MARMARA
MEDICAL JOURNAL

Original Article

https://dergipark.org.tr/tr/pub/marumj

Healing effects of L-carnitine on experimental colon anastomosis wound

Emel KANDAS[1], Mustafa EDREMITLIOGLU[1], Ufuk DEMIR[1], Guven ERBIL[2], Muserref Hilal SEHITOGLU[3]

[1] Department of Physiology, Faculty of Medicine, Canakkale Onsekiz Mart University, Canakkale, Turkey
[2] Department of Histology and Embryology, Faculty of Medicine, Dokuz Eylul University, Izmir, Turkey
[3] Department of Biochemistry, Faculty of Medicine, Canakkale Onsekiz Mart University, Canakkale, Turkey

Corresponding Author: Mustafa EDREMITLIOGLU
E-mail: gymedr@yahoo.com

Submitted: 13.10.2022 **Accepted:** 16.01.2023

ABSTRACT

Objective: The purpose of this study is to examine the effects of L-carnitine on healing of experimental colon anastomosis injury in early and late period.

Materials and Methods: Forty female Wistar-Albino rats were used in this study. The rats were divided into 4 groups (CONT-3, CONT-7, CARN-3, and CARN-7). Injury healing was evaluated for CONT-3 group on the 3rd day and for CONT-7 group on the 7th day following the anastomosis. Following the operation, CARN-3 and CARN-7 groups were intraperitoneally administered with 100 mg/kg/day L-carnitine and injury healing was evaluated on the 3rd and 7th days. Injury strength, histological evaluation and antioxidant enzyme activities and oxidant damage were determined in tissue samples of anastomosis area.

Results: Bursting pressure levels and histological scoring values of CARN-3 group were found to be higher than the CONT-3 group ($p<0.05$). Antioxidant enzyme activities were found to be high in groups which were administered with L-carnitine, and oxidant damage was found to be significantly low in CARN-7 group ($p<0.05$).

Conclusion: It was seen that L-carnitine speeds up the injury healing process and increases the injury strength and antioxidant capacity in early period. Increase in antioxidant enzyme activities was observed to be continued in late period as well.

Keywords: L-carnitine, Colon anastomosis, Antioxidant, Bursting pressure

1. INTRODUCTION

Colorectal surgery is being widely used in colorectal cancers as well as ischemic colitis, ulcerative colitis, Crohn disease, mechanical intestinal obstruction, trauma, and recurrent diverticulitis [1]. Many serious and fatal complications can be seen in colon surgeries as well as in other major surgeries. Incidence of complications is 10-30% [2]. There are still cases which end up with death after taking out the tumor and performing end-to-end anastomosis for the remaining parts when the anastomosis area cannot heal completely and there is a leakage. Therefore, full and rapid healing of the anastomosis is very important.

One of the molecules effective in healing of injuries is L-carnitine [3,4]. L-carnitine is an aminoacid that has the structure of 3-hydroxy-4-N-trimethylaminobutyric acid. It facilitates the entry of long chain fatty acids into the mitochondrion and provides β-oxidation and consequently revealing of energy [5]. It was shown that L-carnitine restores membrane lipid bilayer by an indirect antioxidant effect [6,7]. Furthermore, it was suggested that L – carnitine is also effective in directly inactiviating superoxide and hydrogen peroxite [3,8,9]. It was determined that L-carnitine speeds up healing of skin injuries even under suppression of the immune system [10].

There are several studies suggesting that antioxidant activity is important in injury healing [11-17]. It was shown that antioxidant activity may also be important in surgical injury healing after colon anastomosis [12]. It may be suggested that L-carnitine contributes to injury healing after colon anastomosis by taking into consideration the positive effects of antioxidant

How to cite this article: Kandas E, Edremitlioglu M, Demir U, Erbil G, Sehitoglu HM. Healing effects of L-carnitine on colon anastomosis wound on experimental colon anastomosis. Marmara Med J 2023: 36(2):215-222. doi: 10.5472/marumj.1307971

ISSN: 1309-9469

http://doi.org/10.5472/marumj.1307971
Marmara Med J 2023;36(2): 215-222

activity on injury healing together with the fact that L-carnitine has an antioxidant effect.

Therefore, we examined how L-carnitine affects injury healing after colon anastomosis. For this purpose, we administered L-carnitine intraperitoneally to the rats, which were applied colon anastomosis, beginning from the date of anastomosis. We examined the effects of the treatment on healing of surgical injury in early period (3rd day after anastomosis) and late period (7th day after anastomosis).

2. MATERIALS and METHODS

Experimental Animals and Environment

The project was found in compliance with ethical board directives and approved by Canakkale Onsekiz Mart University Ethical Board of Animal Testing (Approval No. Of the Ethical Board: 2013/09-10).

In this study, 40 female Wistar-Albino rats of 6-8 weeks old were used. During the experiment period, the rats were observed in the laboratory with 21±3 °C constant room temperature, 60% humidity, and 12 hours day and night cycles. For their nutrition standard animal feed and tap water were used.

Experimental Groups

The rats were randomly divided into 4 groups, each group having 10 rats. The number of subjects in the groups used in the study was determined according to the "resource equation" method [18,19]. The groups were formed as follows:

Control 3 days (CONT-3): The rats were applied colon anatomosis and were injected with physiological saline solution intraperitoneally for 3 days. Injury healing was observed after 3 days (Control 3 days).

Control 7 days (CONT-7): The rats were applied colon anatomosis and were injected with physiological saline solution intraperitoneally for 7 days. Injury healing was observed after 7 days (Control 7 days).

L-carnitine-3 (CARN-3): The rats were applied colon anatomosis and were injected with 100 mg/kg L-carnitine [10] intraperitoneally for 3 days. Injury healing was observed after 3 days (L-carnitine 100 mg/kg 3 days).

L-carnitine-7 (CARN-7): The rats were applied colon anatomosis and were injected with 100 mg/kg L-carnitine intraperitoneally for 7 days. Injury healing was observed after 7 days (L-carnitine 100 mg/kg 7 days).

Performing Colon Anastomosis

Animals used in this study were anesthetized by subcutaneous injection of a mixture of Xylazine (5 mg/kg Rompun ®) and Ketamine (50 mg/kg-Ketalar®) following a 12-hour fasting [20,21].

Anesthetized animals were lied down on their back on the table with their abdomens facing up. For hygienic reasons, their abdominal region was shaved and cleaned with 10% povidone iodine. Anastomosis in the left colon region was performed in accordance with our previous study [22].

Actions Carried Out After the Anastomosis

The rats in CONT-3 group and CONT-7 group were injected with physiological saline solution intraperitoneally for 3 days and 7 days, respectively. The rats in CARN-3 group and CARN-7 group were injected with 100 mg/kg L-carnitine intraperitoneally for 3 days and 7 days, respectively.

Subjects were anesthetized by using either on the 3rd (CONT-3 and CARN-3) and the 7th (CONT-7 and CARN-7) day. The animals were sacrificed after blood samples were taken by cardiac puncture. Abdomens of the subjects were opened and 2 cm distal and 2 cm proximal of the anastomosis and intestine including the anastomosis line were resected and anastomosis bursting pressure at the anastomosis line was measured. After this measurement, colon including 0.5 cm distal and 0.5 cm proximal of the anastomosis was resected. After that, the samples taken were put into a – 80 °C freezer for measurement of malondialdehyde (MDA) level, superoxide dismutase (SOD) activity, catalase activity, and hydroxyproline level.

Anastomosis Bursting Pressure Measurement

After the fecal content in the intestinal segment which was resected as described above, was washed with physiological saline solution and removed, one end of the colon was attached to the infusion pump and the other end was attached to the pressure transducer (Biopac MP 35 Data Acquisition System, USA) by 2/0 silk suturing. After the distal catheter was attached to the pressure transducer in the data collecting system, physiological saline solution infusion was made from the proximal catheter at a 4 ml/min speed via infusion pump. Meanwhile, pressure level reached during the bursting of anastomosis location was determined by recording changes in pressure in the intestinal segment continuously.

Measurement of Hydroxyproline (HPR) Level

Hydroxyproline in tissue samples was measured by hydroxyproline kit (Sigma-Aldrich® Hydroxyproline Assay Kit MAK008, St. Louis, MO, USA) spectrophotometrically at 560 nm as defined in the kit prospectus. Briefly, hydroxyproline concentration is determined by the reaction of oxidized hydroxyproline with 4-(dimethylamino)benzaldehyde (DMAB), which results in a colorimetric (560 nm) product, proportional to the hydroxyproline present. We homogenized aproximately 10 mg tissue in 100 µL of distiled water and added 100 µL of 12M hydrochloric acid to hydrolyze at 120 °C for 3 hours. After centrifugation at 10,000 g for 3 minutes, we transferred 50 µL of supernatant to a 96-well plate. We added 100 µL of the Chloramine T/Oxidation Buffer Mixture to each sample and standard well. Then, the sample plate was incubated at room temperature for 5 minutes. After adding 100 µL of the diluted DMAB Reagent to each sample and standard well and incubate for 90 minutes at 60 °C. Measurement was carried out the absorbance at 560 nm (A560).

http://doi.org/10.5472/marumj.1307971
Marmara Med J 2023;36(2): 215-222

Measurement of Malondialdehyde (MDA) Level

Tissue MDA levels were determined according to the spectrophotometric method defined by Yagi [23]. The samples were kept frozen at – 80 °C until the working day. After the tissues dissolved at 4°C were weighed on a precision scale, homogenization was performed. 10% TCA (Trichloroacetic acid) and 0.675% TBA (Thio barbituric acid) were used in the determination. MDA was determined by spectrophotometric measurement at 532 nm of the pink colored complex formed by MDA, which is the end product of lipid peroxidation, which occurs as a result of incubation of tissue homogenate in a boiling water bath at pH: 3.5 in an aerobic environment for one hour.

Measurement of Superoxide Dismutase (SOD) Activity

Superoxide dismutase activity in tissue samples obtained was measured spectrophotometrically at 440-460 nm as defined in the kit prospectus by using SOD kit (Cayman Chemical Company Superoxide Dismutase Assay Kit 706002, USA). The tissues firstly were ringed by phosphate buffer saline (PBS – pH=7.4). After adding 10mM, pH=7.2 of cold HEPES buffer containing 1mM EDTA, 210mM mannitol and 70mM sucrose per gram tissue, we homogenized the tissue by Retsch MM400 (Haan, Germany). Then all samples were centrifugated at 1500 g, 5 minutes at 4°C. We obtained the supernatant containing extracellular and cytosolic SOD for assay. The pellet contains mitochondrial SOD. We diluted 20 µL of SOD standard with 1.98 mL of sample buffer. From the stock solution we prepared, we pipetted 10 µL of standard at various concentrations into the empty wells of the plate. Likewise, 10 µl of each sample was pipetted. Then 200 µl of radical detector was added to them. 20µl of diluted xanthine oxidase was added to all wells. The plate was incubated for 30 minutes at room temperature with gentle shaking. Absorbances were measured at 460 nm with a plate reader. SOD activities of all samples were obtained from the standard graph.

Measurement of Catalase (CAT) Activity

Catalase activity in tissue samples obtained was measured spectrophotometrically at 540 nm as defined in the kit prospectus by using catalase kit (Cayman Chemical Company Catalase Assay Kit 707002). Briefly, we washed the tissues in phosphate buffer with pH=7.4. Then, 50mM, pH=7.0, potassium phosphate buffer containing 1mM EDTA per gram tissue was added. Supernatants were taken by centrifugation at 10000 g for 15 minutes at 4°C, and the study was carried out by preparing the reagents in the kit as specified in the procedure. Different concentrations of formaldehyde were prepared as standard and 20 ml of both standard and samples were added to the empty wells of the plate. 20µl of hydrogen peroxide was added to all wells to initiate the reaction. The plate was covered and incubated for 20 minutes at room temperature. Then 30 µl of potassium hydroxide and then 30 µl of catalase purpald as chromogen were added. After incubation for 10 more minutes, 10 µl of catalase potassium periodate was added and incubation was performed for 5 minutes at room temperature. Absorbances were obtained at 540 nm using a plate reader.

Histopathological Examination

Histopathological evaluation was performed by a histologist who did not have the knowledge of which tissue belonged to which experimental group.

Tissues taken for histopathological examination were fixed in 10 % of formaldehyde. Tissues obtained were washed under tap water for one night long and then were embedded into paraffin after being subject to routine histological procedures. Sections of 5 µm taken from the paraffin blocks were taken onto slides. Tissue samples prepared were dyed with hematoxylin-eosin and evaluated under light microscope according to the criteria based on the method of Chiu et al. as shown in Table I [24].

Table I. *Histopathological evaluation criteria* [24]

(0)	No injury healing/adherence microscopically.
(+)	Non-adherent areas at the microscopic level at the anastomosis line or low level of healing
(++)	There is scar tissue at the anastomosis line. Anastomosis ends are active (Edema in tissue, congestion, hypercellular scar reaction, mononuclear cellular infiltration)
(+++)	Granulation tissue has formed at the microscopic level in the anastomosis area and there is a healthier appearance.
(++++)	Full injury healing, healthy look with epithelialisation

Statistical Analysis

Data obtained were indicated as average ± standard error (SE). Statistical significance levels of data were determined by using statistical package software "SPSS for Windows version 16" (Chicago, IL, USA). Multiple group comparisons were done by Kruskal-Wallis test. Mann Whitney U-test was used for comparison of two groups. For the interpretation of the result found, $p<0.05$ value was accepted as statistically significant.

3. RESULTS

No animals died or were excluded throughout the experiment.

Anastomosis Bursting Pressure Levels

Anastomosis bursting pressure levels (mmHg) were recorded as 16.43±4.86, 282.41±39.76, 48.42±7.60, and 261.66±20.69 for groups CONT-3, CONT-7, CARN-3, and CARN-7 respectively (Figure 1). There was a significant difference between bursting pressure levels of CONT-3 and CARN-3 groups ($p<0.05$). On the other hand, the difference between bursting pressure levels of CONT-7 and CARN-7 groups was not significant ($p>0.05$).

Biochemical Evaluation Results

Results of Hydroxyproline (HPR) Level Measurement

Hydroxyproline levels (µg/mg wet tissue) of scar tissue in groups were recorded as 2.32±0.27, 1.13±0.17, 1.96±0.47, 4.48±0.76 for groups CONT-3, CONT-7, CARN-3, and CARN-7 respectively

http://doi.org/10.5472/marumj.1307971
Marmara Med J 2023;36(2): 215-222

(Figure 2). It was seen that application of L-carnitine did not cause a significant change in hydroxyproline levels compared to the control groups on the 3rd day of the operation. However, hydroxyproline level of CARN-7 group was significantly higher than CONT-7 group (p<0.05).

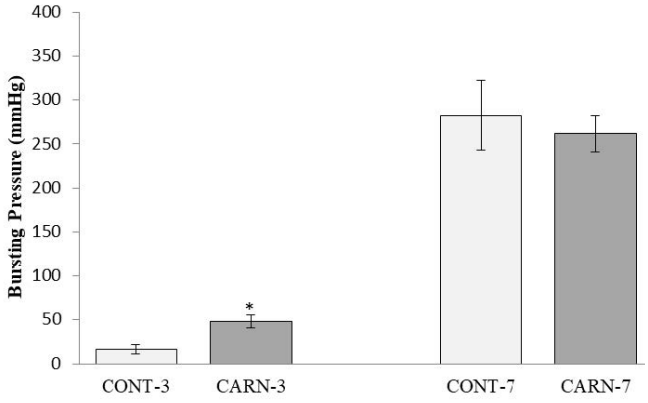

Figure 1. *Bursting pressure levels of groups.*
CONT-3: Control, anastomosis of 3 days,
CONT-7: Control, anastomosis of 7 days,
CARN-3: L-carnitine, anastomosis of 3 days,
CARN-7: L-carnitine, anastomosis of 7 days.
Columns show average and standard error.
**: p<0.05 versus CONT-3 group.*

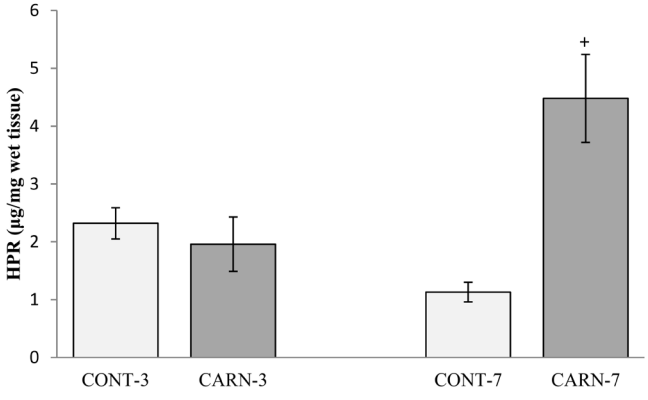

Figure 2. *Hydroxyproline (HPR) levels of groups.*
CONT-3: Control, anastomosis of 3 days,
CONT-7: Control, anastomosis of 7 days,
CARN-3: L-carnitine, anastomosis of 3 days,
CARN-7: L-carnitine, anastomosis of 7 days.
Columns show average and standard error.
+: p<0.05 versus CONT-7 group.

Results of Malondialdehyde (MDA) Level Measurement

MDA levels (nmol/mg wet tissue) of tissue samples were recorded as 6.49±1.09, 10.52±1.53, 4.87±0.90, 3.23±0.51 for groups CONT-3, CONT-7, CARN-3, and CARN-7 respectively (Figure 3). Three-day application of L-carnitine did not cause a significant change in MDA level compared to CONT-3 group. However, MDA level of CARN-7 group was significantly lower than CONT-7 group (p<0.05).

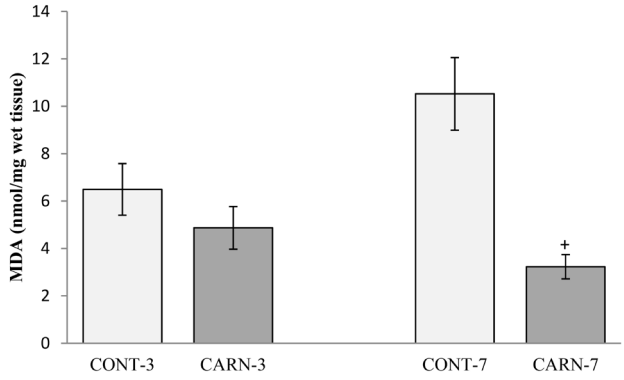

Figure 3. *Malondialdehyde (MDA) levels of groups.*
CONT-3: Control, anastomosis of 3 days,
CONT-7: Control, anastomosis of 7 days,
CARN-3: L-carnitine, anastomosis of 3 days,
CARN-7: L-carnitine, anastomosis of 7 days.
Columns show average and standard error.
+: p<0.05 versus CONT-7 group.

Results of Superoxide Dismutase (SOD) Activity Measurement

SOD activity levels (U/mg wet tissue) of scar tissue in groups were recorded as 4.63±0.51, 3.70± 0.33, 6.82±0.34, 8.59±0.50 for groups CONT-3, CONT-7, CARN-3, and CARN-7 respectively (Figure 4). A significant difference was observed between CONT-3 and CARN-3 groups, as well as between CONT-7 and CARN-7 groups (p<0.05).

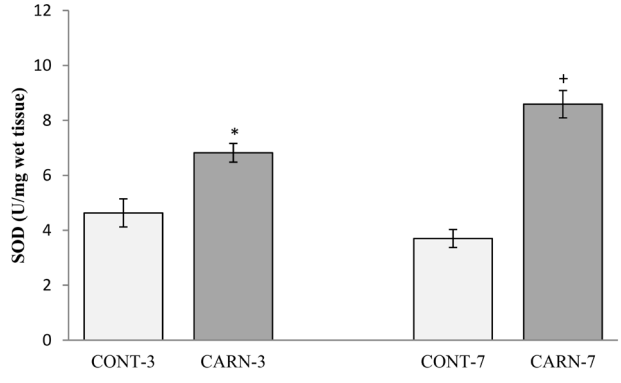

Figure 4. *Superoxide dismutase (SOD) activity levels of groups.*
CONT-3: Control, anastomosis of 3 days,
CONT-7: Control, anastomosis of 7 days,
CARN-3: L-carnitine, anastomosis of 3 days,
CARN-7: L-carnitine, anastomosis of 7 days.
Columns show average and standard error.
**: p<0.05 versus CONT-3 group, +: p<0.05 versus CONT-7 group.*

http://doi.org/10.5472/marumj.1307971
Marmara Med J 2023;36(2): 215-222

Results of Catalase (CAT) Activity Measurement

Average values and standard errors for catalase activity levels (U/mg wet tissue) of tissue samples were recorded as 10.61±0.99, 6.52±0.97, 17.12±1.66, 22.07±1.34 for groups CONT-3, CONT-7, CARN-3, and CARN-7 respectively (Figure 5). When we compared CONT-3 group and CARN-3 group, we found a significant diffference in favor of CARN-3 group with respect to injury healing ($p<0.05$). The same result was seen in comparison of CONT-7 group and CARN-7 group as well ($p<0.05$).

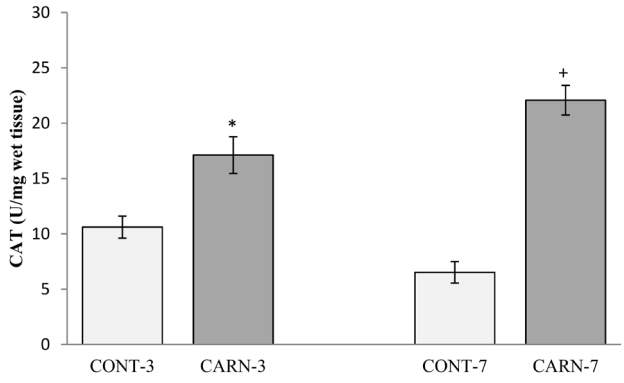

Figure 5. *Catalase (CAT) activity levels of groups.*
CONT-3: Control, anastomosis of 3 days,
CONT-7: Control, anastomosis of 7 days,
CARN-3: L-carnitine, anastomosis of 3 days,
CARN-7: L-carnitine, anastomosis of 7 days.
Columns show average and standard error.
**: p<0.05 versus CONT-3 group, +: p<0.05 versus CONT-7 group.*

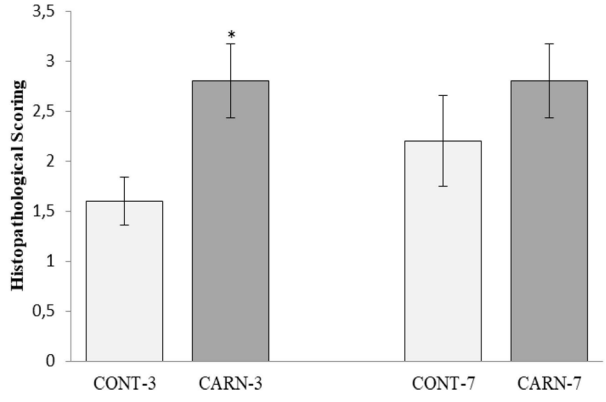

Figure 6. *Histopathological scoring of groups.*
CONT-3: Control, anastomosis of 3 days,
CONT-7: Control, anastomosis of 7 days,
CARN-3: L-carnitine, anastomosis of 3 days,
CARN-7: L-carnitine, anastomosis of 7 days.
Columns show average and standard error.
**: p<0.05 versus CONT-3 group.*

Results of Histopathological Scoring

Average values and standard errors for histopathological scoring of tissue samples were recorded as 1.60±0.24, 2.20±0.45, 2.80±0.37, 2.80±0.37 for groups CONT-3, CONT-7, CARN-3, and CARN-7 respectively (Figure 6). A significant difference was observed between CONT-3 and CARN-3 groups ($p<0.05$). Histopathological sections stained with hematoxylin eosin belonging to different groups are shown in Figure 7.

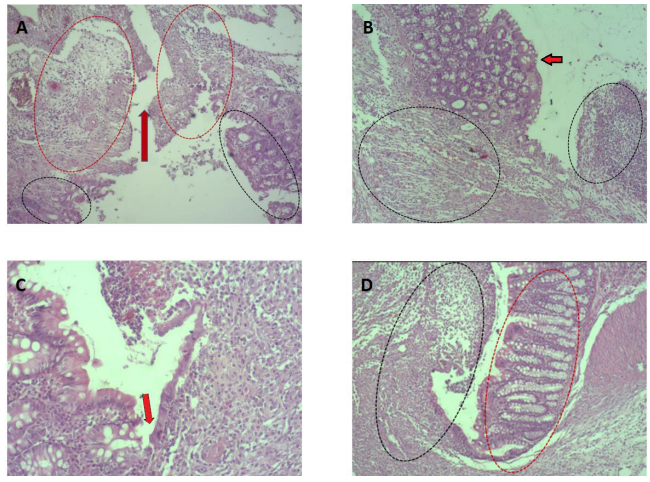

Figure 7. *Histopathological sections stained with hematoxylin eosin belonging to different groups.*
A: Histological section of the anastomosis site on the 3rd post-operative day in a rat belonging to the CONT3 group. The red arrow indicates the anastomosis line. Wound healing begins in the areas surrounded by red dots on both sides of the anastomosis line, but it is observed that there is no fusion between the ends. The two cut ends of the colon are also seen in the black dotted areas. Wound healing has begun in these areas, but fusion between the anastomotic ends has not yet occurred (Mag. 4x).
B: Histological section of the anastomosis site on the 3rd postoperative day of a rat belonging to the CARN3 group. The black dotted areas show the hypercellular healing tissue in the surgical incision area. There is intact epithelial tissue in the area marked with the red arrow (Mag. 4x).
C and D: Histological sections of the anastomosis site on the 7th postoperative day of a rat belonging to the CARN7 group. In C, the junction of the intact epithelium and the newly formed tissue is seen in the area marked with the arrow (Mag. 10x). In D, the colonic epithelium and glandular structure can be observed in the red dotted areas. The black dotted area shows the newly formed tissue at the anastomosis line. Significant mononuclear cell infiltration is seen in this multicellular area (Mag. 4x).

4. DISCUSSION

The main result of our study is that L-carnitine speeds up the anastomosis healing in the early period. High bursting pressures were recorded in CARN-3 group, which show the injury strength. This finding is also supported by the high level of histopathological scores of CARN-3 group. Another important finding of our study is that application of L-carnitine increases antioxidant capacity and significantly decreases the oxcidant damage, especially in the long term (CARN-7 group).

http://doi.org/10.5472/marumj.1307971
Marmara Med J 2023;36(2): 215-222

Surgical intervention has an important role in treatment of colorectal cancers. Colorectal surgeries are applied widely, and the most common complication of these surgeries is anastomotic leaks [25]. Morbidity and mortality rates of colon anastomotic leaks are higher than the other anastomotic leaks [26]. Despite the developments in surgical techniques, these rates increase when there is a leakage from the surgical area after taking out the tumor and performing end-to-end anastomosis of the remaining parts and the anastomosis area cannot heal completely. Therefore, full and rapid healing of the anastomosis is very important. Although we did not evaluate whether there is a leakage from the anastomosis area, the healing of the injury site and injury strength are important signs of a healthy process. One of the most frequently used measurement methods of injury strength in experimental anastomosis studies is bursting pressure [27]. We also used bursting pressure for measuring injury strength in our study. We determined that bursting pressure value increased 3 days after the anastomosis in the group to which L-carnitine was applied but there was no significant change on the 7th day (Figure 1). By looking at these results, it is possible to say that application of L-carnitine in the early period increases injury strength in the model we used. Furthermore, as a result of the evaluation of injury healing in the anastomosis area by histopathological scoring, we determined a distinct healing in the CARN-3 group which represents the early period (Figure 6). According to the protocol we used in our study, a high histopathological score indicates that anastomosis healing is better. The higher histopathological scoring value compared to the CONT3 group explains the increase in bursting pressure in the CARN3 group.

One of the factors that affect injury healing is oxidative stress. Reactive oxygen species (ROS), which cause oxidative stress, are produced by nicotinamide adenine dinucleotide phosphate (NADPH) oxidase during normal metabolic incidents. The superoxide anion produced by the effect of this enzyme can cause tissue damage besides leading other ROS formations. Hydrogen peroxide (H_2O_2) is one of these and it is not a radical. However, it can cause serious damages in cells [28]. The existence of iron and copper ions in the environment causes H_2O_2 to form hydroxyl radicals. Another radical that comes out in this process is hypochloride, which occurs by the effect of myeloperoxidase. Majority of the formation of ROS is realized by phagocytal cells. ROS is an important defense mechanism for the injury area against pathogen microorganisms. In addition, superoxide anion occured in the injury area and H_2O_2 stimulate vascularization by regulating the microcirculation. By this means, healing is accelerated by satisfying the nutrition and oxygen needs of the injury area. It was also stated that ROS in low levels has a role in signal transmission in cells [29]. However, ROS can also cause tissue damage if its amount exceeds the level where the favourable effects are seen [30]. As a result of this, ROS and oxidants occurred in the injury area can deteriorate healing. They break down the proline and hydroxyproline which constitute the collagen and can change the adhesion, reproduction, and vitality of fibroblasts. Besides, they cause serious damages in fibroblasts by inhibiting the migration of H_2O_2 keratinocytes and signal transmission of epidermal growth factor (EGF) [31]. Increased inflammatory infiltration in the injury area indicates the existence of oxidative stress. For this reason, reducing the effects of ROS is very critical for injury healing. Thus, the antioxidant substances stand out. Today antioxidants are used for injury healing [32]. There are numerous studies that examine the contribution of antioxidants to healing of colon anastomosis injuries [12,13,15-17]. In these studies molecules such as acetylcysteine, resveratrol, and simvastatin were used as antioxidants. It was shown that acetylcysteine, which is one of the antoxidant molecules, increased the fibroblast proliferation [33]. Fibroblasts synthesize collagen and glycosaminoglycan, which is very important for injury healing. Existence of adequate fibroblast proliferation and collagen synthesis in injury tissue speeds up healing [34].

In our study, we examined the effects of L-carnitine, which is an antioxidant molecule, on injury healing in early and late period. It was already shown that L-carnitine application has positive effects on colon anastomosis healing [35]. Caecal ligation, formation of peritonitis by drilling, and colon anastomosis were made simultaneously in the mentioned study. The effects of L-carnitine on injury healing were evaluated by determining the bursting pressure values of the anastomosis area on the 5th day following the operation. Injury healing was positively affected by L-carnitine for both rats with and without peritonitis. We, on the other hand, compared the effects of L-carnitine in early and late period in our study. Evaluation of injury healing in the early period following the anastomosis is important as anastomosis leakages occur especially in the early period [36].

L-carnitine can decrease oxidant damage by increasing antioxidant capacity [3,37]. In the comprehensive compilation of Fathizadeh H, it is stated that although L-carnitine does not affect total antioxidant capacity, it causes an increase in SOD activity and a significant decrease in MDA levels, which is an indicator of oxidant damage [37]. Considering the same systematic review, the existence of studies in which the total antioxidant capacity increases in regard to sample size and dose of L-carnitine draws attention. Similar with these results, we also found that the antioxidant capacity in intestinal tissue was increased by L-carnitine. The findings show that SOD and catalase activities were significantly higher in CARN-3 group than CONT-3 group, and also higher in CARN-7 group than CONT-7 group. It can be said that the increase in SOD and catalase activities improves the defense capability of the tissue against oxidant damage.

Another finding of our study is that the increase in antioxidant enzyme activities causes a decrease in MDA levels in the tissue obtained from the anastomosis area. Although the difference between MDA levels of CARN-3 and CONT-3 groups was not significant, a tendency to decrease was determined. On the other hand, we determined a significant decrease in the MDA levels of CARN-7 group following a long-term L-carnitine application. This shows us that L-carnitine decreases oxidant damage in the long run. By looking at these results, it is possible to suggest that the balance between oxidative stress and antioxidant capacity does not fully change in favour of antioxidant capacity in the

http://doi.org/10.5472/marumj.1307971
Marmara Med J 2023;36(2): 215-222

early period of injury healing and that antioxidant capacity can reach a level where it can overcome oxidative stress only in the late period of injury healing.

In our study, finding high bursting pressure levels in CARN-3 group, which was formed to determine the effects of L-carnitine in injury healing in the early period, is an important indicator of injury strength. However, hydroxyproline levels of CARN-3 group were not different from CONT-3 group. In a previous study, Cronin et al. stated that the measurements of anastomosis bursting pressure showed that the applied force increased gradually following the 3rd day of anastomosis and reached its maximum on the 7th and 10th days; hydroxyproline concentration in the injury area decreased by 40% during the first 3 days, approached to normal after approximately the 5th day, and reached a level above normal on the 10th and 14th days [38]. This shows that some factors other than hydroxyproline play an important role in improving injury strentgh. The results of our study are coherent with the study of Cronin et al [38]. Moreover, we determined that hydroxyproline levels of CARN-7 group, which represents the late period of injury healing, increasead significantly. Considering that injury healing is a long process which may take weeks, it is possible to say that L-carnitine application in the late period increases the hydroxyproline levels in the long run. Besides, one of the important findings of our study is that L-carnitine increases injury strength in the early period without an increase in hydroxyproline levels. However, more studies are needed to determine the molecular mechanisms of the increase in injury strength in the early period caused by L-carnitine.

Consequently, it is possible to say that L-carnitine application is useful in preventing anastomosis leakage, which is one of the most important complications that increase mortality rates after intestinal surgery. It is seen that L-carnitine speeds up injury healing, especially in the early period. Moreover, increase of injury strength in the early period comes out without a significant increase in hydroxyproline levels. With the application of L-carnitine, hydroxyproline levels increase and also oxidant damage decreases significantly in the late period of injury healing. It can be said that, for this effect to come out, the increase in SOD and catalase activities, which are antioxidant enzymes, and decrease in oxidant damage caused by L-carnitine play an important role. However, additional studies are needed to determine how L-carnitine affects the molecular mechanisms of injury healing process.

Compliance with Ethical Standards

Ethical Approval: The study protocol was approved by the Animal Experimentation Ethical Committee of Canakkale Onsekiz Mart University (approval number: 2013/09-10)

Financial Support: This study was supported by Canakkale Onsekiz Mart University Scientific Research Projects Coordination Unit. Project No: TYL-2014-186

Conflict of Interest Statement: There is no conflict of interest.

REFERENCES

[1] Kirchhoff P, Clavien PA, Hahnloser D. Complications in colorectal surgery: risk factors and preventive strategies. Patient Saf Surg 2010; 4:5. doi: 10.1186/1754-9493-4-5

[2] Ruiz-Tovar J, Morales-Castiñeiras V, Lobo-Martínez E. Postoperative complicactions of colon surgery. Cir Cir 2010; 78:283-91.

[3] Gülçin I. Antioxidant and antiradical activities of L-carnitine. Life Sci 2006; 78:803-11. doi: 10.1016/j.lfs.2005.05.103

[4] Kutluay Köklü AH, Küpeli Akkol E, Uğar Çankal DA. Biochemical and biomechanical assessment of effects of L-carnitine on oral mucosal wounds. Clin Oral Investig 2015; 19:1101-6. doi: 10.1007/s00784.014.1329-8

[5] Bell FP, Vidmar TJ, Raymond TL. L-carnitine administration and withdrawal affect plasma and hepatic carnitine concentrations, plasma lipid and lipoprotein composition, and in vitro hepatic lipogenesis from labeled mevalonate in normal rabbits. J Nutr 1992; 122:959-66. doi: 10.1093/jn/122.4.959

[6] Mayes PA. Lipids of physiologic significance. In: Murray RK. Granner DK. Mayes PA. Rodwell VW, eds. Harper's Biochemistry. Appleton and Lange: Stamford, 2000: 160,171.

[7] Arduini A. Carnitine and its acyl esters as secondary antioksidants? Am Heart J 1992; 123:1726-7. doi: 10.1016/0002-8703(92)90850-u

[8] Sabry MM, Ahmed MM, Maksoud OMA, et al. Carnitine, apelin and resveratrol regulate mitochondrial quality control (QC) related proteins and ameliorate acute kidney injury: role of hydrogen peroxide. Arch Physiol Biochem 2020; 13:1-10. doi: 10.1080/13813.455.2020.1773504

[9] Zhao T, Chen S, Wang B, Cai D. L-Carnitine Reduces Myocardial Oxidative Stress and Alleviates Myocardial Ischemia-Reperfusion Injury by Activating Nuclear Transcription-Related Factor 2 (Nrf2)/Heme Oxygenase-1 (HO-1) Signaling Pathway. Med Sci Monit 2020; 26:e923251. doi: 10.12659/MSM.923251

[10] Akkus A, Aydinuraz K, Daphan C, et al. Effect of carnitine on cutaneous wound healing in immunosuppressed rats. J Surg Res 2009; 155:301-5. doi: 10.1016/j.jss.2008.06.010

[11] Choi BS, Song HS, Kim HR, et al. Effect of coenzyme Q10 on cutaneous healing in skin-incised mice. Arch Pharm Res 2009; 32:907-13. doi: 10.1007/s12272.009.1613-3

[12] Cakmak GK, Irkorucu O, Ucan BH, et al. The effects of resveratrol on the healing of left colonic anastomosis. J Invest Surg 2009(a); 22:353-61. doi: 10.1080/089.419.30903214701

[13] Kabali B, Girgin S, Gedik E, Ozturk H, Kale E, Buyukbayram H. N-Acetylcysteine prevents deleterious effects of ıschemia/reperfusion ınjury on healing of colonic anastomosis in rats. Eur Surg Res 2009; 43:8–12. doi: 10.1159/000210673

[14] Zeytin K, Çiloğlu NS, Ateş F, Aker VF, Ercan F. Resveratrolün diyabetik sıçanlarda tendon iyileşmesi üzerine etkileri. Acta Orthop Traumatol Turc 2014;48(3):355-62. doi: 10.3944/AOTT.2014.13.0096

[15] Cakmak GK, Irkorucu O, Ucan BH, et al. Simvastatin improves wound strength after intestinal anastomosis in the rat. J Gastrointest Surg 2009(b); 13:1707-16. doi: 10.1007/s11605.009.0951-2

[16] Kurahashi T, Fujii J. Roles of Antioxidative Enzymes in Wound Healing. J Dev Biol 2015; 3:57-70. https://doi.org/10.3390/jdb3020057

[17] Xu Z, Han S, Gu Z, Wu J. Advances and Impact of Antioxidant Hydrogel in Chronic Wound Healing. Adv Healthc Mater 2020; 9:e1901502. doi: 10.1002/adhm.201901502

[18] Charan J, Kantharia ND. How to calculate sample size in animal studies? J Pharmacol Pharmacother 2013; 4:303-6. doi:10.4103/0976-500X.119726

[19] Arifin WN, Zahiruddin WM. Sample Size Calculation in Animal Studies Using Resource Equation Approach. Malays J Med Sci 2017; 24:101-5. doi: 10.21315/mjms2017.24.5.11

[20] Demir U, Edremitlioğlu M, Kandaş E, Şehitoğlu MH, Kılınç N. Quercetin associated with dimethylsulfoxide has a curative effect on experimental colon anastomosis injury. Acta Cir Bras 2020; 35:e202000602. doi: 10.1590/s0102.865.0202000.600.00002

[21] Goel SA, Nagpal P, Nagarajan P, Panda A K, Chhabra HS. Lateral approach to the lumbar spine of sprague dawley rat: development of a novel animal model for spine surgery. Indian Spine J 2019; 2:134-37.

[22] Irvin TT, Hunt TK. Pathogenesis and prevention of disruption of colonic anastomoses in traumatized rats. Br J Surg 1974; 61:437-39. doi: 10.1002/bjs.180.061.0605.

[23] Yagi K. Assay of lipid peroxidation in blood plasma or serum. Methods Enzymol 1984; 105: 328-31. doi: 10.1016/s0076-6879(84)05042-4

[24] Chiu CJ, McArdle AH, Brown R, Scott HJ, Gurd FN. Intestinal mucosal lesion in low-flow states. I. A morphological, hemodynamic, and metabolic reappraisal. Arch Surg 1970; 101:478-83. doi: 10.1001/archsurg.1970.013.40280030009

[25] Poon P, Law WL, Chu KW, Wong J. Emergency resection and primary anastomosis for left-sided obstructing colorectal carcinoma in the elderly. Br J Surg 1998; 85:1539-42. doi: 10.1046/j.1365-2168.1998.00903.x

[26] Thornton FJ, Barbul A. Healing in the gastrointestinal tract. Surg Clin North Am 1997; 77: 549-73. doi: 10.1016/s0039-6109(05)70568-5

[27] Oines MN, Krarup PM, Jorgensen LN, Agren MS. Pharmacological interventions for improved colonic anastomotic healing: a meta-analysis. World J Gastroenterol 2014;20:12637-48. doi: 10.3748/wjg.v20.i35.12637.

[28] Hensley K, Kotake Y, Sang H, et al. Dietary choline restriction causes complex I dysfunction and increased H(2)O(2) generation in liver mitochondria. Carcinogenesis 2000; 21:983-9. doi: 10.1093/carcin/21.5.983

[29] Blokhina O, Virolainen E, Fagerstedt KV. Antioxidants, oxidative damage and oxygen deprivation stress: a review. Ann Bot 2003; 91:179-94. doi: 10.1093/aob/mcf118

[30] Bayir H. Reactive oxygen species. Crit Care Med 2005; 33(12 Suppl):S498-501. doi: 10.1097/01.ccm.000.018.6787.64500.12

[31] Yager DR, Kulina RA, Gilman LA. Wound fluids: a window into the wound environment? Int J Low Extrem Wounds 2007; 6:262-72. doi: 10.1177/153.473.4607307035

[32] Moradi M, Moradi A, Alemi M, et al. Safety and efficacy of clomiphene citrate and L-carnitine in idiopathic male infertility: a comparative study. Urology J 2010; 7:188-93. doi:10.22037/UJ.V7I3.750

[33] Kunnavatana SS, Quan SY, Koch RJ. Combined effect of hyberbaric oxygen and N-acetylcysteine on fibroblast proliferation. Arch Otolaryngol Head Neck Surg 2005;131:809-14. doi: 10.1001/archotol.131.9.809

[34] Broughton G II, Janis JE, Attinger CE. The basic science of wound healing, Plast Reconstr Surg 2006;117: 12-34. doi: 10.1097/01.prs.000.022.5430.42531.c2

[35] Ercan U, Kiraz A, Çikman Ö, et al. The effect of systemic carnitine administration on colon anastomosis healing in an experimental sepsis model. J Invest Surg 2015; 28:334-40. doi: 10.3109/08941.939.2015.1029652

[36] Fang AH, Chao W, Ecker M. Review of colonic anastomotic leakage and prevention methods. J Clin Med 2020; 9:4061. doi: 10.3390/jcm9124061

[37] Fathizadeh H, Milajerdi A, Reiner Ž, et al. The effects of L-carnitine supplementation on indicators of inflammation and oxidative stress: a systematic review and meta-analysis of randomized controlled trials. J Diabetes Metab Disord 2020; 19:1879-94. doi: 10.1007/s40200.020.00627-9

[38] Cronin K, Jackson DS, Dunphy JE. Changing bursting strength and collagen content of the healling colon. Surg Gynecal obstent 1968; 126 : 747 – 53.