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Synergistic Inhibition of Nasopharyngeal Carcinoma by Biyan Qingdu Granule and Micro-Radiation: An Experimental Study

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Abstract: *Objective:* To investigate the role of Biyan Qingdu Granule combined with micro-radiation in suppressing nasopharyngeal carcinoma and to explore its mechanism of action. *Methods:* FAT cells of nasopharyngeal carcinoma were injected into the hind limb axilla of F344 rats to establish a rat nasopharyngeal carcinoma tumor model. X-ray radiation was applied to the oral cavity of the rats, delivering a micro-dose of radiation to the distant hind limb axilla tumor. The general condition of the rats, including oral mucosal inflammation, the size of the nasopharyngeal tumor mass, pathological changes in the tumor tissue, and the levels of TNF-α, IL-1β, IL-6, IL-18, IL-12, ICAM-1, and VCAM-1 in the tumor tissue, were assessed. *Results:* Oral administration of Biyan Qingdu Granule improved the rats' overall condition and reduced oral mucosal inflammation. Pathological examination revealed tumor cell degeneration and necrosis. ELISA analysis of tumor tissue showed significant reductions in TNF-α, IL-1β, IL-6, ICAM-1, and VCAM-1 levels, along with a notable increase in IL-18 levels. *Conclusion:* Biyan Qingdu Granule, when combined with micro-radiation, can inhibit tumor growth and reduce tumor volume. Its mechanism of action may involve enhancing immune function and suppressing inflammatory factors.

Keywords: Biyan Qingdu Granule; Nasopharyngeal carcinoma; Radiotherapy; Tumor necrosis factor; Interleukin; Vascular endothelial cell adhesion molecule

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1. Introduction

Nasopharyngeal carcinoma (NPC) is a squamous cell carcinoma originating from the epithelial lining of the

nasopharynx, with clinical symptoms such as nasal obstruction, blood-streaked sputum, ear fullness, hearing loss, and headache [1]. The distribution of NPC cases is highly uneven, with over 70% occurring in East and Southeast Asia [2]. NPC has the highest incidence among malignant tumors of the ear, nose, and throat, with a five-year survival rate of only 30%. It is also highly prone to recurrence, posing a significant threat to patients' health and safety [3]. Studies have found that NPC is highly sensitive to radiotherapy, making it the preferred treatment in clinical practice [4]. Although radiotherapy significantly extends the survival of NPC patients, it also causes adverse reactions, such as radiation rhinitis, which negatively affect prognosis [5]. Consequently, identifying drugs that enhance antitumor efficacy while reducing the side effects of radiotherapy is of critical importance.

Traditional Chinese medicine (TCM) has a long history and offers unique advantages in treating tumors and alleviating secondary symptoms caused by them. Biyan Qingdu Granule is a TCM formula comprising eight herbs, including Chrysanthemum, Xiangerzi, Zhonglou, Maobai Root, Liangmianzhen, Xiakucao, Longdan, and Dangshen. This formula functions to clear heat and detoxify, resolve phlegm, and disperse nodules. It is used for chronic inflammation of the nasopharynx caused by heat, toxins, blood stasis, and increased secretions following radiotherapy for NPC. Studies have demonstrated that Biyan Qingdu Granule can induce apoptosis in nasopharyngeal carcinoma cells, alter cell cycle distribution, and inhibit cell proliferation [6]. Additionally, it has been shown to mitigate oral mucosal damage caused by radiotherapy [7]. Building on previous research, this experiment combines Biyan Qingdu Granule with micro-radiation therapy to evaluate its effects on nasopharyngeal carcinoma tumors in a rat model.

2. Materials and methods

2.1. Materials

2.1.1. Experimental animals

F344 (CDF) rats, 80 males aged 6–7 weeks with body weights of 120–140 g, SPF grade, were provided by Beijing Weitonglihua Laboratory Animal Technology Co., Ltd. (animal license No. SCXK (Beijing) 2021-0006). The rats were housed in a standardized barrier environment with a temperature of 22–25°C and relative humidity of 45%–60%. This experiment was approved by the Ethics Committee of Tianjin Tiancheng New Drug Evaluation Co., Ltd. (approval Nos. 2023031602 and 2023032302).

2.1.2. Experimental cell lines

The mouse nasopharyngeal carcinoma cell line (FAT), obtained from Uniscal (Shanghai) Life Science Co., Ltd., was cultured in the laboratory.

2.1.3. Experimental reagents

(1) Biyan Qingdu Granules (product of Guangzhou Baiyunshan Hutchison Whampoa Chinese Medicine Ltd., batch No. C22A004, approval No. Z44022139, 20 g per bag)
(2) Rat vascular endothelial cell adhesion molecule 1 (VCAM-1/CD106) ELISA kit
(3) Rat tumor necrosis factor α (TNF-α) ELISA kit
(4) Rat interleukin-12 (IL-12) ELISA kit
(5) Rat intercellular adhesion molecule-1 (ICAM-1) ELISA kit

(6) Rat interleukin-1β (IL-1β) ELISA kit
(7) Rat interleukin-6 (IL-6) ELISA kit
(8) Rat interleukin-18 (IL-18) ELISA kit (all kits from Shanghai Lanpai Biotechnology Co., Ltd., batch No. 202303)

2.1.4. Experimental instruments

(1) CO_2 Cell Incubator (311, Thermo Fisher Scientific)
(2) -80°C Cryogenic Storage Box (907, Thermo Fisher Technology (China) Co., Ltd.)
(3) Biological Microscope (WYS-41XDY, Tianjin Microyi Optical Instrument Co., Ltd.)
(4) Multifunctional Enzyme Marker (M5, Berthold, Germany)
(5) High-speed Refrigerated Centrifuge (Sorvall ST 8R, Thermo Fisher Technology (China) Co., Ltd.)

2.2. Methods

2.2.1. Cell culture

Frozen FAT cells were removed from liquid nitrogen and thawed in a 37°C water bath with gentle agitation for rapid melting. Fresh DMEM high-glucose culture medium containing 10% fetal bovine serum and 1% antibiotics was added to a centrifuge tube. The thawed cell suspension was gently pipetted, transferred to the centrifuge tube, centrifuged at 1,000 rpm for 3 minutes, and the supernatant discarded. Fresh DMEM was added to prepare a cell suspension, which was seeded into T25 culture flasks with 5 mL of culture medium. CNE-2 cells were cultured at 37°C in 5% CO2 and saturated humidity using DMEM containing 10% fetal bovine serum, 100 U/mL penicillin, and 100 μg/mL streptomycin. When cells reached 90% confluence, they were digested with 0.25% trypsin (containing 0.02% EDTA), passaged at a 1:3 ratio, and cultured further. FAT cells were similarly digested at 90% confluence, collected via centrifugation (1000 rpm, 10 minutes), and resuspended in PBS to prepare a single-cell suspension with a density of 4×10^7 cells/mL.

2.2.2. Establishment of FAT rat model of nasopharyngeal carcinoma

SPF male F344 rats weighing 120–140 g were shaved in the axillary region of the hind limbs, disinfected with iodine, and subcutaneously injected with 0.5 mL of FAT cell suspension (4×10^7 cells/mL) during the logarithmic growth phase.

2.2.3. Grouping and treatment of experimental animals

Tumor-bearing F344 rats were randomly divided into four groups using the random number table method: model group, low-dose group, medium-dose group, and high-dose group (10 rats per group). Rats in all groups were anesthetized and irradiated with X-rays targeting the nasopharynx and oral cavity at a dose of 10 Gy with a dose rate of 1 Gy/min for five consecutive days. Tumors in the hind limbs received low-dose radiation due to X-ray exposure. The model group received distilled water (10 mL/kg) via intragastric administration, while the low-, medium-, and high-dose groups were administered Biyan Qingdu Granules at doses of 1, 2, and 4 g/kg, respectively, for 28 days.

2.2.4. General state observation

The diet, coat condition, behavior, activity levels, and oral mucosal changes were observed before and after X-ray

irradiation. Detailed results of oral mucosal changes have been published previously [7].

2.2.5. Observation of tumor size changes

Rats were euthanized 24 hours after the final treatment. Tumor tissues were excised, and tumor length and width were measured. Tumor volume was calculated using the formula: Tumor volume (mm^3) = (length × $width^2$) ÷ 2

The tumor suppression rate was determined as follows: Tumor suppression rate (%) = (Average tumor weight of model group – Average tumor weight of treated group) ÷ Average tumor weight of model group × 100%.

2.2.6. Pathological observation

Tumor tissues were stored at -80°C or fixed in formalin for seven days. Fixed tissues were processed via routine paraffin embedding, sectioned, and stained with hematoxylin and eosin. Sections were dewaxed, rehydrated, stained, differentiated with hydrochloric acid ethanol, counterstained with eosin, dehydrated with ethanol, cleared with xylene, and sealed with neutral gum. Pathological changes were examined microscopically.

2.2.7. ELISA for inflammatory indicators

Frozen tumor tissues were thawed, rinsed with pre-cooled PBS to remove impurities, and weighed. Tissue samples were homogenized with pre-cooled PBS and protease inhibitor (PMSF) at a 1:9 ratio using a tissue homogenizer on ice. Homogenates were centrifuged at 12,000 rpm for 10 minutes, and supernatants were collected. ELISA kits were used to measure levels of TNF-α, IL-1β, IL-6, IL-18, IL-12, ICAM-1, and VCAM-1, following the manufacturer's instructions.

2.2.8. Statistical methods

Data were analyzed using SPSS 26.0 software. One-way analysis of variance (ANOVA) was used for comparisons among groups, with $P < 0.05$ or $P < 0.01$ considered statistically significant. GraphPad Prism 9.0 was used for plotting analysis results.

3. Results

3.1. General status observation

Before X-ray irradiation, the rats exhibited normal diets, quick movements, smooth and glossy coats, and good oral conditions. After X-ray irradiation, the model group rats appeared sluggish, with yellowed fur, reduced activity, poor spirits, reddened oral mucosa, and significantly decreased food and water intake. Following the administration of Biyan Qingdu Granules, the mental state, oral mucosal inflammation, and food intake of rats in the low-, medium-, and high-dose groups improved, with the high-dose group showing the most pronounced improvement in oral mucosal inflammation.

3.2. Changes in tumor volume

After 28 days of treatment with Biyan Qingdu Granules, there was no significant difference in tumor size between the low-dose group and the model group ($P > 0.05$). However, tumor size decreased significantly in the medium- and high-dose groups ($P < 0.05$), as shown in **Table 1**.

Table 1. Effect of Biyan Qingdu Granules on tumor size and inhibition rate

Group	Dose (g/kg)	Tumor size (cm^3)	Tumor inhibition rate (%)
Model group	-	2.80 ± 0.89	-
Biyan Qingdu Granules group	2	2.47 ± 1.13	11.78
	4	1.89 ± 0.98*	32.50
	8	1.78 ± 0.81*	36.43

Note: Compared with the model group, *$P < 0.05$, **$P < 0.01$.

3.3. Pathological changes in tumors

Hematoxylin and eosin (HE) staining revealed distinct pathological changes among the groups. Tumors in the model group exhibited closely arranged tumor cells with abundant cytoplasm, irregular nuclei, coarse chromatin, and visible mitotic figures. The interstitium contained sparse lymphocyte infiltration and extensive multicentric hemorrhage.

In the low-dose group (**Figure 1B**), tumor cells were loosely arranged, with notable deformation, vacuolization, and disorganization. Nuclear shrinkage and necrosis in the tumor center were observed, accompanied by fibrocyte proliferation and a large infiltration of lymphocytes with minimal neutrophil presence.

In the medium-dose group (**Figure 1C**), there was widespread tumor necrosis, with residual tumor cells exhibiting degeneration and contraction. More lymphocytes and a few neutrophils were observed infiltrating the tissue, alongside fibrocyte proliferation.

In the high-dose group (**Figure 1D**), large-scale tumor necrosis was noted, accompanied by pronounced lymphocyte infiltration, sparse neutrophils, fibrocyte proliferation, and multinucleated giant cells engaged in phagocytosis.

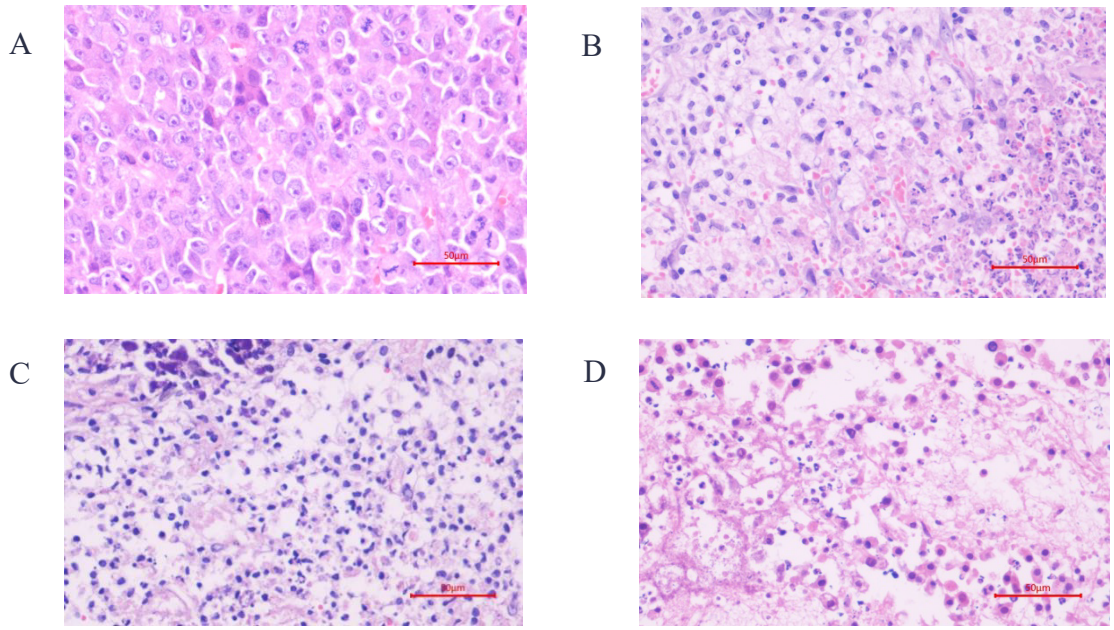

Figure 1. Effects of Biyan Qingdu Granules on tumor pathology. **(A)** Model group; **(B)** Low-dose group (2 g/kg); **(C)** Medium-dose group (4 g/kg); **(D)** High-dose group (8 g/kg)

3.4. Tumor inflammatory factors

Compared with the model group, TNF-α and IL-1β levels significantly decreased in the low-dose group ($P < 0.05$). In the medium-dose group, TNF-α, IL-1β, IL-6, ICAM-1, and VCAM-1 levels significantly decreased ($P < 0.05$). The high-dose group showed significant reductions in TNF-α, IL-1β, IL-6, ICAM-1, and VCAM-1 levels ($P < 0.01$, $P < 0.05$).

IL-12 and IL-18, cytokines that enhance antitumor immunity, exhibited different trends. IL-18 levels significantly increased in the treatment groups ($P < 0.05$), whereas IL-12 levels showed no significant differences ($P > 0.05$). Detailed results are shown in **Tables 2** and **3**.

Table 2. Effects of Biyan Qingdu Granules on TNF-α, IL-1β, and IL-6

Group	Dose (g/kg)	TNF-α (pg/mg)	IL-1β (pg/mg)	IL-6 (pg/mg)
Model group		73.1 ± 17.2	11.7 ± 3.3	47.1 ± 7.0
Biyan Qingdu Granules group	2	57.3 ± 15.1*	8.5 ± 2.2*	42.5 ± 7.9
	4	54.5 ± 13.2*	8.3 ± 2.4*	40.5 ± 6.4*
	8	53.7 ± 11.5**	7.9 ± 2.2**	38.9 ± 6.4*

Note: Compared with the model group, *$P < 0.05$, **$P < 0.01$.

Table 3. Effects of Biyan Qingdu Granules on ICAM-1, VCAM-1, IL-12, and IL-18

Group	Dose (g/kg)	ICAM-1 (pg/mg)	VCAM-1 (ng/mg)	IL-12 (pg/mg)	IL-18 (pg/mg)
Model group		20.9 ± 3.3	656.7 ± 120.0	5.2 ± 1.2	47.2 ± 8.9
Biyan Qingdu Granules group	2	18.3 ± 2.4	591.4 ± 79.1	6.3 ± 2.0	58.9 ± 14.8*
	4	18.0 ± 2.3*	551.7 ± 122.6*	6.4 ± 1.9	60.3 ± 15.9*
	8	17.4 ± 2.0*	517.4 ± 83.8*	6.4 ± 1.6	62.2 ± 14.2*

Note: Compared with the model group, *$P < 0.05$, **$P < 0.01$.

4. Discussion

Nasopharyngeal carcinoma is one of the most common head and neck malignancies in southern China, accounting for approximately 80% of global cases [8]. Due to its high sensitivity to radiotherapy, radiation therapy is the preferred radical treatment for nasopharyngeal carcinoma. Radiotherapy exerts its effects by damaging the genome of cancer cells, thereby interfering with their normal division and proliferation. However, it also causes damage to normal tissues and organs. Common side effects include damage to the nasal and oral mucosa, increased inflammatory secretions, skin reactions (such as redness, desquamation, and itching), changes in taste, throat discomfort, altered smell and hearing, dry mouth, dysphagia, and fatigue.

In this study, Biyan Qingdu Granules combined with micro-radiotherapy demonstrated significant therapeutic effects on nasopharyngeal carcinoma. This combination reduced the required radiation dose while decreasing the tumor volume of nasopharyngeal carcinoma.

IL-18 is known for its anti-tumor properties, primarily mediated by natural killer (NK) cells, which enhance their activity to exert anti-tumor effects. IL-18 also induces the production and proliferation of cytotoxic T lymphocytes (CTLs), which effectively kill tumor cells and provide anti-tumor immunity. Studies have shown

that tumor-bearing mice pretreated with IL-18 exhibit strong anti-tumor activity, including immune memory, as evidenced by their resistance to reinoculated tumors. This resistance is mediated by cytotoxic $CD4^+$ cells [9]. Tumor cells often evade immune responses by reducing lymphocyte infiltration. Additionally, overexpression of IL-12 in hepatocellular carcinoma cells has been observed to promote splenic lymphocyte recruitment, likely due to its regulation of chemokine profiles. Intratumoral injection of IL-12 has also been reported to induce the expression of chemokines such as CCL2, CCL3, and CCL4 in breast cancer models, suggesting that localized IL-12 administration can effectively enhance anti-tumor activity [10].

In this study, Biyan Qingdu Granules significantly increased IL-18 levels but did not significantly affect IL-12 levels. These findings suggest that Biyan Qingdu Granules may enhance anti-tumor immunity by promoting IL-18 secretion, thereby activating NK cells or inducing the production of CTLs to effectively kill tumor cells.

Zhu *et al.* successfully developed a mouse model of AOM/DSS-induced rectal cancer (CAC) and treated it with Huangqin Decoction. The results showed that rectal cancer caused weight loss, reduced colon length, increased pro-inflammatory cytokines (IL-6, IL-1β, TNF-α), and larger and more numerous tumors [11]. Similarly, this study found that Biyan Qingdu Granules reduced the levels of IL-6, IL-1β, and TNF-α, inhibited tumor growth, and mitigated radiation-induced inflammation in normal tissues.

5. Conclusion

In conclusion, Biyan Qingdu Granules promote IL-18 secretion, enhance immune function, and may increase the activity of NK cells or induce CTL production. Additionally, they inhibit the secretion of pro-inflammatory cytokines (IL-6, IL-1β, TNF-α), reduce tumor size, and alleviate radiation-induced inflammation in normal tissues.

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Disclosure statement

The authors declare no conflict of interest.

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