The Efficacy of Vitamin E in the Prevention of Lung Ischemia-Reperfusion Injury After Cardiopulmonary Bypass in Open Heart Surgery

Açık Kalp Cerrahisi Sırasında Kardiyopulmoner Baypas Sonrası Oluşan Akciğer İskemisi-Reperfüzyon Hasarını Önlemede E Vitamininin Etkinliği

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ABSTRACT Objective: The purpose of the study was to investigate the effects of vitamin E in the protection of the lung from potential ischemia-reperfusion injury during elective coronary artery bypass graft surgery. **Material and Methods:** This controlled randomized single-center study included patients who underwent elective coronary bypass grafting (CABG) operation. Forty-nine patients were randomly divided into 2 groups. Water soluble Vitamin E (100 mg) in tepid saline (n=25) or tepid saline alone (n=24) was administered via the jugular vein before the aortic cross clamping. Serum total antioxidant capacity (TAC) levels and serum malonedialdehyde levels (MDA) were measured. Pulmonary biopsies were obtained before the aortic cross clamping and 60 minutes after removing the cross clamp. Biopsies were examined histopathologically under electron microscopy. **Results:** Serum MDA levels at T1 (15 minutes after removal of the cross clamp) and T2 (30 minutes after removal of the cross clamp) were higher in the control group compared to the Vitamin group. Serum TAC levels at T1, T2 and T3 (60 minutes after removal of the cross clamp) were higher in the Vitamin E group compared to the control group. Histopathologic injury grade was lower in the Vitamin E group than in the control group. **Conclusion:** Vitamin E was found to be protective against reperfusion induced oxidative injury in the early operative period.

Key Words: Vitamin E; cardiopulmonary bypass; lung injury; reperfusion injury

ÖZET Amaç: Bu çalışmanın amacı, elektif koroner baypas cerrahisi sırasında, akciğerdeki potansiyel iskemi reperfüzyon hasarını önlemede E vitamininin etkinliğini değerlendirmektir. **Gereç ve Yöntemler:** Bu kontrollü, randomize, tek merkezli çalışma, elektif koroner arter baypas greftleme (KABG) operasyonu geçiren hastalarda yapıldı. Çalışmaya toplam 49 hasta alındı ve hastalar rastgele 2 gruba ayrıldı. Yirmi beş olguya aortik kross klemp yerleştirilmeden önce suda eriyen 100 mg E vitamini içeren serum fizyolojik, 24 olguya ise sadece serum fizyolojik juguler ven yoluyla verildi. Serum total antioksidan kapasitesi (TAK) ve serum malondialdehit (MDA) düzeyleri ölçüldü. Aortic kross klemp yerleştirimeden önce ve kross klempin alınmasını takiben 60. dakikada akciğer biyopsisi alındı ve elektron mikroskopu altında histopatolojik olarak değerlendirildi. **Bulgular:** Serum MDA değerleri karşılaştırıldığında, T1 (kross klemp kaldırıldıktan 15 dakika sonra) ve T2 (kross klemp kaldırıldıktan 30 dakika sonra) değerleri kontrol grubunda, E vitamini grubunun değerlerine göre daha yüksek saptandı. Serum TAK değerleri karşılaştırıldığında, T1, T2 ve T3 (kross klemp kaldırıldıktan 60 dakika sonra) değerleri E vitamini grubunda, kontrol grubuna kıyasla daha yüksek saptandı. E vitamin grubunda histopatolojik hasarlanmanın, kontrol grubundakine göre daha az olduğu saptandı. **Sonuç:** E vitamininin erken operatif dönemde oksidatif hasarın indüklediği reperfüzyona karşı koruyucu olduğu tespit edilmiştir.

Anahtar Kelimeler: Vitamin E; kardiyopulmoner baypas; akciğer hasarı; reperfüzyon hasarı

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Soon after the advent of cardiopulmonary bypass (CPB) in the 1950s, it became apparent that a substantial proportion of deaths after cardiac surgery was related to a syndrome of acute respiratory failure referred

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as "pump lung".[1] The "pump lung" refers to a complex phenomenon involving inflammatory responses mainly related to ischemia reperfusion injury. A burst of reactive oxygen species (ROS) appears immediately after reperfusion, which is responsible for cellular damage via lipid peroxidation, DNA loss and inactivation of many proteins.[2,3] The production of malonedialdehyde (MDA) is used as a biomarker to evaluate the level of oxidative stress in tissue.[4] Antioxidants terminate these chain reactions by removing free radical intermediates and inhibit other oxidation reactions.[5] We measured serum MDA levels for lipid peroxidation and serum total antioxidant capacity (TAC) levels, which reflected the oxidative injury caused by ischemia and reperfusion.

Although Vitamin E is well known to be a potent antioxidant which interrupts and neutralizes lipid peroxidation and free oxygen radical formation on the membrane, little is known about its role in lung protection during open heart surgery.[6,7] The purpose of this study was to investigate the effects of vitamin E in the protection of the lung from potential ischemia-reperfusion injury during elective coronary artery bypass graft (CABG) surgery by determining serum MDA, TAC levels and histopathologic examination by electron microscopy.

MATERIAL AND METHODS

This controlled randomized single-center study included patients who underwent elective CABG operation. Forty-nine EUROscoreII-matched patients were randomly allocated into two groups. Group 1 (n=25) received 100 mg water-soluble vitamin E (alpha-tocopherol;6-hydroxy-2,5,7,8-tetramethyl-chroman-2-carboxylic acid, 97% Sigma-Aldrich Chemie GmbH, Steinheim, Germany) and Group 2 (n=24) received only 100 mL tepid saline administered via the jugular vein. The dosage of vitamin E was determined after consulting with the Department of Pharmacology. The surgeon and the laboratory staff were blinded to the group members. Previous intake of vitamin E or other antioxidant, history of emergent surgery, valve surgery, cross clamp time less than 20 minutes, chronic renal failure, and the presence of hepatic or lung disease were the exclusion criteria. The study protocol was in accordance with the Helsinki Declaration of Human Rights and was approved by the local ethics committee. All patients were informed about the study protocol and informed consents were taken.

OPERATIVE TECHNIQUE

The anesthetic and surgery techniques were the same in all patients. CPB was established with an ascending aortic and a single right atrial cannula. After heparinization by bolus injection of 3 mg/kg, cardiopulmonary bypass circuit was initiated with a roller pump and non-pulsatile flow technique. Moderate hemodilution (hematocrit value 22-24%) and moderate hypothermia (nasopharyngeal temperature 28°C) were used during CPB. Pump flow rate during CPB was maintained at 2.4 l/m^2/min and mean arterial pressures were kept above 60 mmHg. CPB was performed using a membrane oxygenator (D 708 Simplex adult fiber oxygenator, Dideco, Mirando, Italy). Pump prime was a volume of 1 liter of 0.9% sodium chloride solution. Antegrade 10 ml/kg crystalloid cardioplegic solution (Plegisol, Abbot Laboratories, Chicago, IL,USA) at 4°C was delivered into the aortic root and further cardioplegic solution was administered in antegrade direction at intervals of about 20 minutes. Ringer's lactate solution at 4°C was used for topical hypothermia. Left internal mammary grafts were reconstructed to the left anterior descending arteries as the final anastomosis. Proximal anastomoses were implemented under partial aortic clamping on the beating heart.

Before the aortic cross-clamping, 100 mg water soluble vitamin E (alpha-tocopherol;6-hydroxy-2,5,7,8-tetramethyl-chroman-2-carboxylic acid, 97 % Sigma-Aldrich Chemie GmbH, Steinheim, Germany) in 100 ml tepid saline in the vitamin E group, and only 100 ml tepid saline in the control group were administered via the jugular vein.

SAMPLE COLLECTION AND ANALYSES

Blood samples were obtained from the radial artery at predetermined time points. Time points for collecting blood samples were:

T0: Before the aortic cross clamping

T1:15 minutes after removal of the cross clamp

T2:30 minutes after removal of the cross clamp

T3:60 minutes after removal of the cross clamp

The blood samples were transported to the laboratory on ice. Samples were immediately centrifugated (3000 x g) (Rotofix 32, Hettich Zentrifugen, Germany). Plasma was extracted and was kept at -80°C until the analysis time. Routine analyses were performed within 1 hour.

ASSAYS

Malondialdehyde (MDA) levels were measured as described by Gerritsen et al.[8] Total antioxidant capacity kits licensed by RANDOX (Randox Laboratories Ltd., UK) were used to measure the total antioxidant capacity with the Troloks equivalent antioxidant capacity (TEAC) method.

ELECTRON MICROSCOPY

All lung tissues were prefixed with 2.5% gluteraldehyde and were post-fixated with 1% osmium tetra oxide(OsO_4). Tissues were then washed with propylene oxide, were dehydrated in a graded series of ethanol, cleared in propylene oxide, and embedded in Epon for 24 h at 60°C. Thin slices were obtained by Raychert ultra microtome; they were stained with uranylacetate and Jem 100B and were analyzed under Jeol-10-10 electron microscope (Jeol Inc.,USA).

STATISTICAL ANALYSIS

Minitab software program (S0064 Minitab Release 13; Minitab Inc., UK) was used for statistical analyses. Categorical values were expressed as numbers and percentages and the numerical values were expressed as median (minimum-maximum). Following the Kolmogorov-Smirnov test to assess the distribution pattern of the continuous variables, Mann Whitney U and Wilcoxon Signed Rank test were used to compare groups. For the comparison of categorical variables, chi-square (χ^2) test and Fisher's exact test, where appropriate, were used. A p value less than 0.05 was considered significant.

TABLE 1: The clinical and operative characteristics of the patients.

Parameters	E vitamin group (n=25)	Control group (n=24)	p
Male	14(56.0%)	13(54.2%)	1.000
Age (year)	58.3±10.7	55.3±8.3	0.551
DM	5 (20.0%)	4 (16.7%)	1.000
HT	11 (44.0%)	8 (33.3%)	0.636
EUROScoreII	1.3±0.2	1.1±0.2	0.561
Smoking	13 (52.0%)	11 (45.8%)	0.884
Alcohol	7 (28.0%)	4 (16.7%)	0.543
EF (%)	53.3±10.9	56.5±9.1	0.450
Graft number	2.8±0.5	2.8±0.4	0.340
CCT (minute)	56.2±17.4	54.1±17.2	0.643
CPBT (minute)	105.1±26.3	94.9±26.8	0.714

CCT: Cross clamp time; CPBT: Cardiopulmonary bypass time; DM: Diabetes mellitus; EF: Ejection fraction; HT: Hypertension.

RESULTS

The preoperative and operative clinical characteristics of the patients were presented in Table1. There was no difference in age, diabetes mellitus (DM), hypertension (HT), smoking, alcohol use, ejection fraction, graft per operation, aortic cross-clamp time (CCT), and cardio-pulmonary bypass times.

BIOCHEMICAL ASSAYS

Serum MDA assay: The serum MDA levels of groups according to time were shown in Figure 1 and Table 2. T0 values were similar between groups [9.2 (6.8-14.3) vs. 11.2 (6.6-18.9), p=0.051]. In the control group, T1 levels were significantly higher than T0 levels [15.2 (10.4-17.1) vs 9.2 (6.8-14.3), p<0.001], T2 levels were higher than both T0 and T1 levels [19.6 (14.3-22.3) vs. 9.2 (6.8-14.3), 15.2 (10.4-17.1) respectively, p<0.001 and p<0.001] and T3 levels were higher than all T0, T1 and T2 levels [23.8 (18.7-26.5) vs. 9.2 (6.8-14.3), 15.2 (10.4-17.1), 19.6 (14.3-22.3); all p<0.001]. On the other side, there was significant difference between T0 and T1 levels of the Vitamin E group [11.2 (6.6-18.9) vs 12.9 (10.2-18.6), p=0.003]. T2 levels of the Vitamin E group was higher than T0 and T1 levels [16.4 (12.2-19.9) vs. 11.2 (6.6-18.9), 12.9 (10.2-18.6) re-

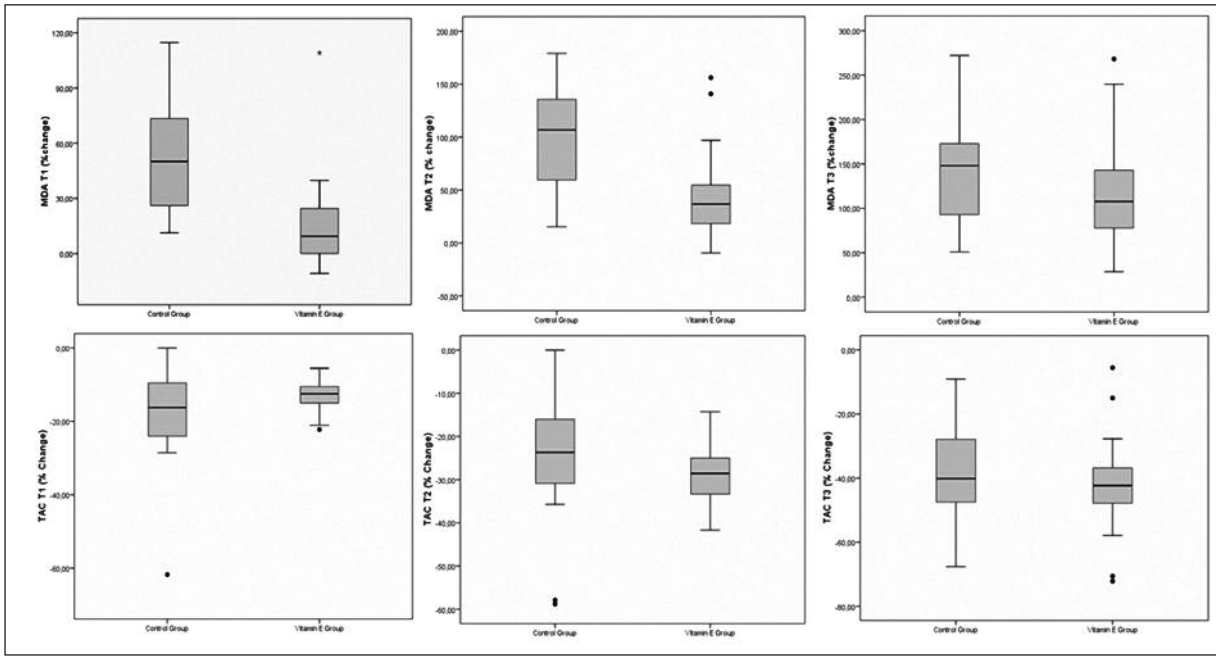

FIGURE 1: Box-plot diagrams of serum MDA and TAC values in the Vitamin E and control groups according to time.
MDA: Malonedialdehyde; TAC: Total antioxidant capacity.

TABLE 2: Serum levels of MDA and TAC according to time in the control and Vitamin E groups.

	T0	T1	T2	T3	p
MDA	Median (Min-max)	Median (Min-max)	Median (Min-max)	Median (Min-max)	p1=(T1-T0) p2=(T2-T0) p3=(T3-T0)
Control Group (n=24)	9.2 (6.8-14.3)	15.2 (10.4-17.1)	19.6 (14.3-22.3)	23.8 (18.7-26.5)	p1<0.001 p2<0.001 p3<0.001
E Vitamin Group (n=25)	11.2 (6.6-18.9)	12.9 (10.2-18.6)	16.4 (12.2-19.9)	24.3 (20.8-26.3)	p1=0.003 p2<0.001 p3<0.001
TAC					
Control Group (n=24)	1.7 (1-3.5)	1.3 (0.8-3.3)	1.2 (0.8-3.2)	1 (0.5-2.6)	p1<0.001 p2<0.001 p3<0.001
E Vitamin Group (n=25)	1.9 (1.4-2.6)	1.7 (1.2-2.3)	1.3 (1.0-1.9)	1.1 (0.5-1.7)	p1<0.001 p2<0.001 p3<0.001

T0: Baseline; T1:15 minutes; T3: 30 minutes
MDA: Malonedialdehyde; TAC: Total antioxidant capacity.

spectively, p<0.001 and p<0.001]. Similar to the control group, T3 levels in the Vitamin E group were higher than all the T0, T1 and T2 levels [24.3 (20.8-26.3) vs. 11.2 (6.6-18.9), 12.9 (10.2-18.6), 16.4 (12.2-19.9), respectively; for all parameters p<0.001]. T0 and T3 levels were not different between groups [for T0, 9.2 (6.8-14.3) vs. 11.2 (6.6-18.9), p=0.051; for T3, 23.8 (18.7-26.5) vs. 24.3

(20.8-26.3), p=0.302] but T1 and T2 levels of the control group was higher than T1 and T2 levels of the Vitamin E group [for T1, 15.2 (10.4-17.1) vs. 12.9 (10.2-18.6), p=0.001; for T2, 19.6 (14.3-22.3) vs. 16.4 (12.2-19.9), p<0.001]. Regarding the timely percent changes, considering T0 as the reference, all percent changes (% Change 15 minutes, % Change 30 minutes, % Change 60 minutes,) were significant in both groups according to time (Table 3).

Serum TAC assay: The serum TAC values according to the groups were shown in Figure 1 and Table 2. T0 values were similar between groups [1.7 (1.0-3.5) vs. 1.9 (1.4-2.6), p=0.058]. In the control group, T1 levels were significantly lower than T0 values [1.3 (0.8-3.3) vs 1.7 (1.2-2.3), p<0.001], T2 values were lower than both T0 and T1 values [1.2 (0.8-3.2) vs. 1.7 (1.0-3.5), 1.3 (0.8-3.3), respectively; p<0.001 and p<0.001] and T3 values were lower than all T0, T1 and T2 values [1.0 (0.5-2.6) vs. 1.7 (1.0-3.5), 1.3 (0.8-3.3), 1.2 (0.8-3.2); for all parameters p<0.001). On the other hand, there was significant difference between T0 and T1 values of the Vitamin E group [1.9 (1.4-2.6) vs 1.7 (1.2-2.3), p<0.001]. T2 values of the Vitamin E group was lower than T0 and T1 values [1.3 (1.0-1.9) vs. 1.9 (1.4-2.6), 1.7 (1.2-2.3), respectively; p<0.001 and p<0.001]. T3 values were lower than all the T0, T1 and T2 values [1.1 (0.5-1.7) vs. 1.9 (1.4-2.6), 1.7 (1.2-2.3), 1.3 (1.0-1.9); for all parameters p<0.001] in the vitamin E group. Comparing the two groups, T0 and T3 values were not different [for T0, 1.7 (1.0-3.5) vs. 1.9 (1.4-2.6), p=0.055; for T3, 1.0 (0.5-2.6) vs. 1.1 (0.5-1.7), p=0.059] but T1 values of the control group were lower than the T1 values of the Vitamin E group [1.3 (0.8-3.3) vs. 1.7 (1.2-2.3), p:0.001], whereas the T2 values of the control group were similar to T2 values of the Vitamin E group [1.2 (0.8-3.2) vs. 1.3 (1.0-1.9), p=0.076]. Regarding the timely percent changes, considering T0 as the reference, only % Change 30 minutes was significantly higher in the Vitamin E group (Table 3).

ELECTRON MICROSCOPY FINDINGS

Both semi-thin and thin sections were used and elecron microscopic results were scored by a histopathologist blinded to the groups, with a score of 0 representing normal histologic appearance; 1, vascular congestion; 2, vascular congestion and interstitial edema; 3, alveolar structural disturbance and infiltration of inflammatory cells; and 4, massive alveolar structural disturbance and infiltration of inflammatory cells.[9] The electron microscopic findings of the baseline biopsies of both groups revealed normal morphology of the lung tissue. Pulmonary biopsies at 60 minutes indicated a high degree of lung injury as outlined in table (Table 4) (Figure 2). When compared with the control group the Vitamin E group revealed less injury in terms of histopathological score (Table 4).

DISCUSSION

In this study, we showed that one dose of perioperative 100 mg i.v. Vitamin E was protective

TABLE 3: The percentage of change of serum MDA and TAC levels according to time in the control and Vitamin E groups.

	% Change T1 (baseline-15 minutes)	% Change T2 (baseline-30 minutes)	% Change T3 (baseline-60 minutes)
MDA	**Median (Minimum/Maximum)**	**Median (Minimum/Maximum)**	**Median (Minimum/Maximum)**
Control Group (n=24)	50,1 (11.3/114.7)	106.8 (15.3/178.1)	148 (50.8/272.1)
Vitamin E Group (n=25)	9.4 (-10.8/109.1)	36.7 (-9.5/156.2)	107.7 (28.6/268.2)
P	<0.001	<0.001	0.093
TAC			
Control Group (n=24)	-16.2 (-61.8/0)	-23.67 (-58.8/0)	-40.2 (-67.7/-9.1)
Vitamin E Group (n=25)	-12.5 (-22.2/-5.6)	-28.6 (-41.7/-14.3)	-42.31 (-72.2/5.6)
P	0.072	0.043	0.441

T0: Baseline; T1:15 minutes; T3: 30 minutes

MDA: Malonedialdehyde; TAC: Total antioxidant capacity.

TABLE 4: Results of histopathological examination.

		Histopathological Grade				
	n	0	1	2	3	4
CONTROL						
Baseline	24	24				
60 minutes	24			7	15	2
VITAMIN E						
Baseline	25	25				
60 minutes	25		2	15	7	1

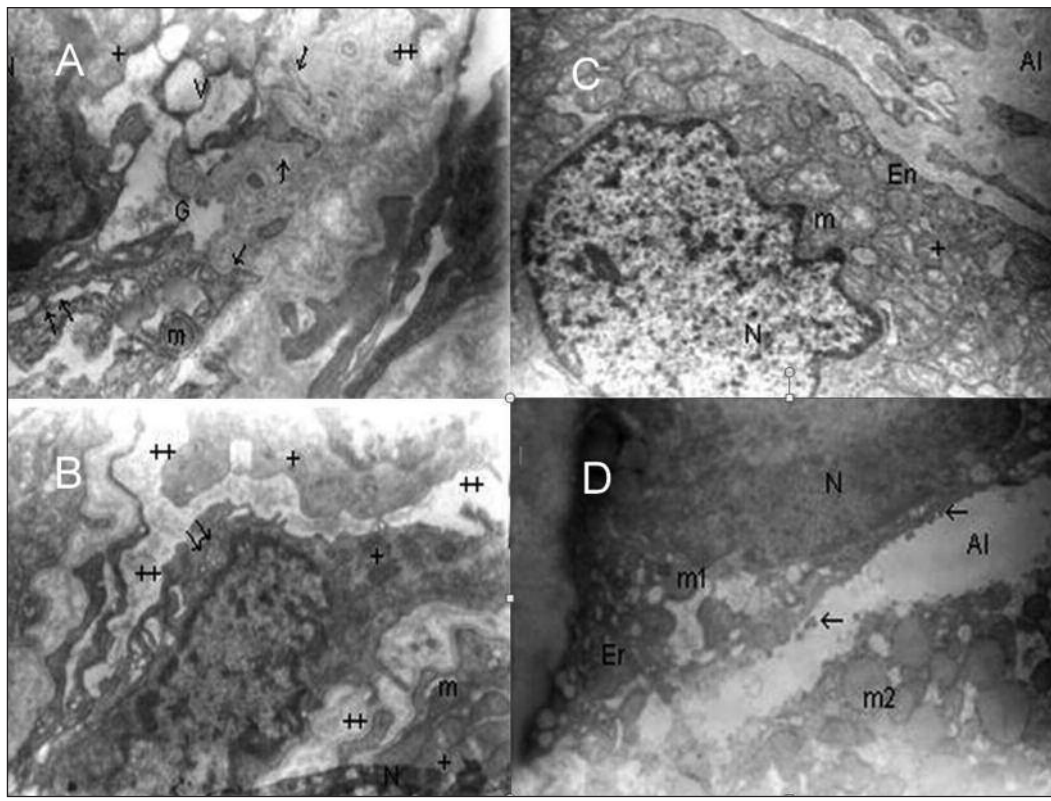

FIGURE 2: Electron microscopic views (x10.000) of pulmonary tissues.

A. Sixty minutes after removal of the cross clamp in the control group; (↓) an alveolocyte with hydrops in its microvillus; enlargement, distruction and combining into vacuoles (V) in the channels of the endoplasmic reticulum, interceptal edema and hydrops is seen. (+) alveolar epithelium; (++) interceptal region; (m) mitochondria; (G) golgi; (↓ ↓) ribosomes; (N) nucleus; B. Sixty minutes after removal of the cross clamp in the control group; (+) alveolar epithelial cells hydrops, (↓) protrusions hydrops, (↓ ↓) vacuolusations, (m) mitochondria & other intact. (++) Enlargement and edema of the septal lumens. (N) nucleus. C. Sixty minutes after removal of the cross clamp in the Vitamin E group. (+) alveolar epithelium (x 10.000) with interstitial edema. Mitochondria (m), endoplasmic reticulum (En), and other organelles in the cytoplasm are uneffected. N: Nucleus; Al: alveolar lumen D. Sixty minutes after removal of the cross clamp in the Vitamin E group. Two large alveolar epithelial cells (+) with their fragments and the lumen in between. Small processus (↓) and organelles in the cytoplasm of the alveolocyts are protected. Some of the mitochondria (m1) are normal while some are hydropsed (m2) but their outer membrane remained intact.

against reperfusion induced lipid peroxidation in early phases of reperfusion but not protective in late phases. In our knowledge, this is the first study to show the protective effects of iv. Vitamin E to protect the lung from CPB related injury in the literature.

Despite the improvements in CPB techniques, as well as the postoperative intensive care, impaired pulmonary function is a well known clinical entity resulting in increased morbidity and mortality.[1,10,11] There are many contributing factors such as anaesthesia, temporary cardiac dys-

function, infused catecholamines and altered mechanical of thoracic cage. A major contribution to pulmonary injury after CPB is attributed to free oxygen radicals arising from ischemia and reperfusion.[12,13] The rise of free radical levels is more intense in the early reperfusion period. After reperfusion, contact of the ischemic tissue with the molecular oxygen results in the formation of free oxygen radicals in a high proportion, and the longer the ischemic period lasts, the more the free radicals are produced.[14] In a healthy organism, oxidative stress and total antioxidation capacity is in a regular balance.[15] If the oxidative burden exceeds the antioxidant capacity of the tissue, it causes injury in the cells.[16-18]

An important chemical property of Vitamin E is its antioxidant activity, and its ability to neutralise peroxides and free oxygen radicals.[19] It is reported that Vitamin E has more powerful antioxidant capacity to prevent lipid peroxidation in the cells than other self-antioxidation systems (Vitamin C, glutathione peroxidase and beta carotene).[6,7,20] Although there is no report concerning the protective effects of Vitamin E against oxidative injury in humans, cardioselective analogs of alpha-tochopherol are used successfully in ischemic rat heart, where it reduced the infarct area and the enzymatic markers.[16] It is well known that antioxidant levels are seriously depressed during ischemia and reperfusion period.

In the present study, we found that vitamin E was protective against oxidative damage during the early reperfusion period. This may be attributable to its high antioxidant capacity and the high proportion of free oxygen radicals produced in early reperfusion. However, these protective effects decreased as the time passed. It is not clear wheather this effect might be related to the preoperative dosage given, because there is no report on a standardized scheme of dose for the parenteral use of vitamin E.[21] The TAC levels in the vitamin E group were higher than in the control group in the early phase of the operation, which implied that vitamin E had a major role in the serum TAC level. Although the levels of TAC and MDA were similar at 60 minutes, electron-microscopic examination revealed that there were more patients with grade 2 injury than those with grade 3 injury at 60 minutes in the vitamin E group, whereas in the control group, there were more patients with grade 3 injury. Actually, the early stage of reperfusion injury may have more detrimental effects on the organelles. This can be the proper explanation of increased damage in the control group compared to the Vitamin E group.

LIMITATION

One of the most important limitations of the present study was the small sample size in both groups. The dosage of vitamin E administered perioperatively was not based on a standardized scheme due to lack of data in the literature. Besides, the effective serum vitamin E level was not measured, which may be another limitation.

CONCLUSION

This study suggested that vitamine E histologically and biochemically was effective in ischemia and reperfusion injury in the lung depending on cardiopulmonary bypass in the early operative period. The patients undergoing cardiopulmonary bypass may benefit from i.v. perioperative vitamin E administration in terms of lung protection against oxidative injury.

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