figure 3). This suggests that surgery or preoperative chemotherapy combined with surgery obviously upregulated CD4$^+$CD25$^+$Foxp3 Treg cells distribution more significantly in the combination group.

Impact of preoperative chemotherapy combined with surgery on cytokines section

Blood samples were collected to test IL-2, IL-17, IL-4, and IL-5 expression changes after preoperative chemotherapy combined with surgery. IL-2 secretion increased, while IL-4, IL-5, and IL-17 secretion reduced in the treatment group compared with controls ($P < 0.05$). Levels were apparently different between the surgical group and preoperative chemotherapy group ($P < 0.05$) (Figure 4). This indicates that surgery or preoperative chemotherapy combined with surgery can change cytokines secretion to impact immune function.

Influence of preoperative chemotherapy combined with surgery on immunoglobulin section

Blood samples were collected to test IgG, IgA, IgM, and IgE expression changes after preoperative chemotherapy combined with surgery. IgA and IgM secretion increased, while IgG and IgE secretion exhibited no statistical changes in the treatment group compared with controls ($P < 0.05$). Levels of IgA and IgM were apparently different between the surgical group and preoperative chemotherapy group ($P < 0.05$) (Figure 5). This indicates that surgery or preoperative chemotherapy combined with surgery...
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Discussion

The exact pathogenesis of oral cancer remains unclear. The entire pathogenesis and process is quite complex. It not only includes external factors, such as diet, environment, and human spirit, but also includes internal factors, such as immunity and endocrine, which leads to reduced tolerance to high-risk factors [17]. Immune status not only affects occurrence and development of tumors, but is closely related to therapeutic effects and prognosis [18, 19]. Immune response first recognizes the antigens on the surface of tumor cells. It activates immune cells, such as T-cells and B-cells, and releases immunoglobulins, cytokines, and other related effector molecules, thereby killing tumor cells or inhibiting tumor cell proliferation and metastasis, ultimately eliminating tumor cells [20, 21]. On the other hand, tumor cells can also escape from the surveillance and killing of immune cells, leading to immune escape and rapid growth, metastasis, and even relapse [22].

T-cells play an important role in the cellular immune response. CD4⁺ T-cells and CD8⁺ T-cells should be in dynamic equilibrium in the normal immune state. Furthermore, both CD4⁺ T-cells and CD8⁺ T-cells can participate in the process of tumor immunity. CD4⁺ T-cells can further transform into helper T-cells involved in the humoral immune response process, while CD8⁺ T-cells are mainly cytotoxic T-cells that can specifically target and kill abnormal cells, including tumor cells [23]. In general, the two subpopulations of T-cells are mainly immunosuppressed during tumorigenesis [24]. Treg cells in T-cell subsets generally exert immunosuppressive effects, thereby promoting immune escape of tumor cells [25]. Humoral immunity also plays an important role in tumorigenesis [26]. At present, surgery is the main treatment for oral cancer. It has been considered that preoperative chemotherapy is beneficial in controlling metastasis and recurrence of oral cancer [27]. However, the effects of preoperative chemotherapy on immune function have not been elucidated. This study confirmed that CD4⁺ T-cells and CD8⁺ T-cells were in a lower ratio range in OSCC before surgery. CD4⁺ T-cells increased, while CD8⁺ T-cells and Treg cells decreased after surgical treatment. Moreover, it promoted IgA and IgM expression in humoral immunity and regulated relevant cytokines secretion, thereby participating in the cytokine network and immune function. Results suggest that preoperative chemotherapy is beneficial in controlling metastasis and recurrence of oral cancer [27].

Figure 5. Influence of preoperative chemotherapy combined with surgery on immunoglobulin section. A. IgG, IgA, and IgM secretion analysis; B. IgE secretion analysis. *P < 0.05, compared with control; †P < 0.05, compared with surgical group.

can change immunoglobulin secretion to impact humoral immune function.
Conclusion

Preoperative chemotherapy combined with surgery can promote an increase of CD4⁺ T-cells, decrease of CD8⁺ T-cells and Treg cells, increase of IL-2 secretion, reduction of IL-17, IL-4, and IL-5 secretion, and elevation of IgA and IgM secretion in OSCC to regulate cellular and humoral immune function.

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Disclosure of conflict of interest

None.

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