The Effect of Coenzyme Q_{10} (Ubiquinone) on Random Pattern Skin Flap Survival in Rat Model

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Background: In this study, the effect of coenzyme Q_{10} (CQ_{10}) on flap survival was investigated.

Methods: Fifty Wistar Albino rats were divided into 5 groups. The survival rates of the skin flaps were assessed 10 days after complete elevation of the flaps. Regions of survival and necrosis were drawn on transparent acetate sheets and scanned into a computer. Tissue samples were assessed histopathologically after staining with hematoxylin-eosin, vascular endothelial growth factor staining and terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate-Biotin Nick End-labeling staining. To evaluate the antioxidant effect of CQ_{10}; malondialdehyde, nitric oxide levels were measured.

Results: Viable flaps area was found higher in groups 3 and 4 as compared to groups 1, 2, and 5. In terms of vascular proliferation, elevated angiogenesis was observed in pathological specimens of groups 3 and 4 as compared to groups 1, 2, and 5. Malondialdehyde levels in groups 3 and 4 were found to be significantly decreased as compared to groups 1, 2 and 5 ($P < 0.05$). Moreover, serum levels of CQ_{10} were found significantly increased in groups 3 and 4 ($P < 0.05$).

Conclusions: In conclusion, CQ_{10} significantly improves flap viability in rat model, and the highest levels of serum CQ_{10} can be obtained by oral administration.

Key Words: coenzyme Q_{10}, ubiquinone, flap survival, antioxidant effect, angiogenesis, apoptosis

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Random pattern skin flaps are frequently used for the repair of tissue defects. Length-to-width ratio of the random flaps is limited.[1–3] As the length of the flap increases, survival of the flaps decrease, and this eventually results in distal flap necrosis and tissue loss.[4] To enhance survival rate of flaps, studies focused on antioxidants, which increase the flap survival[5,6] and inhibit apoptosis.[7] Coenzyme Q_{10} (CQ_{10}) has been shown to neutralize the effects of free radicals, especially the superoxide radicals, after ischemia reperfusion.[8]

Coenzyme Q_{10} can directly interact with the free radicals or it can induce the production of other antioxidants by reducing tocopherol and ascorbate, thus showing antioxidant effect and inhibiting membrane lipid peroxidation. The role of CQ_{10} in the flap survival has not been assessed yet. In the present study, we aimed to investigate the effect of CQ_{10} on flap survival in a classic model of random pattern rat dorsal flap. We compared the results of oral, parenteral, and local administrations of CQ_{10} to determine the most useful application.

MATERIALS AND METHOD

Approval for this study was taken from Local Ethics Committee. The study was held by using total of 50 healthy, adult, and male Wistar Albino rats 105 to 120 days old, 250 to 350 g in weight. Subjects were randomly divided into 5 groups as each group received 10 subjects.

Rats were anesthetized by 30 mg/kg intramuscular ketamine hydrochloride and 10 mg/kg xylazine hydroklorid. After anesthesia application, 4 × 10 cm (ie, 1:2.5 ratio) axial flaps were raised by dissecting the areolar tissue at the level of the panniculus carnosus and deep fascia of the rats' dorsal musculatures by the method of "Mcfarlane flap."[9] Then, the flap was repositioned in its original position and was sutured with 4–0 monofilament nylon single stitches at 0.5-cm intervals (Figure 1). The dressings and wound care of rats were made carefully by daily cleaning with povidone-iodine. For the prophylaxis of infection, 150 mg/kg of single dose subcutaneous Ampicillin was administered to all groups.

Experimental groups were generated as follows:

Group 1 (control group): only wound care was done and no additional application was held.
Group 2 (stress group): after standard wound care, daily intraperitoneally 1 mL of physiological saline was administered to mimic stress.
Group 3 (intraperitoneal): 1 mL 10 mg/kg CQ_{10} solution was applied intraperitoneally by 27 gauge syringe once per day after wound care and 1 hour before surgery.
Group 4 (gavage): starting 3 hours before surgery, capsule form of CQ_{10} was dissolved in preheated physiological saline and administered as 150 mg/kg per day by 24 G branul (Mediflo) (by gavage) once per day.
Group 5 (local): starting 4 hours before surgery, after standard wound care with the help of cotton swabs, liquid form of CQ_{10} was mixed with olive oil, 1:1 ratio and drops containing CQ_{10} (100 mg) were applied to the entire surface of the flap.

At the 10th day of the experiment, rats were prepared for surgical operation and anesthetized by the same method and 0.5 × 0.5 cm of tissue samples were taken from distal, middle, and proximal one third portions of dorsal flap regions. Also, intracardiac 5 mL blood samples were taken, and the experiment was terminated. Serum of the blood samples were separated by centrifuging at 5000 rpm for 5 minutes and subjected to biochemical analysis. To evaluate tissue malondialdehyde (MDA) and nitric oxide (NO) levels, 1 × 1 cm of tissue samples were taken from the middle portion of the dorsum of subjected to biochemical analysis.

Clinical Evaluation

In clinical evaluations, the survival rates of the skin flaps were assessed 10 days after complete elevation of the flaps. Regions of survival and necrosis were drawn on transparent acetate sheets and scanned into the computer. Regions of survival and necrosis were dyed in different colors, and survival rates were assessed by planimetric analysis as follows:

$$\text{Flap survival rate} = ((\text{Area of survival})\%(\text{Total flap area})) \times 100$$

Histomorphological Examination

Tissue samples were fixed in 10% formalin and embedded in paraffin blocks. Four-micron sections were obtained by microtome. Sections were stained by the methods of hematoxylin-eosin, vascular endothelial growth factor (VEGF) and terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate-biotin nick end-labeling (TUNNEL) and examined under light microscope. In histopathological evaluations of tissue samples, parameters of polymorphonuclear

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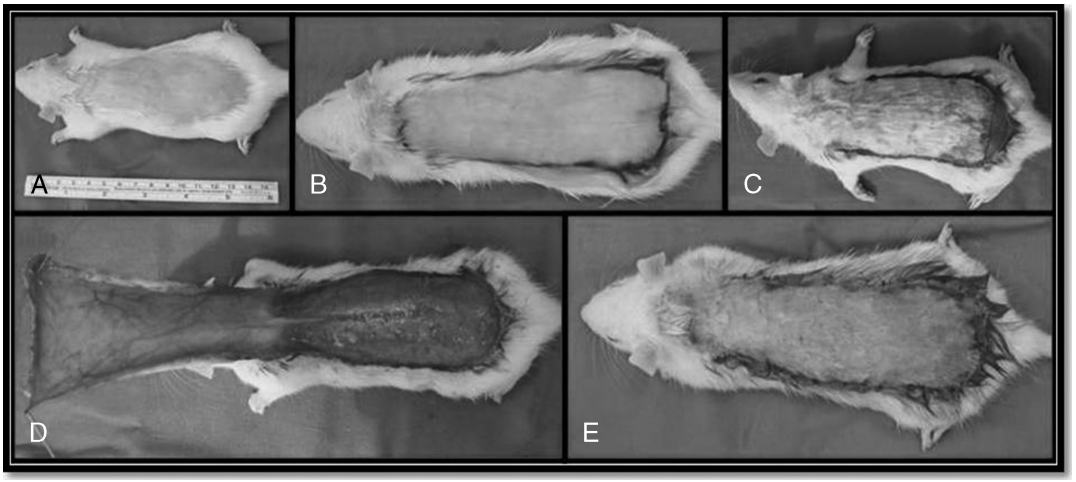

FIGURE 1. A, After anesthesia application, the back of rats is trimmed. B, Drawing of the Mcfarlane flap was performed. C, Then, 4 × 10 cm flaps were removed including "Pannikulus Karnosus" muscle. D, Flaps were then separated from the skin and "Pannikulus Karnosus" muscle which is located under skin by moving on loose anatomical structures of muscle. E, Anatomical structures were sutured by 4–0 nonabsorbable nylon monofilament suture with sharp needle.

leukocyte (PMNL), lymphocyte and fibroblast proliferation, collagen density were assessed by hematoxylin-eosin. To demonstrate differences in the angiogenesis, VEGF staining was used, and the total numbers of vessels were counted. Numbers of cells undergoing apoptosis in tissue sections were detected by TUNNEL staining. All these cell numbers was evaluated by using the scoring system numbered 0 through 3. The average results for each subject for proximal, middle, and distal portions of the flap were evaluated, and comparisons between groups were made.

Biochemical Analysis

At the end of the experiment, serum levels of CQ_{10} were evaluated. For all groups, the relationship between the amount of CQ_{10} and the flap necrosis was assessed. To evaluate the antioxidant effect, tissue samples were homogenized, and MDA and NO were determined.

Statistical Analysis

For comparison of the clinical, histopathological and biochemical data SPSS (The Statistical Package for Social Sciences) 15.0 statistical package software was used. A χ^2 test was used for comparisons between categorical variables. The distributions of the continuous variables were tested using the Kolmogorov-Smirnov test. Comparisons of continuous variables between more than 3 groups were performed by using 1-way analysis of variance (post hoc Tukey method). *P* values of less than 0.05 were considered statistically significant at 95% confidence interval.

RESULTS

Clinical Findings

Study was completed in 10 days after the operation. In postoperative follow-up period, infection was observed in only 1 subject of group 4 and that study subject was excluded from the study and substituted with new one. No complication was encountered during the experiment.

Flap suture line healing was faster in parenteral and gavage groups as compared to other groups. Average viable and necrotic flap area in groups were presented in percentages (%) (Table 1). Because of our clinical observations, viable flap areas were higher in groups 3 and 4 as compared to groups 1, 2, and 5 (Fig. 2).

Histomorphological Findings

All data were determined as mean and standard deviation. The results of PMNL, TUNNEL, and VEGF, which indicate the inflammatory reaction, the total number of apoptotic cells, angiogenesis, and vascular proliferation, respectively, are given in Table 2.

In terms of PMNL and apoptosis in tissue samples, increased levels of inflammation and apoptosis were observed in groups 3 and 4 as compared to groups 1, 2, and 5. There was no significant difference between groups 1, 2, and 5. In terms of vascular proliferation, elevated angiogenesis was observed in pathological specimens of groups 3 and 4 as compared to groups 1, 2, and 5. Also, there was no significant difference between groups 1, 2 and 5 in terms of angiogenesis. No significant difference was observed between the groups with respect to amounts of collagen, fibroblasts, and lymphocytes infiltration. Also, no significant levels of difference were observed in between groups 3 and 4 in terms of all histopathological examinations (Fig. 3).

Biochemical Findings

In 1 g of tissue specimens, NO levels were statistically similar in groups 1, 2, and 5, these results were higher compared to the serum levels of groups 3 and 4 (Table 3). The MDA levels were found to be significantly decreased in groups 3 and 4 as compared to groups 1, 2, and 5 (Table 3). The CQ_{10} levels were significantly higher in groups 3 and 4 compared to the serum levels in groups 1, 2, and 5 (Table 3).

DISCUSSION

Despite recent advancements in flap surgery to improve the viability of flaps, it is well known that every tissue has regions with random patterns and insufficient levels of circulation. Thus, efforts to increase

TABLE 1. Average Viable and Necrotic Flap Area in Groups (Values in %)

Subject Group	Average Viable Flap Area, %	Average Necrotic Flap Area, %
Group 1	49.75	50.25
Group 2	50.15	49.85
Group 3	74.45	25.55
Group 4	73.05	26.95
Group 5	59.64	40.36

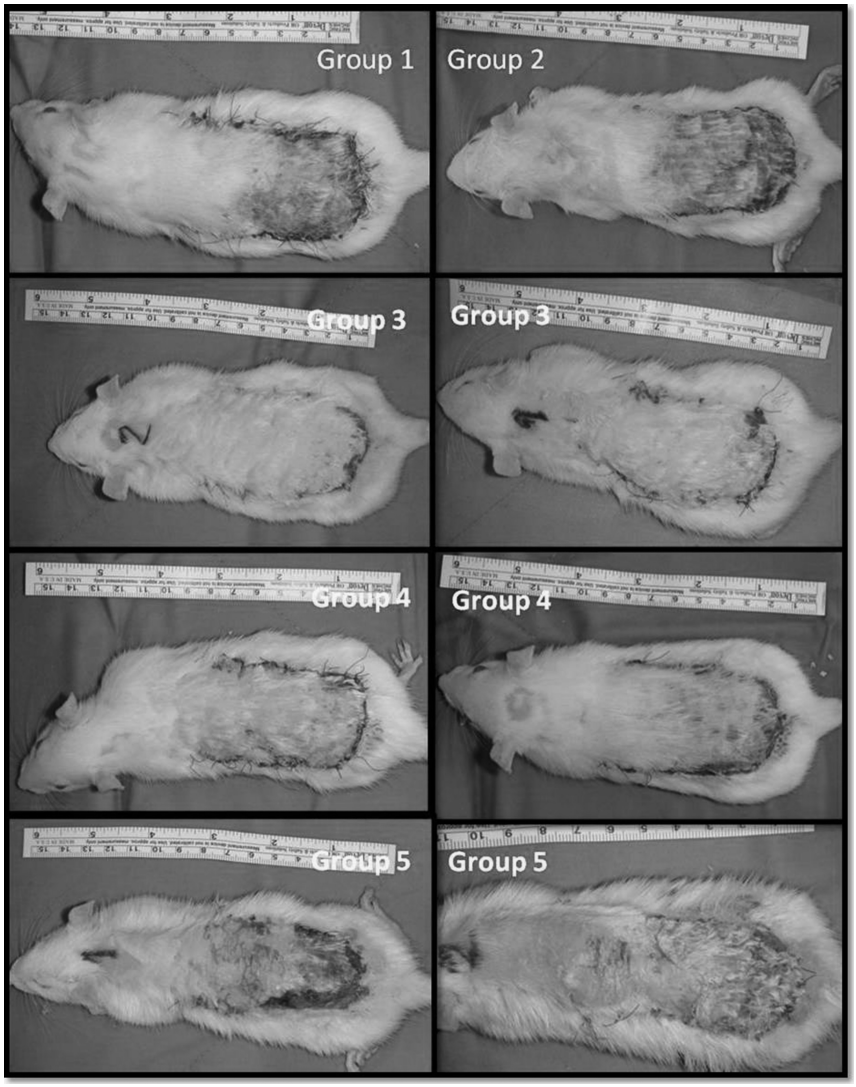

FIGURE 2. Group 1: after the flap is removed; only wound care was held. Group 2: intraperitoneally physiological saline injected group. Group 3: coenzyme Q10 is administered intraperitoneally. Group 4: coenzyme Q10 is administered orally. Group 5: coenzyme Q10 is administered locally.

the blood supply and increase viability of flaps are still continuing. In many hemodynamics studies after flap elevation, even if the blood flow maintains in flap pedicle, the flow in the flap tip decreases 20% below the normal levels within first 6 to 12 hours. During the first 12 to 18 hours, leukocyte-mediated endothelial damage, release of sympathetic vasoconstrictors, and combination of decreased perfusion pressure flow dramatically decreases especially in distal flap regions. When tip regions are exposed to severe ischemia for 6 to 12 hours, flow return causes reperfusion damage, and tissue necrosis results in cellular changes in micro levels.[10,11] During reoxygenation that occurs after ischemia period, xanthine dehydrogenase enzyme gains oxidase activity and initiates the formation of toxic oxygen radicals (superoxide anion). The toxic superoxide radicals may directly show cytotoxic effects, or more importantly, they are involved to trigger adhesion and accumulation of leukocytes in which the following events lead to local acute inflammation, endothelial damage, and failure of microcirculation by cellular events.[12]

Rapid neutrophil accumulation in the arteries causes progressive decrease in perfusion and leads to ischemia-reperfusion–associated "no-reflow phenomenon."[13] There are studies in which increased levels

TABLE 2. The Results of PMNL, TUNNEL, VEGF

	Group 1	Group 2	Group 3	Group 4	Group 5
PMNL, mean ± SD	1.50 ± 0.97	1.50 ± 0.85	0.40 ± 0.69	0.50 ± 0.70	1.50 ± 0.70
TUNNEL, mean ± SD	1.60 ± 0.69	1.70 ± 0.82	0.50 ± 0.52	0.30 ± 0.48	1.60 ± 0.96
VEGF, mean ± SD	0.90 ± 0.73	1.00 ± 0.66	2.10 ± 0.73	2.00 ± 0.66	0.80 ± 0.78

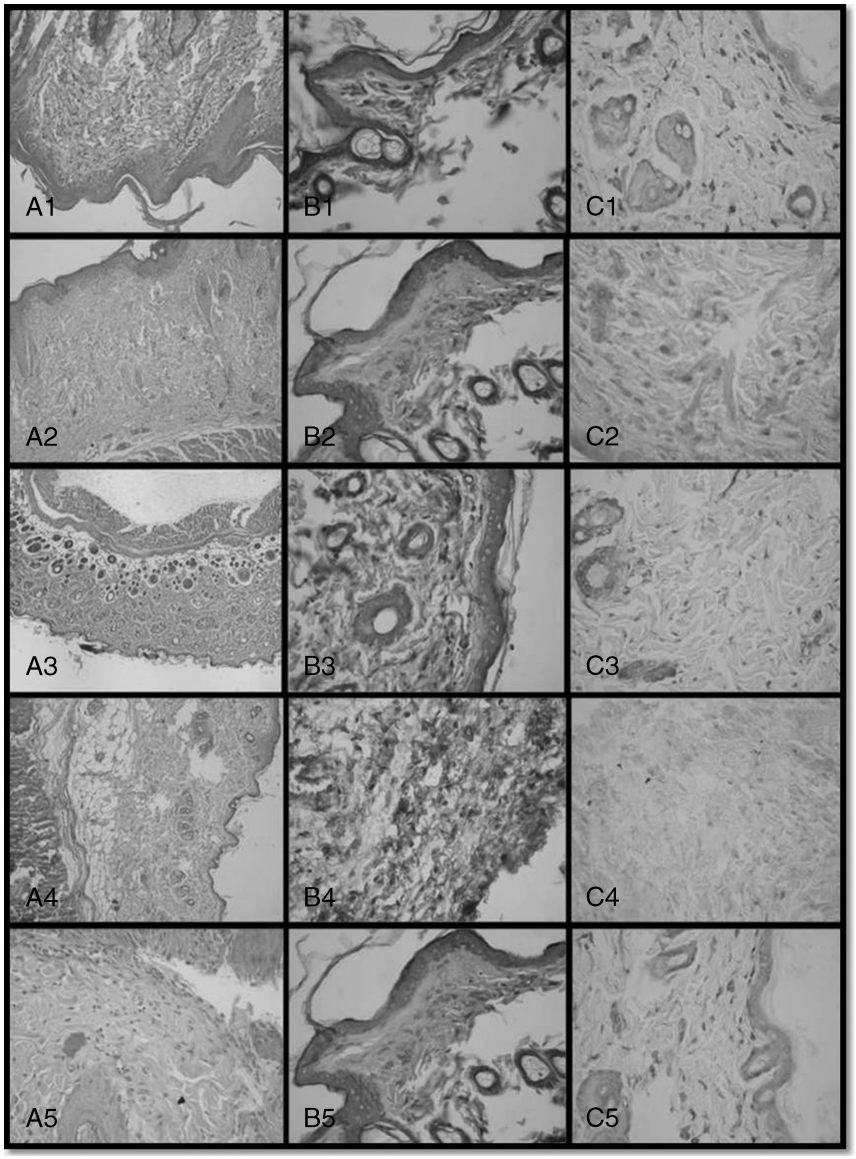

FIGURE 3. A1–5 (groups 1–5), In histopathological evaluations of tissue samples under light microscope, parameters of PMNL, lymphocyte, and fibroblast proliferation, collagen density were assessed by hematoxylin-eosin. B1–5 (groups 1–5), To demonstrate differences in the angiogenesis, VEGF, staining was used and total numbers of vessels were counted. C1–5 (groups 1–5). Number of cells undergoing apoptosis in tissue sections was detected by TUNNEL staining.

of malonyldialdehyde, an indicator of the formation of xanthine oxidase and another free radical, was observed in the distal parts of the flap.[14–16] A hemodynamic, anatomic, and metabolic change in flap removal determines the flap viability. In our study, the decrease in the levels of MDA in groups 3 and 4 were probably because of the antioxidant effect of CQ_{10}.

TABLE 3. Evaluation of MDA, NO, and CQ_{10} Values Within Groups

	MDA*, nmol/g tissue	**NO***, nmol/g tissue	**CQ_{10}***, ng/mL
Group 1	72.60 ± 7.25	72.60 ± 7.25	234 ± 75.25
Group 2	73.83 ± 8.98	73.83 ± 8.98	212.40 ± 65.49
Group 3	52.67 ± 7.67	52.67 ± 7.67	401.4 ± 134.85
Group 4	53.59 ± 8.44	53.59 ± 8.44	683.1 ± 142.44
Group 5	70.20 ± 9.88	70.20 ± 9.88	159.4 ± 58.62

*1-way analysis of variance test $P < 0.0001$ within groups.

In light of this information, many studies were conducted to increase flap viability by reducing superoxide radicals. Manson et al[15] demonstrated that flap survival is increased by superoxide dismutase and analogs. In a study conducted by Bekerecioğlu et al,[5] vitamin E, vitamin C, and desferrioxamine and ginkgo biloba extract were administrated to caudal dorsal flap in rats, and they found significantly decreased levels of flap necrosis in vitamin C and desferrioxamine treated groups. Suzuki et al[17] reported that superoxide dismutase is effective in the prevention of distal flap necrosis.

Coenzyme Q_{10} is a powerful antioxidant that plays a role in stabilizing the membrane by participating in the structure of lipid membranes that surround the cells.[17] Coenzyme Q_{10} prevents peroxidation of lipids and proteins.[18] Reduced CQ_{10} form functions as scavengers of hydrogen peroxide and hydroxyl radical.[19,20] Coenzyme Q_{10} regulates membrane fluidity as a lipid antioxidant and makes radical forms of E and C vitamins available again and protects membrane lipids against peroxidation. Some investigators believe that antioxidant activity of CQ_{10} is similar to vitamin E,[21] and some investigators suggest that CQ_{10} shows its effect synergistically by acting with vitamin E and C.[22] Moreover, CQ_{10} has been found to be effective in the treatment of ischemia reperfusion injury in experimental models.[23]

In the present study, in contrast to the literature, we detected that highest level of CQ_{10} was in gavage-applied group and the second highest level of CQ_{10} was in intraperitoneally applied group. Also, in locally applied group, no such significant increase was observed as compared to control group. The high concentration levels of CQ_{10} in the oral gavage group may be because of the high liposolubility of CQ_{10} in which it undergoes enterohepatic circulation. Local administration of CQ_{10} failed to provide adequate blood levels. To increase absorption in local administration, there is a need for new investigations. After ischemia, macrophages and neutrophils produces NO and superoxide radicals in high amounts. In ischemia, NO shows bidirectional functions. First, with the activity of endothelial-derived nitric oxide synthase I, it plays role in tissue protection, maintaining of baseline blood flow, inhibition of platelet aggregation, and neutrophil adhesion. On the other hand, excessive NO production by the stimulation of nitric oxide synthase II (NOSII) leads to cytotoxicity. The excess NO production because of the increased activity of NOSII causes tissue damage by generating free radicals, such as peroxynitrite.[24] Also, NO inhibits apoptosis by protecting against effects of apoptosis-stimulating factors, such as tumor necrosis factor. Nitric oxide increases the proliferation and migration of endothelial cells. thus[25] increasing angiogenesis by playing a central role. In higher amounts of NO, cellular respiration is inhibited irreversibly by the production of peroxynitrite. This in turn leads to oxidative stress and vascular pathologies.[17,25]

In our study, tissue levels of NO were lower in groups 3 and 4 as compared to groups 1, 2, and 5. In this case, we believe this can be because of the antioxidant and radical scavenging effect of CQ_{10}. Especially, we believe that tissue damage can be reduced by the inhibition of NO which is produced by the activity of NOSII as a result of PMNL activation in ischemia. Previous studies revealed that sufficient levels of NO affect angiogenesis and flap viability by increasing VEGF.[26] In our study, to evaluate the levels of angiogenesis, histopathological tissue biopsies were stained with VEGF. The NO levels were significantly lower in groups 3 and 4 than the other 3 groups. In groups 3 and 4, angiogenesis levels were found to be increased as compared to the other groups. This can be resulted from high levels of NO which is induced by ischemia. The high levels of NO increased the levels of free oxygen radicals, and inflammation therefore, negatively affected tissue damage and neovascularization.[24] Nitric oxide plays an important role in the modulation of apoptosis of vascular cells. Although their low level protects endothelial cells against apoptosis stimulating factors, such as tumor necrosis factor, it causes apoptosis in high doses.[25] The decreased levels of NO in groups 3 and 4 when compared with the other groups caused less apoptosis than the others (groups 1, 2, and 5). Nitric oxide also plays a central role in angiogenesis by increasing proliferation and migration of endothelial cells. It has been proven that the oxidative mechanism plays a major role in apoptotic cell death.[25] In a study conducted by Alleva et al,[7] it was reported that CQ_{10} prevented the apoptosis triggered by chemical mechanisms using its antioxidant effects in mitochondria. Mitochondrial permeability transition pore function provided by CQ_{10} and ultimately the collapse of the mitochondrial membrane has been shown to cause apoptosis.[7,27] As consistent with these results, we used TUNNEL staining for the examination of apoptosis, thus we revealed that apoptosis was significantly reduced in groups 3 and 4 as compared to other experimental groups.

Choi et al[28] studied wound healing in rats by performing skin incisions and evaluated the effect of CQ_{10} in wound healing. They have shown that myeloperoxidase activity was significantly reduced in CQ_{10} administrated group. Thus, it has been suggested that CQ_{10} had anti-inflammatory effects. In our study, the histopathologic evaluations revealed reduced PMNL levels observed in groups 3 and 4 as compared to the other groups. In other words, the high levels of CQ_{10} in groups 3 and 4 resulted with lower degrees of inflammatory reactions. In the same study, they indicated that collagen levels and fibroblast proliferation were found to be significantly increased as compared to control group. Yet, in our study, there was no significant difference between groups in terms of collagen levels and fibroblast proliferation. Because of the anti-inflammatory effects of CQ_{10}, we also histopathologically examined lymphocytic infiltration, and no significant difference was observed between groups, suggesting that anti-inflammatory effect can only be effective on cells of myeloid lineage.

Coenzyme Q_{10} behaves like an antioxidant and prevents peroxidation of lipids and proteins.[18] Reduced CQ_{10} form functions as scavengers of hydrogen peroxide and hydroxyl radical.[19,20] Puntel et al[29] used MDA levels as an indicator of lipid peroxidation in their study. Based on this finding, we used MDA levels as an indicator of antioxidant activity and lipid peroxidation. The MDA levels were significantly lower in groups 3 and 4 as compared to groups 1, 2, and 5. Our study results proved the earlier findings.

As consistent with results of the histopathological and biochemical examinations, necrosis areas were significantly reduced in groups 3 and 4 which have high levels of CQ_{10} as compared to other groups. In addition, although blood values of gavage CQ_{10} administrated group and intraperitoneally CQ_{10} administrated group did differ, there was no such difference in terms of flap viability, suggesting that CQ_{10} can be administrated orally in lower doses. There was no significant difference found in serum MDA and NO levels between gavage and intraperitoneal application of CQ_{10}

Also, CQ_{10} reduces the PMNL numbers thus, exerts anti-inflammatory effect. In addition, by keeping NO levels at the physiological level, it increases positive features of NO and reduces membrane lipid peroxidation and apoptosis by lowering MDA levels. As a result, this study has shown that CQ_{10} can potentially be used to increase the success in flap surgery. However, further clinical studies are needed to prove it. Also, optimal range of drug dose and way of administration should be investigated in humans.

CONCLUSIONS

In conclusion, CQ_{10} significantly improves flap viability in rat model, and the highest levels of serum CQ_{10} can be obtained by oral administration.

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