TARGETED TREATMENT OF ISCHEMIA-REPERFUSION INJURY IN ABDOMINAL FLAPS OF RATS BY MAGNETIC ALPROSTADIL LIPID MICROSPHERE

YANJIN WANG¹, YIQING XIAO², CHENG GANG LEI¹, ZHENYU CHEN¹*, JIZHEN REN¹
¹Department of plastic surgery, the Affiliated Hospital of Qingdao University - ²Department of Obstetrics and Gynecology, Qing Economic & Technological Development Area First People’s Hospital - ³Department of Hepatobiliary Surgery, Qingdao Municipal Hospital - ⁴Department of plastic surgery, the Affiliated Hospital of Qingdao University

Abstract

Objective: To investigate the effects of magnetic alprostadil lipid microsphere in targeted treatment of ischemia-reperfusion injury in abdominal flaps of rats.

Methods: An animal model of ischemia-reperfusion injury in abdominal flaps of rats was developed. The study group was injected with magnetic alprostadil lipid microsphere via the opposite femoral artery, while the control group injected with physiological saline, and the blank group was treated with no vessel blockage or medication after skin flap preparation. At different time points of ischemia and reperfusion of the skin flaps, the blood flow volume and the content of MDA and NO in the flap tissues were determined by a laser Doppler Flowmetry, thiobarbituric acid method, and nitrate reductase method, respectively. At 7h after suture in situ of the skin flaps, the designed area and survived area of the skin flaps were determined by coordinate decal method, based on which, the survival rate of the skin flaps was calculated. Meanwhile, routine histopathologic slides of the flap tissues were taken for immunohistochemical staining to identify the expression of vascular endothelial growth factor (VEGF).

Results: The study group had significantly higher micro-circulation volume, NO synthesis quantity, VEGF expression density, and 7-d survival rate and significantly lower MDS level of the skin flaps than the control group and blank group.

Conclusion: As a high-performance bioactive compound, alprostadil can improve the micro-circulation and lighten the ischemia-reperfusion injury by increasing NO content and protecting the vascular endothelium of skin flaps.

Keywords: Alprostadil lipid microsphere, Magnetic targeted treatment, Ischemia-reperfusion injury.

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Introduction

Skin flap technique is important in surgical field, and is widely applied in professional fields including plastic surgery, orthopaedics, microsurgery, oral and maxillofacial surgery, and ophthalmology. But skin flap transplantation usually experiences a long time of ischemia and anoxia, and consequently it inevitably damages the tissues when ischemia-reperfusion, which affects the survival of the skin flap. Once the skin flap is dead, uncorrectable corporal injury and economic loss inevitably come to the patients. Alprostadil is a high-performance bioactive compound, and can significantly dilate blood vessel, inhibit platelet aggregation, and improve micro-circulation.

In this study, alprostadil was enclosed in lipid microspheres with diameter of 0.2um. In the external magnetic field, the drugs are to be delivered to the flap ischemia-reperfusion injury part in rat abdomen using the carrier features of lipid microspheres. Based on the observation of blood flow of rat island flap for different times upon the application of magnetic liposome prostaglandin, malondi-
adehyde (MDA) content upon cell injury, change of NO content in tissue, and determination of flap survival rate, our study aimed to further understanding for the increase of island flap survival rate under the application of magnetic liposome prostaglandin, which provided the theoretical and experimental basis for the improvement of flap ischemia-reperfusion injury and the promotion of flap survival under the clinical application of magnetic liposome prostaglandin.

Materials and methods

Materials

Laboratory animals: Totally 30 healthy SD rats (Shandong Lukang Company, SPF) with body weight of 250-300g, were randomly divided into group A, B, and C (with 10 rats in each group). They were fed complying with the experimental requirements in the animal laboratory of the scientific-educational building.

Drugs and reagents: Alprostadil; self-made alprostadil lipid microsphere; bovine serum albumin (Shanghai Tiancheng Tech. Co., Ltd); magnetic fluid- rubidium-ferrum-boron magnet; castor oil (Guangzhou Danwang Trade Co., Ltd); diethyl ether (Shenzhen Duoyuan Chemical Industry Co., Ltd); dehydrated alcohol (Shanghai Haoran Biological Technology Co., Ltd); paraformaldehyde (America Sigma Company); VEGF antibody (America Santa Cruz Company); and instantly-available SABC immunohistochemical kit (Wuhan Boster Bioengineering Co., Ltd)

Instruments: Laser Doppler flowmetry (Japan Omega Company); 4 oC refrigerator (Qingdao Aucma); -80 oC refrigerator (Qingdao Haier); light microscope (German Leica); fluorescent invert microscope (German Leica); thermostatic water-bath kettle (China Shexin Laboratory Apparatus Co., Ltd); and high-speed centrifuge (America Beckman Company).

Methods

Preparation of alprostadil lipid microspheres: 250 mg of bovine serum albumin was dissolved in 10 g/L alprostadil distilled water solution by emulsifying - hot curing technique, and then supplemented with 0.2 ml of magnetic fluid and mixed with 100 ml of 10% castor oil. The solution was stirred for 10 min, and then emulsified with ultrasound. Another 100 ml of castor oil was taken and heated to 160 oC, and supplemented with the above-mentioned emulsion when stirring. The emulsion was incubated at 160 oC for 10 min, and then stirred for 6 h. The emulsion was supplemented with 200 ml of diethyl ether for degreasing, and then centrifuged. The oil phase was abandoned, and the sediment was rinsed with diethyl ether and ethanol in order. Finally, the sediment was replaced in distilled water and lyophilized for 48 h, and microsphere powder was obtained.

Preparation of hypogastric island flap model of rats: SD female rats, with body weight of 180-300g, were fasted at 1d before the surgery. It was to perform the moul of abdominal skin flap, with 10% Na2S in reserve, and general anesthesia with the intraperitoneal injection of 3% pentobarbital sodium (40 mg/kg). The abdominal island skin flap of rate was to be designed, referring to the method of Manson et al, and upon routine disinfection, lifted up the island skin flap of 3 cm * 6 cm, predicted with superficial inferior epigastric vessels. The level of flap tissue should reach to subcutaneous tunica intima.

Group of experiments: After the skin flap preparation, a microvascular clip was used for a temporary interruption of blood vessel flow, before abdominal wall resulting in ischemia of skin flap and reperfusion 5h later. Meanwhile, it was injected with magnetic alprostadil lipid microsphere (experiment group), or physiological saline (control group), at their administration of 20m g/kg and 10m g/kg /m in for 2h, via the opposite femoral artery. After normal skin flap preparation, the blank group was treated with no vessel blockage or medication. Nd-Fe-B rare-earth magnet was to be covered out of the skin flap in the experiment group. Suture in situ of the skin flaps would appear, 2h for reperfusion of skin flap in the experiment group and control group, and 7h after preparation of skin flap in the blank group.

Observation of magnetic response of alprostadil lipid microsphere: It was to add the alprostadil lipid microsphere to 0.1% normal saline, at the concentration ratio of 1:10, after ultrasonic dispersion, and observed the dispersion of microsphere under an invert microscope; meanwhile, took a drop of suspension onto the microslide, and then put a Nd-Fe-B magnet, with the surface strength of 3000 Gauss at the end of microslide, to observe the motion and aggregation of microsphere under the microscope.

Detection of blood flow volume of skin flap (nl/s): It was to be detected by Laser Doppler
flowmetry before ischemia, ischemia for 1, 2, 5h and reperfusion for 5min, 30min and 2h, respectively.

Content of MDA and NO in the skin flap tissues: As taking 100mg of tissues of full thickness on the edge of skin flaps respectively, after reperfusion 0.5h and 2h in the experiment group and control group, as well as 2h after skin flap preparation in the blank group, the content of MDA and NO was to be detected by the method of Yan et al[2]. After homogenate preparation, the detection kits were to be purchased from BEIJING Bio-lab Materials Institute.

The content of MDA was to be detected by thiobarbituric acid method. As a red product formed upon the combination of MDA with thio-barbituric acid, the specimen absorbance A was detected at 532nm. The content of specimen MDA was calculated, based on the the specimen absorbance A The specific operations should follow the description of kit.

The content of NO was to be detected by nitrate reductase method. It was available for the nitrate reductase with its specificity, to revert NO3- to NO, and determinate A at 540nm through coloration. The content of specimen NO was calculated based on the the specimen absorbance A. The specific operations should follow the description of kit.

Detection of survival rate of skin flap: At 7d after suture in situ of the skin flaps, the designed area and survived area of the skin flaps were detected by coordinate decal method[5], based on which, the survival rate of the skin flaps was calculated (survived/designed area * 100%).

Detection of VEGF: Elimination, POM fixation, dehydration, paraffin embedding, 5um of continuous slides of the flap tissues, after 7d were taken for immunohistochemical method to detect the expression of VEGF.

Statistical analysis
All data was to be expressed as mean ± standard deviation (x±s), and t-test was applied for statistical analysis.

Results

Blood flow volume of skin flap
Detected by a Laser Doppler flowmetry, micro-circulation blood flow volume at ischemia phase of the skin flap gradually decreased. The blood flow detected under ischemia for 5h decreased to about 30% of that before ischemia.

After reperfusion, the blood flow volume for micro-circulation of skin flap in the three groups increased gradually, and the experiment group had significantly higher micro-circulation volume than the control group and blank group (p < 0.01, Table 1)

<table>
<thead>
<tr>
<th>Group</th>
<th>Before ischemia (nl/s)</th>
<th>After ischemia (nl/s)</th>
<th>After reperfusion (nl/s)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1h</td>
<td>2h</td>
<td>5h</td>
</tr>
<tr>
<td>Experiment group</td>
<td>27.4±0.3</td>
<td>20.1±0.7</td>
<td>15.4±0.6</td>
</tr>
<tr>
<td>Control group</td>
<td>26.1±0.5</td>
<td>21.3±0.5</td>
<td>16.2±0.7</td>
</tr>
<tr>
<td>Blank group</td>
<td>28.6±0.2</td>
<td>19.8±0.6</td>
<td>15.3±0.4</td>
</tr>
</tbody>
</table>

Table 1: Change of micro-circulation blood flow volume in abdominal island skin flaps of rats (x±s, n=10).
*p < 0.01 compared to the control group, # p < 0.01 compared to the control group blank group

Contents of MDA and NO
When reperfusion for 0.5h, the experiment group had no significant increase for the content of MDA in the skin flap tissues, compared to the control group; when reperfusion for 2h, the experiment group and control group had higher content of MDA than the blank group, but the control group had significantly higher content of MDA than the experiment group (p < 0.01, Table 2). When reperfusion for 0.5h and 2h, the experiment group had no significant difference in NO content, comparing to that in the control group and blank group at the same time (p > 0.05, See Table 2).

<table>
<thead>
<tr>
<th>Group</th>
<th>MDA</th>
<th>NO</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reperfusion for 0.5h</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Experiment group</td>
<td>16.65±4.37</td>
<td>4.86±0.55</td>
</tr>
<tr>
<td>Control group</td>
<td>21.38±6.28</td>
<td>3.41±0.95</td>
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<tr>
<td>Reperfusion for 2h</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Experiment group</td>
<td>19.25±6.49*#</td>
<td>6.73±0.24</td>
</tr>
<tr>
<td>Control group</td>
<td>30.68±7.84*</td>
<td>4.85±0.38</td>
</tr>
<tr>
<td>Blank group</td>
<td>13.39±4.21</td>
<td>6.58±0.34</td>
</tr>
</tbody>
</table>

Table 2: change of content of MDA and NO in in abdominal island skin flap tissues of rats (x±s, n=10).
*p < 0.01 compared to the control group, # p < 0.01 compared to the blank group

Survival rate of skin flap
The experiment group had significantly higher 7d survival rate than the control group and blank group, and the difference had statistically significance (p < 0.01 Table 3).
Results of immunohistochemistry

The observation for immunohistochemical staining and HE staining of VEGF showed that, at the local abdominal skin flaps of rats, the experiment group had a mass of newly born capillaries, with VEGF expression density (126.89±7.59), while the control group and blank group had a very few of capillaries (Figure 1), with VEGF expression density (52.46±5.36 and 48.62±6.41 respectively). Compared of the control group and blank group to the experiment group, the difference had statistical significance via t-test ($p \leq 0.01$).

Discussion

In plastic surgery field, skin flap transplantation is common and important means for tissue defect repair and organ reconstruction\(^7\). Ischemia-reperfusion injury has been identified as the important reason of skin flap necrosis, however, its pathogenesis has been still unclear so far\(^8\).

The studies show that it is associated with the cascade reaction, induced by the increase of oxygen radical, while the main observational indexes reacting the level of oxygen radical are SOD, MDA and NO\(^9\). MDA is the product generated by the decomposition of injury of cytomembrane under lipid peroxide, and its content are available to represent the severity of ischemia and anoxia in tissues\(^10\). The higher the content of MDA, the more severe the anoxia in tissues is. NO is free radical presenting in all tissues obviously acting as vasodilatation. It has been demonstrated in studies that NO was released by vascular endothelial cell (VEC), which has an important protective effect in the pathophysiologic process of local micro-circulation ischemia and reperfusion\(^11\).

Alprostadil is a kind of antural prostaglandin having high-performance bioactive activity, and can significantly dilate blood vessel, inhibit platelet aggregation, and protect VEC, as well as protect blood vessel endothelium, and improve micro-circulation through increasing serum NO and reducing endothelin. While as the lipid microsphere has a fairly high affinity for the blood vessel in the diseased region, it has been demonstrated in studies that applied magnetic field is available to increase the targeted distribution of magnetic drugs in the diseased region.

Therefore, alprostadil lipid microsphere was enclosed in lipid microspheres with diameter of 0.2ug, meanwhile, it had the following advantages acting as the applied magnetic field:

- high specificity: the studies demonstrated that the lipid microspheres, upon intravasation, were to flow on the edge of blood vessel mainly together with platelet and available to be adhered to VEC. The alprostadil lipid microsphere is available to be aggregated in targeting diseased region, delivered the drugs to diseased tissues and organs, selectively dilate the blood vessel in diseased region, increase to build the collateral circulation, and improve the local blood supply\(^12\);

- Long efficacy duration: As alprostadil lipid microsphere carrier preparation was under the barrier
protection of lipid microspheres, lung has significantly reduced the action of fire extinction for it\(^{(10)}\);

- Reduction of occurrence rate of adverse effects: Under the protection of lipid microspheres, the alprostadil lipid microspheres have significantly decreased the stimulation for blood vessel and inflammatory reaction\(^{(14)}\);
- It is to decrease for better the generation of free radical and avoid the reperfusion injury. Therefore, alprostadil lipid microsphere carrier preparation showed small side effects and obvious advantages for the treatment of ischemia-reperfusion injury.

In this study, the alprostadil lipid microspheres with high specificity, was injected into the ischemia-reperfusion injury in abdominal flaps of rats, using the applied magnetic field, and the observation of micro-circulation blood flow volume in the skin flaps, NO and MDA in the skin flap tissues, and the survival rate of the skin flaps, were to identify the effect of alprostadil lipid microspheres on the ischemia-reperfusion injury of island skin flaps. It indicated that the micro-circulation blood volume of the abdominal island skin flaps of rats, had increased in a short time upon the use of alprostadil lipid microspheres, meanwhile, its long-term survival rate performed significantly increased as well. While, the synthesis of NO had increased and level of MDA decreased, under the sampling detection of skin flap tissues, which suggested that, the effect of alprostadil lipid microspheres on the micro-circulation and survival rate of the skin flaps were to be realized, by the increase of NO and decrease of MDA. These results further verified, the experiment results of Cordeiro et al\(^{(15)}\), and meanwhile, the mechanism of alprostadil was to be deep discussed.

**Conclusion**

Under magnetic field, alprostadil lipid microspheres can improve the drug concentration in the diseased region, reduce the drugs’ fire extinction in lung, reduce toxic and side effects for other normal parts, improve the micro-circulation of skin flaps, protect the ischemia-reperfusion injury, and increase the survival rate of the skin flaps.

**References**


Corresponding author
ZHENYU CHEN