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Research Article

Efficacy of Zhuling Decoction with BCG Intravesical Therapy on Inhibiting Bladder Carcinoma

Guowei Zhang[1]*, Guifang Qin[1], Yanhong Chen[2], Honggai Yang[1], Jiatao Bei[3], Tian Yu Li[1], Yu Wang[1], Zhen Yin[1], Wei Miao[1], Yi Liu1and Feng LYU[1]

[1]College of Chinese Medicine, Hebei University, China
[2]Huabei Oil of Chinese Medicine Hospital, China
[3]Health School, China

*Corresponding author
Guowei Zhang, College of Chinese Medicine, Hebei University, Yuhua Road, Baoding 071002, China, Tel: 863125078515; Fax: 863125078515; E-mail: xxzgw@126.com

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- TNF-α
- N-methyl-N-nitrosourea

Abstract

Background: Intravesical administration of Bacille Calmette–Guérin (BCG) has been successfully used for bladder carcinoma for over 30 years. However, there are many significant local side effects. Zhuling decoction, a classical recipe from Chinese medicine recorded in Shang Han Lun as a diuretic, has been used for cystitis, hematuresis, and other urinary system diseases, which are some of the side effects of BCG therapy. In the present study, we evaluated the efficacy of Zhuling decoction on the attenuation of side effects of BCG therapy in vivo.

Methods: Rats were induced with bladder carcinoma by intravesical instillation of N-methyl-N-nitrosourea. Rats were orally administered Zhuling decoction or combinations of its components along with intravesical administration of BCG. Bladder, thymus, and spleen indices were calculated in all groups. Levels of serum tumor necrosis factor –α (TNF-α) and intercellular adhesion molecule 1 were assayed by ELISA and histological examination was performed.

Results: The bladder index was significantly higher in rats that received Zhuling decoction or its components in groups 2, 4 and 5 compared with model rats. Histological examination indicated that Zhuling decoction exerts an antagonistic role against BCG intravesical administration and that levels of serum TNF-α were lower. Our findings show that Zhuling decoction and components inhibit the antitumor effect of BCG by lowering TNF-α level.

Conclusions: Zhuling decoction exerts an antagonistic role by down-regulating the level of TNF-α when combined with BCG. Moreover, a certain level of TNF-α is essential for BCG intravesical administration to successfully treat bladder carcinoma.

ABBREVIATIONS

BCG: Bacille Calmette–Guérin; TNF-α: Tumor Necrosis Factor-α; TLR4: Toll-Like Receptor 4; IL-1β: Interleukin-1β; IL-6: Interleukin-6; PPS: Polyporus Polysaccharides; NF-κB: Nuclear Factor Kappa-B; MNU: N-methyl-N-nitrosourea; ICAM-1: Intercellular Cell Adhesion Molecule-1

INTRODUCTION

Bladder carcinoma is one of the most common urologic tumors and has the highest recurrence rate of any malignancy. In 2015, 80500 Chinese were diagnosed with bladder cancer and 32,900 died [1]. Intravesical chemotherapy or immunotherapy has been widely used as adjuvant treatments to prevent recurrence and progression of bladder tumors after transurethral resection. Bacille Calmette–Guérin (BCG) treatment is usually the primary therapeutic schedule for superficial bladder carcinoma [2]. However, BCG has severe local side effects including fever, gross hematuria, and sepsis during bladder carcinoma treatment, which limits its clinical application [3]. Several mechanistic studies have been conducted in an attempt to reduce the side effects of BCG, while maintaining its therapeutic effect.

Zhuling decoction, a classical recipe from Chinese medicine, is composed of *Polyporus umbellate* (Zhuling), *Alisma plantago-aquatica* (Zexie), *Wolfiporia cocos* (Fuling), talcum powder (Huashi), and *Colla Corii Asini* (Ejiao). Zhuling decoction has a long history of use for kidney and bladder diseases. *Polyporus umbellate*, which is the Jun drug in this decoction, plays the lead role in the formula. *Alisma plantago-aquatica* and *Wolfiporia*

cocos are called the Chen drugs, and talcum powder and *Colla Corii Asini* are the Zuo drugs. Studies showed that Zhuling decoction could significantly inhibit bladder cancer [4].

We hypothesize that the Zhuling decoction is likely to regulate some inflammatory factors that are related to the occurrence and development of bladder carcinoma during BCG intravesical therapy. To explore if the Zhuling decoction has efficacy or synergism during BCG intravesical therapy, we investigated the effect of the Zhuling decoction combined with BCG intravesical therapy in bladder carcinoma. We assayed the levels of TNF-α and intercellular adhesion molecule 1 (ICAM-1) in the serum of all experimental groups.

MATERIALS AND METHODS

Experimental animals

Female Sprague–Dawley rats were purchased from of the Academy of Military Medical Science Animal Laboratories (Beijing, China), and used after 3 days of acclimation. All animals were handled in accordance with the Principles for Care and Use of Experimental Animals from Hebei University and approved by the institutional committee on animal care. All animals were maintained under standard environmental conditions (23 ± 2°C, 55 ± 5% humidity and 12-h/12-h light/dark cycle). All animals were allowed free access to tap water and standard laboratory rat food.

Materials

The compounds found in the Zhuling decoction, Zhuling, Zexie, Fuling, Huashi, and Ejiao, were purchased from Wan Shun-da Pharmaceuticals Limited. (Anguo, Hebei, China). Except for Ejiao, other herbs were mixed with water 1:8 (g/mL), and the mixture was boiled at 100°C for 30 min under reflux. The four products obtained were centrifuged, filtered, concentrated, and then spray dried. The products were dissolved in water before administration at the stated doses. Ejiao was melted for treatment in the respective groups before administration.

Experimental design

Animals were assigned randomly to eight groups (Table 1). After 3 days of acclimation, rats in groups 1–7 were given N-methyl-N-nitrosourea (MNU, dissolved in pH 6.0 sodium citrate, 2 mg each rat) four times intravesically (weeks 0, 2, 4 and 6). BCG (60 mg/3 mL), dissolved in physiological saline, was given intravesically to groups 1–6 (2 mg/100 g) in weeks 3, 5 and 7. Rats in group 8 received the same procedure, except for 0.9% NaCl instead of MNU or BCG (Figure 1). Daily oral doses of Jun, Chen, and Zuo drugs were given to groups 1–5 (0.15 g/100 g) once a day from week 0 through to week 7. Group 1 received only the Jun drug; group two received Jun and Chen drugs; group 3 received Jun and Zuo drugs; group 4 received Chen and Zuo drugs; and group 5 received Jun, Chen, and Zuo drugs. Throughout the study, body weight and food intake were recorded. The rats were observed daily for signs of toxicity, weighed weekly, and palpated for urinary bladder lesions weekly. To assess food intake, rats were weighed weekly before the end of the experiment, and bladder, spleen, and thymus indices determined. At sacrifice, urinary bladders were weighed and then half inflated with 10% buffered formalin and the other half bladders immediately frozen in liquid nitrogen at −80°C until analysis. After fixation, bladders were observed under a high-intensity light for gross lesions, and each lesion was dissected and processed (hematoxylin and eosin stained) for histological classification. Levels of TNF-α and ICAM-1 in serum were assayed by ELISA kits (Boster, Wuhan, China).

Table 1: Groups and treatments.

Group	Treatment
1	Jun+ BCG + MNU
2	Jun and Chen+ BCG + MNU
3	Jun and Zuo+ BCG + MNU
4	Chen and Zuo + BCG + MNU
5	Jun, Chen and Zuo+ BCG + MNU
6	BCG + MNU
7	MNU
8	Physiologic saline

Abbreviations: BCG: Bacille Calmette–Guérin treatment; Chen: *Alisma plantago-aquatica* and *Wolfiporia cocos*; Jun: *Polyporus umbellate*; MNU: N-methyl-N-nitrosourea; Zuo: Talcum powder and *Colla Corii Asini*

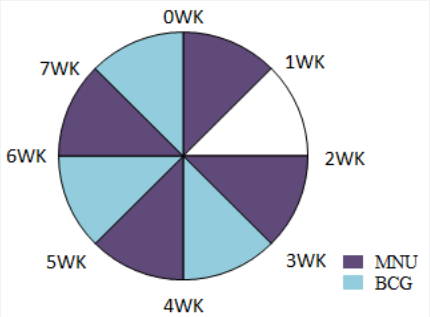

Figure 1 Bladder cancer was induced in groups 1–7 by intravesical instillation of N-methyl-N-nitrosourea (MNU) (2 mg/rat) four times (weeks 0, 2, 4, and 6). Group 1–6 received Bacille Calmette–Guérin (BCG) (2 mg/100 g) three times (weeks 3, 5, and 7). Group 8 received the same treatment, except for 0.9% NaCl instead of MNU and BCG. Groups 1–5 were orally administered with the Zhuling decoction and its decomposed recipes (0.15 g/100 g).

Statistical analysis

Results are expressed as means ± S.D. Statistical significance of differences were evaluated by one-way analysis of variance followed by the Dunnett's *t*-test (SPSS15.0, IBM, Chicago, IL, USA). Radit analysis was used to analyze differences in bladder carcinoma histological classification. A value of $P < 0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

Results

General observations: The mean body weights or food intake in each group did not differ compared with the control group (Figure 2). The average bladder, spleen, and thymus index after adjustment for body weight (mg tissue weight per g body weight) were determined for all groups. There was no significant

difference in the spleen and thymus index among the groups. The bladder index was higher in groups 2, 4, and 5, compared with the control group (Table 2).

Histological examination: Tumors were classified as hyperplasia, pTa (noninvasive superficial tumors), pT1 (tumors invading the lamina propria), and pT2 (tumors invading the muscularis) (Figure 3). Histologic diagnosis was performed twice by the same pathologist. Histological diagnosis showed that the bladder carcinoma model was successful. BCG could inhibit the development of bladder cancer. We found that the Zhuling decoction or its components increased bladder tumor development when combined with BCG (Table 3).

Data of bladder cancer histological classification were analyzed by the Raddit method. If the curative effect of A is better than B, then G < 0. Otherwise, the opposite is true. Group 6 was remarkably better than groups 1–5 (G > 0). Compared with group 6, the therapeutic effect was ranked as: group 8 > group 4 > group 3 > group 7 > group 2 > group 5 > group 1. These findings suggest that Zhuling decoction and its decomposed recipes inhibit the antitumor effect of BCG (Table 4).

Levels of TNF-α and ICAM-1: The expressions of serum TNF-α and ICAM-1 were assayed by ELISA (Table 5). There were no significant differences in ICAM-1 levels compared with the control group except in group 7, which had higher levels after receiving MNU. Our results showed that the level of TNF-α was significantly lower after administration with the Zhuling decoction and its component recipes with the BCG therapy group (group 6). This phenomenon was consistent with the result of histological examination (Table 3,4), which indicated that the Zhuling decoction and its decomposed recipes could weaken the curative effect of BCG.

Discussion

For bladder carcinogen experiments, MNU is widely used to induce a high-grade and invasive bladder carcinoma model [5]. The induced bladder cancer is similar to that induced by N-butyl-

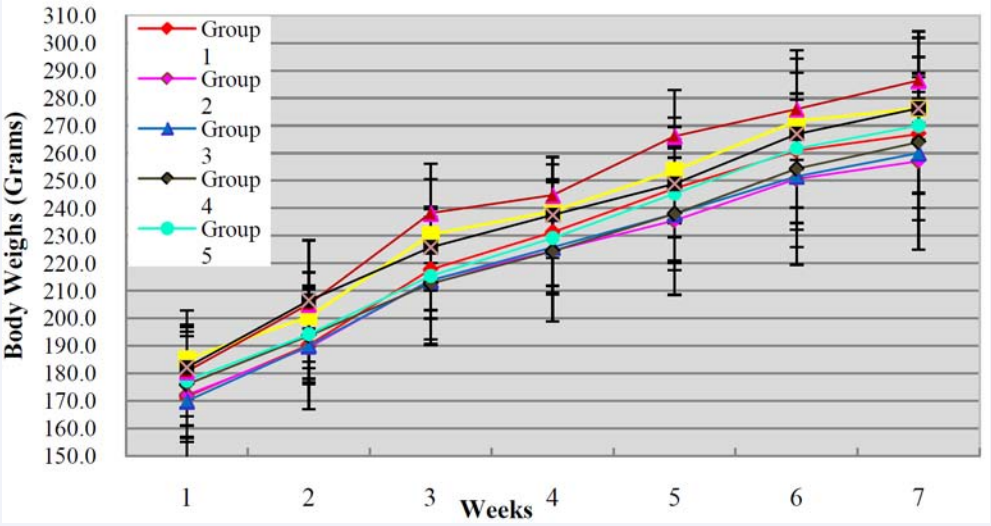

Figure 2a Body weight of each rat was recorded weekly throughout the experiment regularly and mean body weight of all groups was plotted as a function of every week. Values are expressed as mean ± S.D.

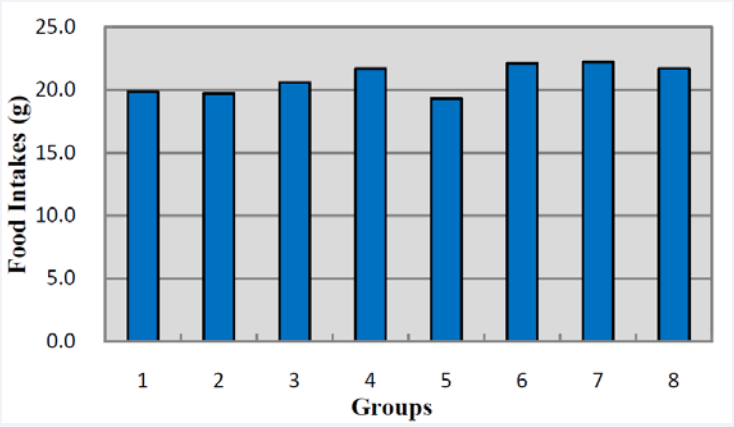

Figure 2b Mean food intake of each group in the last week. *P < 0.05, **P < 0.01, compared with the control group (group 8) using the Dunnett's t-test.

Table 2: Bladder, spleen, and thymus indices of rats.

Group	Number	Value(mean±S.D, mg/g)		
		Thymus index	Spleen index	Bladder index
1	10	1.00±0.29	2.63±1.37	0.57±0.09
2	10	0.96±0.38	2.61±0.49	0.69±0.10**▲
3	8	1.12±0.26	2.42±0.43	0.59±0.12
4	9	1.15±0.26	2.56±0.24	0.61±0.17*
5	9	0.98±0.15	2.40±0.57	0.62±0.09*
6	8	1.11±0.45	2.28±0.68	0.62±0.11
7	8	1.06±0.41	2.39±0.40	0.57±0.10
8	8	1.22±0.19	2.24±0.21	0.49±0.07

Values are expressed as mean ± S.D. of rats in each group. *P < 0.05, **P < 0.01, compared with the control group (8) using the Dunnett's *t*-test. ▲P < 0.05, compared with the model group (7) using the Dunnett's *t*-test.

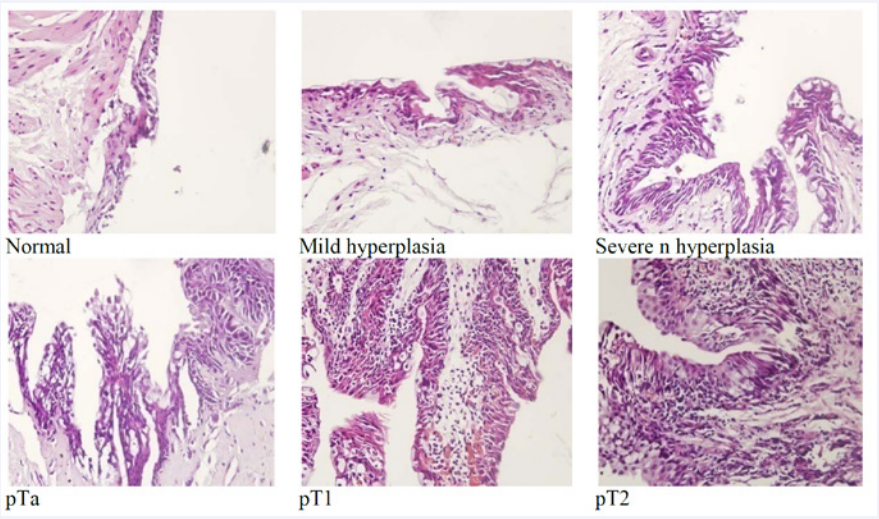

Figure 3 Microscopic images were obtained after hematoxylin and eosin staining of paraffin-embedded bladder tissues. pTa, pT1, and pT2 refer to noninvasive superficial tumors, invading the lamina propria, and invading the muscularis propria.

Table 3: Bladder cancer histological classification.

Group	N	Normal	Hyperplasia		Bladder cancer		
			mild	Severe	pTa	pT1	pT2
1 Jun +BCG+MNU	6	0	1	2	1	2	0
2Jun+Chen+BCG+MNU	6	1	0	3	1	0	1
3 Jun +Zuo+ BCG+MNU	6	2	0	2	0	1	1
4Chen+Zuo+BCG+MNU	6	2	2	0	1	1	0
5Jun+Chen+Zuo+BCG+MNU	6	1	0	1	2	1	1
6 BCG + MNU	6	3	1	0	1	1	0
7 MNU	6	2	0	1	1	1	1
8 Physiologic saline	6	6	0	0	0	0	0

BCG: Bacille Calmette–Guérin treatment; Chen: *Alisma plantago-aquatica* and *Wolfiporia cocos*; Jun: *Polyporus umbellate*; MNU: N-methyl-N-nitrosourea; Zuo: Talcum powder and *Colla Corii Asini*

N-(4-hydroxy-butyl) nitrosamine [6]. In our present study, four of six rats were successfully induced with bladder carcinoma. The results indicate that MNU is highly effective for inducing bladder carcinoma.

The mechanism of intravesical BCG has not been fully elucidated, but some experiments showed that BCG curative

Table 4: Analysis of bladder cancer histological classification.

(A) Group	Number	(B)Value of G		
		Compared with group 6	Compared with group 7	Compared with group 8
1	6	0.50	0.08	1.00**
2	6	0.33	-0.03	0.83*
3	6	0.25	-0.06	0.67*
4	6	0.11	-0.22	0.67*
5	6	0.44	0.14	0.83*
6	6	—	-0.28	0.50
7	6	0.28	—	0.67
8	6	-0.5	-0.67	—

Values are expressed as the size of G in each group. *P < 0.05, **P < 0.01, compared with blank controls using the Raddit method. Data of bladder cancer histological classification were analyzed by the Raddit method. If G < 0, the curative effect of A is better than B. The size of G indicates the degree of difference between A and B.

Table 5: Levels of TNF-α and ICAM-1.

Group	Number	TNF-α(pg/mL)	ICAM-1(pg/mL)
1	10	25.28±36.99	550.58±651.48
2	10	6.75±24.41#	254.25±133.60
3	8	9.86±29.85#	357.51±252.88
4	9	5.84±11.91#	420.43±409.95
5	9	9.47±24.18#	721.05±831.33
6	8	38.47±27.11*	307.80±296.74
7	8	26.43±31.24	758.76±838.41
8	8	9.17±26.21	541.67±602.23

Values are expressed as mean ± S.D. of rats in each group. #P < 0.05, compared with rats in group 6, *P < 0.05, compared with rats in group 8, using the Dunnett's *t*-test

efficacy depends on the activation of TLRs, especially active TLR2 and TLR4 [7]. The activation of these TLRs induces macrophage cells to release proinflammatory cytokines including TNF-α [8]. The production of several inflammatory molecules is associated with BCG therapy [9], and the immune response may defend the host by suppressing neoplastic growth, including macrophages and T lymphocytes [10]. Meanwhile, severe local side effects result from the cytokine induction by intravesical instillation of BCG.

Zhuling is the most important herb within the Zhuling decoction. Our previous study indicated that Zhuling and its main compound, PPS, is highly effective in inhibiting bladder carcinogenesis in rats [11]. The pharmacological inhibition of bladder carcinogenesis is mediated by the immune system, including TLR4-mediated macrophage activation and increased antibody production both *in vitro* and *in vivo* [12,13]. *Polyporus umbellate* has *Polyporus* polysaccharides (PPS), which are the primary active substance of Zhuling decoction and were found to inhibit the expression of inflammatory factors through the NF-κB signaling pathway [11,14]. PPS strongly reduced the side effects and displayed synergistic effects during BCG instillation in rat bladder cancer models, which may result from direct activation of DC TLR4 [15]. Therefore, because PPS combined with BCG showed synergistic effects, we hypothesized that the Zhuling decoction could also significantly inhibit bladder cancer (Figure 4) or reduce the side effects.

In the present study, histological examination showed that BCG could inhibit the development of bladder cancer. However, it was unexpected that the Zhuling decoction and its components did not prevent bladder tumor development while combined with BCG (Table 2). Therefore, Zhuling decoction was antagonistic to BCG treatment.

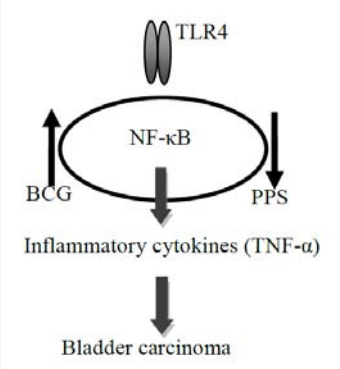

Figure 4 The attenuation mechanism of PPS combined with BCG.

In further study, the levels of serum TNF-α and ICAM-1 were assayed by ELISA. Compared with controls, there was no significant difference among all treatment groups in the levels of serum TNF-α and ICAM-1. Nevertheless, it was evident that BCG intravesical instillation could elevate TNF-α level. In contrast, the concentration of TNF-α was significantly lower after administration with the Zhuling decoction and its components compared with the BCG group (Table 5). This demonstrated that TNF-α, a proinflammatory cytokine, might contribute to inhibiting bladder tumor development [9]. Moreover, this phenomenon was consistent with the result of histological examination (Table 3). Therefore, Zhuling decoction and its components can inhibit the antitumor effect of BCG via a reduction in TNF-α level.

The Zhuling decoction, a classical recipe of Chinese medicine, has a diuretic effect according to *Shang Han Lun*, a classical book of Chinese Medicine. *Polyporus umbellate* (Zhuling), *Alisma plantago-aquatica* (Zexie), *Wolfiporia cocos* (Fuling), and talcum powder (Huashi) also have a long history of use as diuretics according to *Shen Nong Ben Cao Jing*. Histological examination and measurement of TNF-α verified indirectly the effects of the Zhuling decoction. Meanwhile, we confirmed that maintaining a certain level of TNF-α is necessary for BCG to inhibit bladder carcinoma. A previous study showed that urinary TNF-α level might be essential in the antitumor activity after BCG therapy and might play an important role in the prevention of bladder tumor recurrence [16].

CONCLUSION

Our present study suggested that the Zhuling decoction and its components reduced the level of TNF-α through diuresis and systemic immune regulation. A certain level of TNF-α is indispensable for BCG intravesical instillation to inhibit the onset and progression of bladder carcinoma.

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REFERENCES

1. Chen W, Zheng R, Baade PD, Zhang S, Zeng H, Bray F, Jemal A. Cancer statistics in China, 2015. CA Cancer J Clin. 2016; 66: 115-132.
2. Melekos MD, Moutzouris GD. Intravesical therapy of superficial bladder cancer. Curr Pharm Des. 2000; 6: 345-359.
3. Weintraub MD, Li QQ, Agarwal PK. Advances in intravesical therapy for the treatment of non-muscle invasive bladder cancer (Review). Mol Clin Oncol. 2014; 2: 656-660.
4. Yazawa Y, Yokota M, Sugiyama K. Antitumor promoting effect of an active component of Polyporus, ergosterol and related compounds on rat urinary bladder carcinogenesis in a short-term test with concanavalin A. Biological & pharmaceutical bulletin. 2000; 2: 1298-1302.
5. Kunze E, Gassner G. Modification of N-methyl-N-nitrosourea-induced urinary bladder carcinogenesis in rats following stimulation of urothelial proliferation by a partial cystectomy. Journal of cancer research and clinical oncology. 1986; 112: 11-18.
6. Ozono S, Babaya K, Sasaki K, Okamoto S, Momose H, Fujimoto K, et al. Rat urinary bladder carcinomas induced by N-butyl-N-(4-hydroxybutyl)-nitrosamine and N-methyl-N-nitrosourea. Urological research. 1990; 18: 323-326.
7. Simons MP, Moore JM, Kemp TJ, Griffith TS. Identification of the mycobacterial subcomponents involved in the release of tumor necrosis factor-related apoptosis-inducing ligand from human neutrophils. Infection and immunity. 2007; 75: 1265-1271.
8. Kim JS, Kim WS, Choi HG, Jang B, Lee K, Park JH, et al. Mycobacterium tuberculosis RpfB drives Th1-type T cell immunity via a TLR4-dependent activation of dendritic cells. J Leukoc Biol. 2013; 94: 733-749.
9. Jackson AM, Ivshina AV, Senko O, Kuznetsova A, Sundan A, Clinton S, et al. Prognosis of intravesical bacillus Calmette-Guerin therapy for superficial bladder cancer by immunological urinary measurements: statistically weighted syndromes analysis. The Journal of urology. 1998; 159: 1054-1063.
10. Thompson DB, Siref LE, Feloney MP, Hauke RJ, Agrawal DK. Immunological basis in the pathogenesis and treatment of bladder cancer. Expert Rev Clin Immunol. 2015; 11: 265-279.
11. Zhang GW, Zeng X, Li CX, Li JJ, Huang Y, Han l, et al. Inhibition of urinary bladder carcinogenesis by aqueous extract of sclerotia of Polyporus umbellatus fries and polyporus polysaccharide. The American journal of Chinese medicine. 2011; 39: 135-144.
12. Li X, Xu W. TLR4-mediated activation of macrophages by the polysaccharide fraction from Polyporus umbellatus (pers.) Fries. J Ethnopharmacol. 2011; 135: 1-6.
13. Li X, Xu W, Chen J. Polysaccharide purified from Polyporus umbellatus (Per) Fr induces the activation and maturation of murine bone-derived dendritic cells via toll-like receptor 4. Cell Immunol. 2010; 265: 50-56.
14. Wei JA, Zeng X, Han L, Huang Y. The regulatory effects of polyporus polysaccharide on the nuclear factor kappa B signal pathway of bladder cancer cells stimulated by Bacillus Calmette-Guerin. Chinese journal of integrative medicine. 2011; 17: 531.
15. Zhang GW, Qin GF, Han B, Li CX, Yang HG, Nie PH, et al. Efficacy of Zhuling polyporus polysaccharide with BCG to inhibit bladder carcinoma. Carbohydr Polym. 2015; 118: 30-35.
16. Shintani Y, Sawada Y, Inagaki T, Kohjimoto Y, Uekado Y, Shinka T. Intravesical instillation therapy with Bacillus Calmette-Guérin for superficial bladder cancer: Study of the mechanism of bacillus Calmette-Guérin immunotherapy. International journal of urology. 2007; 14: 140-146.

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