ORIGINAL RESEARCH

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# The Effect of Carnitine on Doxorubicin Induced Extravasation Injuries: A New Treatment Method for Extravasation Injuries

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**ABSTRACT Objective:** Doxorubicin is an anthracycline antibiotic widely used as an antineoplastic agent, therefore extravasation injuries due to doxorubicin is common. The incidence of necrosis due to extravasation has been reported to occur between 0.5%-6%. In pediatric patients, this rate increased up to %11 in various studies. **Material and Methods:.** An experimental study was designed and 56 rats were randomly divided into 7 groups. Doxorubicin induced extravasation injury was created in all rats. Standard dose carnitine was used in all groups with different methods and periods. Necrosis sizes were measured weekly for each group and results were analyzed statistically. **Results:**: Significant difference were found between each groups necrosis sizes ($p<0.001$). Necrosis sizes were evaluated weekly for each group. Significant difference were seen each week in necrosis sizes. The difference was $p>0.001$ between 1st and 4th week, $p=0.006$ between 2nd and 3rd week, $p>0.001$ between 2nd and 4th week and $p>0.001$ between 3rd and 4th week. **Conclusion:** We aimed to investigate the effect of carnitine on doxorubicin induced extravasation injuries. This experimental study revealed that carnitine was associated with decreased progression of skin necrosis and improved wound healing. It is possible to say that carnitine might decrease the necessity for surgical intervention for doxorubicin induced extravasation injuries.

**Key Words:** Doxorubicin; carnitine; extravasation of diagnostic and therapeutic materials

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Leakage of intravenous infusions from the intravascular region into the interstitial space is called extravasation. Necrosis of cutaneous tissue, subcutaneous soft tissue and deeper seated anatomic structures after extravasation is a common problem which usually requires surgical intervention.[1] Large series in the literature demostrates that the incidence of necrosis owing to extravasation has been reported to occur between 0.5%-6%. In pediatric patients, this rate was even higher and increased up to 11%.[1,2]

Doxorubicin is an anthracycline antibiotic widely used as an antineoplastic agent.[3] It is also a vesicant drug, which has the potential to induce severe tissue damage after extravasation. The toxicity of doxorubicin is also resistant to all of the metabolic events in the surrounding tissues, that prevents the removing of the toxins in injured tissues.[4] Extravasation of doxorubicin alters wound healing processes. The direct toxic effect of doxorubicin on living cells leads to cellular death that is perpetuated by the release of doxorubicin-DNA complex from dead cells. This also causes increased rates of free oxygen radicals and it prevents the release of cytokines and growth factors which leads to wound healing problems. The local re-

actions enlarge and cause an ulceration zone surrounded by a zone of indurated inflammation.[5]

Extravasation injuires mostly occur in regions with thin skin such as dorsal side of the hand, antecubital fossa, forearm and the dorsal side of the foot. Superficial lesions deepen in time and results extremity dysfunctions by effecting deep seated tendons, muscles, nerves, vascular structures and even joints.[6] Most of the patients who received chemotherapy is already immune-compromised. Therefore, skin ulcers caused by extravasation play an important role in the etiology of sepsis in immune-suppressed patients, who show high morbidity and mortality. By analysing histopathological pattern of extravasation injuries we see collagen necrobiosis, vascular failure with thrombosis, erythrocyte extravasation and absence of an inflammatory response in the dermis. There is also a vascular necrotic chronic ulceration and decreased wound contraction.[7] Several studies were performed on doxorubicin extravasation injuries, yet there is no treatment developed that we can consider as ideal.[8,9]

Carnitine is a micronutrient used by the body to transport long chain fatty acids to the mitochondria in cells where fatty acids are converted to adenosine triphosphate. The possible protective mechanisms of carnitine include the inhibition of mitochondrial membrane permeability transition, a decrease of oxidative stress, and the prevention of proapoptotic protein expression.[10]

Recent studies also revealed that carnitine can induce vasodilatation of subcutaneous human arteries involving the endothelium through the effect related to the synthesis of PGs, especially PGI2.[11,12]

In this study, the authors aimed to investigate the effect of carnitine on doxorubicin induced extravasation, therefore an experimental study was designed.

## MATERIAL AND METHODS

This study has been approved by the appropriate ethics committee and has therefore been performed in accordance with the ethical standards set forth in the 1964 Declaration of Helsinki and its later amendments. The animals were kept in individual cages with free access to food and water and under alternating 12-hour periods of light and darkness. Fifty six female Spradue-Dawley rats weighting 260 gr ± 20 gr were randomized into 7 experimental groups.

10 mg doxorubicin (Adriablastina® 10 mg, Carlo-Erba) and L-carnitine 1gr (Carnitene® 1 g/ 5 ml, Sigma-Tau) were used. For evaluating the time when doxorubicin would form maximum skin necrosis, we performed a preliminary study. We found that intradermal injection of 2 mg of doxorubicin created maximum skin necrosis on day 14 of the preliminary study within a 45 day observation period.

Following intra peritoneal pentobarbital anesthesia, 2 mg of doxorubicin in 0.5 ml saline was injected subcutaneously into the dorsal skin of all animals in order to create extravasation injury. In group I, extravasation injury was created and no carnitine injections were performed in order to observe normal would healing. In the other groups standard dose carnitine was injected subcutaneously, intraperitoneally or both. Groups and treatment protocols were shown in Table 1.

The development and progression of skin necrosis were monitored for four weeks. Necrosis size was measured by Scion Image Programme®. The area of necrosis was measured every 7 days by an investigator who was blind to group allocation. In the second week, one rat were sacrificed from each group. In the fourth week, all rats were sacrificed. Tissue samples were harvested and sent to histopathological evaluation. Histopathological specimens were evaluated for necrosis development and progression such as necrosis size, necrosis depth, capillary necrosis and thrombosis. The groups were also evaluated for would healing parameters such as granulation, collagen formation, oedema,reepithelialization, inflammatory reaction, fibrosis, mitotic activity and angiogenesis.

Statistical analyses were performed using SPSS 15.0 for Windows (SPSS Inc., Chicago, Illinois), and the results were analyzed using Student T test and ANOVA test. One way ANOVA test was used for variance analyses between groups. Subgroup

**TABLE 1:** Experiment groups and treatment protocol.

| Group | Doxorubicin | Carnitine | Interval |
|---|---|---|---|
| I | Sc | None | |
| II | Sc | Sc | Single dose first day |
| III | Sc | Sc | Daily for seven days |
| IV | Sc | Ip | Single dose first day |
| V | Sc | Ip | Daily for seven days |
| VI | Sc | Sc + Ip | Singe dose first day |
| VII | Sc | Sc + Ip | Daily for seven days |

Sc: Subcutaneously; Ip: Intraperitoneal.

analyses were performed with the Bonferroni correction after initial tests.

## RESULTS

Skin necrosis was developed in all animals after doxorubicin injection. Macroscopically, the necrosis progression continued to 14$^{th}$ day. In carnitine injected groups, the skin necrosis progression was moderate compared to control group. On the 14$^{th}$ day, soft tissue necrosis with a distinct demarcation line was visible in all animals. In group I, the demarcation line was distictive in 10th day, which was the earliest on-set among all groups. Between 2$^{nd}$ and 4$^{th}$ week, necrosis size decreased in all carntine injected groups, most significant decrease was in group VII. In group VII, skin necrosis resolved and the wound reepithelised completely for some animals.

The size of necrosis was measured weekly in all groups and the results were analyzed statistically. The necrosis was evaluated weekly for each group. There were significant difference in all weeks for necrosis size between carnitine injected groups (group III-VII) and the control group ($p<0.001$). Among carnitine injected groups, group VII was significantly better than all other carnitine injected groups ($p<0.001$). The progression of the size of necrosis was shown in Table 2.

In group I; a full thickness tissue necrosis was observed from epidermis to underlying muscles. There were severe capillary necrosis, vascular thrombosis and lumen obliteration, minimal regulation of collagen fibrils, minimal inflammatory response. No or minimal granulation formation, re-epithelialization or angiogenesis were seen in histopathological evaluation. On the 4$^{th}$ week, no or minimal re-epithelialization and granulation were seen around the wound.

In group II, tissue necrosis were limited within subcutaneous adipose tissue and the skin. There were mild capillary necrosis and vascular thrombosis. Collagen formation was greater than group I beneath the wound. There were visible re-epithe-

**TABLE 2:** The size of necrosis progression ($cm^2$).

| | First Week | | Second Week | | Third Week | | Forth Week | |
|---|---|---|---|---|---|---|---|---|
| Groups | Mean Size | SD | Mean Size | SD | Mean Size | SD | Mean Size | SD |
| I | 1.0571 | 0.13288 | 1.2643 | 0.21832 | 1.121 | 0.1054 | 0.9486 | 0.19334 |
| II | 0.9729 | 0.37959 | 1.0757 | 0.48308 | 0.831 | 0.1835 | 0.7486 | 0.21106 |
| III | 0.435 | 0.0905 | 0.6067 | 0.23304 | 0.408 | 0.1564 | 0.1367 | 0.03724 |
| IV | 0.5213 | 0.31493 | 0.4163 | 0.24342 | 0.421 | 0.2018 | 0.26 | 0.12917 |
| V | 0.29 | 0.11045 | 0.44 | 0.16021 | 0.403 | 0.19 | 0.265 | 0.1121 |
| VI | 0.254 | 0.2881 | 0.306 | 0.8792 | 0.186 | 0.0305 | 0.142 | 0.08136 |
| VII | 0.3129 | 0.14396 | 0.3443 | 0.11984 | 0.227 | 0.0918 | 0.01814 | 0.07128 |
| Mean | 0.582 | 0.37556 | 0.6602 | 0.44047 | 0.537 | 0.3545 | 0.3891 | 0.35276 |

SD: Standart deviation.

lization and mitotic activity. On the 4th week, collagen formation was wider and re-epithelizastion was observed on the wound edges.

In group III, the size and depth of necrosis were similar to group II. On the 4th week,necrosis was partially resolved in subcutaneous tissue. A thin layer of necrosis was present in epidermis and dermis. There were visible mitotic activity, granulation formation and angiogenesis in the wound. group III showed similar wound healing like group II.

In group IV, the depth of necrosis was similar to group II and III. On the 4th week, visible granulation formation were seen in the wound. Mitotic activity and re-epitelitation were significantly better than only subcutaneous carnitine injected groups.

Group IV and V showed similarities. Only difference was the mitotic activity which was more in group V.

In group VI and VII, the depth of necrosis was present in the deep dermis and epidermis on the 2nd week. On the 4th week, a thin necrosis layer was present in group VI where as the necrosis was completely resolved in group VII. The wound was re-epithelized and healed completely in some rats in group VII. Reepithelization, angiogenesis, mitotic activity and granulation were significantly greater in group VII compared to group VI. In group VI and VII (intraperitoneal + subcutanous injections); all wound healing parameters were significantly greater than all other groups.

Carnitine injected groups for 7 consecutive days (group III, V, VII) were significantly better in would healing compared to single dose carnitine injected groups (II, IV, VI).

The necrosis progression and wound healing parameters were listed in Table 3 and 4.

**TABLE 3:** Necrosis progression parameters.

| Groups | Necrosis depth | Capillary necrosis | Trombosis |
|---|---|---|---|
| Group I | | | |
| 2nd week | +++ | +++ | +++ |
| 4th week | +++ | +++ | ++ |
| Group II | | | |
| 2nd week | +++ | ++ | ++ |
| 4th week | ++ | ++ | ++ |
| Group III | | | |
| 2nd week | ++ | +++ | +++ |
| 4th week | + | - | - |
| Group IV | | | |
| 2nd week | ++ | +++ | +++ |
| 4th week | + | - | - |
| Group V | | | |
| 2nd week | ++ | +++ | +++ |
| 4th week | + | - | - |
| Group VI | | | |
| 2nd week | ++ | +++ | +++ |
| 4th week | + | - | - |
| Group VII | | | |
| 2nd week | ++ | - | - |
| 4th week | - | - | - |

Necrosis depth:
(-): No necrosis, (+): Dermis only, (++): Dermis and panniculus carnosus, (+++): Fascia and deeper structures.
Other parameters:
(-): No reaction, (+): Light, (++): Mild, (+++): Severe.

## DISCUSSION

Doxorubicin is widely used in different chemotherapy protocols, therefore extravasation of this antineoplastic agent is common. Doxorubicin is one of the most potential agents causing tissue necrosis when extravasated. It inhibits DNA replication by binding between successive base pairs in the DNA dobule helix. That leads to production of free oxygen radicals and cell death.[13] After cell death, doxorubicin-DNA complexes release. These complexes affect other cells, leading to further tissue necrosis.

Doxorubicin-induced extravasation injury is more severe than any other chemotherapeutic agent because of its severe toxicity, resistance to metabolism by local tissues, inability to be removed from subcutaneous tissue, painful ulceration, and progressive tissue necrosis characterized by the recruitment of proinflammatory cytokines, oxygen free radicals, and release of cellular enzymes.

Various antioxidants such as hyperbaric oxygen, tocopherol, N-acetylcysteine and EGb761 (gingko biloba extract) have been used as antioxidants in DXR-induced extravasation.[9,14] However, no agent could be suggested for clinic use. Thus, we evaluated the effects of carnitine, a potent antioxidant, on doxorubicin induced extravasation. Various agents have

**TABLE 4:** Wound healing parameters.

| Groups | Granulation | Collagen | Odema | Re-epitelization | Inflamation | Fibrosis | Mitotic activity | Angiogenesis |
|---|---|---|---|---|---|---|---|---|
| Group I | | | | | | | | |
| 2nd week | + | + | ++ | + | - | + | - | + |
| 4th week | + | + | + | + | + | + | - | + |
| Group II | | | | | | | | |
| 2nd week | + | + | + | ++ | + | + | - | + |
| 4th week | +++ | +++ | ++ | ++ | +++ | +++ | ++ | +++ |
| Group III | | | | | | | | |
| 2nd week | ++ | ++ | +++ | ++ | + | ++ | + | ++ |
| 4th week | ++++ | +++ | + | ++ | +++ | +++ | + | +++ |
| Group IV | | | | | | | | |
| 2nd week | ++ | ++ | +++ | ++ | + | ++ | + | ++ |
| 4th week | ++++ | ++++ | + | ++++ | ++++ | ++++ | + | +++ |
| Group V | | | | | | | | |
| 2nd week | + | + | ++ | + | - | - | - | - |
| 4th week | ++++ | ++++ | - | +++ | ++ | ++++ | ++ | +++ |
| Group VI | | | | | | | | |
| 2nd week | ++ | ++ | ++ | + | ++ | ++ | ++ | ++ |
| 4th week | ++++ | ++++ | + | +++ | ++ | ++++ | +++ | ++++ |
| Group VII | | | | | | | | |
| 2nd week | ++++ | ++++ | + | + | +++ | ++++ | ++ | ++++ |
| 4th week | ++++ | ++++ | - | ++++ | ++ | ++++ | +++ | ++++ |

(-): No reaction; (+): Low; (++): Mild; (+++): High; (++++): Very high.

been tested for their ability to reduce the tissue damage that results from doxoru-bicin extravasation. All these agents has different effect mechanisms such as scavenging free oxygen radicals, reducing inflamatory responce, increasing new vessles , inhibiting coagulation, increasing endothelial cell proliferation and angiogenesis.[6,8,15,16] Several clinical and surgical approaches have been tested as well such as VAC, hyperbaric oxygen therapy and surgical intervention.[17-19]

We tested carnitine for doxorubicin induced extravasation injuries in our study. Carnitine is a micronutrient used by the body to transport long chain fatty acids to the mitochondria incells where fatty acids are converted to adenosine triphosphate. The possible protective mechanisms of carnitine include the inhibition of mitochondrial membrane permeability transition, a decrease of oxidative stress, and the prevention of proapoptotic protein expression.[10] Several studies mentioned that carnitine can induce vasodilatation of subcutaneous arteries involving endothelium through the effect related to the synthesis of prostoglandines.[11,12]

Carnitin treatment were applied in various methods such as subcutaneous, intraperi-toneal and subcutaneous + intraperitoneal in this study in order to compare the results of each group. It was observed that the carnitine injected groups (group III-VII) were significantly better in skin necrosis development and progression compared to control group ($p<0.001$). Among the carnitine injected groups, group VII was significantly better than other carnitine injected groups ($p<0.001$). As it was considered before, L-carnitine demostrated a positive effect on necrosis development and progression. Moreover, the combined use of carnitine (in-traperitoneally+ subcutaneous) for seven consecutive days demostrated the best outcome statistically. Eventhough it is not significantly meaningful, intraperitoneal carnitine injected groups (group VI-V) were slightly better than subcutaneous carnitine injected groups (group II-III) in tissue necrosis development and progression. In our study, group VI and group VII shows better wound healing paramaters and decremental tissue necrosis paramaters by the time. On the other

hand, when we compared the results between group III and IV, we handle nearly same paramaters. As a result of this findings, we assumed that both systemic and local application of L-carnitine for a week is more effective way of treatment of tissue necrosis rather than application local or systemic way alone.

Histopathological parameters were measured on the 2nd and 4th weeks in order to evaluate the carnitine's effect on wound healing. It was observed that carnitine use demostrated a positive effect in would healing in the first, second, third and forth weeks. All carnitine injected groups had better values compared to the control group. It was also observed that combined carnitine use for 7 consecutive days showed greater results compared to other groups. Group VII had the greatest results for would healing in our study.

In our study, it was observed that Lcarnitine showed a positive effect in wound healing for doxorubicin induced extravasation injuries. It was also observed that L-carnitine decreased tissue necrosis development and progression. We acquired data that might decrease the necessity of surgical intervention and morbidity of doxorubucin induces extravasation injuries. Further studies are needed in order to use carnitine in clinical use.

There is not a certain treatment protocol for extravasation injuries. We aimed to investigate the effect of carnitine on doxorubicin induced extravasation injuries. It was seen that carnitine increased wound healing and decreased necrosis progression in our experimental study. It is possible to claim that carnitine might decrease the necessity for surgical intervention for doxorubicin induced extravasation injuries. We hope that our study might set a good example for further human or dose studies.

### *Conflict of Interest*

*Authors declared no conflict of interest or financial support.*

### *Authorship Contributions*

***Idea and concept of Study:*** *Vedat Menderes;* ***Design of Study:*** *Burak Ersen, İsmail Aksu;* ***Consultancy:*** *Selçuk Akın;* ***Data Collection and Processing:*** *Mustafa Özyurtlu;* ***Analysis and Comments:*** *Gökhan Ocaklıoğlu, Hülya Öztürk Nazlıoğlu;* ***References Crosshatching:*** *Kemal Karaca;* ***Article Writing:*** *Mehmet Can Şakı, Orhan Tunalı.*

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