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

Ahmet KORKMAZ*, Şükrü ÖTER*

*Dept. of Physiology, Medical School of Gülhane Military Medical Academy, Ankara, TURKEY

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 Spraque Daw/ey (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 intraperitoneal!)'. 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 7fold 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 this three enz)'ine levels may be useful tools of predicting the flap survey on experimental skin flap.

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

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Özet

Antioksidan vitaminler ve/veya hiperbarik oksijen (HB02) ile tedavi edilen epigastrik ada deri fleplerinde, flep yaşayabilirliği ile plazma kreatin kinaz (CK), laktat dehidrojenaz (LDH) ve aspartat aminotransferaz (AST) düzeyleri arasındaki ilişki araştırılmıştır. 40 dişi Sprague-Dawley ratta 6x3.5 cm boyutlarında epigastrik ada flepleri kaldırıldı. Inferior epigastrik pedikülün klempe edilmesi ile oluşturulan 8 saatlik total iskemi sonrasında dokunun reperjüz)'onu 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 dksüreyle ve günde bir kez verilerek 7 gün boyunca devam edildi. Plazma enzim seviyelerini ölçmek için kuyruk veninden operasyon öncesinde ve postoperatif 2., 3. ile 7. günde 0.5 ml kadar kan alındı. Enzim düzeyleri kan alındıktan sonraki 3 saat içerisinde çalışıldı. Postoperatif 3. günde, aynı zamanda en yüksek nekroz oranlarının da görüldüğü kontrol grubunda, her üç enzim düzeyi de operasyon öncesine göre pik seviyelerine ulaştı (CK 35 kat, LDH 6 kat ve AST 7 kat arttı). En düşük nekroz oranları ile birlikte olan vitamin grubunda CK, LDH ve AST düzeylerindeki artış sırasıyla yalnızca 5, 1.4 ve 2 kat olarak gerçekleşti (kontrol grubu ile karşılaştırıldığında p<0.01). Bu bulgulara dayanarak, söz konusu üç enzim düzeyinin takibinin deneysel deri flebi yaşayabilirliğinin izlenmesi için iyi birer ölçüt olabileceği öne sürülebilir.

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

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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 (repcrfusion injury) (1). The most important

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Correspondance: Ahmet KORKMAZ
Dept. of Physiology, Medical School of Gulhanc Military Medical Academy
Ankara, TURKEY

changes induced by ischacmia-repcrfusion 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 sequental necrosis of myocytes, smooth muscle cells, fat cells and endotelial cells lining the vasculature (5). Endotelial damage may then result in exposure of subendotelial collagen, leading to platelet aggregation and thrombus formation after revascularization. A11 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 HB0$_2$ 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 ascorbatc 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-tocophcrol known as vitamin E. a-tocopherol, a lipid soluble antioxidant, has the ability to prevent ROS-induced lipid peroxidation by reacting with organic pcroxyl 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 extend 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-spesific, are certain to reflect any ischaemic damage to the vasculature (11,12).

This study was performed whether flap survival, on epigastric island skin flap treated with antioxidant vitamins and/or HB0$_2$, could be predicted by the changes in these three enzyme plasma levels.

Materials and Methods

Forty adult female Spraquc Dawlcy rats, weighing 200-225g, were divided into 4 equal groups by the simple random sampling method: Control and 3 treatment groups [HB0$_2$, Vitamin (E+C), HB0$_2$ + Vitamin (E+C)]. The rats were placed separately under normal laboratory condition and room temparature 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 kctaminc hydrochloride (65 mg/kg) plus xylazinc hydrochloride (0.65 mg/kg) abdomen was shaved and prepared with povidone-iodine (betadinc) 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), ischaemia was provided and flaps were sutured back down to their beds over Stcri-Drape sheets placed before to prevent plasmatic imbibition (13). After eight hours of ischaemia, flaps were repcrfuscd 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% 0$_2$ 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-tochopherol 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. Postopertive 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 $HB0_2$ and HBO, + Vit (E+C) groups significant decrease were mostly observed, except 2nd and 7th day AST level for $HB0_2$ group. LDH 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-repcrfusion injury in rat epigastric island flap model (1). Vitamin E supplementation also reduces ischaemia-repcrfusion 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 (%)

Groups	Flap necrosis	Enzymes	Preop	Postop 2nd day	Postop 3rd day	Postop 7th day
		CK	1200±188	29720±2917	42700±7293	5218±558
Control	71.4±6.9	LDH	550±112	2400±326	3150±416	653±66
		AST	74±7	350±43	540±64	82+11
		CK	1150±186	6180±982 *‘§	5800±869 *‘	1743Ü58 î‘§
Vitamin	18.0±3.9J	LDH	530±109	941±92 t^§	730±70 *İ§	456±61 î‘‘
		AST	74±15	220+21 *‘§	155+18	52±6 î‘§
		CK	1220±118	10325±918 *	6784±738 *	2152±303 İ
$HB0_2$	40.8±3.8J	LDH	571±85	1198=l=185 f	1127Ü44 *t*	743±83 ‘
		AST	76±9	345±34	231±33 *	94±14
		CK	1182±137	8900±633 t‘	6359±1028I	2029±79 *t*
$HB0_2$+Vit	33.7±5.9$	LDH	580±91	1105+76 *‘	892±98 İ‘	523±61 *‘
		AST	81±9	297±26 *	193±26 *‘	68±10‘‘

All numeric data are reported as the mean ± standart error of the mean. n=10 for all groups.
Statistically significance: compared with control; fp<0.01, ‘p<0.05 /compared with $HB0_2$; °p<0.01, "p<0.05 /compared with $HB0_2$+vitamin; §p<0.01, ‘p<0.05.

cently 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 p02 level (6-9).

A possible relation between increased release of certain tissue-spesific 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 postischacmia 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; preopcratively and in days 2nd, 3rd ,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 ct 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 hi 4th day 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 skletal 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 skletal 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 cyctoplasm 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.

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