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ORIGINAL ARTICLES

Protective effect of *Radix Astragali* injection on immune organs of rats with obstructive jaundice and its mechanism

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Abstract

AIM: To observe the protective effect of *Radix Astragali* injection on immune organs (lymph nodes, spleen and thymus) of rats with obstructive jaundice (OJ) and its mechanism.

METHODS: SD rats were randomly divided into sham-operation group, model control group and *Radix Astragali* treatment group. On days 7, 14, 21 and 28 after operation, mortality rate of rats, pathological changes in immune organs, expression levels of Bax and nuclear factor (NF)-κB p65 proteins, apoptosis indexes and serum tumor necrosis factor (TNF)-α level in spleen and thymus were observed, respectively.

RESULTS: Compared to model control group, the number of dead OJ rats in *Radix Astragali* treatment group decreased ($P > 0.05$). The TNF-α level (27.62 ± 12.61 *vs* 29.55 ± 18.02, 24.61 ± 9.09 *vs* 31.52 ± 10.95) on days 7 and 21, the pathological severity score for spleen [0.0 (0.0) *vs* 0.0 (2.0) on days 7 and 14 and for lymph nodes [0.0 (1.0) *vs* 1.0 (2.0), 1.0 (0.0) *vs* 2.0 (1.0)] on days 21 and 28, the product staining intensity and positive rate of Bax protein in spleen [0.0 (0.0) *vs* 1.0 (2.0), 0.0 (1.0) *vs* 2.0 (1.5) and thymus [0.0 (0.0) *vs* 1.0 (2.0), 0.0 (1.0) *vs* 2.0 (1.5)] on days 14 and 28, the apoptotic indexes [0.0 (0.0) *vs* 0.0 (0.01)] in spleen and thymus [0.0 (0.0) *vs* 0.0 (0.01) on days 14 and 21 were significantly lower in *Radix Astragali* treatment group than in model control group ($P < 0.05$).

CONCLUSION: *Radix Astragali* has protective effects on immune organs of OJ rats by relieving the pathological changes in immune organs, reducing TNF-α level and inhibiting Bax expression and apoptosis in spleen and thymus.

Key words: *Radix Astragali*; Traditional Chinese medicine; Obstructive jaundice; Rat; Immune organ; Tumor necrosis factor-α; Bax; Nuclear factor-κB; Apoptosis; Tissue microarry

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INTRODUCTION

Obstructive jaundice (OJ) is a kind of common clinical manifestation. The pathogenesis and treatment of OJ have been a hot topic in medical field for a long time[1-3]. Since

systemic inflammatory response syndrome and multiple organ dysfunction syndrome were studied in recent years, immune function impairment concomitant with OJ has gradually attracted wide attention and is considered as a cause of death in OJ patients[4-7]. Therefore, one of the important approaches to treatment of OJ is to restore the functions of immune organs[8-10].

Development and utilization of traditional Chinese medicine have good prospects in therapy for OJ since it has a lower cost, more extensive pharmacological effects and fewer side effects. *Radix Astragali,* dried root of *Astragalus membranaceus*, is sweet in taste with a warm property and mainly produced in Inner Mongolia Autonomous Region, Shanxi, Gansu and Heilongjiang Provinces of China. The raw *Radix Astragali* can be used to consolidate the exterior of body. *Radix Astragali* can regulate sweating, warm muscles, strengthen striae, invigorate Qi (vital energy), and alleviate heat in muscles due to Qi deficiency. *Radix Astragali* can also be used in treatment of chicken pox or other diseases with vesicular-papules due to inadequate "dispersing of the evils". *Radix Astragali* invigorates Qi, ascends the Yang-Qi, protects Qi and consolidates the exterior of body, promotes diuresis, relieves edema (generalized swelling of the body), supports Qi to promote skin wound/ulcer healing and tissue/muscle regeneration. *Astragalus* injection is made of extraction from *Radix Astragali*. Since *Astragalus* injection contains polysaccharide, saponin, flavone and trace elements, it has a variety of pharmacological effects and increases the immunity and protects the liver and kidneys[11-15]. It has been shown that cellular immune function decreases in OJ rats[16], which can be successfully treated with *Astragalus* injection.

At present, studies about the effects of *Astragalus* injection on immune organs during OJ are not available. This study was to investigate the protective effect of *Astragalus* injection on immune organs of OJ rats and its mechanism. The results may provide an experimental basis for its application in clinical practice.

MATERIALS AND METHODS

Materials

Healthy male SD rats of clean grade, weighing 270-330 g, were provided by Laboratory Animal Research Center, Zhejiang University of Traditional Chinese Medicine (China). Sodium pentobarbital was purchased from Sigma Corporation (USA). *Radix Astragali* injection (10 mL vial contains active components equivalent to 20 g of the original medicine) was purchased from Chiatai Qingchunbao Pharmaceutical Co, Ltd (China). Serum tumor necrosis factor (TNF)-α ELISA kits were purchased from Shanghai Senxiong Technological Company (China). Anti-nuclear factor (NF)-κB P65 and anti-Bax antibodies were purchased from Santa Cruz Biotechnology, Inc (USA). TUNEL assay kits were purchased from Takara Bio Inc (Jingdu, Japan).

Animal grouping and preparation of OJ models

One hundred and eighty OJ rats, enrolled in this study, were randomly divided into sham-operation group, model control group, and treatment group (n = 60), which were further subdivided into 7, 14, 21 and 28 d groups (n = 15) according to the time after operation. After the rats were anesthetized with intraperitoneal injection of 2.5% sodium pentobarbital (0.2 mL/100 g), their abdominal cavity was opened to identify and dissociate the common bile duct along the hepatoduodenal ligament. The proximal end of the common bile duct of rats in the model control and treatment groups was double-ligated with surgical threads, the common bile duct was cut off, and a layered suture of the abdominal wall was performed to close the abdominal cavity. The common bile duct of rats in the sham-operation group was dissociated but not ligated, and a layered suture of the abdominal wall was also performed to close the abdominal cavity. Rats in the treatment group received intraperitoneal *Radix Astragali* injection at a dose of 0.75 mL/100 g per day, while those in the sham-operation and model control groups received an equal volume of physiological saline solution until the end of 7-, 14-, 21- and 28-d observation periods in the corresponding groups.

Determination of experimental parameters

Mortality rates of rats in different groups were recorded. Rats were killed after anesthesia with sodium pentobarbital in batches, serum was collected to measure TNF-α level by ELISA, and pathological changes in immune organs (lymph nodes, spleen and thymus) were observed. Pathological severity of immune organs was scored according to related standards. Tissues of spleen, thymus and lymph nodes were cut into sections, but the sections of lymph node were not stained. Changes in expression levels of Bax and NF-κB P65 proteins, as well as apoptosis index of spleen and thymus were observed, respectively.

Immunohistochemical staining of Bax and NF-κB P65 proteins in intestinal mucosa

Envision two-step method was used to detect the expression levels of Bax and NF-κB P65 proteins in intestinal mucosa. The staining intensity was evaluated according to the extent of cell coloration: "-" represents negative staining; "+" represents mild staining with positively stained cells showing a yellow pigment; "++" represents moderate staining with positively stained cells showing a brown pigment; "+++" represents intense staining with positively stained cells showing a dark brown pigment; (-) indicates no positive cells; (+) indicates less than 25% of positive cells; (++) indicates 26%-50% of positive cells; and (+++) indicates over 50% of positive cells, each of which was scored as 0, 1, 2 and 3 points, respectively.

Detection of apoptotic index in intestinal mucosa with TUNEL staining

DNA nicking in tissue sections was observed with *in situ* end-labeling (TUNEL) staining. In brief, sections were

Table 1 Comparison of serum TNF-α levels in different groups (ng/L, mean ± SD)

Group	7 d	14 d	21 d	28 d
Sham-operation group	12.89 ± 1.63	12.25 ± 3.37	14.21 ± 3.24	15.61 ± 4.84
Model control group	29.55 ± 18.02[b]	34.10 ± 8.94[b]	31.52 ± 10.95[b]	57.66 ± 12.99[b]
Treatment group	27.62 ± 12.61[a,b]	27.20 ± 9.34[b]	24.61 ± 9.09[a,b]	39.01 ± 9.62[b]

[a]$P < 0.05$ *vs* model control group; [b]$P < 0.01$ *vs* sham-operation group.

baked at 60℃ for 30 min, deparaffinaged, and washed with Milli-Q for 5 min. Tissue was processed with protease K (10 μg/μL) at room temperature for 15 min and washed with phosphate-buffered saline (PBS) for 5 min. A 3% H_2O_2 solution was used to block endogenous peroxydase for 5 min, washed twice with PBS, 5 min each time. Thirty microliters of reaction solution at freezing condition (TdT enzyme : labeling safe buffer = 1:10) was added, incubated at 37℃ for 90 min, and washed twice with PBS, 5 min each time. Fifty microliters of anti-FITC HRP conjugate was added, incubated at 37℃ for 30 min, and washed twice with PBS, 5 min each time. DAB was colored, washed with Milli-Q, counterstained with hematoxylin, and washed with water after differentiation till it became blue. The DAB was routinely dehydrated and mounted onto neutral gum. Apoptotic index was calculated following the equation: Apoptotic index = apoptotic cell count/total cell count × 100%.

Statistical analysis

Data were input into the Excel sheet and read into SPSS 15.0 for further analysis. Normal data were expressed as mean ± SD while abnormal data were expressed as median (interquartile range). Analysis of variance and pairwise comparison were used for normal data, whereas abnormal data were subjected to non-parametric tests, of which Kruskal-Wallis H test was used for pairwise comparison and Mann-Whitney *U* test was used for multiple comparisons. Yates' χ^2 test was used for inter-group comparisons of mortality rates.

RESULTS

Comparison of mortality rates

All rats in the sham-operation group were alive after operation. Two rats in the model control group and one rat in the treatment group died on day 7 after operation. Four rats in the model control group and three rats in the treatment group died on day 14 after operation. Four rats in the model control group and four rats in the treatment group died on day 21 after operation. Seven rats in the model control group and six rats in the treatment group died on day 28 after operation. The total mortality rate of rats in the model control and treatment groups on day 28 was significantly higher than that of rats in the sham-operation group ($P < 0.001$). No significant difference was found in mortality rate between the model control and treatment groups.

Comparison of serum TNF-α levels

The serum TNF-α level was significantly lower in sham-operation group than in model control and treatment groups at different time points after operation ($P < 0.01$), and was significantly lower in treatment group than in model control group on days 7 and 21 after operation ($P < 0.05$, Table 1).

Pathological changes in spleen

In the sham-operation group, the morphology of spleen was normal with no gross pathological changes under light microscope.

In the model control group, the size of spleen increased by 1.2-1.5 folds and the texture of spleen became fragile with no change in color on day 7 after operation. The spleen became enlarged with a thickness of above 0.6 cm and a deeper color and its texture became fragile on day 14 after operation. The spleen was 4 cm × 1 cm × 1 cm in size and its texture became fragile with a purple black color on days 21 and 28 after operation. Under light microscope, the spleen of all rats was grossly normal on day 7 after operation. Fusion, enlargement or spotty necrosis of follicular germinal center in the white pulp of spleen, hyperplasia of fibrous tissue in sinus, and arteriolar sclerosis in spleen of most rats were observed on day 14 after operation. The spleen of few rats was grossly normal. Fusion, enlargement or spotty necrosis of follicular germinal centers in the white pulp of spleen, hyperplasia of fibrous tissue in sinus, and arteriolar sclerosis in spleen of few rats were seen on days 21 and 28 after operation. The spleen of some rats was grossly normal (Figure 1A).

In the treatment group, no significant difference was found in pathological changes at all time points after operation compared to the model control group. Under light microscope**,** no significant difference in pathological changes was noted at each time point after operation. The spleen of most rats was grossly normal. Arteriolar sclerosis in spleen of few rats was seen (Figure 1B).

The pathological scoring standards for spleen are listed in Table 2. The pathological scores were significantly lower for sham-operation group than for model control group on day 14 after operation ($P < 0.05$), and were significantly lower for treatment group than for model control group on days 7 and 14 after operation ($P < 0.05$, Table 3).

No significant difference was found in product staining intensity and in positive rate of NF-κB protein in spleens of all groups (Table 3).

The product staining intensity and positive rate of Bax protein were significantly lower in sham-operation and treatment groups than in model control group on day 28 after operation ($P < 0.05$, Table 3).

The apoptosis index was significantly lower in sham-

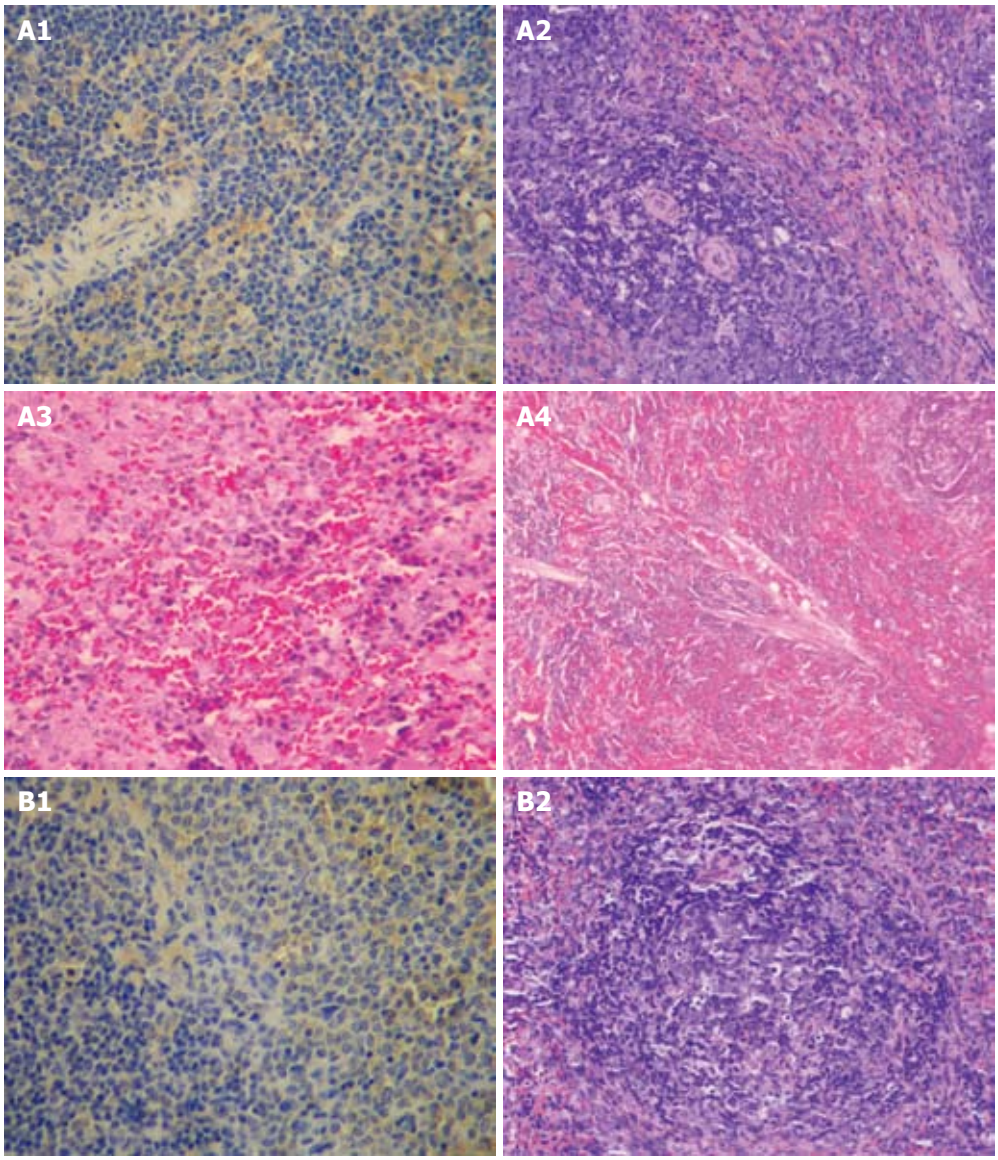

Figure 1 Pathological changes in spleen of model control group (A) and treatment group (B). A1: 21 d. Spleen (++) (Bax, × 200); A2: 28 d. Thickening of the wall of small spleen arteries as well as expansion and congestion of the red pulp (HE, × 200); A3: 28 d. Enlargement of spleen sinusoid, hyperplasia of cells in the spleen sinus as well as inflammatory cell infiltration and hemorrhage (HE, × 200); A4: 28 d. Enlargement of spleen sinusoid and hyperplasia of fibrous tissue (HE, × 100); B1: 21 d. Spleen (+) (Bax, × 200); B2: 28 d. Focal necrosis in spleen lymphoid follicles (HE, × 100).

Table 2 Pathological severity scoring standards for spleen

Score standards	Observation indexes
0	Normal
1	Necrosis of follicle center
2	Blood sinus dilation or arteriolar sclerosis
3	Necrosis of follicle center, blood sinus dilation and arteriolar sclerosis

operation group than in model control group on days 7, 14 and 28 after operation ($P < 0.05$), and was significantly lower in treatment group than in model control group on day 14 after operation ($P < 0.05$, Table 3).

Pathological changes in lymph nodes

In sham-operation group, the gross morphology of lymph nodes was normal. Under light microscope, no significant difference was observed in pathological changes at different time points after operation. The morphology and structure of lymph nodes were grossly normal. Enlargement of follicular germinal centers and hyperplasia of sinus cells were seen in few rats (Figure 2A).

Table 3 Comparison of different pathological indexes for spleen, M (Q_R)

Index	Time (d)	Sham-operation group	Model control group	Treatment group
Pathological severity score	7	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)[c]
	14	0.0 (1.0)[c]	0.0 (2.0)	0.0 (0.0)[c]
	21	0.0 (0.0)	1.0 (2.0)	0.0 (0.0)
	28	0.0 (0.0)	0.0 (1.0)	0.0 (0.0)
Product staining intensity and positive rate of Bax	7	0.0 (0.0)[c]	1.0 (2.0)	0.5 (1.0)[a]
	14	0.0 (1.0)	1.0 (2.0)	0.0 (0.0)[c]
	21	0.0 (0.0)[c]	1.0 (2.0)	0.0 (2.0)
	28	0.0 (0.0)[c]	2.0 (1.5)	0.0 (1.0)[c]
Apoptosis index	7	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
	14	0.0 (0.0)	0.0 (0.01)	0.0 (0.0)[c]
	21	0.0 (0.0)[c]	0.0 (0.01)	0.0 (0.0)
	28	0.0 (0.0)[c]	0.01 (0.02)	0.0 (0.0)
Product staining intensity and positive rate of NF-κB	7	0.0 (0.0)	0.0 (1.0)	0.0 (0.0)
	14	0.0 (1.0)	0.0 (2.0)	0.0 (0.0)
	21	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
	28	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)

[a]$P < 0.05$ *vs* sham-operation group; [c]$P < 0.05$ *vs* model control group.

In model control group, lymph nodes became yellow in 50% of the rats on day 7 after operation, but no

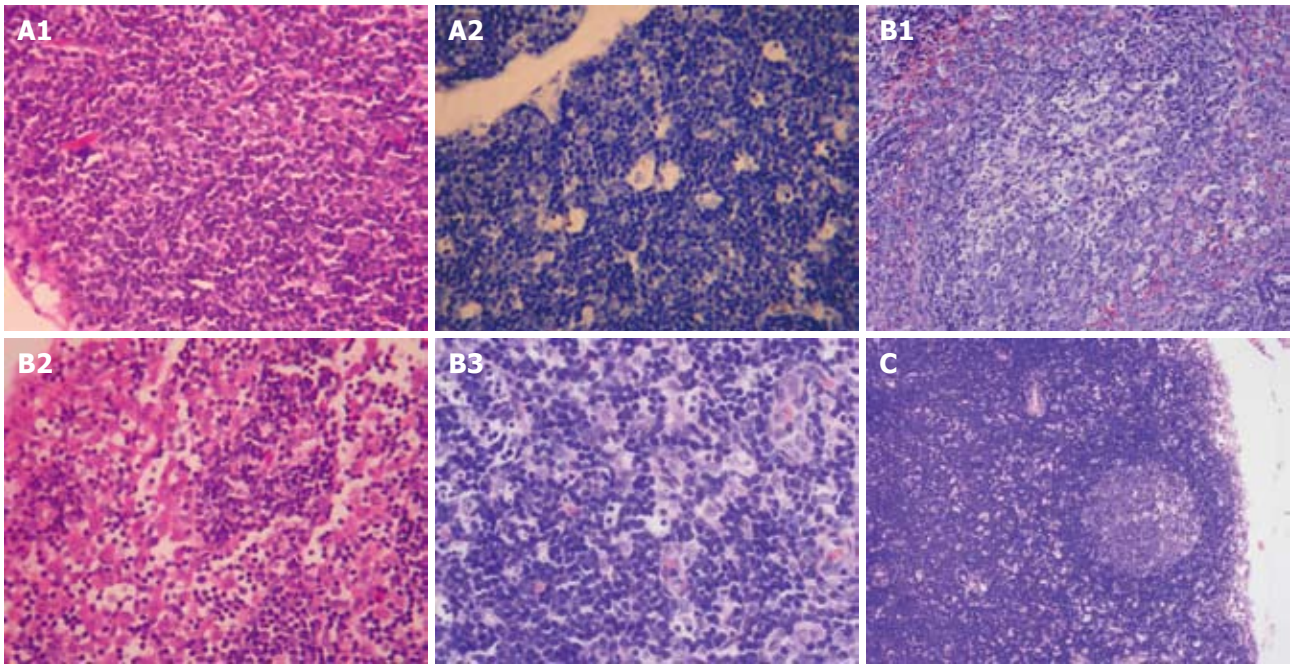

Figure 2 Pathological changes in lymph nodes of sham-operating group (A), model control group (B), and treatment group (C). A1: 28 d. Mainly normal lymph nodes (HE, × 200); A2: 28 d. No apoptotic cells in lymph node (TUNEL, × 200); B1: 21 d. Focal necrosis in lymphoid follicles and formation of germinal centers (HE, × 200); B2: 21 d. Expansion of lymph sinus, sinus cell hyperplasia and inflammatory cell infiltration (HE, × 200); B3: 28 d. Enlargement and spotty necrosis in germinal centers of lymph nodes, expansion of lymph sinus, hyperplasia of sinus cells and infiltration of neutrophils in lymph sinus (HE, × 200); C: 28 d. Clear follicular structure and fewer necrotic spots in lymph nodes (HE, × 100).

Table 4 Comparison of pathological severity scores for lymph nodes

Groups	7 d	14 d	21 d	28 d
Sham-operation group	0.0 (2.0)[c]	0.0 (1.0)[c]	0.0 (1.0)[c]	0.0 (1.0)
Model control group	1.0 (2.0)	1.0 (1.0)	1.0 (2.0)	2.0 (1.0)
Treatment group	0.0 (0.0)[a]	0.0 (1.0)	0.0 (1.0)[c]	1.0 (0.0)[c]

[a]$P < 0.05$ *vs* sham-operation group; [c]$P < 0.05$ *vs* model control group.

changes were found in the texture of lymph nodes at all time points after operation. Under light microscope, no significant difference in pathological changes was observed at all time points after operation. Enlargement of follicular germinal centers and hyperplasia of sinus cells were seen in most rats and few rats showed no obvious pathological changes in lymph nodes with spotty necrosis in the mantle zone and germinal centers on days 7, 14, 21 and 28 after operation (Figure 2B).

In the treatment group, no significant difference in pathological changes was observed at all time points after operation compared to the model control group. Under light microscope, no obvious difference was found in lymph node pathological changes at all time points after operation. In most rats, enlargement of lymph nodes in germinal centers and hyperplasia of cells in lymph sinus were observed with spotty necrosis of lymph nodes in the mantle zone and germinal centers of (Figure 2C).

The pathological scoring standards for lymph nodes have been described elsewhere[17]. The pathological score was significantly lower for sham-operation group than for model control group on days 7, 14 and 21 after operation ($P < 0.05$). The pathological score was significantly lower for sham-operation group than for treatment group on day 7 after operation ($P < 0.05$) and was significantly lower for treatment group than for model control group on days 21 and 28 after operation ($P < 0.05$, Table 4).

Pathological changes in thymus

In sham-operation group, no significant difference was found in thymus pathological changes at all time points after operation compared to model control group, and the thymus tissue of all rats was grossly normal (Figure 3A).

In model control group, the thymus of rats was mildly shrunken on day 7 after operation, moderately shrunken and jaundiced on day 14 after operation, and severely shrunken and jaundiced on days 21 and 28 after operation. Under light microscope, no significant difference was noted in thymus pathological changes at all time points after operation. The thymus tissue of most rats was grossly normal. An obscure boundary between thymus cortex and medulla was occasionally seen. The thymus tissue was grossly normal on day 14 after operation. The thymus pathological changes were similar on days 14, 21 and 28 after operation (Figure 3B).

In treatment group, no significant difference in thymus pathological changes was observed on days 7 and 14 after operation compared with model control group. The thymus became mildly jaundiced with no obvious shrinkage on days 21 and 28 after operation. Under light microscope, the thymus tissue of most rats was grossly

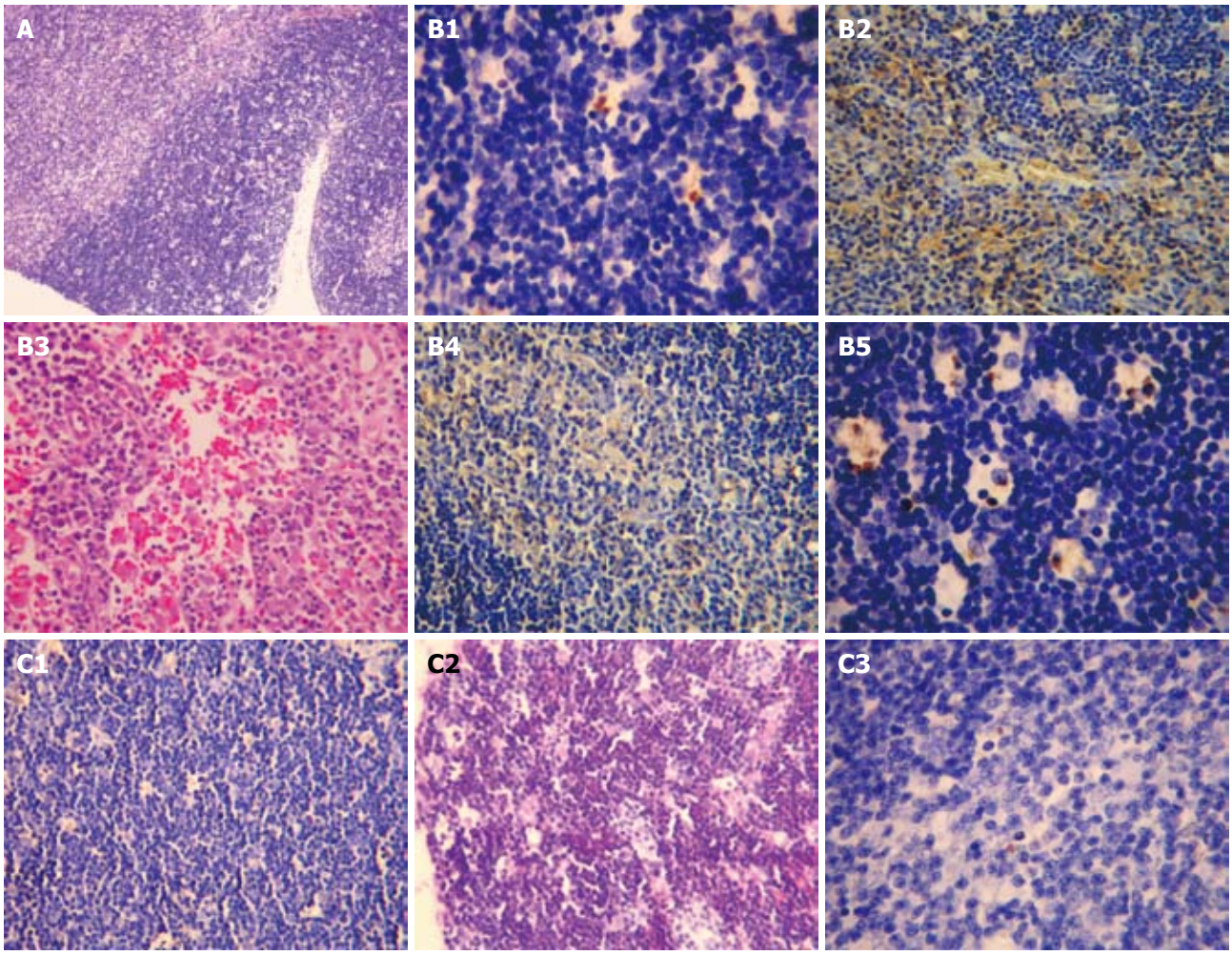

Figure 3 Pathological changes in thymus of sham-operated group (A), model control group (B), and treatment group (C). A1: 28 d. Clear structure of thymus lobules and a clear boundary between thymus cortex and medulla (HE, × 100); B1: 7 d. Sporadic apoptotic cells in thymus (TUNEL, × 400); B2: 21 d. Thymus (++) (NF-κB p65, × 200); B3: 28 d. An obscure boundary between the thymus cortex and medulla with hemorrhage in the medulla (HE, × 200); B4: 28 d. Thymus (++) (Bax, × 200); B5: 28 d. Sporadic apoptotic cells in thymus (TUNEL, × 400); C1: 21 d. Thymus (not shown) (NF-κB p65, × 200); C2: 28 d. Normal thymus (HE, × 200); C3: 28 d. Sporadic apoptotic cells in thymus (TUNEL, × 400).

Table 5 Comparison of different pathological indexes for thymus, *M* (Q_R)

Index	Time (d)	Sham-operation group	Model control group	Treatment group
Product staining intensity and positive rate of Bax	7	0.0 (0.0)[c]	1.0 (2.0)	0.5 (1.0)[a]
	14	0.0 (1.0)	1.0 (2.0)	0.0 (0.0)[c]
	21	0.0 (0.0)[c]	1.0 (2.0)	0.0 (2.0)
	28	0.0 (0.0)[c]	2.0 (1.5)	0.0 (1.0)[c]
Apoptosis index	7	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
	14	0.0 (0.0)	0.0 (0.01)	0.0 (0.0)[c]
	21	0.0 (0.0)[c]	0.0 (0.01)	0.0 (0.0)[c]
	28	0.0 (0.0)[c]	0.01 (0.02)	0.0 (0.0)
Product of the staining intensity and positive rate of NF-κB	7	0.0 (0.0)	0.0 (2.0)	0.0 (0.0)
	14	0.0 (4.0)	2.0 (4.0)	0.0 (2.0)
	21	0.0 (2.0)	0.0 (2.0)	0.0 (0.0)
	28	0.0 (1.0)	0.0 (3.0)	0.0 (2.0)

[a]$P < 0.05$ *vs* sham-operating group; [c]$P < 0.05$ *vs* model control group.

normal. An obscure boundary between thymus cortex and medulla was seen in few rats (Figure 3C).

The pathological scoring standards for thymus have been described elsewhere [18]. No significant difference was observed in pathological scores for different groups (Table 5).

No significant difference was found in product staining intensity and positive rate of NF-κB protein in thymus of different groups (Table 5).

The product staining intensity and positive rate of Bax protein were significantly lower in sham-operation group than in model control group on days 7, 21 and 28 after operation ($P < 0.05$), in sham-operation group than in treatment group on day 7 after operation ($P < 0.05$), and in treatment group than in model control group on days 14 and 28 after operation ($P < 0.05$).

The apoptosis index for thymus was significantly lower in sham-operation group than in model control group on days 21 and 28 after operation ($P < 0.05$), and in treatment group than in model control group on days 14 and 21 after operation ($P < 0.05$).

DISCUSSION

It has been shown that the incidence rate of endotoxemia in OJ patients is as high as 39.3%[19], which is mainly

due to insufficient intestinal bile salt that leads to excessive multiplication of intestinal bacteria and decreased inactivation of endotoxins. Since the function of reticuloendothelial system is inhibited, gut-derived endotoxins are not effectively eliminated. As a result, large amounts of endotoxin enter into the blood resulting in endotoxemia. Endotoxin is a main factor for immune function impairment during OJ since it can directly stimulate Kupffer cells to release inflammatory mediators, including oxygen free radicals, TNF-α, IL-6 and IL-8, and thereby aggravates the inflammatory response of body[19-24]. TNF-α is the most important factor for mediating toxic effects of endotoxin. Excessive release of TNF-α can induce multiple organ injuries. We speculate that immune function impairment results from the damage to immune organs. The results of this study show that the serum TNF-α level and pathological scores for spleen and lymph nodes were significantly lower in sham-operation and treatment groups than in model control group, suggesting that TNF-α is involved in OJ-induced damage to immune organs, and *Astragalus* injection can significantly lessen the toxic effects of TNF-α on and improve the pathological changes in immune organs. We think that *Astragalus* injection exerts, to a certain degree, its protective effects on immune organs by suppressing the production of TNF-α. Although no statistically significant difference was observed in pathological scores for thymus, the pathological changes in thymus of *Astragalus* treatment group showed varying degrees of improvement compared with model control group. In model control group, the thymus showed varying degrees of jaundice and atrophy at all time points after operation, and an obscure boundary between the thymus cortex and medulla in parts of thymus tissue under light microscope. In contrast, the thymus in treatment group became jaundiced with no atrophy on days 21 and 28 after operation, and the boundary between the thymus cortex and medulla was obscure in few parts of thymus tissue under light microscope, suggesting that *Astragalus* has protective effects on immune organs.

NF-κB p65, a protein that is extensively distributed in cytoplasm of many cells, can regulate gene transcription in nuclei. It is a member of Rel family of transcriptional regulatory proteins and is involved in gene expression regulation of many inflammatory factors. When the body is under stress, NF-κB p65 is activated and binds to specific κB gene sequences, and thereby promotes gene transcription and protein synthesis of pro-inflammatory molecules, causes strong expression of inflammatory cytokines such as TNF-α and IL-6mRNA, accelerates toxic effect on cells in multiple organs, eventually leading to multiple organ dysfunction. Based on the expression levels of NF-κB p65 protein in spleen and thymus, we speculate that *Astragalus* has no inhibitory effects on the expression of NF-κB p65 protein in spleen and thymus of OJ rats.

Apoptosis is a self-protective strategy employed by the body for removal of the destroyed cells through initiating programmed gene expression under certain pathophysiological conditions[25]. In contrast to cell necrosis, apoptosis is an active and spontaneous process and does not induce dramatic inflammatory reaction. However, apoptosis as a mode of cell loss can also induce functional impairment of immune organs. Bax, a soluble protein encoded by a recently discovered apoptosis-promoting gene, shares the same protein family as Bcl-2 and is able to promote cell apoptosis[26,27]. In this study, the expression level of Bax protein was higher in spleen and thymus of model group than in those of sham-operation group. As a result, the apoptosis index was increased and pathological injury was aggravated, suggesting that Bax protein is involved in physiological or pathological cellular apoptosis of spleen and thymus. After treatment with *Astragalus* injection, the pathological changes in immune organs were improved and the expression level of Bax protein in spleen and thymus, apoptosis index and pathological scores for spleen and thymus were significantly lower in treatment group than in model control group, indicating that *Astragalus* injection can down-regulate the expression of Bax protein, suppress cell apoptosis and exert protective effects on immune organs.

In summary, *Astragalus* injection can improve pathological changes in immune organs, reduce serum TNF-α level, down-regulate expression of Bax protein in spleen and thymus, and suppress cell apoptosis, thereby exerting its protective effects on immune organs of OJ rats. Since *Astragalus* has diverse pharmacological actions, low cost and few side effects, it has a better application prospect and economic value.

COMMENTS

Background

Obstructive jaundice (OJ) is a kind of common clinical manifestation. The pathogenesis and treatment of OJ have been a hot topic in medical field for a long time. As systemic inflammatory response syndrome and multiple organ dysfunction syndrome were studied in recent years, immune function impairment concomitant with OJ has gradually attracted wide attention and is considered as a cause of death in OJ patients. Therefore, one of the important approaches to treatment of OJ is to restore the functions of immune organs.

Research frontiers

Development and utilization of traditional Chinese medicine have good prospects in therapy for OJ since it has lower cost, more extensive pharmacological effects and fewer side effects. Since *Astragalus* injection contains polysaccharide, saponin, flavone and trace elements, it has a variety of pharmacological effects and plays an important role in increasing the immunity of body and protecting the liver and kidney. This study demonstrated that *Radix Astragali* could exert its protective effects on immune organs of OJ rats by relieving the pathological changes in immune organs, reducing tumor necrosis factor (TNF)-α level, and inhibiting Bax expression and apoptosis in spleen and thymus.

Innovations and breakthroughs

At present, no studies about the effects of *Astragalus* on immune organs during OJ are available. In the present study, we investigated the protective effect of *Astragalus* injection on immune organs of OJ rats and its mechanism, which may provide an experimental basis for its application in clinical practice.

Applications

Astragalus has diverse pharmacological actions, low cost and few side effects, and thus can be applied in clinical practice.

Terminology

Nuclear factor (NF)-κB p65, a protein that is extensively distributed in cytoplasm of many cells, is able to regulate gene transcription in nuclei. TNF-α

is a most important factor for mediating the toxic effects of endotoxins. Bax, a soluble protein encoded by a recently discovered apoptosis-promoting gene, shares the same protein family as Bcl-2 and is able to promote cell apoptosis.

Peer review

The manuscript describes the protective effects of *Radix astragali* injection on immune organs of rats with OJ. *Radix astragali* injection could reverse elevated TNF-α level, and spleen, thymus and lymph node lesions. Bax immunoreactivity and apoptosis could be observed after obstructive jaundice. This manuscript is largely descriptive by providing novel insights into the mechanism underlying the beneficial effect *of Radix astragali* on obstructive jaundice.

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