Prediction of Experimental Skin Flap Survival by Observing Plasma Enzyme Levels; CK, LDH and AST

DENEYSEL DERİ FLEBİ YAŞAYABİLİRLİĞİNİN PLAZMA CK, LDH VE AST ENZİM DÜZEYLERİNİN İZLENMESİ YOLUYLA TAKİBİ

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Summary

The relationship between flap viability and plasma creatine kinase (CK), lactate dehydrogenase (LDH) and aspartate aminotransferase (AST) levels on epigastric island skin flap treated with antioxidant vitamins and/or hyperbaric oxygen (HB02) was investigated. Epigastric island skin flaps in the dimensions of 6.0x3.5 cm were raised from 40 female Sprague Dawley (SD) rats. The tissues were reperfused following 8 hours of total ischaemia obtained by clamping the inferior epigastric pedicle. Administration of Vitamin E and C combination initiated 3 days before the operation and then were given on operation day, and postoperatively 3rd and 6th days intraperitoneally. HB02 treatments were performed at 2.5 atm abs for 60 minutes once a day, for a total of 7 days. 0.5 cc blood was taken from tail veins to measure plasma enzyme levels preoperative and on days 2, 3 and 7 days postoperatively. The enzyme levels were studied in 3 hours after taken the blood samples. When compared with preoperative levels, in control group, which showed also the highest necrosis ratio, all three enzymes reached peak levels on 3rd day postoperatively (CK 35, LDH 6, AST 7 fold increased). In vitamin group, which showed the lowest necrosis ratio, CK, LDH and AST increased only 5, 1.4 and 2 fold, respectively (p<0.01 as compared with controls). According to these results, it could be suggested that these enzyme levels may be useful tools of predicting the flap survival on experimental skin flap.

Key Words: Skin flap. Creatine kinase, Lactate dehydrogenase. Aspartate aminotransferase


An inevitable increase of ischaemia in skin tissue occurs following the treatment of open wounds by skin flaps. Tissue hypoxia is the most common cause of ischaemia pathogenesis resulting in flap necrosis. Restoring blood flow in flaps reperfused following ischaemia is insufficient for providing flap survival. On the contrary, when blood flow is reestablished in such kind of flap, further damage, caused by the oxygen entering the flap tissue, occurs (reperfusion injury) (1). The most important

Özet

Antioksidan vitaminler ve/veya hiperbarik oksijen (HB02) ile tedavi edilen epigastrik ada deri fleplerinde, flep yaşayabilirliği ile plazma kreatin kinaz (CK), lactat dehidrojenaz (LDH) ve aspartat aminotransferaz (AST) düzeyleri arasında ilişki araştırılmıştır. 40 dişi Sprague-Dawley kırmızı (SD) rata, 6x3.5 cm boyutlarındaki epigastrik ada flepleri kaldırıldı. Inferior epigastrik pedikülün klempe edilmesi ile oluşturulan 8 saatlik total iskemi sonrasında dokunun reperfüzyonu sağlandı. Antioksidan E ve C kombinasyonu, operasyondan 3 gün önce başlanarak, operasyon günü ve postoperatif 3. ve 6. günlerde intraperitoneal olarak verildi. HB02, 2.5 ATA’da 60 dk sürede ve günde bir kez verilen 7 gün boyunca devam etti. Plazma enzim seviyelerini ölçmek için kuyruk veninden operasyon öncesinde ve postoperatif 2., 3., 6. ve 7. gününde 0.5 ml kadar kan alınmıştı. Enzim düzeyleri operasyonun tamamlanması ve postoperatif 3. ve 6. gününde aştıktan sonra 3 saat içerisinde ölçüldü. Postoperatif 3. ve 6. gününde, aynı zamanda en yüksek nöroksor oranlarının da görüldüğü kontrol grubunda, her üç enzim düzeyi de operasyon sonrası forik seviyelerine ulaştı (CK 35 kat, LDH 6 kat ve AST 7 kat arttı). Daha düşük nöroksor oranlarında ise birlikte olan vitamin grubunda, CK, LDH ve AST düzeylerindeki artış sırada yaltura yansıtılmadı, 1.4 ve 2 kat olarak gerçekleşti (kontrol grubu ile karşılaştırıldığında p<0.01). Bu bulgulara dayanarak, söz konusu üç enzim düzeyinin deney serileri deri flebi yaşayabilirliğinin izlenmesi için iyi birer ölçüt olabileceği öne sürülmüşdür.

Anahtar Kelimeler: Deri flebi, Kreatin kinaz, Laktat dehidrojenaz, Aspartat aminotransferaz

changes induced by ischamcia-reperfusion injury are cellular swelling, edema, vasoconstriction, reactive oxygen species (ROS), and thrombosis (2,3).

Results of these changes are breakdown of the sodium/potassium pump and inflammation of the perivascular tissue, with a concomitant of the number of for example, superoxide radicals (4). Edema may cause constriction of lumen, particularly in the microvasculature. ATP and oxygen are gradually depleted, leading to sequential necrosis of myocytes, smooth muscle cells, fat cells and endothelial cells lining the vasculature (5). Endotelial damage may then result in exposure of subendotelial collagen, leading to platelet aggregation and thrombus formation after revascularization. All of these factors affect tissue viability, and tissue necrosis is inevitable if proper precautions are not taken (2,5).

Hyperbaric oxygenation (HBO?), is a method to obtain high plasma concentrations of oxygen (6,7). Either HBO, and antioxidant agents including vitamin E or C are commonly used in experimental skin flap studies (1,8,9). Water-soluble vitamin C (L-ascorbic acid), which is present as ascorbate in the most of biologic medium, is reported to be the most important antioxidant of extracellular fluids and an agent decreasing the vascular permeability. The most effective and popular tocopherol molecule is alpha-tocopherol known as vitamin E. a-tocopherol, a lipid soluble antioxidant, has the ability to prevent ROS-induced lipid peroxidation by reacting with organic peroxyl radicals in the tissues. This is the most important biochemical function of this molecule representing the antioxidant activity of vitamin E (10).

Certain tissue-specific enzymes are released during tissue damage and have been used in the past to quantify the nature and extent of tissue damage. Especially creatine kinase (CK) plasma levels, during myocardial infarction, is used as a specific measure of cardiac damage, although this enzyme has a variety of distribution in the body such as skeletal muscle and in smaller quantities in brain, intestine and other minor tissue. Similarly, aspartate aminotransferase (AST) has its highest catalytic activity in the heart and lactate dehydrogenase (LDH) is distributed ubiquitously. Both LDH and AST are present in the cytoplasm of several types of cells and, while not tissue-specific, are certain to reflect any ischamcia damage to the vasculature (11,12).

This study was performed whether flap survival, on epigastric island skin flap treated with antioxidant vitamins and/or HBO, could be predicted by the changes in these three enzyme plasma levels.

**Materials and Methods**

Forty adult female Spraque Dawley rats, weighing 200-225g, were divided into 4 equal groups by the simple random sampling method: Control and 3 treatment groups [HBO, Vitamin (E+C), HBO + Vitamin (E+C)]. The rats were placed separately under normal laboratory condition and room temperature and were fed with rat chow and water ad libitum.

The epigastric island skin flap was used as a model (1). After anesthesia with intramuscular ketamine hydrochloride (65 mg/kg) plus xylazine hydrochloride (0.65 mg/kg) abdomen was shaved and prepared with povidone-iodine (betadine) solution. Then, in the dimensions of 6.0x3.5 cm epigastric island skin flaps including panniculus carnosus were elevated on the left side. The epigastric nerve was carefully dissected out and left intact to minimize autocannibalization. By clamping inferior epigastric pedicle (artery and vein), ischamia was provided and flaps were sutured back down to their beds over Steri-Drape sheets placed before to prevent plasmatic imbibition (13). After eight hours of ischamia, flaps were reperfused by removing clamps. Re-establishing the blood flow was evaluated by performing the Acland test on the epigastric vessels (1).

HBO, at 2.5 ATA, 100% O2 for 60 minutes initiated in the first 4 hours following reperfusion, and applied once a day during postoperative 7 days. A total of 4 doses of the combination of antioxidant vitamins a-tocopherol acetate (vitamin E, 40 mg/kg) and Na-ascorbate (vitamin C, 200 mg/kg) were given intraperitoneally once in three days; 3 days before the operation, on the operation day, postoperative 3rd and 6th days (14,15).

0.5 cc blood samples were taken four times from tail vein preoperative and in days postoperative 2nd, 3rd, 7th and studied in three hours after
taken. Enzyme levels at the same day for all groups were compared with each other. The percentages of flap viability were determined by Sasaki's paper template technique in postoperative 7th day (16).

The Kruskal-Wallis 1-Anova test first was performed to find whether there is significant difference among all groups and then Maim-Whitney U test used, only for enzymic values, to observe the differences between two groups consecutively. Differences were considered significant when p<0.05.

Results

The plasma level of three enzymes CK, LDH and AST and flap necrosis ratios are shown with details in Table 1. The level of enzymes were dramatically increased up to postoperative 3rd day for control group which showed the highest necrosis ratio (71.4±6.9%) at the end of the study. Compared with preoperative levels CK 35, LDH 6, and AST 7 fold increased. On the contrary, in vitamin E+C group that the lowest necrosis (18.0±3.9%) ratios obtained; CK, LDH and AST increased only 5, 1.4 and 2 fold at the same day respectively and as compared with controls the differences were found statistically significant (p<0.01).

All of the treatment groups had shown higher flap viability ratios (compared with control p<0.01) and the enzyme levels of these three groups had reached their top level in 2nd day. In 3rd day, the day control group enzymes peaked, these levels were observed tending to decrease. Postoperative 2nd day the increase for CK, LDH, AST were found 25, 4, 5 fold for control, 5, 1.7, 3 fold for vitamin E+C group, respectively (p<0.01).

Enzyme levels of both HB0 and HB0, + Vit (E+C) groups significant decrease were mostly observed, except 2nd and 7th day AST level for HB0 group, L DH and AST but not CK had recovered to preoperative levels even by postoperative 7th day for all groups.

Discussion

It was reported that oxidant damage occurred during ischacmia-reperfusion injury can be reduced by different antioxidant vitamins such as E and C. Zaccaria et al, have shown that vitamin C reduces ischaemia-reperfusion injury in rat epigastric island flap model (1). Vitamin E supplementation also reduces ischaemia-reperfusion injury caused by myocardial infarction in rabbits (17). The combination of vitamin E+C were also used to prevent ischaemia-reperfusion injury (18) and, as shown re-

### Table 1. Enzyme levels (IU/1) and ultimate flap necrosis ratios (%)

<table>
<thead>
<tr>
<th>Groups</th>
<th>Flap necrosis</th>
<th>Enzymes</th>
<th>Preop</th>
<th>Postop 2nd day</th>
<th>Postop 3rd day</th>
<th>Postop 7th day</th>
</tr>
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<tbody>
<tr>
<td>Control</td>
<td>CK 1200±188</td>
<td>29720±2917</td>
<td>42700±7293</td>
<td>5218±558</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>LDH 550±112</td>
<td>2400±326</td>
<td>3150±416</td>
<td>653±66</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>AST 7±4</td>
<td>350±43</td>
<td>540±64</td>
<td>82±11</td>
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<td></td>
</tr>
<tr>
<td></td>
<td>CK 1150±186</td>
<td>6180±982  *§</td>
<td>5800±869  *</td>
<td>1743±58  I §</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vitamin</td>
<td>LDH 530±109</td>
<td>941±92  ℓ  $</td>
<td>730±70  ℓ  $</td>
<td>456±61  I ℓ $</td>
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<td></td>
</tr>
<tr>
<td></td>
<td>AST 74±15</td>
<td>220±21  *§</td>
<td>155±18</td>
<td>52±6  I $</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>CK 1220±118</td>
<td>10325±918 *</td>
<td>6784±738  *</td>
<td>2152±303  I ℓ $</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HB0</td>
<td>LDH 57±85</td>
<td>1198±185  ℓ</td>
<td>1127±44  r</td>
<td>74±3  §</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>AST 76±9</td>
<td>345±34</td>
<td>231±33  *</td>
<td>94±14</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>CK 1182±137</td>
<td>8900±633  t</td>
<td>6359±1028 I</td>
<td>2029±79  r</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HB0, +Vit</td>
<td>LDH 580±91</td>
<td>1105±76  * $</td>
<td>892±98  I $</td>
<td>523±61  * $</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>AST 81±9</td>
<td>297±26  * $</td>
<td>193±26  *</td>
<td>68±10</td>
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</tr>
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</table>

All numeric data are reported as the mean ± standard error of the mean. n=10 for all groups. Statistically significance: compared with control; ℓp<0.01, $p<0.05 /compared with HB0;  $p<0.01, "p<0.05 /compared with HB0;vitamin;  $p<0.01,  p<0.05.
ciently in our department, lipid peroxidation (14,19). Another useful tool for skin flap treatment is HB02, a method to obtain high plasma oxygen levels. It was shown in many studies that HB02 could increase flap survey by reducing the tissue edema and enhancing the depleted tissue ATP and pO2 level (6-9).

A possible relation between increased release of certain tissue-specific enzymes (CK, LDH and AST) during tissue damage and skin flap survey has been claimed by Knight et al (20). In this rabbit epigastric free flap study, changes of these three enzyme plasma levels during 7 days postischemia were observed and demonstrated a huge release of enzymes from failed flap. The authors have concluded that, to predict flap survey via postischaemic enzyme levels in plasma, especially CK, were reliable parameters.

In our study, enzyme levels were measured four times; preoperatively and in days 2nd, 3rd and 7th postoperatively. The 2nd day all three enzyme plasma levels were dramatically increased and all treatment groups but not control reached peak plasma levels. One day later, only enzymes of control group continued to increase and the others initiated to decrease. The most impressive finding of this procedure was the 35 fold increase of CK in 3rd day for control flaps resulting with higher necrosis ratio in 7th day. This finding has been supported by Knight et al, who reported 68 fold increase of CK and enzyme peaks in 2nd and 3rd days (20).

According to these results, enzymes' peak day and degree of increase may be important to predict flap survey. If enzyme peaks disappear at 3rd day, it seems logically true to say that flaps survive. Because 4th day enzyme levels were not measured, it is impossible to know whether the enzyme levels continued to increase or reached peak levels in 3rd day for failed flaps such as controls in this study. However, it must be emphasized that these values apply only to this experimental model, and the enzyme release in human clinical case or other experimental studies would have to be characterized.

The tissue sources of these three enzymes is a matter of conjecture; some likely candidates are vascular endothelial cells, smooth and skeletal muscle. Muscle necrosis is known to be one of the earliest changes observed in ischaemia (12,21). Because of epigastric skin flap contains relatively small amounts of skeletal muscle compared with some other musculocutaneous flaps (22), the source of the huge release of CK cannot be explained only with flap skeletal muscle. Flap vascular smooth muscle damage, platelets during clotting (23), inflamed striated muscle in the abdominal tissue surrounding the flap are possible sources of CK. This explanation is also possible for the source of LDH and AST, that is, not only the release of these enzymes from the cytoplasm of damaged vascular endothelial cells but release from inflamed tissue surrounding the flap.

These enzymic changes are probably due to a combination of ischaemic changes in the flap vasculature, ischaemic changes in the flap muscle, and inflammatory changes in the surrounding abdominal tissue. These enzyme plasma levels, especially CK, may be used as parameters in the control of total flap failure and predict ultimate flap condition.

REFERENCES
Ahmet KORKMAZ et al.  PREDICTION OF EXPERIMENTAL SKIN FLAP SURVIVAL BY OBSERVING PLASMA ENZYME LEVELS; CK, LDII AN^AST


