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ORIGINAL ARTICLE

Biochemical and biomechanical assessment of effects of L-carnitine on oral mucosal wounds

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Abstract
Objectives The present study aimed to investigate the oral mucosal wound healing potential of L-carnitine in a rat model.
Materials and methods Twenty-four Wistar-albino rats were divided into 4 groups: control group (group I), L-carnitine groups (100 and 200 mg/kg/day, intraperitoneally) (groups II and III), and vitamin E group (100 mg/kg/day, intraperitoneally) (group IV). A 1.5-cm linear incision was created on the buccal mucosa of each rat and was left to heal by secondary intention. On the tenth day, rats were anesthetized and sacrificed. The tensile strength of wound was measured with a tensiometer. Hydroxyproline (HYP) and malondialdehyde (MDA) levels in wound were assayed by spectrophotometry. Results were statistically analyzed using a one-way ANOVA analysis ($p \leq 0.001$).
Results In the analysis of tissue samples, there was a statistically significant decrease in MDA levels in group II ($p < 0.01$) and group IV ($p < 0.001$). Wound tension strength that was seen in groups II (57.88 %) and IV (48.71 %) was better than group III (33.39 %). Hydroxyproline levels in group II (46.98 ±1.37) was higher than groups III (29.40±1.64) and IV (38.83±1.41).
Conclusion Although there was a tendency toward faster healing in the groups receiving L-carnitine, it may have a dose-related positive effect for wound healing.
Clinical relevance With the advantages of having positive effects on wound healing, being a natural substance in the body, being easy to procure, and having a practical usage, L-carnitine may be clinically feasible for human oral mucosal wounds.

Keywords L-carnitine · Vitamin E · Wound healing · Oral mucosa · Antioxidant

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Introduction

Conditions such as wound, trauma, and ischemia increase oxidative stress. Free oxygen radicals, which appeared following injury and formed after local tissue damage, create a lipid peroxidation reaction by interacting with fatty acid radicals in the injured cell membrane. Malondialdehyde (MDA) is a secondary product of lipid peroxidation and is an essential parameter used in determining the peroxidation of lipids, and it is accepted to be a specific indicator of oxidant stress in biological systems [1, 2].

Antioxidants are postulated to help prevent, limit, or partially repair oxidative damage. In several tests conducted on animals, it has been demonstrated that antioxidants increase the speed of scarring and experimentally disrupt the dynamics of granulation tissues by preventing and limiting the oxidative damage [3].

L-carnitine, an endogenous molecule found on all mammals, exhibits a significant antioxidant activity. Although primary supply is from dietary intake (75 %), it is also synthesized in the liver and the kidneys using lysine and methionine. Its main function is to transfer fatty acids to the mitochondria and ATP synthesis through beta oxidation. In addition, L-carnitine serves an important role in mitochondrial and peroxisomal metabolism [4, 5]. In the studies conducted by Gomez-Amores et al. [6] and Sushamakumari et al. [7], it was

observed that L-carnitine cleansed the free radicals and protected the cells against oxygen radicals. In a study conducted by Thangasamy et al. [8], for aged rats that were administered L-carnitine, the macromolecules (DNA, proteins, lipids) within the cell were protected against oxidative stress by increases in the antioxidant enzyme and glutathione levels and by reduction in the lipid peroxide levels. Furthermore, L-carnitine has a radioprotective effect on several tissues and organs including oral mucosa of rats or guinea pigs [9].

In recent years, studies have shown that in addition to carnitine's anti-inflammatory functions, it also performs strong antioxidant activity. In rat models, reduced level of MDA along with the decreased level of neutrophil infiltration has shown that L-carnitine is acting as an anti-inflammatory agent [2, 10].

On the basis of this input, this study aimed to experimentally investigate the effect of L-carnitine on the treatment of soft tissue defect in oral mucosa.

Material and methods

Animals

Twenty-four male Wistar-albino rats, weighing 160–180 g each, were used in this study. Animals were acclimatized to the study environment 1 week before the start of the study. The animals were kept in stainless-steel cages in the animal room, which was maintained on a 12:12 h light/dark cycle at 21–22 °C.

Rats were allowed free access to food and water ad libitum and fed a standard pellet diet. The study was approved by the animal ethics committee and institutional guidelines for the care and treatment of laboratory animals were followed.

Experimental design

All rats underwent creation of linear incisional wounds on the buccal mucosa and received the following treatments: (1) group I (controls): no pharmacologic agent treatment; (2) group II: L-carnitine 100 mg/kg/day intraperitoneally; (3) group III: L-carnitine 200 mg/kg/day intraperitoneally; (4) group IV: vitamin E 100 mg/kg/day intraperitoneally. The dose of L-carnitine was determined in accordance with the daily dose in humans. L-carnitine (Carnitene® 1 g 5 ml−1 ampule, Sigma Tau, Roma, Italy) and vitamin E (Evigen ampule, Aksu Farma, Istanbul, Turkey, 300 mg/2 ml dl-alpha tocopherol acetate) were used in the study once daily for 10 days, starting the day of surgery.

Anesthesia management

All surgical interventions were performed under anesthesia induced by intraperitoneal injection of 2 mg/kg xylazine hydrochloride (Rompun, Bayer, Istanbul, Turkey) and 10 mg/kg ketamine hydrochloride (Ketalar, Parke Davis, Eczacıbaşı, Turkey).

Surgical procedure

After achieving an adequate visual field by cleaning the area of operation with an antiseptic solution, using a scalpel (2-mm depth marked) and a 1.5-cm tip mouth spatula, two linear incisional wounds of 1.5 cm, parallel to the occlusal plane, on both right and left buccal mucosa, were made on 5 mm of the upper side of the mucogingival junction [11]. All surgical interventions were performed under clean conditions.

On the tenth day of the experiment, a cut was made to the wound using surgical scissors at 1 cm from the edges of the wound. One of these wounds was reserved for the hydroxyproline experiment. The tensile strength of the other wound was measured with a tensiometer. On the wound for which the tensile strength had been measured using the tensiometer, a thiobarbituric acid reactive substances (TBARS) assay was performed. The increase of collagen formation and tensile strength as a result of the substances used in the linear incision wound model were evaluated by employing the methods of Lodhi et al. [12] and Suguna et al. [13].

Tensile strength

Tensile strength of previously wounded and treated skin was measured by using a tensiometer (Zwick/Roell Z0.5, Germany) [12, 13]. The tissue was gradually stretched manually by a vernier mechanism. The breaking strength at the time of wound dehiscence was noted (Fig. 1).

Lipid peroxidation determination

The lipid peroxidation level in tissue samples is expressed by malondialdehyde (MDA). It was measured according to the procedure of Ohkawa et al. [14]. This method was used to

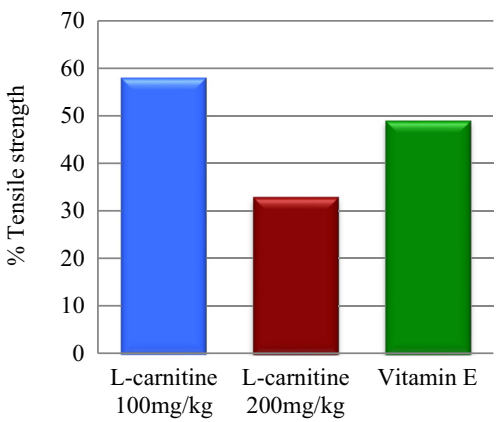

Fig. 1 Comparison of the effect of L-carnitine and reference substance on linear incision wound model

measure spectrophotometrically the color produced by the reaction of thiobarbituric acid (TBA) with MDA at 532 nm. Results were expressed as nanomole TBARS per gram of wet tissue [14]. In the wound model created in this study, the L-carnitine's antioxidant activity quantization was carried out by measuring MDA using the TBARS assay.

Hydroxyproline estimation

For this purpose, the method developed by Woessner [15] has been used by implementing various modifications to the method [16]. Standard hydroxyproline was run and values reported as microgram per milligram dry weight of tissue [17].

Statistical analysis of the data

The data on percentage wound healing was statistically analyzed using one-way analysis of variance (ANOVA). The values of $p \leq 0.001$ were considered statistically significant. Statistical analysis was performed with "InStat" (Windows).

Results

Infection and mortality were not observed during our experimental study. In the L-carnitine linear incision wound model, it was found that a dose of 100 mg/kg increased the wound strength by 57.88 %, and a dose of 200 mg/kg increased the wound strength by 33.39 %. The effect of L-carnitine and vitamin E, which was used as the reference substance on the linear incision wound model, is shown in Fig. 1.

The animals receiving the 100 mg/kg/day L-carnitine supplements healed more rapidly, with almost complete restoration of mucosa by 10 days. In the analysis of tissue samples, there was a statistically significant decrease in MDA levels in group II. The mean±SEM of MDA levels were 204.1±9.3 nmol/g in group I, 145.4±7.2 nmol/g in group II, 193.8±8.7 nmol/g in group III, and 121.5±6.4 nmol/g in group IV. TBARS measurement results of L-carnitine and vitamin E (as the reference substance) is presented in Fig. 2.

In observing the hydroxyproline levels in the tissues that had been treated with L-carnitine, it was determined that a dose of 100 mg/kg L-carnitine significantly increased the hydroxyproline in tissues. In the analysis of tissue samples, the mean±SEM of hydroxyproline levels were 8.27±2.03 μg/mg in group I, 46.98±1.37 μg/mg in group II, 29.40±1.64 μg/mg in group III, and 38.83±1.41 μg/mg in group IV. The amount of hydroxyproline in tissues that were treated with L-carnitine and vitamin E (as the reference substance) on the linear incision wound model is presented in Fig. 3.

Discussion

Any method and substance that has a positive effect on wound healing, such as accelerating the healing process, attracts the heightened attention of researchers. In literature, many research studies have investigated the effect of topical insulin administration [18], epidermal growth factor [19], fibroblast growth factor, vitamin E [20, 21], selenium [22], and taurine [16] on the healing process of tissue defects that have been created in oral mucosa and on oxidative stress. There are also other research studies that have examined the effects of L-carnitine on the healing of defects developed on the skin [1, 23–26]. However, in our survey of the literature to date, there have been no research studies published that have investigated the effects of L-carnitine on the wound healing in oral mucosa.

In this present research, the in vivo activity method has been used in order to evaluate the wound healing activity. The linear wound model was studied using rats to determine whether the materials that contribute to wound healing by exhibiting proliferative effects in the construction of epithelium have a wound healing effect in the sublayers of the wounded tissue, which in turn provides information on collagen formation and collagen strength. In this experiment, after treatment of 10 days on the test samples, strength against the stress intensity was measured on the linear incision wounds created in the oral mucosa of the rats.

In wound healing, the reorganization of collagen fibers stored in the wound area was evaluated by measuring the tensile strength, a basic parameter for both matrix formation and collagen synthesis. Though collagen can be produced, it

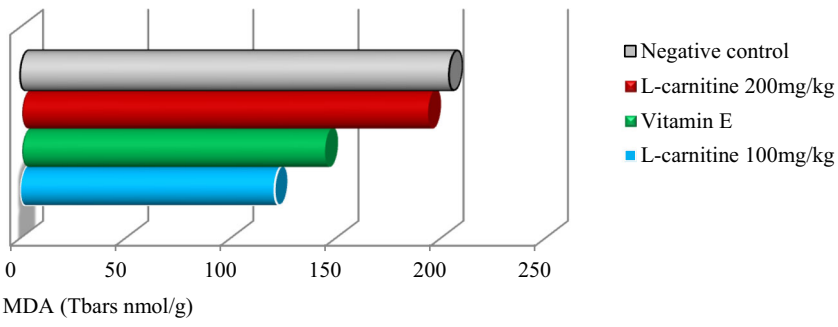

Fig. 2 Comparison of the effects of L-carnitine and the reference substance in TBARS measurement experiment

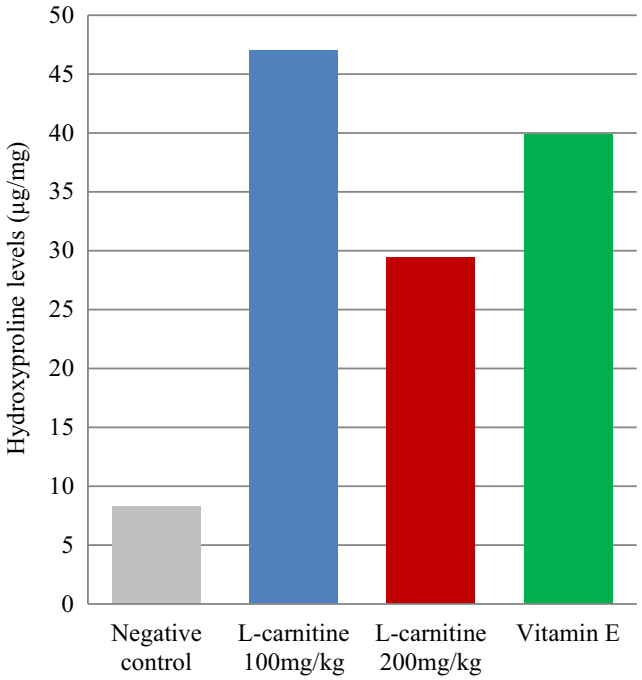

Fig. 3 Comparison of hydroxyproline measurement results on the L-carnitine and reference substance treated tissues

may reorganize in an improper way, resulting in a decrease in tensile strength [12, 13]. In this study, adequate tensile strength in wound was reached in all of the linear incision wound model groups, and it was found that L-carnitine was responsible for increasing the wound strength.

Karsidag et al. [25] have investigated, from a clinical and pathological perspective, the effects of carnitine administered locally and systemically on secondary healing full-thickness skin defects created on the back of rats. As the result of this study, L-carnitine was found to have positive effects on wound healing speed and on tensile strength of the wound in rats. Akkus et al. [23] also found that, when compared to the control groups, due to the increase in hydroxyproline levels related to carnitine treatment, there was a significant increase in tensile strength of the wounds created on the back of rats that were immunosuppressed through chronic steroid administration.

Collagen is an important extracellular matrix protein that is necessary for a wound to undergo a fast healing process. The quality and quantity of collagen content in the wound flaps and inter- and intracellular molecular bonds in collagen fibers have an impact on tensile strength and strength of the wound. In this study, in order to determine the collagen amount in wounded area, the amount of hydroxyproline (one of the amino acids that form collagen) in the tissue was measured. Looking at the hydroxyproline levels in the tissues that had been treated with L-carnitine, it was determined that a dose of 100 mg/kg L-carnitine significantly increases the hydroxyproline in tissues. This result is in agreement with the study that was conducted by Akkus et al. [23].

The administration of antioxidants is effective in decreasing the levels of reactive oxygen types [23]. Many studies propose that vitamin E and L-carnitine have an antioxidant effect and that they sweep free radicals away [5, 6, 21, 27–30]. Vitamin E and L-carnitine protect the cell from peroxidative stress by inhibiting the formation of reactive oxygen species. In this study, the wound healing effect of two potent antioxidants, L-carnitine and vitamin E, was investigated.

In the literature, administered doses of vitamin E are approximately 5–100 mg/kg/day, and it is administered orally using the i.p. and i.m. methods. In this study, vitamin E is administered as a dose of 100 mg/kg/day through the i.p. method.

While L-carnitine's oral dose varies between 50 and 200 mg/kg [24, 25], the intravenous (IV) dose is 15–140 mg/kg [24, 26]. The intraperitoneal dose is parallel with the IV dose. Since, to date, L-carnitine does not have a specified dose for rats, in the studies observed in the literature, it has been administered at 50 mg/kg/day [26, 31], 100 mg/kg/day [1, 24, 26, 28], and 200 mg/kg/day [26] as daily doses; these are the same as the human doses and they were generally administered using the i.p. and i.m. methods, intralesionally, and orally. In this study, L-carnitine was administered at 100 and 200 mg/kg/day through the i.p. method in order to examine the dose-dependent effects as well.

Side effects are rarely observed with L-carnitine, and in cases where there are side effects, they generally impact the gastrointestinal system. High plasma concentrations with IV administration are obtained in a shorter period than with oral administration [26]. In this study, there were no observable side effects of L-carnitine. The reason for selecting the i.p. as the administration method was to overcome the variability in gastrointestinal absorption and its easy administration on rats [26].

In some rat models, reduced level of MDA, which is an indicator of lipid peroxidation along with the decreased level of neutrophil infiltration, has shown that L-carnitine is acting as an anti-inflammatory agent [31, 32]. In this study, MDA levels after wound healing were statistically lower in the group for which L-carnitine had been administered at a dose of 100 mg/kg/day. In comparison to the control group as well as in the wounds for which L-carnitine was administered at a dose of 200 mg/kg/day, in wounds for which vitamin E and L-carnitine were administered at a dose of 100 mg/kg/day, higher degree of reduction of MDA occurred, and this indicated the oxidative stress decrease and wound healing. In this study, it was determined that L-carnitine contributed to the healing of the wound through its strong antioxidant effect. The data obtained complies with the results of the similar studies previously conducted [31, 32].

In this study, the biological activity of L-carnitine has been investigated using in vivo tests, and it was observed that L-carnitine affected wound healing positively in a dose-

dependent way and was able to accelerate the wound healing. With the advantages of being a natural substance in the body, having less side effects, being easy to procure, and having a practical usage, L-carnitine is a substance whose prospect for wound healing in the future can be further secured by conducting additional studies.

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Conflicts of interest The authors declare no conflicts of interest.

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