European Review for Medical and Pharmacological Sciences 2020; 24: 11865-11870

Protective effect of dexmedetomidine against renal injury in diabetic nephropathy rats through inhibiting NF-κB pathway

Y.-T. LIU[1], W. LIU[2], Z.-H. WAN[1], X.-Q. WANG[3], F.-X. GU[3]

[1]Department of Anesthesiology, First Hospital of Lanzhou University, Lanzhou, China
[2]Institute of Pathology, School of Basic Medical Sciences, Lanzhou University, Lanzhou, China
[3]Department of Anesthesiology, First Hospital of Lanzhou University, Lanzhou, China

Abstract. – OBJECTIVE: The aim of this study was to investigate the protective effect of dexmedetomidine (Dex) against renal injury in diabetic nephropathy (DN) rats by inhibiting the nuclear factor-κB (NF-κB) pathway.

MATERIALS AND METHODS: A total of 36 Sprague-Dawley rats were randomly divided into three groups, including: normal group (n=12), model group (n=12) and Dex group (n=12). The rats underwent no treatment in normal group. In model group, the diabetes model was successfully established, and normal saline was intraperitoneally injected after operation. In Dex group, the diabetes model was established as well, and Dex was intraperitoneally injected after operation. After intervention for 2 weeks, the samples were taken for use. Blood urea nitrogen (BUN) and serum creatinine (Cr) were detected using a full-automatic biochemical analyzer. The expression of Caspase-3 was detected via immunohistochemistry. Western blotting was conducted to detect the protein expression of NF-κB. The apoptosis was detected via terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling (TUNEL) assay. In addition, the levels of interleukin-6 (IL-6) and tumor necrosis factor-α (TNF-α) were determined via enzyme-linked immunosorbent assay (ELISA).

RESULTS: The levels of BUN and Cr were significantly higher in model group and Dex group than those in normal group ($p<0.05$). However, they were significantly lower in Dex group than those in the model group ($p<0.05$). Immunohistochemistry results showed that the mean optical density of Caspase-3 positive expression increase remarkably in model group and Dex group when compared with normal group ($p<0.05$). However, it significantly declined in Dex group when compared with the model group ($p<0.05$). The results of Western blotting revealed that model group and Dex group exhibited evidently higher relative protein expression of NF-κB than normal group ($p<0.05$). However, Dex group displayed notably lower relative protein expression of NF-κB than model group ($p<0.05$). TUNEL assay demonstrated that the apoptosis rate increased significantly in the model group and Dex group when compared with normal group ($p<0.05$). However, it remarkably declined in Dex group in comparison with the model group ($p<0.05$). Finally, ELISA assay indicated that model group and Dex group had markedly higher levels of IL-6 and TNF-α than normal group ($p<0.05$). However, the levels of IL-6 and TNF-α were significantly lower in Dex group than model group ($p<0.05$).

CONCLUSIONS: Dex inhibits inflammation and apoptosis by suppressing the NF-κB signaling pathway, thereby exerting a protective effect against renal injury in DN rats.

Key Words:
Diabetic nephropathy, NF-κB signaling pathway, Dexmedetomidine, Apoptosis, Inflammation.

Introduction

Diabetes mellitus is a clinically common endocrine system disease. The resulting hyperglycemia is an important predisposing factor of a variety of diseases, including microangiopathy and renal tissue lesions[1,2]. Particularly, the latter is an important pathological cause of diabetic nephropathy (DN). In recent years, DN is considered as one of the major complications of diabetes, whose clinical incidence rate rises with increased incidence of diabetes[3,4]. DN seriously damages renal function and even induces renal failure, seriously threatening the life and health of patients.

Currently, the exact pathological mechanism of DN remains unclear. The pathological reactions of diabetes-induced renal tissue damage are extremely complicated, including inflammation, apoptosis and necrosis[5-7]. Many studies have indicated that excessive inflammation and apoptosis

Corresponding Author: Yatao Liu, MD; e-mail: doctorliuyt@163.com

are important pathological reactions after the occurrence of DN. Meanwhile, they are important causes of aggravating renal tissue damage and affecting renal function.

As a kind of commonly used α_2 receptor agonist in clinic, dexmedetomidine (Dex) possesses sedative and anxiolytic effects. It is also able to improve cardiovascular and renal function[8]. Therefore, Dex has been used for a wide range of indications. The aim of this study was to explore the protective effect of Dex against renal injury in DN rats by inhibiting the nuclear factor-κB (NF-κB) pathway.

Materials and Methods

Laboratory Animals and Grouping

A total of 36 Sprague-Dawley rats (half-male and half female) weighing (220±20) g were purchased from Shanghai SLAC Laboratory Animal Co., Ltd. [license No.: SCXK (Shanghai, China) 2014-0003]. All rats were divided into three groups using a random number table, including: normal group (n=12), model group (n=12) and Dex group (n=12). This study was approved by the Animal Ethics Committee of Lanzhou University Animal Center.

Experimental Reagents and Instruments

Streptozotocin (Sigma-Aldrich, St. Louis, MO, USA), anti-Caspase-3 (Abcam, Cambridge, MA, USA) and anti-NF-κB primary antibodies (Abcam, Cambridge, MA, USA), secondary antibodies (Abcam, Cambridge, MA, USA), quantitative Polymerase Chain Reaction (qPCR) kits (Vazyme, Nanjing, China), terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling (TUNEL) apoptosis kit (Sigma-Aldrich, St. Louis, MO, USA), and enzyme-linked immunosorbent assay (ELISA) kit (Sigma-Aldrich, St. Louis, MO, USA).

Establishment of Diabetes Model in Rats

Streptozotocin solution at a concentration of 1% was prepared and intraperitoneally injected into rats (60 mg/kg). After 3 d, venous blood was drawn from the tail to measure blood glucose. Blood glucose > 16.7 mmol/L indicated successful establishment of diabetes model in rats.

Treatment in Each Group

The rats in normal group were fed normally without any treatment. In the model group, the diabetes model was successfully established in the above way, and 0.9% sodium chloride solution was intraperitoneally injected after operation. In Dex group, the diabetes model was established as well, and Dex was intraperitoneally injected (30 mg/kg/d) after operation. The rats in each group were sacrificed after 2 weeks, followed by sampling.

Sampling

After successful anesthesia, the abdominal aortic blood was first drawn from each rat. Subsequently, 6 rats in each group were fixed with paraformaldehyde. Renal tissues were then collected and fixed in 4% paraformaldehyde solution at 4°C for 48 h. Next, paraffin sections were prepared for immunohistochemistry and TUNEL assay. Besides, samples were directly taken from the remaining 6 rats in each group: renal tissues were taken and placed into Eppendorf (EP, Hamburg, Germany) tubes for Western blotting.

Detection Using a Full-Automatic Biochemical Analyzer

Collected abdominal aortic blood was first centrifuged at 1200 g for 10 min. Then, the levels of blood urea nitrogen (BUN) and serum creatinine (Cr) were detected using a full-automatic biochemical analyzer.

Immunohistochemistry

Paraffin-embedded tissues were sliced into 5 μm-thick sections, flattened in warm water at 42°C, fished up, baked, and prepared into paraffin sections. The sections were then soaked and routinely deparaffinized in xylene solution and gradient alcohol, placed in citric acid buffer, and repeatedly heated in a microwave for 3 times (3 min/time, braised for 5 min each time) for complete antigen retrieval. After washing the sections, the endogenous peroxidase blocker was added drop-wise for 10 min of reaction. Next, the sections were washed again, followed by sealing with goat serum for 20 min. After discarding the goat serum, the sections were incubated with anti-Caspase-3 primary antibody (1:200) at 4°C overnight. On the next day, the sections were washed and incubated with corresponding secondary antibody for 10 min. After fully washing, the sections were reacted with streptavidin-peroxidase solution for 10 min, followed by color development with diaminobenzidine (DAB; Solarbio, Beijing, China). The nuclei were counterstained with hematoxylin, and the sections were finally sealed and observed.

Western Blotting

The cryopreserved renal tissues were lysed with lysis buffer and subjected to ice bath for 1 h, followed by centrifugation at 14000 g for 10 min. The concentration of extracted proteins was quantified using the bicinchoninic acid (BCA) method (Beyotime, Shanghai, China). The absorbance of protein was detected using a micro-plate reader and the standard curve was plotted, based on which the protein concentration was calculated. Protein samples were then separated *via* sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE), and the position of Marker protein was observed. The electrophoresis was terminated when the Marker protein reached the bottom of the glass plate in a straight line. Subsequently, the proteins were transferred onto polyvinylidene difluoride (PVDF) membranes (Millipore, Billerica, MA, USA) and sealed with the sealing buffer for 1.5 h. Next, the membranes were incubated with anti-NF-κB primary antibody (1:1000) and corresponding secondary antibody (1:1000) according to standard instructions. Immuno-reactive bands were finally developed in a dark place using the chemiluminescent reagent for 1 min.

Terminal Deoxynucleotidyl Transferase-mediated dUTP Nick End Labeling (TUNEL) Assay

The apoptosis of renal tissues was detected according to the instructions of TUNEL apoptosis kit.

Enzyme-linked Immunosorbent Assay (ELISA)

The abdominal aortic blood was centrifuged in a high-speed centrifuge at 14000 g for 10 min, and the supernatant was taken. According to the instructions of ELISA kit, the samples were loaded and added with standards, biotinylated antibody working solution and enzyme-linked working solution. Next, the plate was fully washed. Absorbance value at 450 nm was finally measured using a micro-plate reader.

Statistical Analysis

Statistical Product and Service Solutions (SPSS) 20.0 software (IBM Corp., Armonk, NY, USA) was used for all statistical analysis. Enumeration data were expressed as mean ± standard deviation. *t*-test was used for the data in line with normal distribution and homogeneity of variance. Corrected *t*-test was applied for the data in line with normal distribution and heterogeneity of variance. Non-parametric test was conducted for the data not in line with normal distribution and homogeneity of variance. Rank sum test and chi-square test were selected for ranked data and enumeration data, respectively. p-value < 0.05 was considered statistically significant.

Results

Levels of BUN and Serum Cr in Each Group

As shown in Figure 1, the levels of BUN and Cr were significantly higher in the model group and Dex group than those in the normal group (p<0.05). However, they were significantly lower in Dex group than those in model group (p<0.05). The differences were all statistically significant.

Immunohistochemistry Results

As shown in Figure 2A, dark brown indicated positive expression of Caspase-3. The positive expression of Caspase-3 was lower in normal group, whereas was higher in model group and Dex group. According to the statistical results (Figure 2B), the mean optical density of Caspase-3 positive expression rose significantly in the model group and Dex group when compared with normal group (p<0.05). However, it declined markedly in Dex group compared with model group (p<0.05), showing statistically significant differences.

Western Blotting Results

The protein expression of NF-κB was lower in normal group, but higher in model group and Dex group (Figure 3A). The statistical results (Figure 3B) manifested that the relative protein expres-

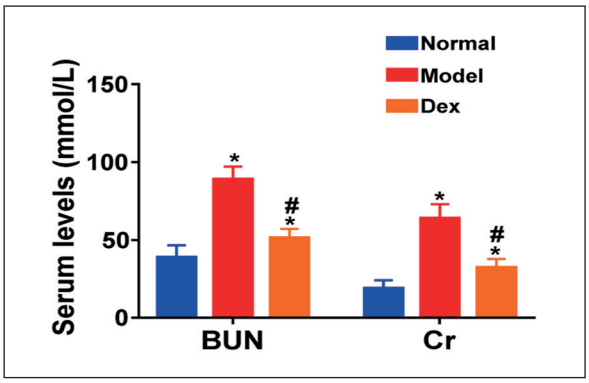

Figure 1. Comparison of BUN and Cr among groups. Note: p^{*}<0.05 *vs.* normal group, $p^{\#}$<0.05 *vs.* model group.

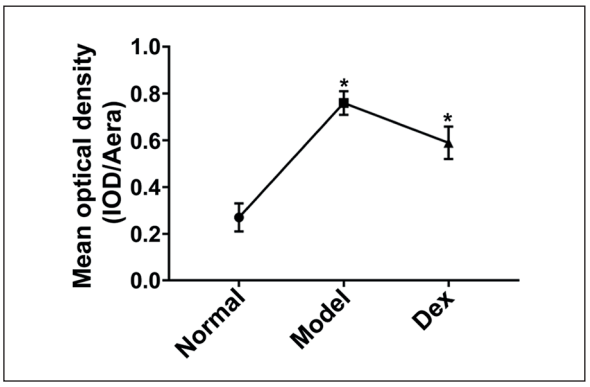

Figure 2. Immunohistochemistry results: Mean optical density of positive expression in each group. $p^{*}<0.05$ *vs.* normal group, $p^{\#}<0.05$ *vs.* model group.

sion of NF-κB increased evidently in the model group and Dex group in comparison with normal group ($p<0.05$). However, Dex group exhibited notably lower relative protein expression of NF-κB than model group ($p<0.05$). The differences were all statistically significant.

TUNEL Apoptosis Assay

Apoptotic cells showed dark brown color. There were fewer apoptotic cells in normal group,

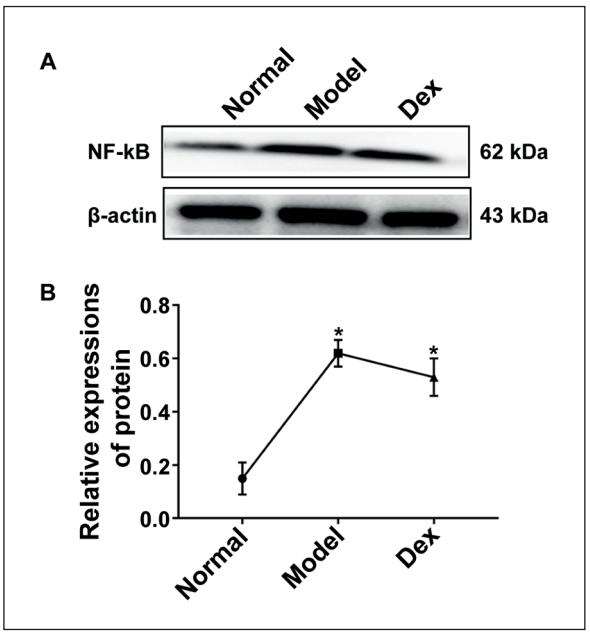

Figure 3. Protein expressions detected *via* Western blotting. Note: **A,** Western blotting results. **B,** Relative protein expressions in each group. $p^{*}<0.05$ *vs.* normal group, $p^{\#}<0.05$ *vs.* model group.

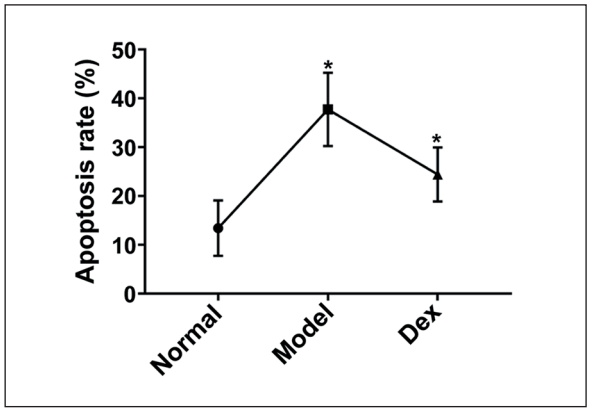

Figure 4. Apoptosis detected *via* TUNEL assay: Apoptosis rate in each group. $p^{*}<0.05$ *vs.* normal group, $p^{\#}<0.05$ *vs.* model group.

but more apoptotic cells in the other two groups (Figure 4A). Subsequent results demonstrated that the apoptosis rate increased significantly in model group and Dex group when compared with normal group ($p<0.05$). However, it was remarkably lower in Dex group than model group, showing statistically significant differences ($p<0.05$) (Figure 4B).

ELISA Results

Model group and Dex group exhibited markedly higher levels of IL-6 and TNF-α than normal group ($p<0.05$). However, Dex group had markedly lower levels of IL-6 and TNF-α than model group ($p<0.05$) (Figure 5). The differences were all statistically significant.

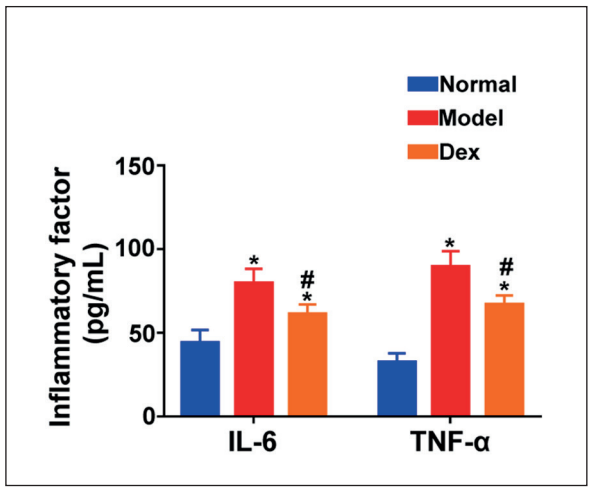

Figure 5. ELISA results. Note: $p^{*}<0.05$ *vs.* normal group, $p^{\#}<0.05$ *vs.* model group.

Discussion

DN, characterized by high incidence rate and great harm, is one of the common complications of diabetes. Clinically, DN may lead to proteinuria, hypoalbuminemia, renal dysfunction and even acute renal failure, seriously threatening the life health and affecting the life quality of patients[9,10]. Currently, there have been no satisfactory treatment methods for DN, and the medical expenses are extremely high. Therefore, DN brings great economic burden to the patient's family and the society. At present, the related pathological mechanism of DN has not been fully elucidated. It is believed that inflammation, apoptosis, necrosis, glucose metabolic disorders and changes in blood flow are involved in the pathogenesis of DN[11-13]. Inflammation and apoptosis are not only common pathological reactions, but also important ones in the occurrence and development of DN; they play critical roles in the occurrence and development of DN. Moreover, inflammation and apoptosis cause great damage to renal tissues and renal function. During DN, a large number of inflammatory factors and cytokines (such as IL-6 and TNF-α) released by damaged renal cells and tissues can lead to inflammatory infiltration of renal cells and tissues. Eventually, this further induces secondary inflammation and apoptosis, aggravating renal tissue damage[14,15]. Meanwhile, inflammation and apoptosis also lead to renal interstitial fibrosis, thereby causing tubular atrophy and glomerular sclerosis. These consequences are all the important causes of renal dysfunction after DN occurrence. The NF-κB signaling pathway is an important signaling pathway with a vital regulatory effect on inflammation and apoptosis. As an important transcription factor in the body, NF-κB binds to its inhibitors and stays in a resting state under physiological conditions[16,17]. After injury, NF-κB will be separated from its inhibitors and activated under the action of inflammatory factors and cytokines, thus activating the NF-κB signaling pathway. Activated NF-κB enters the nucleus and binds to its transcription factors, eventually playing an important role in regulating the expressions of downstream inflammatory factors and apoptotic effector Caspase-3, as well as in regulating inflammation and apoptosis[18].

As a kind of commonly-used α_2 receptor agonist in clinic, Dex shows sedative and anxiolytic effects. It is also able to improve cardiovascular and renal functions. Currently, it is believed[19,20] that Dex can well ameliorate the endocrine system and urinary system, and relieve hyperglycemia. Due to diuretic and natriuretic effects, Dex can protect the kidneys as well.

Conclusions

Our findings demonstrate that Dex shows a good protective effect on kidneys. It can remarkably lower the levels of BUN and serum Cr, and ameliorate renal function in DN. At the same time, Dex can well inhibit inflammation and apoptosis during DN *via* suppressing the NF-κB signaling pathway. In conclusion, Dex inhibits inflammation and apoptosis by suppressing the NF-κB signaling pathway, thereby exerting a protective effect against renal injury in DN rats.

Conflict of Interest

The Authors declare that they have no conflict of interests.

References

1) Qiao Y, Gao K, Wang Y, Wang X, Cui B. Resveratrol ameliorates diabetic nephropathy in rats through negative regulation of the p38 MAPK/TGF-beta1 pathway. Exp Ther Med 2017; 13: 3223-3230.
2) Bramham K. Diabetic Nephropathy and pregnancy. Semin Nephrol 2017; 37: 362-369.
3) Vitova L, Tuma Z, Moravec J, Kvapil M, Matejovic M, Mares J. Early urinary biomarkers of diabetic nephropathy in type 1 diabetes mellitus show involvement of kallikrein-kinin system. BMC Nephrol 2017; 18: 112.
4) El-Horany HE, Abd-Ellatif RN, Watany M, Hafez YM, Okda HI. NLRP3 expression and urinary HSP72 in relation to biomarkers of inflammation and oxidative stress in diabetic nephropathy patients. IUBMB Life 2017; 69: 623-630.
5) Zhu L, Han J, Yuan R, Xue L, Pang W. Berberine ameliorates diabetic nephropathy by inhibiting TLR4/NF-kappaB pathway. Biol Res 2018; 51: 9.
6) Zhang XG, Zhang YQ, Cheng QP, Cao Y, Sun JM, Lv XF. The impact of insulin pump therapy to oxidative stress in patients with diabetic nephropathy. Eur J Med Res 2018; 23: 7.
7) Zabad I, Amin MN, El-Shishtawy MM. Protective effect of vanillin on diabetic nephropathy by decreasing advanced glycation end products in rats. Life Sci 2019; 239: 117088.
8) Tobias JD. Dexmedetomidine: applications in pediatric critical care and pediatric anesthesiology. Pediatr Crit Care Med 2007; 8: 115-131.
9) Wang X, Gao L, Lin H, Song J, Wang J, Yin Y, Zhao J, Xu X, Li Z, Li L. Mangiferin prevents diabetic

nephropathy progression and protects podocyte function via autophagy in diabetic rat glomeruli. Eur J Pharmacol 2018; 824: 170-178.

10) Kim Y, Lim JH, Kim MY, Kim EN, Yoon HE, Shin SJ, Choi BS, Kim YS, Chang YS, Park CW. The adiponectin receptor agonist AdipoRon ameliorates diabetic nephropathy in a model of type 2 diabetes. J Am Soc Nephrol 2018; 29: 1108-1127.

11) Dayem S, Battah AA, Bohy A, Ahmed S, Hamed M, Fattah S. Nitric oxide gene polymorphism is a risk factor for diabetic nephropathy and atherosclerosis in type 1 diabetic patients. Open Access Maced J Med Sci 2019; 7: 3132-3138.

12) Wang D, Zhang G, Chen X, Wei T, Liu C, Chen C, Gong Y, Wei Q. Sitagliptin ameliorates diabetic nephropathy by blocking TGF-beta1/Smad signaling pathway. Int J Mol Med 2018; 41: 2784-2792.

13) Kitada M, Ogura Y, Suzuki T, Monno I, Kanasaki K, Watanabe A, Koya D. A low-protein diet exerts a beneficial effect on diabetic status and prevents diabetic nephropathy in Wistar fatty rats, an animal model of type 2 diabetes and obesity. Nutr Metab (Lond) 2018; 15: 20.

14) Talsma DT, Katta K, Ettema M, Kel B, Kusche-Gullberg M, Daha MR, Stegeman CA, van den Born J, Wang L. Endothelial heparan sulfate deficiency reduces inflammation and fibrosis in murine diabetic nephropathy. Lab Invest 2018; 98: 427-438.

15) Li W, Yang S, Qiao R, Zhang J. Potential value of urinary exosome-derived let-7c-5p in the diagnosis and progression of type II diabetic nephropathy. Clin Lab 2018; 64: 709-718.

16) Yang SM, Ka SM, Wu HL, Yeh YC, Kuo CH, Hua KF, Shi GY, Hung YJ, Hsiao FC, Yang SS, Shieh YS, Lin SH, Wei CW, Lee JS, Yang CY, Chen A. Thrombomodulin domain 1 ameliorates diabetic nephropathy in mice via anti-NF-kappaB/NLRP3 inflammasome-mediated inflammation, enhancement of NRF2 antioxidant activity and inhibition of apoptosis. Diabetologia 2014; 57: 424-434.

17) Yi B, Hu X, Zhang H, Huang J, Liu J, Hu J, Li W, Huang L. Nuclear NF-kappaB p65 in peripheral blood mononuclear cells correlates with urinary MCP-1, RANTES and the severity of type 2 diabetic nephropathy. PLoS One 2014; 9: e99633.

18) Liu P, Li F, Qiu M, He L. Expression and cellular distribution of TLR4, MyD88, and NF-kappaB in diabetic renal tubulointerstitial fibrosis, in vitro and in vivo. Diabetes Res Clin Pract 2014; 105: 206-216.

19) Isik B, Arslan M, Tunga AD, Kurtipek O. Dexmedetomidine decreases emergence agitation in pediatric patients after sevoflurane anesthesia without surgery. Paediatr Anaesth 2006; 16: 748-753.

20) Herr DL, Sum-Ping ST, England M. ICU sedation after coronary artery bypass graft surgery: dexmedetomidine-based versus propofol-based sedation regimens. J Cardiothorac Vasc Anesth 2003; 17: 576-584.