Comparison of the Oxidative Damage That Occurs During the Preparation of the Internal Thoracic Artery and the Saphenous Vein As A Graft and the Effect of the Systemic Total Antioxidant Capacity on This Entity

Safen Ven ve İnternal Torasik Arterin Greft Olarak Hazırlanmasında Ortaya Çıkan Oksidatif Hasarlanmanın Karşılaştırılması ve Buna Sistemik Total Antioksidan Kapasitenin Etkisi

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ABSTRACT Objective: We aimed to demonstrate the differences in terms of oxidative damage during the preparation of the internal thoracic artery (ITA) and the greater saphenous vein (SV) in this study. Relation of this process with the systemic total antioxidant capacity (TAC) was also investigated. **Material and Methods:** Oxidative damage in the SV and the ITA samples was analyzed by the mye-loperoxidase (MPO) and the malondialdehyde (MDA) levels and the histopathologic changes. Blood samples for TAC levels were taken before the induction of anesthesia. **Results:** Endothelial irregularities, separation of the smooth muscle cells and the presence of the mast cells within the interstitial tissue were in conjunction with higher MPO and MDA values in the SV than the ITA (p=0.046 and p=0.001 respectively). Comparison of MPO and MDA values with the serum TAC yielded no statistical significance for both two graft. **Conclusion:** Histological changes induced by the mechanical trauma and hypoxemia during the graft preparation, leads to a higher degree of oxidative damage in the SV. ITA is prepared as a pedicled graft with a non-touch and less traumatic technique while the blood is still running through the lumen. Lack of a correlation between the serum TAC and oxidative indicators may be explained by the effectivity of the constitutional antioxidants.

Key Words: Coronary artery bypass; saphenous vein; mammary arteries

ÖZET Amaç: Yaptığımız çalışmada safen ven (SV) ile internal torasik arter (İTA) arasındaki oksidatif hasar farkını göstermeyi amaçladık. Bunun sistemik total antioksidan kapasite ile olan ilişkisini araştırdık. **Gereç ve Yöntemler:** SV ve İTA örneklerinde oksidatif hasar, myeloperoksidaz (MPO) ve malondialdehid (MDA) düzeyleri ve histopatolojik inceleme ile değerlendirildi. Total antioksidan kapasite (TAC) düzeyi için kan örnekleri anestezi indüksiyonundan önce alındı. **Bulgular:** Safen vendeki endotel düzensizliği, düz kas ayrışmaları ve interstisyel mesafedeki mast hücreleriyle uyumlu olarak MPO ve MDA değerleri internal torasik artere göre yüksekti (sırasıyla p=0.046 ve p=0.001). Her iki greftin MPO ve MDA değerleri ile sistemik TAC arasında bir ilişki yoktu. **Sonuç:** Greftin hazırlanması sırasındaki mekanik travma ve hipoksi ile oluşan histolojik değişiklikler, SV'de daha fazla oksidatif hasara neden olmaktadır. İTA içinde kan akımı devam ederken, pedikküllü olarak minimal travmayla çıkarılmaktadır. Plazma TAC'si ile greftler arasındaki oksidatif göstergeler arasında bir korelasyon olmaması ise greftlerin bünyesindeki antioksidanların etkinliği ile açıklanabilir.

Anahtar Kelimeler: Koroner arter baypas; safenöz ven; meme arterleri

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Cardiovascular diseases are responsible from as much as 50% of all the deaths in the developed countries and 25% in the developing countries. Among these coronary artery diseases (CAD) constitue almost 50% of the deaths. Previsions purport that in 2020 CAD will be the most common cause of death all over the world.[1]

At present, CAD is treated by medical protocols, percutan transluminal coronary angioplasty applications or by coronary artery bypass grefting (CABG) or with combinations of these applications. Arterial and vein grafts are commonly used for a CABG surgery. Although an approach with full arterial revascularization is commonly approved vein grafts still carry an important role and widely used for revascularization purposes of the coronary arteries. The first choice among the arterial grafts is the ITA and its equivalent among the vein grafts is the greater SV. Nevertheless, short and long term results of the SV grafts do not prove to be at a desired level having 30% of occlusion within one year and almost 50% of occlusion within 10 years. In the remaining patent grafts significant atherosclerotic changes are present almost by half. Patency rate of the left internal thoracic artery-left coronary artery (ITA-LAD) grafts during the same time period varies between 85-95%.[2-6] It has been very well demonstrated that the difference in terms of patency rates between the two vascular grafts depends mainly on the structural and functional properties of the two.[7-9] Preparation of the SV as a graft by using the traditional approach exposes the vein to ishemic and mechanical insults until the commencement of the distal anastomosis hence leading to endothelial impairments and dysfunction. This pathological process begins with the oxidative destruction of the free radicals liberated by the activated adhesion molecules and fagocytic cells leading to graft thrombosis within one month, to intimal hyperplasia in the following months and finally to atherosclerotic changes by the end of one year and onwards.[10-15]

During oxidative destruction free radicals, either or not derivatives of oxygen, reacts with unsaturated fatty acids leading to lipid peroxidation which give rise to irreversible damage on the cell membrane. Nevertheless the liberated reactive aldehydes also act on the intracellular organelles. MDA which is a derivative of these reactions is commonly accepted as an indicator of cellular damage.[16,17] Oxidative activation is demonstrated by MPO which is found in the primary granules of the neutrophills and monocytes.[18] Free radicals that are present during the oxidative damage are eliminated from the medium either by enzymatic or non enzymatic antioxidants. All of these antioxidants that are existant within the systemic circulation are named as the TAC.[19]

In this study we aimed to investigate the oxidative damage in traditionally prepared SV and pedicled ITA tissue samples both with biochemical methods in terms of MDA and MPO levels and in terms of histopathologic changes. We also investigated the effect of systemic TAC on the MPO and MDA values.

MATERIAL AND METHODS

STUDY PROTOCOL

This study has been approved by the Ethics Committee of our instution. Meeting date was 11 July, 2005, meeting number was 10 and the decision number is 11.

This study has also been supported by Scientific Investigation Projects Committee of our institution. The code of the project is BAP-TF-CTB (NS) 2005-2.

PATIENT PROPERTIES

Sixteen male patients were included in this study. The mean age was 61 ± 4 years and all of them had three vessel disease in their coronary arteries. All of the operations were carried out under cardiopulmonary bypass by using cardioplegic diastolic arrest. All patients were on acetylsalycilic acid, beta blocker, statin and nitrate therapy before the operation. Patients with peripheral arterial disase, diabetes mellitus, having steroid therapy or non-steroid antiinflammatory therapy were excluded from the study.

OBTAINING AND THE PREPARATION OF THE TISSUE SAMPLES

Blood samples were collected into the tubes before the induction of anesthesia for the CABG surgery.

All of the ITA grafts were prepared by the senior surgeon as a pedicled graft with the no-touch technique. The patients were heparinized a clamp applied to the distal end of the graft just before the initiation of the cardiopulmoner bypass (CPB). The

ITA was transsected and after flushing some blood the distal end was cut before the bifurcation point with the epigastric artery. The distal piece was dissected free from the adventitial tissue and after dividing it into two halves the tissues were placed in tubes for biochemical and histopathologic evaluation.

The SV was exposed by a continuous longitudinal incision by the senior surgeon, the adventitial layer was stripped and the side branches were ligated with 4/0 silk. The vein was removed from the leg immediately after dissection and was manually distended with saline at 300 mmHg for 1 min, using a syringe connected to a manometer. As an adequate length of the SV was harvested it was placed into a solution containing 10 cc of autologous blood, 1 cc of heparin and 50 cc saline at a temperature of + 4°C. They were kept in this solution around 50 ± 5 minutes until the distal anastomoses were completed. After the completion of the proximal anastomoses the left over vein tissues were collected in the tubes for biochemical and histopathological analysis.

MEASUREMENTS OF THE TISSUE MPO, TİSSUE MDA AND TAC

The evaluation of the MPO levels in the tissue samples was done in accordance with the method developed by Golowich and Kaplan. The principle of this method depends on the measurement of reduced o-dianosidine, which is produced by the oxidation of hydrogen peroxide by the homogenate, by a spectrophotometer at a wavelength of 410 nm. Results were expressed in u/g tissue.[20]

The principle of determining the tissue MDA levels depend on the measurement of the pink colour at a wavelength of 532 nm in a spectrophotometer that occurs as a product of the reaction of the thiobarbutiric acid with MDA. The results were expressed as nmol/g tissue.[21]

ABTS (2,2'-azino-bis[3-ethylbenzthiazoline-6-sulphonic acid]), peroxidase (metmyoglobin) and hydrogen peroxide were incubated to form ABTS radical cathion. The formed blue-green colour was measured at a wavelength of 600 nm by a spectrophotometer.[22] The antioxidants existant in the sample prevent the formation of the anticipated colour in conjuction with their concentration.

HISTOLOGIC EVALUATIONS

The tissues were fixed in neutral 10% formaline for 48 h as soon as they were collected. Following fixation they were washed under running water and dehydrated in alcohol of increasing concentrations. Xylol was used to lucidify the tissues which were then embedded in paraffin blocks. Slices of 5 mm thick were cut by using a microtome and they were dyed with Hematoxylene-Eosin (HE) dye. Moreover toluidine blue dying was also performed in order to be able to evaluate the mast cells that were anticipated to be present in the SV tissues. The prepared specimens were evaluated under a light microscope using magnifications of x40 and x200 and were photographed by an Olympus BX50 light microscope.

STATISTICAL ANALYSIS

SPSS (Statistical Package for the Social Sciences) for Windows, Release 11.5.1, Standard Version Package Program was used for the statistical evaluations. Graphics were formed by using Microsoft Office Excell 2003 Module.

RESULTS

TISSUE MPO AND MDA LEVELS

Paired samples t test was used for the statistically analysis of tissue MPO and MDA levels. MPO values in the ITA and SV were 0.83 ± 0.51 and 1.38 ± 0.84 u/g tissue respectively and the difference was statistically significant (p=0.046). The MDA values were 2.25 ± 2.55 and 7.57 ± 3.93 nmol/g tissue respectively and the difference was statistically significant (p=0.001) (Figure 1).

THE CORRELATION BETWEEN THE TAC VALUES IN THE SERA AND THE MPO AND THE MDA VALUES IN THE TISSUES

We were unable to demonstrate a correlation between the TAC in the sera and tissue MPO and MDA values (Table1).

HISTOLOGIC FINDINGS

The examination of the prepared tissues from the SV revealed a patchy disintegration in the endothelial layers, swelling in the media layers and

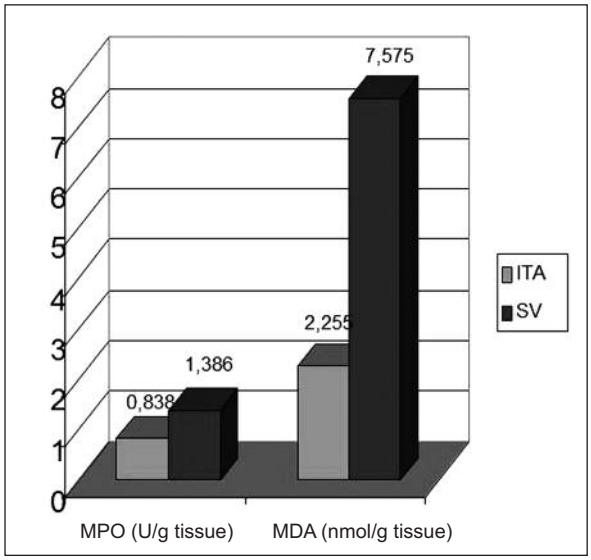


FİGURE 1: Tissue MPO and MDA values. ITA: Internal thoracic artery, SV: Saphenous vein.

splitting and irregularities in the smooth muscle cells. The adventitial tissues were loose and porous and contained some neutrophils. On the other hand when the tissue samples from the ITA's were examined the integrity of the intimal layers were preserved, the elastic fibers and the smooth muscle cells appeared to be normal (Figure 2 and Figure 3 for SV) (Figure 4 and Figure 5, for ITA). A toluidine blue dying was performed especially on the SV tissues with the aim of demonstrating the monocytes and the macrophages that are responsible from the release of MPO however, a normal distribution of these cells along the adventitial and medial layers were observed (Figure 6).

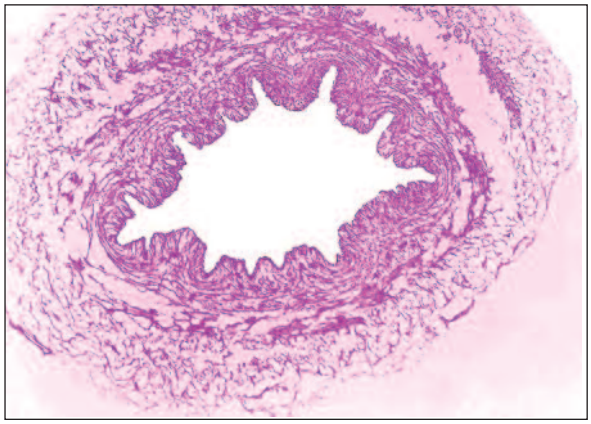
DISCUSSION

The long term patency of the grafts used in the CABG operations depend vastly on the integrity and the functions of the endothelium. SV is commonly harvested by the classical technique in many centers all over the world. With this technique it is affected by being subjected to mechanical trauma by grabbing and applying traction, to hypoxia by being kept in a solution until the distal anastomosis time and by the removal of the vasa vasorum and to tensile trauma by being distended with a solution in order to overcome the present vasospasm and to control the leakage.[10-15] These effects were visible in the histologic examination of the tissue samples taken from the SV that demonstrated disintegration in the endothelial cells and splitting of the elastic and collagen fibers and the smooth muscle cells (Figure 2 and 3). Hinokiyama et al. demonstrated the increased gene expression of the leukocyte adhesion molecules and proinflammatory cytokines together with a 25% endothelial loss in the human SV samples which have been prepared by using the classical technique and that were distended by 100-150 mmHg pressure.[12] Chello et al. demonstrated a 33% endothelial loss and an increase in the adhesion molecules and neutrophil adhesion by applying a 300 mmHg endoluminal pressure to the SV for only 2 minutes.[13] Furthermore, another in vitro study showed the activation of the monocytes and the macrophages displaying also the invasion of all the layers of the SV with these cells.[4] Resting of the SV in heparinized autologous blood for only 60 minutes results in an increase in the levels of the MPO and the adhesion molecules.[15] MPO is an indicator of the activation of the neutrophils, the macrophages and the monocytes.[18,23] The MPO levels in the SV tissue samples were found to be significantly higher than the MPO levels in the ITA tissue samples (p=0.046). The low MPO levels in the ITA tissue samples can be explained by the preserved integrity of the intima and the media of the vessels as can be seen in the histologic sections. Furthermore, as the ITA is harvested as a pedicled graft the vasa vasorum and the adventitial tissue is also preserved and blood

TABLE 1: There was no correlation between the TAC in the sera and tissue MPO and MDA values (p>0.001).

MPO ITA TAC	MPO SV TAC	MDA ITA TAC	MDA SV TAC
Correlation Coefficient:0.031	Correlation Coefficient:-0.004	Correlation Coefficient:0.53	Correlation Coefficient:0.292
p:0.908	p:0.998	p:0.845	p:0.273

MPO: Myeloperoxidase, MDA: Malondialdehyde, TAC: Total antioxidant capacity, ITA: Internal thoracic artery, SV: Saphenous vein.



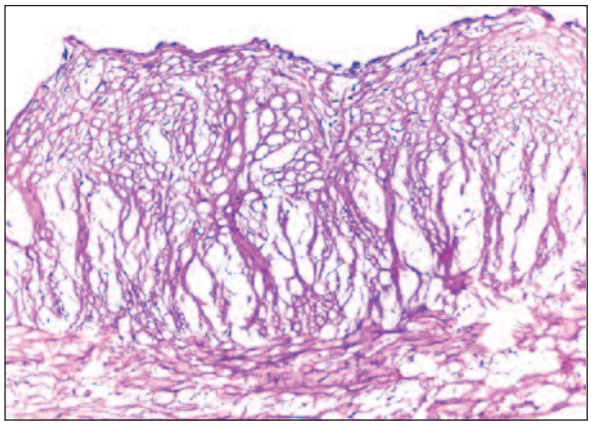
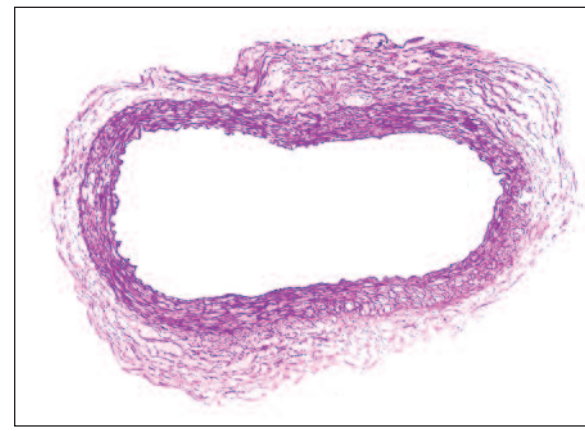


FIGURE 2, 3: A patchy disintegration in the endothelial layers of saphenous vein. Adventitial tissues were loose and porous and contained some neutrophilles (x40 and x200, Hematoxylene-Eosin).

continues to flow through the lumen during the harvesting period (Figure 4 and 5). However, although we demonstrated patchy disintegrations in the intimal layers and splitting in the smooth muscle cells of the SV tissue samples that were dyed with HE we failed to demonstrate leukocyte agglomerations which would be the explanation for the high levels of MPO in these tissues. It seems that this rise in the MPO can be explained by the presence of the monocytes and/or macrophages, the presence of which was demonstrated by the toluidine blue dying (Figure 5). In experimental heart models devoid of blood Keller et al. and Yapıcı et al. described the MPO activation to be related with the monocytes and the macrophages within the interstitial space.[24,25] Furthermore, with the raised MPO levels Crook and coworkers described the activation of the fagocytic cells in the interstitial space by the hypoxic medium after washing the SV tissue samples with saline which were harvested by an endoscopic technique and kept for an hour in a heparinized solution that also contained blood.[26]

As it can clearly be seen, an interaction between the endothelium and the phagocytic cells throughout the harvesting period of the SV is present. Oxygen and nitrogen samples liberated by this interaction reacts with arachidonic acid metabolites and granular enzymes, by the lipids, proteins and the nucleic acids and give rise to the lipid peroxidation of the biological membranes.[27] Lipid peroxidation is a highly injurious chain reaction. It directly harms the cell membrane and by producing reactive aldehydes it indirectly causes damage on the other cellular components. MDA is the resulting product of the peroxidation of the fatty acids containing three or more double links. MDA



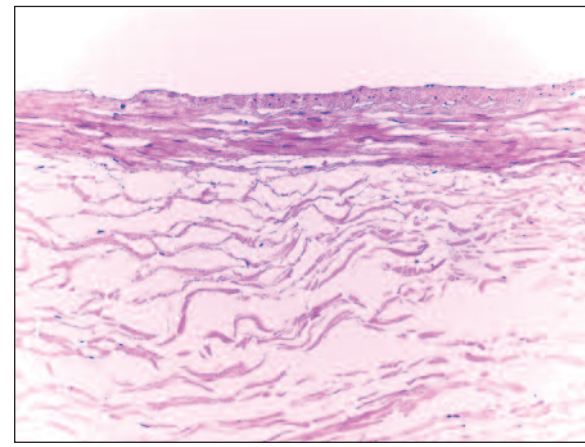


FIGURE 4, 5: The integrity of the intimal layers, elastic fibers and the smooth muscle cells appeared to be normal in ITA (x40 and x200, Hematoxylene-Eosin).

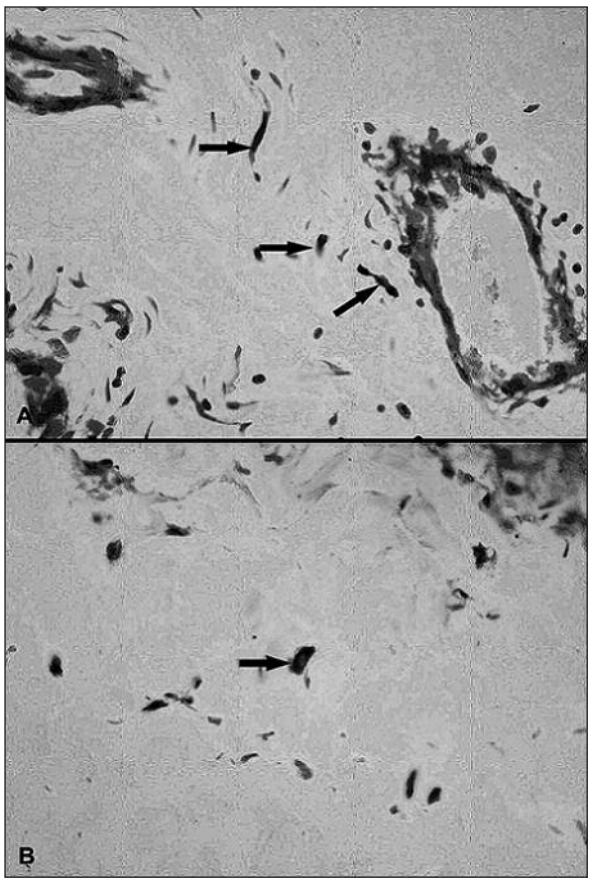


FIGURE 6: The presence of macrophage and/or monocytes in the SV (x40, toluidine blue).

is not the quantitative or specific indicator of lipid peroxidation but is well correlated with the degree of it.[28,29] In the SV tissue samples the MDA levels were consistent with the histopathologic findings and were higher than the MDA levels from the ITA tissue samples (0.001). An interesting estimation in the comparison of the MPO and MDA values were that the MPO values were 1.5 times higher in the SV tissue samples when compared to the ITA tissue samples whereas MDA values were higher by 3.5 times. This dissonance can be explained by the insufficiency of the antioxidant system in the SV and the effects of the free radical sources other than the leukocytes on the lipid peroxidation. Free radicals are eradicated from the medium both by enzymatic and non-enzymatic antioxidants. The TAC in the sera is decreased in patients with cardiovascular system disorders.[30-32] It is commonly accepted that the low molecular weighted antioxidants are effective in the amelioration of the oxidative damage by entering the site where the oxidative stress is present.[33,34] We failed to establish a correlation between the MPO and the MDA levels in the SV and ITA tissues and the TAC in the sera. In an effort to raise an explanation to this one might say that the plasma antioxidants like glutathion reductase, catalase, superoxide dismutase have high molecular weights therefore they might not be entering the suffering tissues hence raising the importance of the tissues constitutional antioxidants.

As a conclusion, we can say that the histological changes taking place due to trauma and hypoxia in the SV in comparison to the ITA is higher. The ITA is preserved better as harvesting is less invasive in comparison to vein harvesting in the classical technique and blood flow is not ceased during the harvesting period. The lack of correlation between the TAC in the sera and the oxidative indicators in the tissues may be explained by the effectivity of the antioxidants within the constitution of these vessels.

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