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Original Research Article

Effect of dexmedetomidine on blood T cell proliferation, T cell subsets and phagocytic function of alveolar macrophages in young rats subjected to splenectomy

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Abstract

Purpose: *To study the effect of dexmedetomidine on blood T cell proliferation, T cell subsets and phagocytosis of alveolar macrophages in young rats undergoing splenectomy.*
Methods: *Fifty-four healthy male rats were used for the establishment of an animal model of splenectomy. The young rats were randomly assigned to control, model (untreated) and medication groups, each with 18 rats. The rats in the control and model groups were given physiological saline at a dose of 10 ml/kg, while those in the treatment group were injected with dexmedetomidine at a dose of 50 μg/kg. All treatments were given intraperitoneally (i.p.). T cell proliferation, T cell subset level, phagocytic index and degree of phagocytosis of alveolar macrophages were compared among the rat groups.*
Results: *Relative to control, CD4+, CD8+ and CD4+/CD8+ levels in model and medication groups decreased significantly ($p < 0.05$). CD4+ and CD8+ levels were lower in the medication group than in model group. Phagocytic index and degree of phagocytosis of alveolar macrophages in model and medication groups were significantly lower than those in the control group, while phagocytic index and degree of phagocytosis of alveolar macrophages in the medication group of rats were smaller than those in model rats ($p < 0.05$).*
Conclusion: *Dexmedetomidine significantly reduces immune function in splenectomy rats. However, it should be used with caution in patients with splenectomy.*

Keywords: *Dexmedetomidine, Splenectomy, Young rats, T cell proliferation, T cell subsets, Alveolar macrophage, Phagocytosis*

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INTRODUCTION

The immune system is composed of immune organs, immune cells and immune molecules, and is responsible for non-specific immune and specific immune responses [1]. The spleen is the largest peripheral immune organ. It filters the blood, removes dead blood cells, phagocytizes viruses and germs, and also activates B cells to produce large amounts of antibodies [2].

Following splenectomy, the ability of the body to remove bacteria from the blood, and the concentration of IgM are significantly reduced, thereby increasing the chances of infection. In children, the immune system is immature and vulnerable to pathogenic microorganisms and foreign antigens, and the possibility of intracranial infection is significantly increased due to incomplete development of the blood-brain barrier [3].

Dexmedetomidine acts on the mesencephalic nucleus of the midbrain, and inhibits the release of synaptic neurotransmitters thereby suppressing the transmission of neural signals [4]. It is often used for sedation in ICU patients due to its pharmacological effects of sedation, analgesia and anti-stress reaction [5]. Moreover, it does not inhibit the respiratory system, and it has a high degree of safety [5]. It has been reported that sedative drugs like dexmedetomidine affect the immune system [6].

The present investigation was carried out to determine the influence of dexmedetomidine on blood T cell proliferation, T cell subsets and phagocytosis of alveolar macrophages in young rats subjected to splenectomy.

EXPERIMENTAL

Animals

Fifty-four (54) male SD rats were purchased from Changsha Tianqin Biotechnology Co. Ltd {production license = SCXK (Xiang) 2016-0001)}. The rats had a mean body weight of 140 ± 10 g, and were aged 5 weeks. They were maintained in a laboratory at 24 ± 1 °C, 55 ± 15 % humidity, and equal daily durations of light and darkness, with *ad libitum* access to feed and water.

This study received approval from the Animal Ethical Committee of The Second People's Hospital of Kunming (approval no. 20187317), and was performed according to "Principles of Laboratory Animal Care" (NIH publication no. 85 - 23, revised 1985) [7].

Main instruments and reagents

Constant temperature water bath (HH-US brand) was product of Shanghai Hetian Scientific Instrument Co. Ltd.); low temperature refrigerator was made by Jinan Chuangxiang Biotechnology Co. Ltd (type: MDF-193), while electronic balance (type: JA2603B) was product of Shanghai Tianmei Tianping Instrument Co. Ltd). High pressure steam sterilization pot (model LDZM-40KCS) was purchased from Shanghai Precision Instrument Co. Ltd. Leica DMI8 Optical microscope was made by Yike Optical Instrument Co. Ltd. Microfuge 16 centrifuge was produced by Beckman Coulter Co. (USA). Fetal bovine serum was purchased from Shanghai Yubo Biotechnology Co. Ltd.

Anhydrous ethanol was supplied by National Pharmaceutical Group Chemical Reagent Co. Ltd, while phosphate buffer was obtained from Shanghai Thermo Fisher Scientific Co. Ltd. Physiological saline injection (0.9 % sodium chlorine) was purchased from Sichuan Kelun Pharmaceutical Co. Ltd (production batch number: 20166625, specification: 100 ml). Dexmedetomidine was product of Jiangsu Enhua Pharmaceutical Co. Ltd (production batch number: 20170085, specification: 1ml: 5mg).

Grouping and establishment of animal model of splenectomy

The young rats were anesthetized and placed on a console to maintain a temperature of 37 ºC. The hair on their chest and abdomen was shaved off. The spleen was excised, and the abdomen was closed. Thereafter, the rats were divided into 3 groups: control, model and medication groups, each with 18 rats. The rats in the control and model groups were injected *i.p*. with 0.9 % sodium chloride at a dose of 10 ml/kg, while rats in treatment group were injected *i.p.* with dexmedetomidine at a dose of 50 µg/kg.

Assessment of T cell proliferation

Blood (3 ml) was taken from the heart of 9 rats in each group at 3, 6 and 9 h after the treatments, and placed in anticoagulation tubes (1 and 2 mL). Lymphocyte separation solution (4 mL) was added to the centrifuge tubes, followed by addition of 2 ml of anticoagulant. The tubes were then centrifuged at 3000 rpm. The second layer containing lymphocytes was taken and mixed with 10 mL of phosphate buffer. The mixture was centrifuged, and the precipitate was collected. The experiment was repeated twice and the concentration of cells in the suspension was 2×10^6/mL.

The cells were inoculated into a 96-well plate at 100 µL per well, and 100 µL of Concanavalin A (ConA) was added to the wells, while 100 µL of RPMI 1640 was added to the control wells. The cells were cultured at room temperature for 48 h, after which 20 µL of MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide) solution was added, and culture was continued for 3 h. Thereafter, the absorbance of each well was measured at a wavelength of 570 nm.

Determination of T cell subsets

Anticoagulant (100 µL) was added to a sterile EP tube, followed by 5 µL APC anti-rat CD3, 0.5 µL FITC anti-rat CD4, and 1.25 µL PE anti-rat CD8a. After standing at 4 °C for 30 min in the dark, 1 ml of RBC Lysis Buffer was added, followed by centrifugation. The sediment was collected and used for flow cytometric determination of changes in T cell subsets (CD4+, CD8+, CD4+/CD8+) of rats in each group.

Assessment of alveolar macrophage phagocytosis

Alveolar lavage fluid was centrifuged to obtain a precipitate of macrophages which was cultured at 37 °C in a T25 cell culture flask containing 5 % carbon dioxide. The alveolar macrophages attached to the walls were isolated and purified into a suspension. Then, 4 mL of healthy cock blood was taken to make a chicken red cell suspension with a volume fraction of 1%. The alveolar macrophage suspension (1 mL) and 0.05 mL of 1 % chicken red blood cell suspension were thoroughly shaken in a test tube, and centrifuged. The supernatant was discarded, and 0.05 mL of the precipitate taken and placed on crossed slides and stained with Wright dye solution. Six fields of view were selected from each high-power field for a total of 200 alveolar macrophages. Phagocytic index and extent of phagocytosis of alveolar macrophages in each group were compared.

Statistical analysis

Measurement data were compared between the two groups using independent sample *t*-test. Comparison between the 3 groups was done with one-way ANOVA, while comparison of the enumeration data was carried out with χ^2 test. Values of $p < 0.05$ were deemed to indicate statistical significance. All analyses were done with SPSS21.0 software package.

RESULTS

Blood T cell proliferation

Table 1 shows that there were marked reductions in absorbance of cell culture supernatants for the model and treatment rats, relative to control ($p < 0.05$). Interestingly, absorbance was significantly lower in the treatment rats than in model rats ($p < 0.05$).

Table 1: Absorbance values of for proliferated T cells in rats (n = 9)

Group	3 h after medication	6 h after medication	9 h after medication
Control	0.23 ± 0.02	0.23 ± 0.02	0.22 ± 0.01
Model	0.07 ± 0.01[a]	0.08 ± 0.02[a]	0.09 ± 0.02[a]
Medication	0.04 ± 0.02[ab]	0.05 ± 0.01[ab]	0.06 ± 0.02[ab]
F-value	313.002	1359.001	217.004
P-value	<0.001	<0.001	<0.001

Values are presented as mean ± SD. [a]$p < 0.05$, vs control rats; [b]$p < 0.05$, vs model rats

Blood T cell subsets of the rats

Relative to control group, CD4+, CD8+ and CD4+/CD8+ ratio of model group and treatment rats group were markedly lower ($p < 0.05$). The amounts of CD4+ and CD8+ in treatment group were lower than those in model group, and the CD4+/CD8+ ratio of treatment rats was elevated, relative to model rats. These results are shown in Table 2.

Table 2: Blood T cell subsets in rats (n = 9)

Group and time after medication		CD_4^+	CD_8^+	CD_4^+/CD_8^+
Control	3h	48.71 ± 1.18	24.33 ± 1.07	2.00 ± 0.05
	6h	47.76 ± 1.99	23.91 ± 1.57	2.01 ± 0.02
	9h	49.43 ± 2.74	25.03 ± 2.68	1.99 ± 0.07
Model	3h	35.94 ± 2.15[a]	19.23 ± 1.02[a]	1.87 ± 0.02[a]
	6h	34.98 ± 2.54[a]	18.68 ± 1.19[a]	1.87 ± 0.05[a]
	9h	35.63 ± 2.15[a]	19.14 ± 1.35[a]	1.86 ± 0.05[a]
Treatment	3h	33.89 ± 3.31[ab]	18.13 ± 1.81[ab]	1.87 ± 0.01[ab]
	6h	33.19 ± 2.78[ab]	18.66 ± 1.78[ab]	1.88 ± 0.08[ab]
	9h	34.31 ± 2.48[ab]	19.08 ± 1.51[ab]	1.87 ± 0.11[ab]

[a]$P < 0.05$, vs control; [b]$p < 0.05$, vs model. Values are presented as mean ± SD

Phagocytic index and phagocytic degree of alveolar macrophages in the young rats

As shown in Table 3, the phagocytic index and degree of phagocytosis of alveolar macrophages in the treatment rats were markedly lower than model and control rat values ($p < 0.05$).

Table 3: Phagocytic index and phagocytosis rate of alveolar macrophages (n = 9)

Group and time after medication		Phagocytic index	The rate of phagocytosis (%)
Control	3 h	5.15 ± 1.18	35.74 ± 2.77
	6 h	5.49 ± 1.91	34.26 ± 3.88
	9 h	4.83 ± 1.17	36.02 ± 4.39
Model	3 h	2.51 ± 0.46[a]	25.21 ± 1.46[a]
	6 h	2.74 ± 0.77[a]	26.03 ± 3.25[a]
	9 h	2.59 ± 0.53[a]	26.66 ± 4.01[a]
Treatment	3 h	1.02 ± 0.25[ab]	20.65 ±2.17[ab]
	6 h	1.26 ± 0.38[ab]	21.28 ± 1.96[ab]
	9 h	1.34 ± 0.55[ab]	21.82 ± 2.87[ab]

[a]$P < 0.05$, vs control, [b]$p < 0.05$, vs model. Values are presented as mean **±** SD

DISCUSSION

The immune system recognizes foreign cells and molecules and removes them to protect the body from pathogenic microorganisms and tumors [8]. The immune system is comprised of non-specific immunity and specific immunity. Non-specific immunity, also known as innate immunity, is a defense system inherent in the body. Various phagocytic cells in the body first recognize foreign components, and then sacrifice themselves together with foreign bodies for killing by lymphocytes and natural killer cells (NK cells) [9].

Specific immunity refers to the immune response of the body during the second exposure to antigen, and it comprises B cell-mediated humoral immunity and T cell-mediated cellular immunity. The T cells are divided into helper T cells (T Helper cells, Th) and cytotoxic T cells (Tc) [10]. It has been suggested that a shift from Th cells to Th1 subsets or Th2 subsets has a significant impact on immune response, and that Th1 cell response has a significant beneficial effect on trauma and infection [11]. The spleen, the largest immune organ in the body, contains a large number of lymphocytes and macrophages, and it is the center of cellular immunity and humoral immunity [12].

Dexmedetomidine is a highly safe and potent α2-adrenoreceptor agonist with an affinity for α2-adrenergic receptors 8 times higher than that of clonidine [13]. This study was aimed at investigating the effects of dexmedetomidine on blood T cell proliferation, T cell subsets and phagocytosis of alveolar macrophages in young rats subjected to splenectomy.

The T lymphocytes arise from bone marrow pluripotent stem cells. In the human embryonic stage and the primary stage, some pluripotent stem cells or pre-T cells in the bone marrow enter the thymus under the influence of thymus hormone, and mature into immunologically active T cells [14]. The immunity produced by T cells is cellular immunity, and its response to foreign components is usually determined through the level of T cell proliferation. Studies have shown that dexmedetomidine induces apoptosis in T lymphocytes, reduces the ability of ConA to bind to T lymphocytes, and suppresses the expression of interleukin-2 (IL-2) receptor on T cell membrane, thereby reducing the level of T cell proliferation [15].

In this study, the blood optical density values of model and medication rats were markedly reduced, relative to those in the model rats, and the blood optical density of rats in the medication group was significantly decreased, relative to that of the model group. These results are consistent with the findings of Nishiyama *et al* [16].

It has been reported that immune function is closely related to the level of T cell subsets [17]. All mature T cell membranes contain CD4+ which is a typical membrane antigen of helper T cells. It helps B cells to secrete antibodies and regulate other T cell immune responses, and it is an important indicator in the diagnosis of immunodeficiency. In contrast, CD8+ is the cytotoxic effector cell which inhibits the immune function of the body. Dynamic balance in CD4+/CD8+ ratio is an important indicator of immune system homeostasis. A reduction in the ratio of CD4+/CD8+ indicates a decrease in immune function, which implies a significant deterioration in the patient's condition or aggravation of infection.

The levels of CD4+, CD8+, CD4+/CD8+ in model and medication groups were markedly reduced, relative to control. The CD4+ and CD8+ levels in the rats treated with dexmedetomidine were decreased, when compared with those in the model group. The level of CD8+ was increased when compared with that of the model group, although both groups were comparable.

Alveolar macrophages are differentiated from monocytes. They are distributed in the interstitial spaces of lungs, and are involved in defense,

phagocytosis, immunity and secretion. Pulmonary macrophages can also engulf senescent red blood cells [18]. Studies have shown that in septic mice, dexmedetomidine reduced the number of sepsis in mouse alveolar macrophages and inhibited their phagocytosis [19].

In the present study, the phagocytic index and the rate of phagocytosis alveolar macrophages in the model group and the control group were markedly decreased, relative to control. Moreover, phagocytic index and the degree of phagocytosis of alveolar macrophages in the medication rats were lowered, indicating that dexmedetomidine significantly inhibited the phagocytic function of alveolar macrophages in young rats subjected to splenectomy.

CONCLUSION

The results of this study indicate that dexmedetomidine significantly reduces immune function in young rats subjected to splenectomy, inhibits T cell proliferation and phagocytosis of alveolar macrophages, but has no significant effect on T cell subsets. Thus, dexmedetomidine should be used with caution in patients with splenectomy.

DECLARATIONS

Conflict of interest

No conflict of interest is associated with this work.

Contribution of authors

All work was done by the author named in this article and the authors accept all liability resulting from claims which relate to this article and its contents. Xiaoyun Hu designed the study and interpreted the results. Yecai Zhang, Jianming Yang, Hao Yang, Decai Wang, Yunrui Guo, Guoyun Zhang, Xiaoyun Hu collected data and drafted the manuscript. Yecai Zhang performed the experiments.

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REFERENCES

1. *Lackey DE, Olefsky JM. Regulation of metabolism by the innate immune system. Nat Rev Endocrinol 2016; 12(1): 15-28.*
2. *Liu Z, Wu Y, Feng Y, Wu F, Liu RF, Wang LF, Liang JY, Liu JH, Sun X, Wu ZD. Spleen atrophy related immune system changes attributed to infection of Angiostrongylus cantonensis, in mouse model. Parasitol Res 2017; 116(2): 1-11.*
3. *Baris HE, Baris S, Karakoc-Aydiner E, Gokce I, Yildiz N, Cicekkoku D, Ogulur I, Ozen A, Alpay H, Barlan I. The effect of systemic corticosteroids on the innate and adaptive immune system in children with steroid responsive nephrotic syndrome. Eur J Pediatr 2016; 175(5): 685-693.*
4. *Zhao WB, Gong SM, Tan RD, Jiang HR, Xu XW. Effect of sevoflurane combined with dexmedetomidine on immune system after surgery in patients with asymptomatic human immunodeficiency virus infection. J Clin Anesthesiol 2018; 11(7): 1068-1071.*
5. *Bulow NM, Colpo E, Pereira RP, Correa EF, Waczuk EP, Duarte MF, Rocha JB. Dexmedetomidine decreases the inflammatory response to myocardial surgery under mini-cardiopulmonary bypass. Braz J Med Biol Res 2016; 49: 4646.*
6. *Khafagy HF, Ebied RS, Mohamed AH, El-said MH, El-haddad AM, Abla S, Samhan YM. Effect of dexmedetomidine infusion on desflurane consumption and hemodynamics during BIS guided laparoscopic cholecystectomy: A randomized controlled pilot study. Egypt J Anaesth 2017; 33(3): 227-231.*
7. *World Health Organization. Principles of laboratory animal care. WHO Chron 1985; 39: 51-56.*
8. *Sherman MP, Zaghouani H, Niklas V. Gut microbiota, the immune system, and diet influence the neonatal gut–brain axis. Pediatr Res 2015; 77(2): 127-135.*
9. *Califano JA, Khan Z, Noonan KA, Rudraraju L, Zhang Z, Wang H, Goodman S, Gourin CG, Ha PK, Fakhry C, et al. Tadalafil Augments Tumor Specific Immunity in Patients with Head and Neck Squamous Cell Carcinoma. Clin Cancer Res 2015; 21(1): 30-38.*
10. *Bouman A, Schipper M, Heineman MJ, Faas MM. Gender Difference in the Non-Specific and Specific Immune Response in Humans. Am J Reprod Immunol 2015; 52(1): 19-26.*
11. *Hiroi T, Iwatani K, Iijima H, Kodama S, Yanagita M, Kiyono H. Nasal immune system: distinctive Th0 and Th1/Th2 type environments in murine nasal-associated lymphoid tissues and nasal passage, respectively. Eur J Immunol 2015; 28(10): 3346-3353.*
12. *Beheshti A, Wage J, McDonald JT, Lamont C, Peluso M, Hahnfeldt P, Hlatky L. Tumor-host signaling interaction reveals a systemic, age-dependent splenic immune*

influence on tumor development. Oncotarget 2015; 6(34): 35419-35432.

13. *Cho JS, Kim HI, Lee KY, An JY, Bai SJ, Cho JY, Yoo YC. Effect of Intraoperative Dexmedetomidine Infusion on Postoperative Bowel Movements in Patients Undergoing Laparoscopic Gastrectomy: A Prospective, Randomized, Placebo-Controlled Study. Med 2015; 94(24): 959.*
14. *Attaf M, Legut M, Cole DK, Sewell AK. The T cell antigen receptor: the Swiss army knife of the immune system. Clin Exp Immunol 2015; 181: 1-18.*
15. *Sethna Z, Elhanati Y, Dudgeon CS, Callan CG Jr, Levine AJ, Mora T, Walczak AM. Insights into immune system development and function from mouse T-cell repertoires [Biophysics and Computational Biology]. Proc Natl Acad Sci U S A 2017; 114(9): 2253.*
16. *Nishiyama Y, Saikawa Y, Nishiyama N. Interaction between the immune system and acute myeloid leukemia: A model incorporating promotion of regulatory T cell expansion by leukemic cells. Bio Systems 2018; 165(1): 99-105.*
17. *Hor JL, Whitney PG, Zaid A, Brooks AG, Heath WR, Mueller SN. Spatiotemporally Distinct Interactions with Dendritic Cell Subsets Facilitates CD4+ and CD8+ T Cell Activation to Localized Viral Infection. Immunity 2015; 43(3): 554-565.*
18. *Goritzka M, Makris S, Kausar F, Durant LR, Pereira C, Kumagai Y, Culley FJ, Mack M, Akira S, Johansson C. Alveolar macrophage-derived type I interferons orchestrate innate immunity to RSV through recruitment of antiviral monocytes. J Exp Med 2015; 212(5): 699-714.*
19. *Tippimanchai DD, Nolan K, Poczobutt J, Verzosa G, Li H, Scarborough H, Huang J, Young C, DeGregori J, Nemenoff RA, et al. Adenoviral vectors transduce alveolar macrophages in lung cancer models. Oncoimmunology 2018; 7(6): 1544-1545.*