ORIGINAL RESEARCH

THE EFFECTS OF DEXMEDETOMIDINE INFUSION ON THE FORMATION OF REACTIVE OXYGEN SPECIES DURING MESENTERIC ISCHEMIA-REPERFUSION INJURY IN RATS

Feyza İnci[1], İ. Varlık Doğan[1], Zeynep Eti[1], Mustafa Deniz[2], F. Yılmaz Göğüş[1] , Feyza Yağmur[3]

[1]Department of Anesthesiology, School of Medicine,Marmara University, Istanbul, Turkey [2]Department of Physiology, School of Medicine, Marmara University, İstanbul, Turkey [3]Department of Biochemistry, School of Medicine,Marmara University, Istanbul, Turkey

ABSTRACT

Objective: The aim of this study was to evaluate the effect of dexmedetomidine infusion on the formation of reactive oxygen species during mesenteric ischemia-reperfusion injury in rats.

Methods: Twenty-eight male Sprague-Dawley rats weighing between 300-320 g were divided into four groups (n=7). The first two groups underwent sham operation; group 1 was the control group and group 2 received 5 µg/kg/h dexmedetomidine iv infusion. Ischemia-reperfusion (I/R) was produced in the remaining two groups (group 3 and 4) by ligation of the superior mesenteric artery for 30 min followed by 60 min of reperfusion period. Group 3 (I/R-S) rats were control I/R rats receiving 0.9% NaCl infusion and group 4 (I/R-D) rats received 5 µg/kg/h dexmedetomidine iv infusion during I/R. Tissue samples were obtained from the ileum for the measurement of luminol and lucigenin enhanced chemiluminescence (CL), tissue myeloperoxidase activity, malondialdehyde (MDA) and glutathione levels.

Results: Luminol CL levels, tissue myeloperoxidase activity ($p<0.05$). and MDA levels ($p<0.05$) were significantly higher in I/R-S group compared with sham-operated groups and I/R-D group. Glutathione levels of I/R-S group was significantly higher than those of I/R-D group ($p<0.05$).

Conclusion: We concluded that dexmedetomidine infusion can prevent the increase in reactive oxygen species during mesenteric ischemia-reperfusion injury in rats.

Keywords: α_2-adrenergic agonists, Dexmedetomidine; Ischemia-reperfusion injury; Reactive oxygen species

DEKSMEDETOMİDİN İNFÜZYONUNUN SIÇANLARDA MEZENTER İSKEMİ-REPERFÜZYON HASARINDA OLUŞAN REAKTİF OKSİJEN TÜRLERİ ÜZERİNE ETKİSİ

ÖZET

Amaç: Bu çalışmanın amacı; sıçanlarda oluşturulan mezenter iskemi-reperfüzyon modelinde intravenöz uygulanan deksmedetomidinin reaktif oksijen ürün düzeyi üzerine etkilerinin araştırılmasıdır.

Yöntem: 300-320 g ağırlığında, 28 adet, erkek, Spraque-Dawley cinsi sıçan dört gruba ayrıldı (n:7). Sham operasyonu geçiren iki gruptan; grup 1'deki sıçanlara (Kontrol grup, Grup K) ilaç uygulanmazken grup 2'deki sıçanlara 5 mg/kg/s deksmedetomidin infüzyonu yapıldı. Diğer iki gruba (grup 3 ve 4) süperior mezenter arter ligasyonu ile 30 dk iskemi ve ardından 60 dk reperfüzyon uygulandı (I/R). İskemi-reperfüzyon sırasında Grup 3'deki (I/R-S) sıçanlara 0.9% NaCl ve grup 4'deki (I/R-D) sıçanlara 5 mg/kg/h deksmedetomidin infüzyonu yapıldı. İleumdan, luminal ve lusigenin kemiluminesans (KL), doku miyeloperoksidaz aktivitesi, malondialdehid (MDA) ve glutatyon seviyesi ölçümleri için doku örnekleri alındı.

Bulgular: Luminol KL seviyesi, doku miyeloperoksidaz aktivitesi ($p<0.05$) ve MDA seviyesi ($p<0.05$) I/R-S grubunda sham grupları ve I/R-D grubuna gore belirgin yüksek bulundu. Glutatyon seviyesi, I/R-S grubunda I/R-D grubundan belirgin yüksek bulundu ($p<0.05$).

Sonuç: Sıçanlarda intravenöz deksmedetomidin infüzyonunun mezenter iskemi-reperfüzyon modelinde oluşan reaktif oksijen türlerindeki artışı engellediği kanısına varılmıştır.

Anahtar Kelimeler: Deksmedetomidin; Iskemi-reperfüzyon hasarı; Serbest oksijen radikalleri

İletişim Bilgileri:
Feyza İnci, M.D.
Marmara University Hospital Anesthesiology Department Altunizade, İstanbul, Turkey
e-mail: feyzainci@hotmail.com

Marmara Medical Journal 2007;20(3);154-160

INTRODUCTION

Ischemia is the condition suffered by tissues when deprived of blood flow resulting in inadequate nutrient and oxygen supply. Depending on the time and intensity of the ischemia, when oxygen is reintroduced to the tissues, tissue injury can be further exacerbated due to reperfusion injury. Reperfusion injury refers to the tissue damage inflicted when blood flow is restored after an ischemic period of more than about ten minutes. Restarting blood flow after ischemia is typically more damaging than the ischemia itself because the ischemia sets the stage for oxygen to generate free radicals rather than to contribute to cellular energy production[1].

Mesenteric ischemia-reperfusion (I/R) injury of the intestine is a common and often devastating clinical occurrence and performs a fundamental role in the pathophysiology of several clinical-surgical conditions[2]. Intestinal ischemia is generally the result of arterial occlusion by thrombi or embolisms and, more frequently, by non-occlusive processes resulting in low mesenteric flow such as hypovolemia, cardiac insufficiency, sepsis, the administration of α-adrenergic agents or digitalis[3]. One of the major factors that induces intestinal injury after reperfusion is the generation of free radicals from oxygen molecules, derived from the electron transport chains of the mitochondria, xanthine-oxidase (XO) metabolism, endothelial cells, prostaglandins and activated neutrophils[4].

Most of these patients with I/R injury are admitted to the intensive care unit (ICU) during the postoperative period and require sedation and analgesia to reduce anxiety, tolerate mechanical ventilation and provide pain relief. In the presence of the I/R injury, the effects of sedative agents on the release of reactive oxygen species becomes an important issue affecting the rate of morbidity and mortality.

The highly selective and potent α2-adrenergic agonist dexmedetomidine is an effective sedative, anxiolytic and analgesic agent used in postoperative patients requiring mechanical ventilation in the ICU and the aim of this experimental study was to evaluate the effects of dexmedetomidine infusion on the formation of reactive oxygen species during a mesenteric ischemia-reperfusion model in rats.

METHODS

The study protocol was approved by the Marmara University School of Medicine Animal Care and Use Committe. The animals were housed individually in a temperature controlled environment (20±1ºC), maintained on a 12h light/ dark cycle, and provided with food and water ad libitum.

Twenty-eight adult, male Sprague-Dawley rats weighing 300-320 g were divided into four groups (n=7). Anesthesia was induced with 100 mg/kg ketamine administered intraperitoneally. An intratracheal cannula was inserted from a midline incision for a tracheostomy to facilitate breathing and a polyethylene catheter was inserted into the right vena jugularis for intravenous infusions. After midline laparotomy, the intestines were carefully removed and the superior mesenteric artery (SMA) isolation was performed in all rats. Ischemia was produced by occlusion of the SMA at its origin from the aorta with atraumatic microvascular clamp. The absence of arterial pulsation distal to the clamp and pallor of the small intestine confirmed the adequate occlusion. After 30 minutes of mesenteric ischemia, the clamp was removed allowing reperfusion of the mesenteric vasculature for 60 min. Adequate reperfusion was confirmed by return of arterial pulsation and recoloration of the small intestine. The first two groups underwent sham operation without clamping SMA and the rats were followed up for 90 min to simulate the I/R interval in the other groups. During the 90 min. Group 1 received no medication (sham operated-control) and Group 2 received 5 µg/kg/h dexmedetomidine infusion (sham operated-dexmedetomidine). Group 3 and 4 underwent intestinal ischemia-reperfusion injury. Group 3 rats were control I/R rats receiving 5 ml/kg/h 0.9% NaCl infusion while Group 4 rats received 5 µg/kg/h

dexmedetomidine infusion during 90 minutes of I/R period. All rats were sacrificed and the tissue samples were obtained from the ileum for measurement of luminol and lucigenin enhanced chemiluminescence (CL), tissue myeloperoxidase activity, lipid peroxidase and glutathione levels.

Chemiluminescence: Ileum specimens were put into vials containing 3 ml PBS-HEPES buffer at room temperature. Reactive oxygen species were quantitated after the addition of enhancers (0.2 mM lucigenin and 0.2 mM luminol). CL was done using a scintillation counter. Counts were obtained at 5 s intervals and the results are given as a counting period of 5 min. Counts were corrected for wet tissue weight and expressed as relative light units per milligram tissue (rlu/mg).

Lipid peroxidation: The degree of lipid peroxidation was determined by monitoring thiobarbituric acid (TBA) reactive substance formation. Tissue samples were homogenized in trichloroacetic acid (TCA) (1g tissue+9 ml 10% TCA). After centrifugation, at 3000 rpm for 15 min, the supernatant was added to an equal volume of 0.67% TBA and rested in a 100ºC water bath for 15 min. The absorbance of the samples were measured at 535 nm after cooling to room temperature. Lipid peroxidation end products levels were determined in terms of malondialdehyde (MDA) from 1.56x105 M/cm extinction coefficient and expressed as nmol MDA/gram tissue.

Tissue myeloperoxidase (MPO) activity: Tissue MPO activity was measured on samples, weighing 0.2-0.3 g. Tissue samples were homogenized in 10 volume of ice-cold potassium phosphate buffer (20 mM K2HPO4; pH:7.4). The homogenate was centrifugated at 12000rpm for 10 min at 4ºC. The supernatant was discarded and the pellet was then rehomogenized with an equivalent volume of 50 mM acetic acid containig 5% hexadecyltrimethylammonium bromide (HETAB). Myeloperoxidase activity was assessed by measuring the H2O2 dependent oxidation of 0-dianizidine 2HCl. One unit of enzyme activity was defined as the amount of the MPO present that caused a change in absorbance of 1.0/min at 460 nm and 37ºC.

Glutathione level : 0.5 ml of the supernatant which was obtained after a centrifugation at 2500 rpm for 10 min, was added to 2 ml 0.3M Na2HPO4. After adequate mixing, 0.5 ml DTNB (5,5-dityobis-2nitrobenzoic acid) was added and the absorbance was measured at 412 nm.

Statistical analysis: The values were represented as mean±SD. Statistical differences were analysed by using one-way analyses of variance (ANOVA) and Tukey test. P values <0.05 were considered significant.

RESULTS

Luminol CL levels were significantly higher in the I/R-S group compared with the sham-operated groups and the I/R-D group ($p<0.001$). There was no difference between the sham-operated control group and the I/R-D group (Table I).

Lucigenin CL levels were not different between groups ($p>0.05$) (Table II).

Tissue myleoperoxidase activity was significantly higher in the I/R-S group compared with the sham operated groups and the I/R-D group ($p<0.001$). There was no difference between the sham operated groups and the I/R-D group (Table III).

The glutathione levels of the I/R-D group was significantly lower than those of the I/R-S group ($p<0.005$) (Table IV).

The MDA levels of the I/R-S group was significantly higher than the sham-operated groups and the I/R-D group ($p<0.005$) (Table V).

Table I: Results of all groups

	Sham-operated	Sham-operated-D	I/R-S	I/R-D
Luminol CL levels	11.41±1.44	12.95±1.60	24.02±1.89**	11.93±1.91
Lucigenin CL levels	22.04±3.92	20.62±2.99	25.36±3.94	20.29±4.97
Myeloperoxidase activity	42.65±4.91	49.03±4.02	89.81±5.35**	53.50±4.41
Glutathione levels	2.08±0.39	2.16±0.37	2.59±0.49*	1.93±0.24
Lipid peroxidation	12.45±8.19	13.37±8.65	21.16±8.10*	16.73±7.15

Values are mean±SD
I/R-S: Ischemia-reperfusion-saline, I/R-D: Ischemia-reperfusion-dexmedetomidine
*p<0.05 compared with other groups
**p<0.001 compared with other groups
I: Results of all groups

Table II: Lucigenin chemiluminescence levels (AUC rlu/mg tissue)

Sham-operated	Sham-operated-D	I/R-S	I/R-D
22.04±3.92	20.62±2.99	25.36±3.94	20.29±4.97

Values are mean±SD, p>0.05
I/R-S: Ischemia-reperfusion-saline
I/R-D: Ischemia-reperfusion-dexmedetomidine
II: Lucigenin chemiluminescence levels (AUC rlu/mg tissue)

Table III: Tissue myeloperoxidase activity (ml/min)

Sham-operated	Sham-operated-D	I/R-S	I/R-D
42.65±4.91	49.03±4.02	89.81±5.35*	53.50±4.41

Values are mean±SD
I/R-S: Ischemia-reperfusion-saline, I/R-D: Ischemia-reperfusion-dexmedetomidine
*p<0.001 compared with other groups
III: Tissue myeloperoxidase activity (ml/min)

Table IV: Glutathione levels (µmol/L)

Sham-operated	Sham-operated-D	I/R-S	I/R-D
2.08±0.39	2.16±0.37	2.59±0.49*	1.93±0.24

Values are mean±SD
I/R-S: Ischemia-reperfusion-saline, I/R-D: Ischemia-reperfusion-dexmedetomidine
*p<0.05 compared with I/R-D group
IV: Glutathione levels (µmol/L)

Table V: Lipid peroxidation (nmolMDA/ g tissue)

Sham-operated	Sham-operated-D	I/R-S	I/R-D
12.45±8.19	13.37±8.65	21.16±8.10*	16.73±7.15

Values are mean±SD
I/R-S: Ischemia-reperfusion-saline, I/R-D: Ischemia-reperfusion-dexmedetomidine
*p<0.05 compared with other groups
V: Lipid peroxidation (nmolMDA/ g tissue)

DISCUSSION

The present data provide evidence that the selective α2-adrenergic agonist, dexmedetomidine may attenuate the excessive release of the reactive oxygen species during mesenteric ischemia-reperfusion injury in rats.

Ischemia-reperfusion injury is a common pathology in surgical patients during general anesthesia as well as postoperative period in ICU. Massive and abrupt release of reactive oxygen species after reperfusion followed by endothelial dysfunction or neutrophil infiltration triggers the oxidative damage[5]. Dysfunction induced by free radicals may thus be a major component of ischemic diseases of the heart, bowel, liver, kidney and brain. In the study of Bhaskar et al[6], tissue activity of myeloperoxidase was increased, α-tocopherol level was decreased and histology indicated morphological changes in the colon during colonic I/R injury in rats. The authors suggested that colonic mucosal damage occurs during I/R and free radicals generated by the infiltrated neutrophils may play a role in this damaging process. Therefore, administration of anesthetic and sedative agents with anti-oxidant capacity during the peroperative period and ICU stay in patients with mesenteric I/R injury might attenuate the harmful consequences of possible ischemic injury.

In recent years; there has been a growing interest in the use of α2-adrenergic agonist agents as useful adjuncts during general anesthesia or ICU sedation. Clonidine, the prototypical α2 agonist and dexmedetomidine, the recently described one, have potent analgesic, sedative and sympatholytic effects. The experimental studies evaluating the effects of these agents on I/R injury, mostly used models of myocardial, brain or retinal ischemia. Reichhalter et al[7] evaluated the area at risk and area of infarction by photometric quantification in a rat myocardial I/R model in which the left anterior descending coronary artery ligation lasted for 30 min and was followed by a 150-min reperfusion. They found that the rats who received iv n-alil clonidine (alinidine) before and after ligation had a significantly smaller area of infarction in relation to the area at risk than the control group and suggested that the cardioprotective effects of alinidine may be explained by a reduction in heart rate and blood pressure. The results of an another rat coronary I/R model suggested that α2-adrenergic receptor stimulation had a potent antiarrhythmic effect on ischemia-reperfusion-induced ventricular arrhythmias and these effects were reversed by the α2-adrenergic antagonist yohimbine[8]. Yuan et al[9] evaluated the effects of adrenoceptor modulation on hypoxic-ischemic brain damage produced by unilateral carotid artery ligation combined with hypoxia (6% or 8% O2 in N2) in immature rats. They found that post-treatment with clonidine reduced brain injury by 45% compared with saline controls and mortality increased after α2-adrenergic antagonist (prazosin, yohimbine) treatment. These results suggested that the activation of central α2-adrenergic receptors provided neuroprotection during reperfusion after hypoxic-ischemic brain injury.

Dexmedetomidine has a relatively high ratio of α2/α1 activity (1620:1) and therefore is considered as a full agonist of the α2 receptor without unwanted cardiovascular and respiratory effects at therapeutic doses[10]. The experimental studies evaluating the effects of this agent on I/R injury are limited to models of global or focal cerebral hypoxia-ischemia. Kuhmonen et al[11] examined the effects of dexmedetomidine on the cerebral infarct volume produced by transient (90 min occlusion and reperfusion) and permanent middle cerebral artery occlusion. They found that the rats receiving 15 μg/kg dexmedetomidine had smaller (20-30%) infarct volume after transient occlusion. In Jolkkonen et al's study[12] assessing the effect of dexmedetomidine and glutamate receptor antagonists on infarct volume in rat focal cerebral ischemia, dexmedetomidine decreased total ischemic volume by 40% compared to NaCl-treated rats and the efficacy was better compared to that of the competitive NMDA receptor antagonist.

It is well known that a variety of mechanisms are important for the development of I/R injury such as the release of excitatory aminoacids (adrenalin, noradrenalin and glutamate), intracellular calcium elevation and reactive oxygen species formation. Although the results of experimental studies suggested that α2-adrenergic agonist agents have a protective effect on ischemia-reperfusion injury especially in cerebral ischemia models, the exact mechanisms by which they reduce ischemic damage are contraversial. At the cellular level, the effects of α2-adrenoceptor activation are inhibition of voltage-operated calcium channels, neuronal membrane hyperpolarization, inhibition of adenylate and guanylate cyclase and at the supracellular level they reduce excitatory neurotransmitter release[13-15]. The most popular theory for the mechanism of neuroprotective effect is the reduction of excitatory neurotransmitter release and alleviation of the potential detrimental effects of metabolizing excessive noradrenaline which can lead to the formation of free radicals. Talke et al[16] evaluated the effects of dexmedetomidine on hypoxia-evoked glutamate release and glutamate receptor activity in the rat hippocampus and demonstrated that dexmedetomidine decreased glutamate release from hippocampal rat brain slices during hypoxic stress, but did not alter calcium changes mediated by the stimulation of glutamate receptors during hypoxic conditions. Similarly, it was demonstrated that dexmedetomidine can selectively attenuate ischemia-induced increases in striatal norepinephrine concentrations[17]. However, recently Engelhard et al[18] investigated whether neuroprotection seen with dexmedetomidine is associated with suppression of peripheral or central sympathetic tone during ischemia and demonstrated that the increase of circulating catecholamine concentrations during cerebral ischemia was suppressed with dexmedetomidine by 95% compared with control animals, but it did not suppress elevation in brain norepinephrine and glutamate concentration suggesting that the neuroprotective effects of dexmedetomidine are not related to the inhibition of presynaptic norepinephrine or glutamate release in the brain. As it was shown that inhibition of oxidative deanimation of catecolamines decreases H_2O_2 production during reperfusion, we tested the hypotheses that α2-adrenergic agonist, dexmedetomidine attenuates the increase in the formation of free radicals during mesenteric I/R injury and we demonstrated that luminol CL level, especially sensitive to hydrogen peroxide and hypochloride, tissue MPO activity, TBARS and glutathione levels in the I/R-D group were significantly lower than those of the I/R-S group. The present study, to our knowledge is the first one that evaluates the effects of dexmedetomidine on the formation of reactive oxygen species during mesenteric I/R injury.

According to our data we concluded that, α2-adrenergic agonist dexmedetomidine can attenuate the increase in the formation of reactive oxygen species due to the mesenteric ischemia-reperfusion injury in rats. Further studies are needed to evaluate the exact mechanism of the anti-oxidant effect of dexmedetomidine.

REFERENCES

1. McCord JM. Oxygen-derived free radicals in postischemic tissue injury. N Engl J Med 1985; 312: 159-163.
2. Stefanutti G, Vejchapipat P, Williams SR, Pierro A, Eaton S. Heart energy metabolism after intestinal ischaemia and reperfusion. J Pediatr Surg 2004; 39: 179-183.
3. Stoney RJ, Cunninghan CG. Acute mesenteric ischemia. Surgery 1993; 114: 489-490.
4. Grace PA. Ischaemia-reperfusion injury. Br J Surg 1994; 81: 637-647.
5. Kharbanda RK, Peters M, Walton B, et al. Ischemic preconditioning prevents endothelial injury and systemic activation during ischemia-reperfusion in humans in vivo. Circulation 2001; 103: 1624-1630.
6. Bhaskar L, Mathan MM, Balasubramanian KA. Oxygen free radical-induced damage during colonic ischemia/reperfusion in rats. Mol Cell Biochem 1995; 151 : 9-14.
7. Reichhalter R, Lillie C, Kobinger W. Effects of alinidine on survival and infarct size in rats with coronary artery occlusion. Eur J Pharmacol 1988; 157: 75-81.
8. Cai JJ, Morgan DA, Haynes WG, Martins JB, Lee HC. á2-adrenergic stimulation is protective against ischemia-reperfusion-induced ventricular arrhythmias

in vivo. Am J Physiol Heart Circ Physiol 2002; 283: 2606-2611.
9. Yuan SZ, Runold M, Hagberg H, Bona E, Lagercrantz H. Hypoxic-ischaemic brain damage in immature rats: effects of adrenergic modulation. Eur J Paed Neurol 2001; 5: 29-35.
10. Virtanen R, Savola JM, Saano V, Nyman L. Characterization of the selectivity, specifity and potency of medetomidine as an á2-adrenoceptor agonist. Eur J Pharmacol 1988; 150: 9-14.
11. Kuhmonen J, Haapalinna A, Sivenius J. Effects of dexmedetomidine after transient and permanent occlusion of the middle cerebral artery in the rat. J Neural Transm 2001; 108: 261-271.
12. Jolkkonen J, Puurunen K, Koistinaho JR, et al. Neuroprotection by the á2-adrenoceptor agonist, dexmedetomidine, in rat focal cerebral ischemia. Eur J Pharmacol 1999; 372: 31-36.
13. Nacif-Coelho C, Correa-Saler C, Chang LL, Maze M. The hypnotic response to the á2-adrenoceptor agonist, dexmedetomidine in the locus coeruleus of the rat. Anesthesiology 1994; 81: 1527-1534.
14. Surprenant A, Horstman DA, Akbarali H, Limbird LE. A point mutation of the á2-adrenoceptor that blocks coupling to potassium but not calcium currents. Science 1992; 257: 977-980.
15. Vulliemoz Y, Shen H, Virag I. á2 Adrenoceptor agonists decrease cyclic guanosine 3’5’ monophosphate in the mouse brain. Anesthesiology 1996; 85: 544-550.
16. Talke P, Bickler PE. Effects of dexmedetomidine on hypoxia-evoked glutamate release and glutamate receptor activity in hippocampal slices. Anesthesiology 1996; 85: 551-557.
17. Matsumato M, Zornow MH, Rabin BC, Maze M. The á2-adrenergic agonist, dexmedetomidine, selectively attenuates ischemia-induced increases in striatal norepinephrine concentrations. Brain Res 1993; 627: 325-329.
18. Engelhard K, Werner C, Kaspar S, et al. Effect of the a2-agonist dexmedetomidine on cerebral neurotransmitter concentrations during cerebral ischemia in rats. Anesthesiology 2002; 96: 450-457.