Comparing the Effect between Oral and Injection Form of Carnitine on Skin Flap Survival in Rats

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Background: *Carnitine is an endogenous cofactor, having a regulatory action on the energy flow from different oxidative sources. Carnitine has been used for ischemic conditions such as coronary heart diseases, peripheral vascular diseases with satisfactory results. So ischemic skin flaps should obtain benefit from carnitine.*
Objective: *To determine the effect of oral and injection form of carnitine on skin flap survival in a rat model.*
Material and Method: *Twenty-one Sprague-Dawley rats were divided into 3 groups, each group had 7 rats; a control group and two carnitine-treated groups. Random skin flap was elevated on the backs of the rats. The control group was not given any pharmacologic agent. Two treated groups, Group 1 received carnitine orally (150 mg/kg/day) for 3 days before flap elevation and continuing to 1 week after the procedure, Group 2 received carnitine intraperitoneally (100 mg/kg/day) for 1 week after flap elevation. The surface area of flap survival was measured in each group.*
Results: *The median areas of flap survival of the control groups and two carnitine treated groups were 65.89%, 69.03%, 77.47%, respectively. There was significant improvement of flaps survival in carnitine-treated groups, especially carnitine injected group was found to be significantly higher than the control group and carnitine-oral group ($p < 0.05$). The carnitine-oral group could slightly increase flap survival compared to the control group but was not statistically significant.*
Conclusion: *Effect of carnitine has increased flap survival in random skin flap. Carnitine injection form is more effective than the oral one.*

Keywords: *Skin flap, Ischemic, Survival*

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Flap is a unit of skin and other tissue that maintain its own vascular circulation that is transferred from the donor site to reconstruct various defects. However, there is a risk of flap necrosis from vascular compromise particularly in the distal part of the flap. If the diminished vascular supply is sufficient to respond to the basic metabolic requirements the tissue can survive until normal vascular condition is established. If not, the flap undergoes necrosis[1]. Now numerous pharmacologic agents are used to increase flap survival, carnitine appears to be a potential one because it provides a more efficient regulation of the energy flow from the different oxidative sources, especially under ischemic conditions[2,3].

Carnitine is a nutrient responsible for the transport of long-chain fatty acids into the mitochondria where they undergo β-oxidation. Carnitine helps the body convert fatty acids into energy, which is used primarily for muscular activities throughout the body. The body produces carnitine in the liver and kidneys and stores it in the skeletal muscles, heart, brain, and sperm[3].

In this shuttle, fatty acids are converted to acylcarnitine by the action of carnitine palmitoyltransferase. Another reaction hydrolyzed by carnitine acetyltransferase involves the reaction of acetyl-coenzyme A (CoA) with carnitine to yield acetylcarnitine and CoA. Here, carnitine produces free CoA for other metabolic reactions and reduces the ratio of acetyl-CoA to CoA, thereby stimulating pyruvate dehydrogenase to enhance oxidative use of glucose, reducing lactate production and acidosis[3,4].

During ischemia the metabolic flux into the Krebs' cycle decreases. As a result, acyl-CoA and acetyl-CoA esters, which are toxic, accumulate in the mitochondria, increasing acetyl-CoA to CoA ratio, thus inhibiting oxidative use of glucose. Here, carnitine serves as a buffer through the action of carnitine

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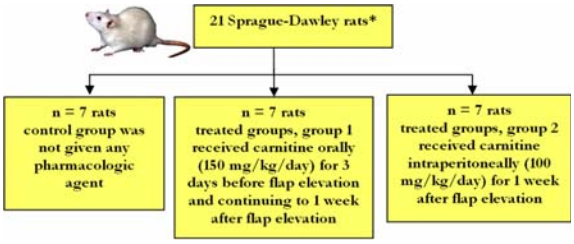
acetyltransferase, it relieves the excess acyl-CoA esters by forming short-chain acylcarnitines that may be transported out of the mitochondria and the cell, thus reducing free-fatty-acid toxicity[4]. However, all these reactions require an adequate pool of carnitine and carnitine acetyltransferase. Thus, carnitine may have a potential in the management of both chronic and acute ischemic conditions[4,5], especially when the reduction of blood flow impairs delivery of substrates and wash-out of metabolites. The carnitines have been used in cardiovascular diseases such as angina, acute myocardial infarction, postmyocardial infarction, congestive heart failure, peripheral vascular disease, dyslipidemia, diabetes, and chronic renal diseases, and satisfactory improvements have been achieved in cellular function and organ performance[7-9].

Now carnitine is available in oral form and injection form, but effects of oral-carnitine on skin flap survival have not been researched. Thus, the objective of the present study was to compare the effect of oral and injection form on skin-flap survival and comparing the effect of oral and injection form.

Material and Method

Twenty one Sprague-Dawley rats weighing 250 to 350 g. They were divided into three test groups. Group l (control group) was not given any pharmacologic agent. Group 2 was given carnitine orally (Acetyl-l-carnitine) (150 mg/kgBW/day) for 3 days before flap elevation and continuing to 1 week after the procedure, Group 3 received carnitine intraperitoneally (Carnitene Sigma Tau, Rome, Italy) (100 mg/kgBW/day) for 1 week after flap elevation (Fig. 1). Animals were anesthetized with intraperitoneal Xylazine 2 mg/kgBW and Zolitil 30-40 mg/kgBW injections.

The dorsal area was scrubbed with providine after hair removal. All surgical processes were conducted under sterile conditions, and in accordance Subcommittee in compliance for the Ethical care and uses of Laboratory Animals from the Natural Research Council of Thailand[12]. A dorsal flap with a size of 3 x 10 cm was elevated on the rats according to the method of Khouri and colleagues[6] and then sutured back into its original site with separate sutures (Fig. 2).



* Sample size was calculated according to IACUC (Institu tional Animal Care and Use Committee) protocol [15]

Fig. 1 Twenty-one Sprague-Dawley rats were divided into three test groups

The rats were followed for 7 days post-operatively. The surface area of flap survival was calculated on the seventh postoperative day by computer-assisted planimetry[10,11] (Sony Cyber-Shot 5.0 MEGAPIXELS MIPEGMOVIE VX DSC-T11) and analysis area of flap survival using Program Image J 1.40 processing. Discoloration of the flap, dry eschar formation, and lack of bleeding were regarded as gross criteria of flap necrosis. Analysis area of flap survival data was processed determine statistic analysis using software provided by SPSS. Flap viability data were analyzed using nonparametric Mann-Whitney U test. The differences between groups of data were assessed by Kruskal-Wallis analysis of variance.

Results

The median areas of flap necrosis of the groups were as follows: group 1 (control group) 10.23 cm^2; group 2 (carnitine-oral group) 9.29 cm^2; group 3 (carnitine injected group) 6.76 cm^2.

The percentage of flap survival of the groups were 65.89%, 69.03%, 77.47% respectively. Infection and mortality were not observed during the present study. The median flap survival area of groups were found significantly different (Kruskal-Wallis analysis of variance, $p = 0.014$). Carnitine-treated group 2 (Mann-Whitney U test, $p = 0.277$) and group 3 (Mann Whitney U test, $p = 0.006$), group 3 had a significantly increased flap survival compared to the control group (Fig. 3).

Discussion

During ischemia, a larger amount of carnitine is required to remove excess acyl-CoA esters that accumulate and secondary carnitine deficiency develops. Ischemic tissues exhibit a marked decrease in the content of carnitine and its esters and a significant reduction in the activity of carnitine acetyltransferase. Tissue carnitine is progressively lost as the ischemic process advances. During increasing severity of ischemia, a depletion of carnitine and a decreased activity of carnitine-acetyltransferase is responsible for noxious accumulation of acyl-CoA esters in ischemic tissues[4,5,7]. The more severe the chemic disease, the

greater the amount of carnitine required to remove the accumulation of acyl-CoA esters[9]. Although carnitine has yet to be studied in flaps, its metabolic actions are expected to augment survival of a random flap, which has a diminished vascular supply. Carnitine supplementation may limit metabolic acidosis and increase adenosine triphosphate synthesis, thus leading to improved flap survival.

A caudally based 10 x 3 cm dorsal rat flap was advocated by Khouri et al[6]. They reported this model as the most suitable choice for experimental flap studies. Several authors have used this flap model for their investigations[3,5]. The authors also used the same flap model in the present study. The areas of flap necrosis in the present control group were found to be similar to those of control groups of other studies.

Various mechanisms of action of carnitine on ischemic tissue have been proposed. Carnitine activates the transport of adenine nucleotides across the inner mitochondrial membrane by preventing adenylate translocase inhibition by long-chain fatty-acid accumulation, resulting in increased adenosine triphosphate concentration in the tissues, lessening cellular injury, and protecting cells from subsequent episodes of ischemia[7-9]. Through this, carnitine improves the circulatory reserve of the ischemic tissue. It might permit the ischemic tissue to utilize its remaining limited oxygen supply more efficiently by improving its functional circulatory reserve[4,5].

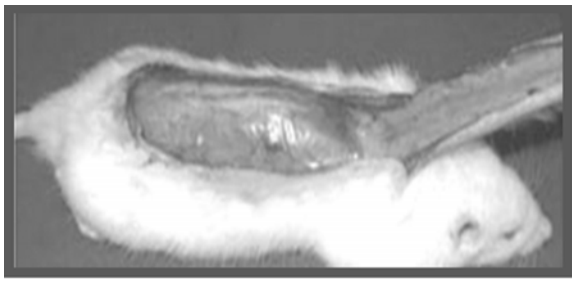


Fig. 2 A dorsal flap cm was elevated on the rats

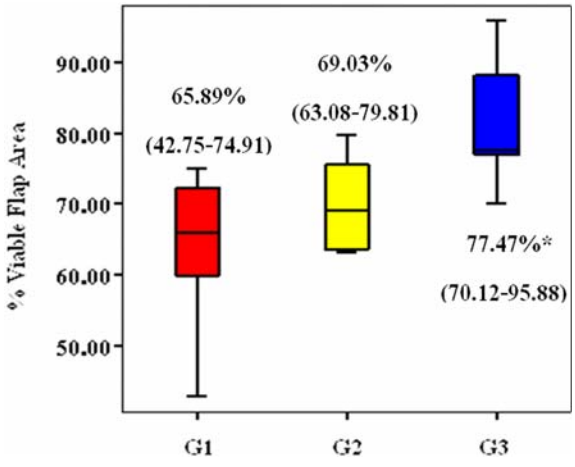


Fig. 3 The Differrence area of flap survival between control group (G1), carnitine-oral group (G2) and carnitine injected group (G3)

The activities of carnitine may be particularly important in the vascular system of the flap because it was demonstrated that vascular endothelial cells and smooth muscle cells include carnitine acetyltransferase[9]. It was reported that lipid droplets in these cells disappear after carnitine administration and that carnitine stimulates oxidation of oleate in endothelial cells. Hence, vascular endothelial and smooth muscle cells may be less vulnerable to ischemia with carnitine.

Furthermore, carnitine may help membrane stabilization in cells. It was shown experimentally that there is a much higher membrane potential in carnitine-treated rats. This effect may be caused by the removal of long-chain acyl-CoA from the mitochondrial membranes[13,14]. According to Pola et al[16] carnitine has the capacity to prevent alterations in endothelial membrane permeability.

Carnitine is a novel pharmacological agent used for flap survival. In the present study of Telliog lu et al[3] and Arslan et al[5] it was shown to have prolonged flap survival. Oral human dose of carnitine ranges between 50 and 200 mg/kg, whereas intravenous dose is 15 to 140 mg/kg. Intraperitoneal dose to 4 g/day, divided into two to three doses, are generally chosen. Dietary carnitine is absorbed by active and passive transfer across enterocyte membranes. Bioavailability of dietary l-carnitine is 54-87%. Absorption of carnitine dietary supplements (0.5-6g) is primarily passive systemic bioavailability is low, 14-28% of dose, reflecting intestinal absorption barrier and hepatic first pass elimination.

Acetyl-l-carnitine is partially hydrolyzed in enterocytes during absorption, circulating acetyl-l-carnitine concentration was increased 43% after oral supplements of 2 g/day[8]. Oral administration of acetyl-l-carnitine is found to be safe, without significant hepatic, renal, or metabolic side effects[13-15]. Carnitine did not produce any observable side effect in the present study groups, either. Oral carnitine rarely produces side effects, and when it does, they are usually gastrointestinal. The majority of intravenous carnitine reaching the blood is rapidly eliminated by

the kidney. Higher plasma concentrations are reached in a shorter time after intravenous administration than after oral administration[8].

In the present study by Tellioglu et al[3] the impact of 50 and 100 mg carnitine injection on flap survival has been evaluated, demonstrating that 100 mg is more effective. Therefore, the authors used the 100 mg dosage in injection form.

In the present study, there was significant improvement of flaps survival in carnitine-treated groups, especially carnitine injected group was found to be significantly higher than controlled group and carnitine-oral groups ($p < 0.05$). The carnitine-oral group can slightly increased flap survival compared to the control group but not statistically significant, the authors believe that oral-carnitine supplement are absorbed to be less efficiently and low systemic bioavailability, resulting less effective than intra-peritoneal injection.

Conclusion

In conclusion, carnitine administration might lead to a significant improvement in flap survival. Carnitine injection form is more effective than the oral form. Further research may be used with oral carnitine supplement used to support flap survival in flap surgery in humans.

Potential conflicts of interest

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การเปรียบเทียบผลระหว่างการกินและการฉีดคาร์นิทีน ต่อการอยู่รอดของ skin flap ในหนูขาว

ปวีณา ลาวัณลักขณา, สุรกานต์ สาหร่ายทอง, ชัยชุมพล สุวรรณเตมีย์, อานนท์ ปีติเสรี

ภูมิหลัง: *คาร์นิทีนเป็นสารที่สร้างขึ้นภายในตับและไตและนำไปเก็บที่กล้ามเนื้อทำหน้าที่เป็นตัวเร่งให้ไมโตคอนเดรียเปลี่ยนไขมันเป็นพลังงาน ปัจจุบันมีการนำคาร์นิทีนมาใช้ในภาวะเนื้อเยื่อขาดเลือด เช่น ใช้รักษาโรคหัวใจขาดเลือด, โรคหลอดเลือดแขนขาขาดเลือด, โรคไตเรื้อรัง ซึ่งได้ผลดี การนำคาร์นิทีนมาใช้ลดภาวะเนื้อเยื่อขาดเลือดจากการผ่าตัดทำ flap น่าจะช่วยเพิ่มการอยู่รอดของ flap มากขึ้น*

วัตถุประสงค์: *เพื่อเปรียบเทียบการเพิ่มการอยู่รอดของ flap ในหนูด้วยวิธีการกินและการฉีดคาร์นิทีน*

วัสดุและวิธีการ: *ทำการศึกษาในหนู Rat สายพันธุ์ Sprague Dawley เพศผู้ จำนวน 21 ตัว โดยแบ่งหนูออกเป็น 3 กลุ่ม กลุ่มละ 7 ตัว กลุ่มแรกเป็นกลุ่มควบคุม และอีก 2 กลุ่ม เป็นที่ได้รับคาร์นิทีน หนูทุกตัวจะได้รับการผ่าตัดทำ flap บริเวณหลัง โดยหนูกลุ่มแรกจะไม่ได้รับคาร์นิทีน กลุ่มที่ 2 ได้รับคาร์นิทีนแบบกิน (150 มก./ กก./วัน) ก่อนผ่าตัด 3 วัน และให้ต่อเนื่องอีก 1 สัปดาห์ กลุ่มที่ 3 ได้รับคาร์นิทีนแบบฉีดเข้าทางช่องท้อง (100 มก./ กก./วัน) หลังผ่าตัด 1 สัปดาห์ และหลังจากนั้นจะทำการวัดบริเวณ flap ที่อยู่รอดในแต่ละกลุ่มและเปรียบเทียบผลที่ได้*

การวิเคราะห์ข้อมูลทางสถิติ: *วิเคราะห์เปรียบเทียบความแตกต่างของการอยู่รอดของ flap ระหว่างกลุ่ม โดยใช้สถิติ Kruskul-Wallis analysis และเปรียบเทียบความแตกต่างเป็นรายคู่โดยใช้สถิติ Mann-Whiney U test*

ผลการศึกษา: *ผลการวัดบริเวณที่อยู่รอดของ flap แต่ละกลุ่ม มีอัตราร้อยละ 65.89, 69.03, 77.4 ตามลำดับ ซึ่งพบว่าการให้คาร์นิทีนแบบกินไม่มีผลต่อการเพิ่มการอยู่รอดของ flap ในทางตรงกันข้ามการให้คาร์นิทีนแบบฉีดพบว่าเพิ่มการอยู่รอดของ flap อย่างมีนัยสำคัญทางสถิติ ($p < 0.05$)*

สรุป: *จากการศึกษาพบว่าคาร์นิทีนมีผลต่อการเพิ่มการอยู่รอดของ flap ในหนู และพบว่าคาร์นิทีนแบบฉีดมีประสิทธิภาพกว่าแบบกิน*