The Effect of Dexmedetomidine on Ischemia Reperfusion Injury in Myocard of Rat

Rat Myokardında Deksmedetomidinin İskemi Reperfüzyon Hasarı Üzerine Etkisi

Abdullah Özer¹, Dilek Erer², Gürsel Levent Oktar³, Gülay Kip³, Mustafa Bilge³, Mustafa Kavuşçu³, Ozlem Erdem⁴ Mustafa Arslan⁵, Yusuf Unal⁵

¹ Gazi University Medical Faculty, Department of Cardiovascular Surgery, Ankara, Turkey
² Gazi University Dentistry Faculty, Departments of Paediatric Dentistry, Ankara, Turkey.
³ Gazi University Medical Faculty, Department of Medical Biochemistry, Ankara, Turkey
⁴ Gazi University Medical Faculty, Department of Pathology, Ankara, Turkey
⁵ Gazi University Medical Faculty, Department of Anaesthesiology and Reanimation, Ankara, Turkey

ABSTRACT

Objective: The aim of this study was to evaluate the effect of dexmedetomidine (100µg/kg-ip) on ischemia reperfusion (IR) injury in myocard of rats.

Methods: Twenty-four Wistar Albino rats were separated into four groups. There were four experimental groups (Group C (Control; n=6), Group IR (ischemia-reperfusion, n=6), Group D (Dexmedetomidine; n=6), Group IR-D (IR-Dexmedetomidine; n=6), underwent coronary artery was occluded for 30 min followed by two hours of reperfusion. However, after the above procedure, the coronary artery was not occluded or reperfused in the control rats. At the end of the study, myocard tissue was obtained for biochemical, histochemical and immunohistochemical determination/analyses.

Results: Myonecrosis, cell infiltration and edema were significantly higher in the IR group than in the C and D groups. In the IR-D group, myonecrosis, cell infiltration and edema were significantly lower than in the IR group. TBARS levels were found to be significantly higher in the IR group than in the C and D groups. TBARS levels in the IR-D group were found to be significantly lower than in the IR group. SOD enzyme activity was found to be significantly lower in the IR group than in the C group. In the IR-D group, SOD enzyme activity was found to be significantly higher than in the IR group.

Conclusion: Dexmedetomidine removed degenerative effects after ischemia reperfusion in myocardia reperfusion group and we may conclude that dexmedetomidine may have regenerative effects on IR injury.

Key Words: Dexmedetomidine, myocardial ischemia reperfusion, SOD, Myonecrosis

Received: 10.17.2017
Accepted: 11.02.2017

ÖZET

Amaç: Bu çalışmanın amacı sıçanların miyokardlarında iskemi reperfüzyon (IR) hasarına deksmedetomidinin (100 µg / kg-ip) etkisini değerlendirmektir.

Yöntem: Yirmi dört adet Wistar Albino sıçan dört gruba ayrıldı. Dört deney grubu vardı (Grup C (Kontrol n = 6), Grup IR (iskemi-reperfüzyon, n = 6), Grup D (Dexmedetomidin, n = 6), sol koroner arter bağlanmadan 30 dakika önce 100µg / kg Dexmedetomidin ip yol ile uygulandı. Küçük bir plastik snare ligaturedan geçirilmiş kalp kıl parçası edecek şekilde yerleştirildi. IR elde etmek için sol koroner arterden 30 dakika boyunca tıkanıp ardından iki saatlik reperfüzyon uygulandı. Bununla birlikte, yukarıdaki prosedürden sonra, kontrol farelerinde koroner arter tıkanmadı veya reperfüzyon uygulanmadı. Çalışmanın sonunda miyokard dokusu, biyokimyasal, histokimyasal ve immunohistokimyasal belirleme / analizler için alındı.

Bulgular: Myokroks, hücre infiltrasyonu ve ödem, IR grubunda C ve D grubuna göre anlamlı derecede yüksekti. IR-D grubunda, miyonekroz, hücre infiltrasyonu ve ödem, IR grubuna göre anlamlı derecede düştü. TBARS düzeylerinin IR grubunda C ve D gruplarına göre anlamlı derecede yüksek olduğu bulundu. IR-D grubundaki TBARS düzeyleri IR grubunda olduğundan daha düşük bulundu. SOD enzim aktivitesinin IR grubunda C grubuna göre anlamlı olarak düşük olduğu bulundu. IR-D grubunda SOD enzim aktivitesinin IR grubundan anlamlı olarak daha yüksek olduğu bulundu.

Sonuç: Deksmedetomidin, iskemi reperfüzyon grubunda iskemi reperfüzyon sonrası dejeneratif etkileri ortadan kaldırdı ve deksmedetomidinin iskemi-reperfüzyon hasarı üzerine rejeneratif etkileri olabilir sonucuna varabiliriz.

Anahtar Sözcüklər: Deksmedetomidin, myokardiyal iskemi reperfüzyon, SOD, Myokroks

Geliş Tarihi: 17.10.2017
Kabul Tarihi: 02.11.2017
INTRODUCTION

Ischemia is defined as the significant reduction of blood flow and the insufficiency of oxygen and nutrients’ provision to the various tissues and organs. Reperfusion is essential for the restoration of the energy needs of the ischemic cells and the removal of toxic products. Nevertheless, it has been proved that reperfusion of ischemic tissues induces damages that frequently exceed the original ischemic insult. This is called ischemia reperfusion injury (I/R) injury. Oxidative damage due to I/R is thought to play an important role(2).

As morbidity and mortality due to ischemic heart disease continue to increase, they are receiving increasing attention. Despite early reperfusion and improvements in antplatelet and anti-thrombotic therapy, the mortality of acute myocardial infarction (AMI) patients remains significant even if undergoing primary percutaneous coronary intervention. One major contributing factor is the inability to protect the heart against the detrimental effects of lethal myocardial reperfusion injury, which occur on reoccurring blood flow to the acutely ischemic myocardium. Therefore, fully understanding the mechanisms of ischemia/reperfusion (I/R) injury and seeking for novel therapeutic strategies is still the focus of intense research(3).

The first study on reperfusion injury was made by Hearse at al in 1973(4). In this study it was demonstrated that in ischemic rat hearts, oxygen related enzyme release has an important role. Toxic injury during ischemic in myocardial or other cells, is essentially related to particulate iron. This is called oxygen paradox. Toxic metabolites are removed when blood flow occurs again in ischemic tissue. But, if toxic metabolites mixes systemic circulation there can be a damage in cell membrane and the other structures. Oxidative radicals cause reperfusion injury in ischemic tissue after reperfusion. It is taught that. Toxic radicals are produced by PNL (poliformo nuclear leukocyte) , during reperfusion(5).

Mortal ischemic reperfusion develops if there is no reperfusion but, toxic oxygen radicals doesn’t seen at that side. Inflammatory response occurs after reperfusion(6).

Symptoms and signs of acute respiratory insufficiency, including cough, expectoration and asthma, may occur during thrombolytic therapy of left ventricular myocardial infarction, and may cause respiratory failure. Therefore, protecting the lungs from injury throughout thrombolytic therapy is becoming a focus of particular interest in cardiovascular research(7).

Dexmedetomidine, a selective and potent α2-adrenoceptor agonist, was approved by the U.S. Food and Drug Administration in 1999 for sedation of patients hospitalized in intensive care settings. Since then, a growing number of research articles have emerged reporting other possible indications, such as regional and general anesthesia(8,9).

The first study on reperfusion injury was made by Hearse at al in 1973(4). The TBARS assay was carried out to determine lipid peroxidation using the thiobarbituric acid method as described by Van Ye et al (15). TBARS measurements were conducted based on the reaction of MDA with thiobarbituric acid (TBA), which form a pink pigment with an absorption maximum at 532 nm in acid pH, and 1,1,3,3-tetraethoxypropane was used as a standard MDA solution. All procedures were performed at 4°C throughout the experiment.

Enzyme activities and TBARS levels were determined by continuously monitoring and end point change in absorbance at 25°C with a Shimadzu UV-1601 spectrophotometer. Results were expressed IU/L for CAT, mIU/L for GST and μg/ml for SOD respectively.

Statistical Analysis

The Statistical Package for the Social Sciences (SPSS, Chicago, IL, USA) 20.0 program was used for the statistical analysis. Variations in oxidative state parameters, and histopathological examination between study groups were assessed using the Kruskal-Wallis test. The Bonferroni-adjusted Mann-Whitney U test was used after significant Kruskal-Wallis to determine which groups differed from the others. Results were expressed as means with standard deviation (Mean ± SD), median (25%-75%). Statistical significance was set at a p value of <0.05 for all analysis.

RESULTS

When the groups were compared in terms of myocardial myonecrosis, there was a significant difference between the groups (p<0.007). Myonecrosis was significantly higher in the IR group compared with the C and D groups (p<0.007, p<0.011, respectively). In addition, myonecrosis in the IR-D group was significantly lower compared with the IR group (p=0.018, Table1). There was a significant difference between the groups in terms of cardiac muscle cell infiltration (p<0.007). Cell infiltration was found to be significantly higher in the IR group compared with the C and D groups (p=0.006, p=0.009, respectively). In addition, cell infiltration in the IR-D group was significantly lower compared with the IR group (p=0.018, Table1). There was a significant difference between the groups in terms of cardiac muscle cell edema (p=0.002). Edema was measured significantly higher in the IR group compared with the C and D groups (p=0.002, p=0.002, respectively). In addition, edema in the IR-D group was significantly lower compared with the IR group (p=0.002, Table1).
Table 1. Histopathological data of the heart muscle tissue of rats [Median (25-75%)]

<table>
<thead>
<tr>
<th></th>
<th>Group C (n=6)</th>
<th>Group D (n=6)</th>
<th>Group IR (n=6)</th>
<th>Group IR-D (n=6)</th>
<th>P**</th>
</tr>
</thead>
<tbody>
<tr>
<td>Myonecrosis</td>
<td>0.00 (0-1)</td>
<td>0.50 (0-1)</td>
<td>2.00 (1-2)*,+</td>
<td>1.00 (0.75-1)    &amp;</td>
<td>0.007</td>
</tr>
<tr>
<td>Cell Infiltration</td>
<td>0.00 (0-0.25)</td>
<td>0.00 (0-0.25)</td>
<td>1.00 (1-2)*,+</td>
<td>0.50 (0-1)       &amp;</td>
<td>0.007</td>
</tr>
<tr>
<td>Edema</td>
<td>0.00 (0-1)</td>
<td>0.50 (0-1)</td>
<td>2.00 (2-2)*,+</td>
<td>0.50 (0-1)       &amp;</td>
<td>0.002</td>
</tr>
</tbody>
</table>

P**: Kruskal-Wallis test significance level p< 0.05 *p<0.05: Compared with group C; +p<0.05: Compared with group D; &p<0.05: Compared with group IR

Images of the histopathological changes of immunohistochemical preparations of myocardial tissues of rats obtained in light microscopy are shown in Figure 1 (a, b, c, d, e).

Figure 1. (a) Control group, Normal rat myocardial tissue(HE x 200); (b) Mild inflammation, Dexmedetomidine Group: Rat myocardial tissue(HE x100); (c) Mild myonecrosis, Dexmedetomidine Group: Rat myocardial tissue, (HEx200);

When the groups were compared in terms of serum TBARS levels, there were a significant difference between the groups (p=0.036). TBARS levels were significantly higher in the IR group than in the C and D groups (p = 0.021, p = 0.015, respectively). In addition, the TBARS levels in the IR-D group were significantly lower than the IR group (p = 0.029) (Table 2).

Table 2. Oxidant and antioxidant status parameters in serum samples of rats [Mean ± Standard Deviation]

<table>
<thead>
<tr>
<th></th>
<th>Group C (n=6)</th>
<th>Group D (n=6)</th>
<th>Group IR (n=6)</th>
<th>Group IR-D (n=6)</th>
<th>P**</th>
</tr>
</thead>
<tbody>
<tr>
<td>MDA (nmol/L)</td>
<td>1.94±0.30</td>
<td>1.91±0.31</td>
<td>3.40±1.09*,+</td>
<td>2.04±0.40&amp;</td>
<td>0.036</td>
</tr>
<tr>
<td>CAT (IU/L)</td>
<td>163.97±92.24</td>
<td>128.10±35.49</td>
<td>99.55±45.30</td>
<td>206.18±115.22</td>
<td>0.203</td>
</tr>
<tr>
<td>SOD (U/L)</td>
<td>29.71±1.87</td>
<td>26.00±11.86</td>
<td>18.72±6.43*</td>
<td>32.34±2.10&amp;</td>
<td>0.008</td>
</tr>
<tr>
<td>GST (mIU/L)</td>
<td>85.40±17.15</td>
<td>84.84±9.58</td>
<td>88.90±18.29</td>
<td>77.70±9.11</td>
<td>0.666</td>
</tr>
</tbody>
</table>

P**: Kruskal-Wallis test significance level p< 0.05 *p<0.05: Compared with group C; +p<0.05: Compared with group D; &p<0.05: Compared with group IR

DISCUSSION

Koçoğlu et al., (16) reported that dexmedetomidine administration decreases the infarct area but does not affect the arrhythmia incidence, on their study about myocardial IR. Kabukçu et al. (17) used dexmedetomidine as an adjunct to general anesthesia for 20 patients posted for coronary artery bypass grafting and concluded that it provided stable hemodynamics in the perioperative period Mangano et al.(18) reported that myocardial ischemia is one of the most important risk factors for adverse cardiac outcome in surgical patients with coronary artery disease. This adverse outcome was reported to be reduced by perioperative infusion of dexmedetomidine(18).
In the literature, studies on the affect of dexmedetomidine a kind of alpha2-receptor agonist on cardiac ischemia reperfusion damage are limited. With the experimental study it is purported to contribute to this subject. Our model was set on; occlusion of LAD that supplies the dominant perfusion of myocard and than opening the occlusion and provide reperfusion and before IR, administrating the drug and observing the histopathologic and biochemical changes on subjects. In myocardial IR studies it has been shown that one of the most important oxygen radicals are lipids. Some authors accepted the lipid peroxidation as a key in the IR damage. Different methods have been used so far to show lipid peroxidation in the tissue but the most popular one is MDA. The level of MDA altitude shows the lipid peroxidation directly. The important determinant in the hypothesis of decreasing the IR is MDA.

At the end of this study MDA was higher in the IR group and lower in the IR-D group, so it means that dexmedetomidine decreases the IR damage. Another determinant used in the hypothesis of decreasing IR damage is GSH-Px glutation, which is a natural cleaner against superoxide anions. It helps cells to maintain their structural integrity and decreases the levels of the hydrogen peroxide and prevents severe cell damage.

SOD, CAT, and GSH-Px are responsible in cellular antioxidant defense mechanisms. These enzymes eliminate superoxide anions and hydrogen peroxides, and prevent free radical production. SOD is the primary defensive enzyme against oxygen derived free radical production and catalyses from O2 to H2O2 conversion reaction. Oxygen radicals generated in response to IR have been implicated in the microvascular dysfunction and parenchymal cell injury of the intestine and liver. Increase of the activity in the glutation peroxidase leads to increase the cleaning activity of hydrogen peroxide so probability of the damage of the cell membrane bind to oxygen radicals may become less.

In our study, there were no differences between the groups in terms of serum GST and CAT enzyme activity. SOD is one of the determinants which reduces IR. Catalase is a common enzyme found in all aerobic cells and catalyses the decomposition of hydrogen peroxide to water and oxygen. SOD enzyme activity increases when oxidative stress increase in the cells. SOD activity shows presence of oxygen radicals and cleaning activity. In our study it is showed that SOD enzyme activity was significantly lower in IR group rather than C group. Additionally SOD enzyme activity was significantly higher in IR-D group rather than IR group. Thus, dexmedetomidine administration before ischemia was found to be protective.

In the basis of these findings it can be said that dexmedetomidine is protective against myonecrosis. Cell infiltration was significantly higher in IR group than C group and edema was significantly lower in IR-D group than IR group. These results show that dexmedetomidine administration before ischemia reduces the edema in rats.

As a result; according to these findings dexmedetomidine has a protective effect on IR damage. Other aspects of these findings, including clinical significance and practical applications, merit further experimental and clinical investigation.

Conflict of interest

No conflict of interest was declared by the authors.

REFERENCES

4. Hearse DJ, Humphrey SM, Chane EB. Abrupte oxygenation of the anoxic potassium arrested perfused rat heart: a study of myocardial enzyme release. Mol Cell Cardiol 1973; S:395-407.