

EFFECT OF DEXMEDETOMIDINE OR PROPOFOL ON ENDOPLASMIC RETICULUM STRESS IN HK-2 CELLS WITH HYPOXIA/REOXYGENATION

Yu Gu, Wenting Fu, Yonghua Yao, Xi Zheng*

Department of anesthesiology, Affiliated Cancer Hospital & Institute of Guangzhou Medical University, Guangzhou, 510030, China

Corresponding E-mail: 9774616@qq.com

Abstract: Objective: To investigate the effects of dexmedetomidine (Dex) or propofol on oxidative stress and the activation of endoplasmic reticulum stress (ERS) in renal cells under hypoxia/reoxygenation (H/R) conditions. **Methods:** Human tubular epithelial cells (HK-2) were selected and divided into Control group, hypoxia 24h and reoxygenation24 (H24R4) group, Dex group and Propofol group. Cell growth was detected by CCK-8 assay kit, malondialdehyde (MDA) and superoxidase dismutase (SOD) were detected by kits, and the expressions of ERS-related key genes such as ER chaperone BiP (GRP78) and C/EBP homologous protein (CHOP) were detected by qPCR. **Results:** Compared with the Control group, the cell growth and SOD activity of HK-2 cells in H24R4 group were significantly decreased, while MDA content and the expressions of GRP78 and CHOP in these cells were significantly increased ($P<0.05$). Pretreatment with Dex or propofol could significantly improve the cell growth and SOD activity of H24R4-treated cells, and reduce intracellular MDA content and GRP78 expression ($P<0.05$). Dex also obviously alleviated CHOP expression in H24R4-treated cells ($P<0.05$). **Conclusion:**s Dex or propofol pretreatment could reduce oxidative stress and attenuates ERS activation in H24R4-treated cells. Dex had a stronger inhibitory effect on the activation of ERS-related apoptosis way compared with propofol.

Keywords: Dexmedetomidine, Propofol, Hypoxia/Reoxygenation, Oxidative Stress, Endoplasmic Reticulum Stress

INTRODUCTION

Acute kidney injury (AKI) is a common clinical complication characterized by acute renal dysfunction. Renal ischemia/reperfusion (I/R) injury is a major cause of AKI[1]. Renal tubular epithelial cells are sensitive to ischemia and hypoxia, which may be triggered by renal hypo-perfusion in many clinical settings, such as renal or heart surgery and so on[2] [3]. Previous studies have reported that the main death modes of renal tubular epithelial cells during ischemia and hypoxia are apoptosis and necrosis[4]. In particular, ischemia can notably induce apoptosis in the early stage. Therefore, clarifying the mechanism of renal cells apoptosis induced by ischemia and hypoxia is vital for the prevention and treatment of AKI.

The mitochondrial pathway and the death receptor pathway are two classical apoptosis pathways. Beside, studies also found that endoplasmic reticulum stress (ERS)-related apoptosis, as a noncanonical apoptosis pathway, is involved in the pathogenesis of glomerulonephritis, acute and chronic kidney disease, and some other renal

diseases[5]. Our previous study also confirmed that ERS over-activation is an important cause of cell death and tissue injury in renal I/R injury, however, no clinical medication have been found to effectively regulate ERS activation[6].

Dexmedetomidine (Dex) is a highly selective α -2 adrenoceptor agonist usually used as a sedative in clinical anesthesia[7]. In addition, Dex protects against organ I/R injury in the heart, brain, and kidney through antioxidant, anti-inflammatory, or anti-apoptotic effects[8]. Researchers further focused on examining several crucial ERS-related proteins and apoptosis indicators in some kinds of cells, such as cardiomyocytes and endothelial cells, and found that Dex interfered in the ERS signaling pathway under I/R or hypoxia/reoxygenation (H/R) conditions[9]. However, few studies have explored the effect of Dex in human tubular epithelial cell (HK-2) under H/R condition. propofol, a commonly used intravenous anesthetic, has also been reported to have anti-apoptotic, antioxidant and anti-inflammatory properties, and could alleviate I/R injury in organs. Su, M., et al have reported propofol

could downregulate the expression of CHOP and caspase-12, two ERS-related proteins, in renal tissue under I/R condition[10]. However, the exact regulatory effect of propofol on ERS remains unknown.

Propofol and Dex are the most commonly used sedative drugs for patients with the highest incidence of AKI, such as renal surgeries or ICU monitoring. The impact of drugs selection in clinical settings with high AKI risk remains an open question. The aim of this study was to investigate the effects of Dex or propofol on renal cells under H/R condition excluding humoral and neural factors, and to explore the relationship between these effects and ERS activation.

MATERIALS AND METHODS

Materials

HK-2 cells were obtained from China Center for Type Culture Collection. Fetal bovine serum (FBS) and trypsin were purchased from Gibco. Cell Counting Kit-8 (CCK8) assay kit was purchased from Dojindo (Tokyo, Japan). Malondialdehyde (MDA) and superoxidase dismutase (SOD) were purchased from Nanjing Jiancheng Bioengineering institute. Trizol reagent were purchased from Invitrogen. The ReverTra Ace qPCR RT Master Mix and SYBR® Green Realtime PCR Master Mix were purchased from TOYOBO. Dex was provided by Jiangsu Hengrui Medicine Co., Ltd.(Jiangsu, China). propofol was purchased from Sigma-Aldrich.

Cell Culture and Treatment

The HK-2 cells were cultured in DMEM with 10% FBS and grown at 37°C in a humidified atmosphere of 5% CO₂ in air. We used H/R model to mimic I/R injury. 70-80% confluent cells were cultured with

low-glucose and serum-deprived DMEM under low-oxygen (95% N₂+5%CO₂) condition in a humidified hypoxia chamber for 24h (Galaxy 48R, Eppendorf, Hamburg, Germany), and then transferred back to normal-oxygen condition (95% air+5%CO₂) for 4 h (H24R4). Cells in the Control groups were incubated in normal-oxygen conditions for 28 h. Cells treated with Dex were incubated in DMEM with 1nM Dex for 1h before H/R stimulation. Cells treated with propofol were incubated in DMEM with 15µM propofol for 1h before H/R stimulation.

CCK8 assay

HK-2 cells were transplanted into 96-well plates at a density of 5000 cells per well. At the end of each stimulation, CCK-8 assay kit was used to test the cell growth using a microplate reader, according to the manufacturer's introduction.

Assessments of intracellular MDA and SOD

After each treatment, the cells were rinsed twice with PBS. Scrape off cells with a cell scratch carefully and the supernatant was removed after centrifugation (3000rpm, 10 min). The intracellular MDA and SOD levels were determined with the relevant kits according to the manufacture's introduction.

Quantitative Real-Time PCR (qPCR)

Total RNA extracted from HK-2 cells were isolated using Trizol reagent. Using NanoDrop-1000 spectrophotometer to detect the quality and concentration of RNA. ReverTra Ace qPCR RT Master Mix was used to perform Reverse transcription. Quantitative analysis of GRP78 mRNA and CHOP mRNA were conducted with qPCR using SYBR® Green Realtime PCR Master Mix with Roche LightCycler 1.1. GAPDH was used as the housekeeping gene.

Table 1 Primer sequences of genes.

Gene	Forward	Reverse
GRP78	CCGAGGAGGAGGACAAGAAGGAG	ACACGCCGACGCAGGAGTAG
CHOP	CTGCTTCTCTGGCTTGCTGAC	CTCCCTTGGTCTTCCTCCTCTCC
GAPDH	GTCATCCCTGAGCTGAACGG	CCACCTGGTGCTCAGTGTAG

Statistical Analysis

Statistical analysis was performed using SPSS 13.0 (SPSS Inc., Chicago, IL) software. Multiple comparisons among different groups were analyzed using one-way ANOVAs followed by Tukey post hoc comparisons. Quantitative data are presented as mean±SD. p< 0.05 was considered as statistically significant different.

RESULTS

Effects of Dex or propofol on HK-2 cells growth under H24R4 condition

The result in Fig. 1 showed that H24R4 treatment significantly reduced HK-2 cells growth. However, Dex or propofol pretreatment significantly alleviated the inhibitory effect of H24R4 on HK-2 cell growth (p<0.05), and there was no significant difference between Dex and propofol

Effects of Dex or propofol on oxidative stress in HK-2 cells under H24R4 condition

H24R4-induced oxidative stress can lead to HK-2 cells death, so the we evaluated the relevant indicators of oxidative stress, including MDA and SOD. As shown in Fig. 2A-B, compared with Control group, H24R4 treatment significantly

increased the content of MDA and decrease the activity of SOD in HK-2 cells ($p < 0.05$). While Dex and propofol markedly ameliorated the increase of MDA content and improved SOD activity in H24R4-treated HK-2 cells ($p < 0.05$).

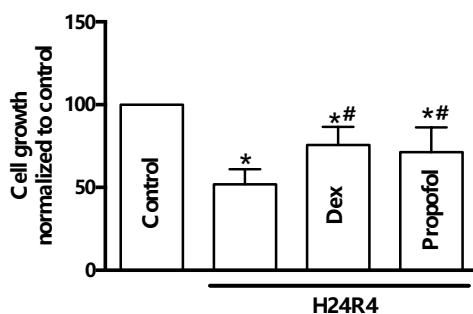


Fig. 1. Effects of dexmedetomidine (Dex) or propofol on HK-2 cells growth under 24h hypoxia and 4h reoxygenation (H24R4) condition. HK-2 cells growth in different groups. Data are presented as the mean \pm SD (n=5). * $p < 0.05$ vs Control; # $p < 0.05$ vs H24R4 group.

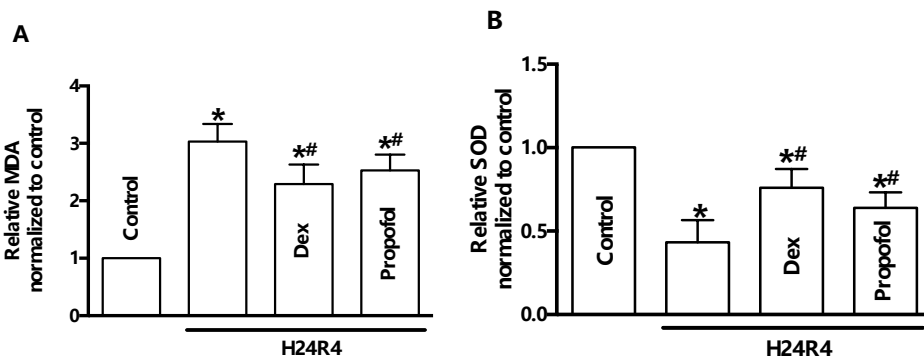


Fig. 2. Effects of dexmedetomidine (Dex) or propofol on oxidative stress in HK-2 cells under 24h hypoxia and 4h reoxygenation (H24R4) condition. A Content of malondialdehyde (MDA). B Activity of superoxide dismutase (SOD). Data are presented as the mean \pm SD (n=5). * $p < 0.05$ vs Control; # $p < 0.05$ vs H24R4 group.

Dex or propofol inhibited the expressions of ERS-related genes in HK-2 cells under H24R4 condition

The expressions of GRP78 and CHOP were analyzed by qPCR. The results showed that H24R4 treatment significantly increased the expressions of GRP78 and CHOP in HK-2 cells ($p < 0.01$). However, Dex

alleviated the increases of GRP78 and CHOP expressions in HK-2 cells induced by H24R4 ($p < 0.05$). Although, propofol could relieve the increased GRP78 expression in HK-2 cells induced by H24R4 ($p < 0.05$), there was no significant difference in CHOP expression between propofol pretreatment and H24R4 treatment HK-2 cells.

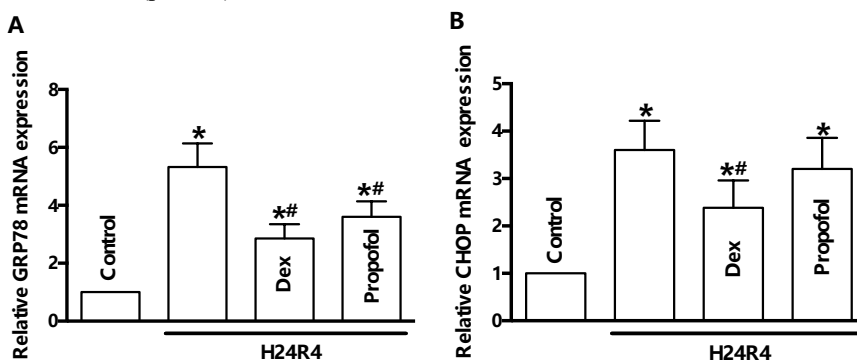


Fig. 3. Effects of dexmedetomidine (Dex) or propofol on the expressions of ERS-related genes in HK-2 cells under 24h hypoxia and 4h reoxygenation (H24R4) condition. A The level of GRP78 mRNA expression. B The level of CHOP mRNA expression. Data are presented as the mean \pm SD (n=5). * $p < 0.05$ vs Control; # $p < 0.05$ vs H24R4 group.

DISCUSSION

I/R-induced AKI may occur in many clinical settings, such as major renal surgery or cardiovascular surgery, which may be caused by transient insufficiency of blood flow in the kidney[3]. Renal tubular epithelial cells are sensitive to ischemia and hypoxia, which may lead to cell death. The main forms of cell death are discrepant in different stages of ischemia and hypoxia. Transient renal ischemia leads to apoptosis, while long-term persistent renal ischemia leads to cell necrosis[11]. Therefore, relieving apoptosis during renal I/R injury may be an effective treatment for AKI.

As a selective alpha2-adrenoceptor agonist, Dex possesses sedative, antalgic, anxiolytic and sympatholytic properties, so it is widely used in clinical anesthesia[12].

Previous studies have demonstrated that Dex could alleviate cells apoptosis and exhibits protective effects against I/R injury in the heart, liver, and kidney[13]. Of particular importance, several clinical studies have shown that Dex has a protective effect on perioperative AKI[14, 15]. Experimental studies also reported that Dex significantly reduced renal oxidative stress and cell apoptosis during early stage of LPS-induced AKI [16]. Nevertheless, the exact role of Dex in renal tubular epithelial cells apoptosis under hypoxia and reoxygenation conditions has not been fully elucidated. Propofol, as a commonly used intravenous anesthetic in clinical anesthesia, has many other functions, such as anti-inflammation, anti-oxidation and anti-apoptosis[10]. It also can reduce I/R injury of brain, liver and kidney, but its effects on apoptosis of renal cells during hypoxia and reoxygenation conditions is not well understood.

Oxidative stress is considered to be one of the important contributors to the pathogenesis of AKI, especially in I/R-induced AKI. MDA is the final product of lipid peroxidation and an effective biomarker for monitoring and measuring oxidative stress. SOD as an antioxidant enzyme involved in the defense mechanism of reducing oxidative stress. They are often used to assess oxidative stress[17]. According to the results of current study, H24R4 treatment could significantly increase MDA content and inhibit SOD activity of HK-2 cells, however, Dex or propofol significantly lowered the content of MDA while restoring the activity of SOD. Meanwhile, Dex or propofol improved the growth of H24R4-treated cells, which may be related to the antioxidant effects of these two drugs.

Our previous studies have found that activation of ERS-related apoptotic pathways induced by oxidative stress is an important cause of renal cell death and renal tissue injury during renal I/R. However, no effective inhibitors of ERS have been found in clinical practice. There's growing evidence that some anesthetic drugs can alleviate oxidative stress and inflammation, and play protective roles in oxidative stress-related pathological processes. Therefore, we selected Dex and propofol, two commonly used anesthetics with antioxidant properties,

to observe their effects on the activation of ERS-related apoptotic pathway in renal cells under H24R4 condition. We examined the expressions of genes involved in ERS activation, including GRP78 and CHOP. We found that the expressions of GRP78 and CHOP were significantly increased in H24R4-treated HK-2 cells. Propofol pretreatment could significantly inhibited GRP78 expression in HK-2 cells under H24R4 condition, but it has no obviously inhibitory effect on CHOP expression. However, Dex pretreatment could not only inhibit GRP78 expression, but also effectively reduce CHOP expression in H24R4-treated cells. CHOP, as a pro-apoptotic transcription factor, plays a crucial role in ERS response, it is the most distinctive regulator in the transition from ERS to apoptosis[18]. So based on the evidence above, we believed that Dex had a stronger inhibitory effect on ERS-related apoptosis in H24R4-treated cells compared with propofol.

In conclusion, pretreatment with Dex or propofol could lead to lower levels of oxidative stress in H24R4-treated cells. Although both Dex and propofol could inhibit ERS activation, Dex had a stronger inhibitory effect on the activation of ERS-related apoptosis way compared with propofol.

ACKNOWLEDGMENTS

This study is supported by Medical Research Foundation of Guangdong Province (Guangzhou, Guangdong Province, China; Grant no. B2019035).

REFERENCES

- [1]Chen W, Xi X, Zhang S, Zou C, Kuang R, Ye Z, Huang Y, Hu H. Pioglitazone Protects Against Renal Ischemia-Reperfusion Injury via the AMP-Activated Protein Kinase-Regulated Autophagy Pathway. *Frontiers in pharmacology* 2018, **9**: 851.
- [2]Mao H, Huang Q, Liu Y. MEG3 aggravates hypoxia/reoxygenation induced apoptosis of renal tubular epithelial cells via the miR-129-5p/HMGB1 axis. *J Biochem Mol Toxicol* 2020: e22649.
- [3]Choi YJ, Zhou D, Barbosa ACS, Niu Y, Guan X, Xu M, Ren S, Nolin TD, Liu Y, Xie W. Activation of Constitutive Androstane Receptor Ameliorates Renal Ischemia-Reperfusion-Induced Kidney

- and Liver Injury. *Molecular pharmacology* 2018, **93**(3): 239-250.
- [4] Shi Y, Melnikov VY, Schrier RW, Edelstein CL. Downregulation of the calpain inhibitor protein calpastatin by caspases during renal ischemia-reperfusion. *American journal of physiology Renal physiology* 2000, **279**(3): F509-517.
- [5] Li X, Zhu G, Gou X, He W, Yin H, Yang X, Li J. Negative feedback loop of autophagy and endoplasmic reticulum stress in rapamycin protection against renal ischemia-reperfusion injury during initial reperfusion phase. *FASEB J* 2018: fj201800299R.
- [6] Gu Y, Huang F, Wang Y, Chen C, Wu S, Zhou S, Hei Z, Yuan D. Connexin32 plays a crucial role in ROS-mediated endoplasmic reticulum stress apoptosis signaling pathway in ischemia reperfusion-induced acute kidney injury. *Journal of translational medicine* 2018, **16**(1): 117.
- [7] Weerink MAS, Struys M, Hannivoort LN, Barends CRM, Absalom AR, Colin P. Clinical Pharmacokinetics and Pharmacodynamics of Dexmedetomidine. *Clinical pharmacokinetics* 2017, **56**(8): 893-913.
- [8] Yao H, Chi X, Jin Y, Wang Y, Huang P, Wu S, Xia Z, Cai J. Dexmedetomidine Inhibits TLR4/NF- κ B Activation and Reduces Acute Kidney Injury after Orthotopic Autologous Liver Transplantation in Rats. *Scientific reports* 2015, **5**: 16849.
- [9] Zhu Z, Ling X, Zhou H, Zhang C, Yan W. Dexmedetomidine Attenuates Cellular Injury and Apoptosis in H9c2 Cardiomyocytes by Regulating p-38MAPK and Endoplasmic Reticulum Stress. *Drug design, development and therapy* 2020, **14**: 4231-4243.
- [9] Su M, Ren S, Zhong W, Han X. Impact of propofol on renal ischemia/reperfusion endoplasmic reticulum stress. *Acta chirurgica brasileira* 2017, **32**(7): 533-539.
- [10] Schumer M, Colombel MC, Sawczuk IS, Gobé G, Connor J, O'Toole KM, Olsson CA, Wise GJ, Buttyan R. Morphologic, biochemical, and molecular evidence of apoptosis during the reperfusion phase after brief periods of renal ischemia. *The American journal of pathology* 1992, **140**(4): 831-838.
- [11] Huang C, Ng OT, Chu JM, Irwin MG, Hu X, Zhu S, Chang RC, Wong GT. Differential effects of propofol and dexmedetomidine on neuroinflammation induced by systemic endotoxin lipopolysaccharides in adult mice. *Neuroscience letters* 2019, **707**: 134309.
- [12] Wang Z, Wu J, Hu Z, Luo C, Wang P, Zhang Y, Li H. Dexmedetomidine Alleviates Lipopolysaccharide-Induced Acute Kidney Injury by Inhibiting p75NTR-Mediated Oxidative Stress and Apoptosis. *Oxidative medicine and cellular longevity* 2020, **2020**: 5454210.
- [13] Cho JS, Shim JK, Soh S, Kim MK, Kwak YL. Perioperative dexmedetomidine reduces the incidence and severity of acute kidney injury following valvular heart surgery. *Kidney international* 2016, **89**(3): 693-700.
- [14] Song Y, Kim DH, Kwon TD, Han DW, Baik SH, Jung HH, Kim JY. Effect of intraoperative dexmedetomidine on renal function after cytoreductive surgery and hyperthermic intraperitoneal chemotherapy: a randomized, placebo-controlled trial. *International journal of hyperthermia : the official journal of European Society for Hyperthermic Oncology, North American Hyperthermia Group* 2019, **36**(1): 1-8.
- [15] Chen Y, Luan L, Wang C, Song M, Zhao Y, Yao Y, Yang H, Ma B, Fan H. Dexmedetomidine protects against lipopolysaccharide-induced early acute kidney injury by inhibiting the iNOS/NO signaling pathway in rats. *Nitric oxide : biology and chemistry* 2019, **85**: 1-9.
- [16] He J, Zhou D, Yan B. Eriocitrin alleviates oxidative stress and inflammatory response in cerebral ischemia reperfusion rats by regulating phosphorylation levels of Nrf2/NQO-1/HO-1/NF- κ B p65 proteins. *Annals of translational medicine* 2020, **8**(12): 757.
- [17] Li J, Zhuo JY, Zhou W, Hong JW, Chen RG, Xie HY, Zhou L, Zheng SS, Jiang DH. Endoplasmic reticulum stress triggers delanzomib-induced apoptosis in HCC cells through the PERK/eIF2 α /ATF4/CHOP pathway. *American journal of translational research* 2020, **12**(6): 2875-2889.