The Effects of Sufentanil Postconditioning on Ischemic-reperfusion Injury in Rats

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Abstract. This study aims to explore the protective effects of sufentanil-postconditioning on myocardial cells in rats. Methods: Choosing 80 healthy rats, all was established in Myocardial Ischemia-reperfusion Model. Dividing them into 8group(n=10):1)SHAM group;2)CONgroup;3)IPC group; 4–8) SUF group. Observe and count:① The Amount of Apoptosis Positive Cell Nucleus, Total Cell Nucleus, and rate of myocardial cell apoptosis. ②Concentration of cTnI. ③Weight of Left Ventricle, Weight of Ischemic Myocardium, Weight of infarction. The areas of myocardial ischemia and myocardial infarction are also calculated. ④HE staining. Results:① The apoptosis index is significantly lower in SHAM Group than others groups (P<0.01), CON group shows obviously increased. The apoptosis index in IPC Group and SUF Group are between SHAM Group and CON Group. The differences are statistically significant (P<0.01).②The concentration of cTnI is lower in SHAM Group than others groups (P<0.01). Compared with CON Group, the cTnI concentrations are decreased in IPC Group and SUF Group (P<0.001). ③For comparison of myocardial ischemia and myocardial infarction areas, the myocardial ischemia area (AAR/LV%) and myocardial infarction area (IS/AAR%) are significantly reduced in IPC Group and SUF Group, the difference is statically significant (P<0.05); while the comparison between the IPC and the SUF group show no statistically significant difference (P>0.05). ④For HE staining, the myocardium tissue of rats in SHAM Group shows no obvious pathological change. But the change is visible in CON group. IPC Group and SUF group with mild pathological change. Conclusion: Post-treatment with sufentanil alleviates myocardial ischemia-reperfusion injury in rats.

Introduction
Myocardial ischemia-reperfusion injury refers to recovery of blood perfusion on the basis of long-term myocardial ischemia. Namely, after reperfusion, ischemia and tissue damage are, instead on being improved, further aggravated and show irreversible injury[1]. This study aims to observe the effects of sufentanil ischemia postconditioning on myocardial apoptosis, in order to provide theoretical basis for clinical practice.

Materials and Methods
Groups and Processing
80 healthy male SD rats was established in myocardial ischemia-reperfusion model, weight 250-300g. These rats laying aside 30 mins after stitching waiting for ligation. Then, dividing them into 8group (n=10), all the rats were subjected to 30 min ischemic and 120 min reperfusion: (1)sham group(SHAM), SHAM group only stitching but not for ligation; (2)the control group(CON), CON group injects 1ml normal saline in intravenous on the time of 5 min before infusion; (3)ischemic-postcondition (IPC) group, IPC group undergo 3 circles of 10 mins and 10 mins reperfusion at the time of reperfusion; (4–8) The Sufentanil postconditioning Group (SUF), SUF group respectively inject sufentanil0.1,0.3,1,3,10μg/kg on the time of 5 min before infusion, diluting drugs to 1ml salt water.
Observational Index

Test of Myocardial Cell Apoptosis

Refer to the instruction of in-site Terminal deoxynucleotidyl Transferase-Mediated dUT P-biotin Nick End Labeling (TUNEL) of myocardial apoptosis for specific steps. Based on observation under light microscope, brown stained cell nucleus is TUNEL positive. Then count the amounts of apoptosis positive cell nucleus and total cell nucleus. The apoptosis index of myocardial cells was calculated according to the formula \[
\text{Apoptosis Index (AI)} = \frac{\text{Amount of Apoptosis Myocardial Cell Nucleus}}{\text{Total Amount of Myocardial Cell Nucleus}} \times 100\%.
\] The average value of AI is taken as the rate of myocardial cell apoptosis.

Concentration of Serum Cardiac Troponin I (cTnI)

Follow the steps indicated in the kit instructions. A total of 2ml blood is sampled and use a centrifuge to centrifugalize serum at 3500r/min under room temperature; after processing for 5min, sandwich-antibody enzyme linked immunosorbent assay and full-automatic immunoassay analyzer are used for analysis.

Areas of Myocardial Ischemia and Infraction

Even’s blue-TTC method is used to determine the scope of myocardial infarction. Picture processing software is used to determine the scopes of AAR and IS; weight of every myocardium slice is used for correction; the total weight of LV is calculated; and the results are expression in percentage. The area of myocardial ischemia refers to the ratio of weight of ischemic myocardium / weight of LV (AAR/LV), and the area of myocardial infarction refers to the ratio of weight of infract myocardium / weight of ischemic myocardium (IS/AAR).

Myocardium HE Staining

Based on routine HE staining, pathological changes of myocardium tissues are observed under light microscope. Myocardial cell breakage is compared among the groups.

Statistical Analysis

The research data was treated with SPSS 13.0. The measurement data was expressed by mean ± SD. The one-way ANOVA analysis or student's t test was employed for the comparison between groups. P < 0.05 indicates the statistical difference, P<0.01 indicates extremely significant difference.

Experiment Result

In the sufentanil post-treatment group, serum cardiac troponin I, and areas of myocardial ischemia and infraction show no statistically significance after iv administration of 0.1, 0.3, 1, 3 ,10 μg/kg sufentanil (P>0.05). Therefore, the median (1μg/kg) is taken as the experiment group for the comparison.

Change of Myocardial Cell Apoptosis

Under light microscope, TUNEL positive cells are all visible in slices of all groups. SHAM Group shows a low-amount distribution, The apoptosis index is significantly lower in the SHAM Group than others three groups (P<0.01), CON group shows obviously increased amount of apoptosis myocardial cell and group-based distribution, and is significantly higher than SHAM Group. The amounts of apoptosis myocardial cell in the IPC Group and the SUF Group are obviously lower than CON Group and between SHAM Group and CON Group. The differences are statistically significant (P<0.01). specific date was shown in table 1.
Table 1. The change of each rat myocardial apoptosis(x±s,n=10).

<table>
<thead>
<tr>
<th>Groups</th>
<th>Infarct area (AI%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SHAM</td>
<td>2.33±1.29</td>
</tr>
<tr>
<td>CON</td>
<td>37.04±9.5**</td>
</tr>
<tr>
<td>IPC</td>
<td>19.88±5.03**ΔΔ</td>
</tr>
<tr>
<td>SUF</td>
<td>24.54±8.87**ΔΔ</td>
</tr>
</tbody>
</table>

Note: compare with SHAM, *p<0.05,**p<0.01,compare with CON, Δp<0.05, ΔΔp<0.01.

Change of the Concentration of Serum Cardiac Troponin I (cTnI)

Using Variance analysis of random design to analyze the concentration of cTnI. The result display, comparing with SHAM group, the concentration of cTnI in the rest of three groups increasing markedly(p<0.001); comparing with CON group, the concentration of cTnI in the SUF group and IPC group decreasing greatly; comparison among groups, the difference was not statistically significant(LSD-t test, p=0.363). the details in table 2.

Table 2. change of the Concentration of Serum Cardiac Troponin I (cTnI) in all groups (x±s , n=10).

<table>
<thead>
<tr>
<th>Groups</th>
<th>The concentration of cTnI</th>
</tr>
</thead>
<tbody>
<tr>
<td>SHAM</td>
<td>5.42±2.698 Δ</td>
</tr>
<tr>
<td>CON</td>
<td>69.02±7.78*</td>
</tr>
<tr>
<td>IPC</td>
<td>39.89±4.25*△</td>
</tr>
<tr>
<td>SUF</td>
<td>38.01±4.90*△</td>
</tr>
</tbody>
</table>

Areas of Myocardial Ischemia and Infraction

SHAM group do not participate in comparison for it has no obvious myocardial ischemic area. Comparing with CON, the areas of Myocardial Ischemia(AAR/LV%) and Infraction(IS/AAR%) decrease to a large degree, the difference was statistically significant. But comparing among groups, there was no statistically significant(p>0.05). see table 3

Table 3. The compare of areas of Myocardial Ischemia and Infraction.

<table>
<thead>
<tr>
<th>items</th>
<th>LV(mg)</th>
<th>AAR(mg)</th>
<th>AAR/LV(%)</th>
<th>IS(mg)</th>
<th>IS/AAR(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>groups</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>CON</td>
<td>579.01±20.98</td>
<td>297.02±12.11</td>
<td>50.96</td>
<td>155.12±4.94</td>
<td>51.98</td>
</tr>
<tr>
<td>IPC</td>
<td>598.10±11.16</td>
<td>302.87±8.12</td>
<td>50.89</td>
<td>105.01±9.98*</td>
<td>35.12Δ</td>
</tr>
<tr>
<td>SUF</td>
<td>590.93±7.89</td>
<td>300.85±7.01</td>
<td>50.87</td>
<td>102.87±5.89*</td>
<td>35.15Δ</td>
</tr>
</tbody>
</table>

Note: left ventricle weight(LV); ischemic myocardial weight(AAR); the weight of infarction area(IS); *p<0.05 compared with CON group; Δp>0.05 compared among two groups.

Myocardium HE Staining

For HE staining, the myocardium tissue of rats in the SHAM (figure 1)Group shows no obvious anthological change. In the CON(figure 2) Group, pathological change of myocardium is obvious, and cardiac muscle fibers are arranged loosely, disorderly and brokenly. Flake-like necrosis and disintegration of myocardial cells are visible. The IPC Group(figure 3) and the SUF(figure 4), with relatively mild pathological change. See below figures
Discussion

Myocardial ischemia reperfusion injury was firstly proposed by Jennings et al. in 1960[2]. Reperfusion injury against the myocardial ultrastructure was irreversible myocardial necrosis, incomplete repair after injury and hyper-plasia of fibrous tissue can cause persistent myocardial damage and gradually progress to chronic cardiac failure, which is serious bad to our health. Myocardial infarction area is the key standard to evaluate the degree of myocardial injury.[3] Therefore, We want to find a good way to against ischemia-reperfusion and to induce infarction area. Zhao proposed a concept of ischemic postconditioning in 2000[4], meaning short repeated many times after ischemia reperfusion/ischemia treatment can effectively alleviate reperfusion injury. Drug post-processing is protecting heart by drug action or replacing organism protective factor on the basis of ischemia postconditioning.

This study through the grouping experiment, observe the change of the indicators, discovering sufentanil postconditioning can relieve myocardial ischemia-reperfusion injury. Studies suggested that phosphatidylinositol-3-kinase serien/threonine kinase (PI3K/Akt) pathway is involved in sufentanil postconditioning on the protection of myocardial ischemia-reperfusion in rats[5,6,7]. A study demonstrates that cardioprotection of sufentanil seems to be ceiling-effective and dose-dependent, which is mediated by the preservation of phosphorylation of Cx43[8]. The above studies are consistent with this article, all confirmed that sufentanil postconditioning can relieve myocardial ischemia-reperfusion injury, even put forward the protection mechanism. But, there a study deems that diabetes mellitus abrogates the cardioprotection of sufentanil against ischemia/reperfusion injury by altering glycogen synthasekinase-3b[9].

In summary, sufentanil postconditioning has protective effects on myocardial ischemia-reperfusion injury in rats.

Acknowledgement

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References


