Cone-beam CT Evaluation of Tea Polyphenols on Postoperative Sinus Tract Healing in Radiation-Induced Osteomyelitis of the Jaw: A Postprint

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Abstract

Objective: To evaluate the effect of tea polyphenols on postoperative sinus tract healing in radiation-induced osteomyelitis of the jaw using cone-beam CT and fistulography.

Methods: Thirty patients with postoperative radiation-induced osteomyelitis of the jaw with oral mucosal sinus tracts were enrolled and randomly divided into 2 groups of 15 patients each. The sinus tracts were treated with daily local irrigation and mouth rinsing using either compound tea polyphenol mouthwash (experimental group) or normal saline (control group). Cone-beam CT fistulography was performed every 3 weeks to calculate changes in sinus tract volume. The MTT assay was used to detect the effect of tea polyphenols on the proliferation of a human immortalized oral mucosal epithelial cell line.

Results: After 12 weeks of treatment, the experimental group showed superior sinus tract reduction compared to the control group (5.40±4.15 cm^3 vs 8.12±5.47 cm^3), with statistical significance (F=4.384, P=0.043). The CCK-8 assay revealed that after treatment with 120 mg/L tea polyphenols, the proliferation rate of the human immortalized oral mucosal epithelial cell line was higher than that of the untreated control group (0.162±0.030 vs 0.075±0.017), with statistical significance (F=5.783, P=0.017).

Conclusion: Tea polyphenols may accelerate healing of postoperative sinus tracts in radiation-induced osteomyelitis of the jaw by promoting proliferation of oral mucosal epithelial cells.

Full Text

Preamble

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Effect of Tea Polyphenols on Sinus Healing in Post-operative Radioactive Osteomyelitis of the Jaws Evaluated by Cone Beam CT

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Abstract

Objective: To evaluate the effect of tea polyphenols on sinus healing in post-operative radioactive osteomyelitis of the jaws (ORN) through cone beam CT and sinus tractography. **Methods:** Thirty ORN patients with post-operative oral mucosal sinus tracts were collected and randomly divided into two groups (n=15 each). Sinus tracts were irrigated and gargled daily with either compound tea polyphenol mouthwash (experimental group) or normal saline (control group). Cone beam CT sinus tractography was performed every three weeks to calculate sinus volume changes. The MTT assay was used to detect the effect of tea polyphenols on the proliferation of a human immortalized oral mucosal epithelial cell line. **Results:** After 12 weeks of treatment, sinus reduction in the experimental group was superior to that in the control group (5.40±4.15 cm^3 vs. 8.12±5.47 cm^3), with statistically significant difference (F=4.384, P=0.043). CCK-8 assay revealed that treatment with 120 mg/L tea polyphenols resulted in a higher proliferation rate of human immortalized oral mucosal epithelial cells compared with the untreated control group (0.162±0.030 vs. 0.075±0.017), which was statistically significant (F=5.783, P=0.017). **Conclusion:** Tea polyphenols may accelerate the healing of post-operative sinus tracts in ORN by promoting oral mucosal epithelial proliferation.

Keywords: tea polyphenols; radioactive osteomyelitis of the jaws; sinus tract; epithelial cells; proliferation

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Introduction

Radioactive osteomyelitis of the jaws is a common oral complication following radiotherapy for head and neck malignant tumors [1]. After radiation, the

jawbone exists in a "three-low" state characterized by hypoxia, low vascular structure, and low cellular activity, making it prone to aseptic necrosis and secondary bacterial infection that leads to osteomyelitis [2]. Previous studies have demonstrated that tea polyphenols possess protective effects against radiation-induced diseases [3] along with antioxidant and anti-infective pharmacological properties [4-5]. The main component of tea polyphenols is catechin (a flavan-3-ol derivative) [6], which exhibits proliferative effects on certain somatic cells. He et al. [7] found that tea polyphenols could promote the in vitro proliferation of periodontal ligament fibroblasts, though no relevant studies have investigated their effects on oral mucosal epithelium. This led to the hypothesis that tea polyphenols might accelerate the healing of post-operative mucosal sinus tracts in radioactive osteomyelitis by promoting oral mucosal epithelial cell proliferation. The present study investigated this effect by comparing sinus volume changes in post-operative ORN patients and assessing the proliferative capacity of a human immortalized oral mucosal epithelial cell line (NOK), providing experimental evidence for adjuvant therapy in radioactive osteomyelitis.

1.1 Study Subjects

Thirty patients with radioactive osteomyelitis of the jaws admitted to Longmen County People' s Hospital, Huizhou, between January 2013 and June 2016 were enrolled. Using a 1:1 case-control design, patients were randomly allocated to experimental and control groups through sequentially numbered, opaque, sealed envelopes. This study was approved by the hospital ethics committee and conducted with informed consent from all participants.

1.1.1 Inclusion Criteria

1. History of head and neck tumor radiotherapy

2. Diagnosis of radioactive osteomyelitis based on clinical and imaging manifestations, using Harris' s criteria [8]: "irradiated jawbone loses vitality, becomes exposed beneath the overlying skin or mucosa, persists for more than three months without healing, and shows no local tumor formation"

3. Formation of oral mucosal sinus tracts with delayed wound healing after conventional surgical treatment (including curettage, jaw resection, etc.), without internal-external fistula communication

1.1.2 Exclusion Criteria

1. Bone destruction caused by other diseases such as tumors or bisphosphonate-related osteonecrosis [9]

2. Residual osteomyelitis lesions or sequestrum confirmed by postoperative imaging

3. Pregnant or lactating women

4. Severe cardiovascular disease or hepatic/renal insufficiency

1.1.3 Dropout Criteria

1. Allergic reactions or severe adverse effects to study medications

2. Non-compliance or incomplete treatment for various reasons

3. Development of severe illness during treatment preventing continuation

4. Cases completing less than half the treatment course were excluded from statistical analysis

Based on these criteria, 30 cases were enrolled and single-blind randomly divided into experimental and control groups (15 cases each). One patient in the control group withdrew at week 9 for further treatment elsewhere, and two patients in the experimental group discontinued treatment due to personal reasons. All three cases had completed more than half the planned treatment and were included in the statistical analysis. Comparison of baseline data showed no statistically significant differences between groups in age, gender, radiation dose, lesion location, or surgical approach, indicating relatively consistent distribution (Table 1).

1.2 Treatment Protocol and Sinus Volume Measurement

Experimental group patients received outpatient treatment every other day, with tea polyphenol mouthwash irrigation of unhealed sinus tracts and instruction to gargle with tea polyphenol mouthwash twice daily for 3 minutes each time. The control group received saline irrigation and gargling. Sinus volume was measured every three weeks as follows: meglumine diatrizoate was injected into the sinus tract until filled, the sinus opening was sealed with medical hydrogel dressing, and cone beam CT imaging was performed using Pax-Uni3D (Vatech, Korea) at 110 kV, 2.0 mA, with 3.6 s exposure time by an experienced radiologist following manufacturer protocols. Following He et al. [10], images were reconstructed three-dimensionally using Ez3D plus software (Vatech, Korea) with maximum intensity projection algorithm. DICOM data were imported into Mimics 14.1 software for three-dimensional volume calculation.

1.3 Cell Culture Experiments

1.3.1 Oral Mucosal Epithelial Cell Culture The NOK cell line was purchased from Shanghai Zhonghua Biotechnology Co., Ltd. Cells were cultured in

RPMI 1640 complete medium with 10% FBS at 37 °C in 5% CO .

1.3.2 Reagents and Instruments Tea polyphenols (98% purity) from Jiangxi Lükang Natural Products Co., Ltd.; CCK-8 from Dojindo Chemical, Japan; RPMI 1640 medium and fetal bovine serum from Gibco, USA; M200 automatic microplate reader from Tecan, Switzerland.

1.3.3 CCK-8 Assay for Relative Cell Growth Rate Logarithmic-phase NOK cells were seeded in 96-well plates at 4×10 cells/well. After cells covered the well bottom, the original medium was removed. The experimental group received complete medium containing 120 mg/L tea polyphenols, while the control group received polyphenol-free medium. Daily, 10 L CCK-8 solution was added to 8 wells, incubated for 4 hours, and absorbance (OD) at 450 nm was measured to generate a 1-5 day growth curve.

1.4 Statistical Analysis

SPSS 23.0 software was used for statistical analysis. Measurement data were expressed as mean ± standard deviation. The Mann-Whitney nonparametric rank-sum test compared baseline data consistency between groups. Repeated measures ANOVA analyzed sinus volume changes and NOK cell growth curves. All tests were two-tailed with $P<0.05$ considered statistically significant.

Results

2.1 Sinus Volume Changes

Cone beam CT-based sinus tractography reconstructed images with clear contour boundaries and enabled precise sinus volume calculation (Figure 1 [Figure 1: see original paper]). After local irrigation and oral care, both groups showed varying degrees of sinus volume reduction over time (Figure 2 [Figure 2: see original paper]). At 12 weeks post-treatment, the experimental group demonstrated significantly smaller sinus volume than the control group (5.40±4.15 cm^3 vs. 8.12±5.47 cm^3), with statistically significant intergroup difference ($F=4.063$, $P=0.033$).

2.2 NOK Cell Growth Curve

NOK cells cultured with tea polyphenols (experimental group) showed higher absorbance after 5 days compared with control cells in normal medium (0.162±0.030 vs. 0.075±0.017), indicating greater proliferation rate (Figure 3 [Figure 3: see original paper]). The difference was statistically significant ($F=5.783$, $P=0.017$), suggesting tea polyphenols promote NOK cell growth.

Discussion

The primary treatment for radioactive osteomyelitis of the jaws involves surgical removal of necrotic bone and diseased soft tissue to achieve radical cure [11]. However, fibrous atrophy or fibrosis of irradiated tissues results in reduced proliferative, anti-infective, and metabolic capacities compared with normal tissue, potentially leading to poor wound healing and secondary sinus tract formation postoperatively [12]. Accurate localization and volume measurement of sinus tracts provide important references for subsequent treatment. In oral and maxillofacial regions, sinus tractography via injection of contrast agents (such as iohexol, iodized oil, or meglumine diatrizoate) followed by X-ray imaging is commonly used for preoperative evaluation of branchial cleft cysts and thyroglossal duct cysts [13-14]. This study employed the water-soluble ionic monomer meglumine diatrizoate, which offers better flow characteristics than oily contrast agents and can be easily removed through routine sinus irrigation afterward. Unlike sinus tracts with cutaneous openings, the cases in this study had intraoral openings that were wide and prone to contrast agent overflow; therefore, alginate hydrogel dressing with good adhesion and biocompatibility [15] was used to seal the sinus openings.

Sinus tract treatment includes surgical and conservative approaches. Large defects are typically repaired with tissue flap transfer [16], while smaller fistulas are managed through local irrigation to maintain hygiene, reduce secondary infection, and promote epithelial and granulation tissue growth for eventual healing. This study found that irrigation with tea polyphenol mouthwash produced ideal adjuvant therapeutic effects, with significantly greater sinus volume reduction compared with the control group, accelerating wound healing. To elucidate the underlying mechanism, we examined the effects of tea polyphenols on epithelial cell proliferation in vitro. Under culture conditions, tea polyphenols exhibit differential effects on epithelial-derived cells—inducing apoptosis in tumor cells [17-18] while promoting proliferation in normal epithelial cells. Hsu et al. [18] demonstrated that tea polyphenols promote proliferation and differentiation of keratinocytes. Our results similarly showed that human immortalized oral mucosal epithelial cells exhibited strong proliferative capacity when induced with 120 mg/L tea polyphenols, suggesting that tea polyphenols may accelerate sinus healing by promoting epithelial cell proliferation.

Furthermore, sinus tracts exposed to the oral cavity are often complicated by bacterial infection, which may impair wound healing. Common pathogens in radioactive osteomyelitis include Staphylococcus aureus and Pseudomonas aeruginosa [19]. Qian et al. [20] verified the antibacterial effects of tea polyphenols against these organisms, suggesting that local application of tea polyphenol mouthwash may inhibit these bacteria in sinus tracts and reduce secondary infection, though this hypothesis requires further experimental verification.

In summary, this study utilized cone beam CT-based sinus tractography to localize and measure post-operative sinus tracts in ORN patients, demonstrating

that tea polyphenols may accelerate sinus healing by promoting oral mucosal epithelial cell proliferation.

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Note: Figure translations are in progress. See original paper for figures.