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ARTICLE

The beneficial effects of subcutaneous *Allium cepa* injection on random flap survival in rats

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ABSTRACT

Random flaps are frequently used in the practice of reconstructive surgery. The aim of this experimental study was to investigate the effects of *Allium cepa* on random flap survival in rats. Fourteen Wistar rats were used for this experimental study. The subjects were divided into experiment and control groups. Rats in the experiment group received daily injections of *A. cepa* extract for 7 d before the elevation of the flaps. Following the treatmentperiod, elevation and reinsertion of the dorsal flaps were performed. Necrotic and total flaps areas were marked and calculated 7 d after the flap elevation. Histological examinations and microangiography were performed to evaluate the results. The mean value of the proportion of necrotic flap areas to the total flap area was 25.06 and 50.6% in the *A. cepa* and control group, respectively ($p = .0079$). In the histological examination, number of vessels identified in the dermis were 23.75 ± 0.7 and 33.75 ± 9 in the *A. cepa* and control group, respectively ($p = .7457$). In angiographic images, vessels formations were more noticeable in the *A. cepa* group. We conclude that preoperative subcutaneous *A. cepa* injection increases dorsal flap survival in rats.

Abbreviations: *A. cepa*: Allium cepad: days

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Allium cepa; random flaps; rat

Introduction

Allium cepa, commonly known as the onion is a vegetable which belongs to the *Allium* genus. It is structure is composed of layers of large epidermal cells. Around 80% of its volume is composed of water. It contains a low amount of carbohydrate, protein and fat. Onions have been shown to contain phytochemicals [1,2]. These compounds have been attributed to its various biological effects. It can cause eye irritation which is a result of syn-propanethial-S-oxide gas. These sulfur-containing molecules are also responsible for its characteristic odor. It can cause allergic reactions which vary from contact dermatitis to anaphylactic reactions.

Dorsch et al. have shown that onion extract can have anti-inflammatory effects [3]. This effect has been attributed to the cepaenes and thiosulfinates. In another study, these molecules were shown to have anti-microbial properties [4–6]. In addition, Draelos has shown that the onion extract can improve the cosmetic appearance of postsurgical scars [7]. This might be partly related to its anti-inflammatory properties. Vasorelaxant and hypotensive effects of *A. cepa* have also been shown in previous studies which might have effects on skin microcirculation and flap survival [8].

This study investigates the effect of subdermal injection of *A. cepa* solution on flap survival. To our knowledge, this is the first study in English literature to evaluate the effect of *A. cepa* on flap survival.

Materials and methods

This study was conducted with the approval of local ethical comity. A number of 14 female Wistar rats weighing at approximately 300 g were used for the study. A common control group was used with another simultaneous study on the effect of incisional surgical delay techniques on random flap survival, where exact flap design was used, to prevent the unnecessary sacrifice of additional animals.

Diethyl ether inhalation followed by intraperitoneal injection of 60 mg/kg Ketamine (Ketalar 500 mg/10 ml; Pfizer® 100 mg/ml; Pfizer Inc., New York City, NY) and 10 mg/kg xylazine (Rompun 2%; Bayer, Leverkusen, Germany) was used for surgical anesthesia. Back skin was shaved with an electric shaver. Skin preparation was performed with the application of a povidone-iodine solution. The study was originally planned with three groups: experiment, sham and control group. However, due to the fact that the effect of trauma during the injections could be minimized with tuberculin needles and due to ethical concerns from the ethical comity with the aim of reducing rat sacrifice, the sham group was excluded from the study. The control and the study groups were matched in terms of flap size. The flap dimensions were 9×3 cm. Weight and the age of the rats were homogenous between the groups. Two groups were formed with seven animals in each:

Group 1: *Allium cepa group.* An amount of 3 ml aqueous solution of *A. cepa* was injected daily under the dorsal skin for 7 d before the elevation of the dorsal skin flaps. The solution was prepared from 3 g of *A. cepa* which was minced, pulverized and mixed in 20 ml of distilled water and was stored following filtration.

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Figure 1. Caudally based dorsal skin flap after flap elevation.

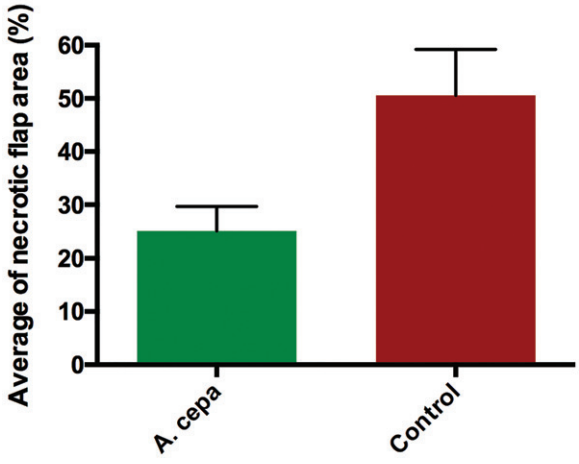

Figure 2. Mean of necrotic flap area percentage for each group (*$p<.05$).

Group 2: *Control group.* This group did not receive any treatments prior to flap elevation and were kept in the same conditions with the animals in the experiment group.

Following a 7-d treatment period caudally based dorsal skin, random flaps were raised and sutured back with 4.0 polypropylene sutures in all of the subjects (Figure 1). Following the operation, a digital camera was used to take daily digital photographs of the flaps (Sony Nex-3n; Sony Corporation, Tokyo, Japan). Necrotic and whole flap areas of the flaps were measured with the Image J software (U. S. National Institutes of Health, Bethesda, MD) following demarcation and the percentage of necrotic flap area to whole flap area was calculated [9,10].

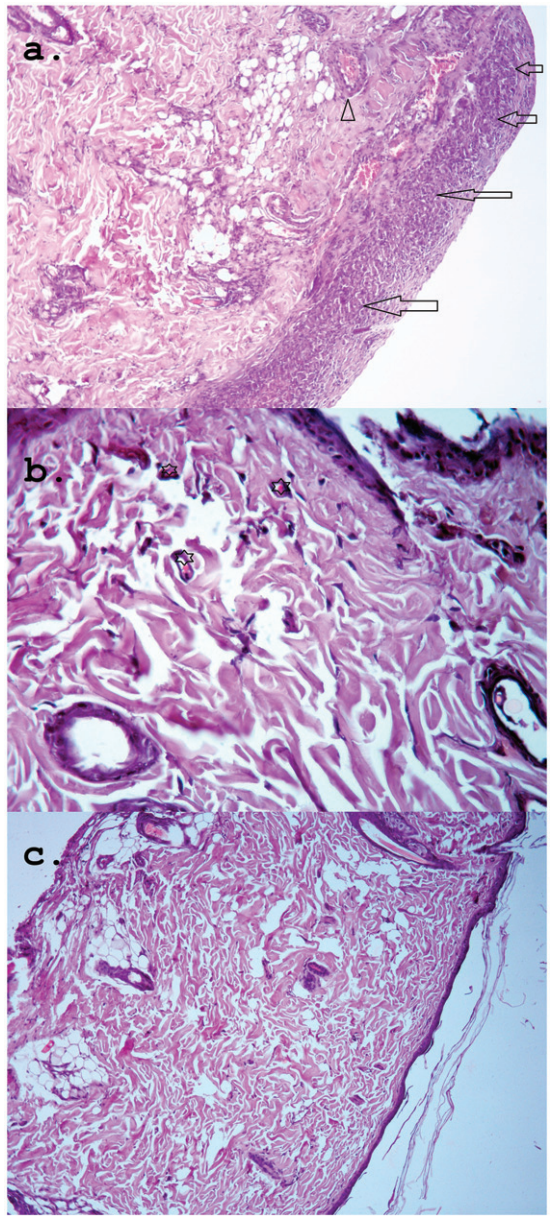

Figure 3. Photomicrographs of histological samples. (a) Inflammatory cells are marked with arrows. An additional vascular structure is marked with an arrow head (*A. cepa* group). (b) Vascular structures are marked with star signs (*A. cepa* group). (c) Control group (Hematoxylin and eosin, A: ×100, B: ×400, C: 100×).

At the end of the 7-d follow-up period following the flap elevation histological samples was taken from the proximal and the distal ends of the flaps under surgical anesthesia. Afterward, the abdominal cavity was exposed to angiographic study. Cannulation of inferior vena cava and abdominal aorta was performed with a 20-gauge and 22-gauge cannulas, respectively. After the cannulation, high dose intravenous Pentothal Sodium (Abbott®, Chicago, IL) was injected to euthanasia the animals. The blood which remained in the vascular bed was removed with serum saline solution injection of 250 ml. An amount of 40 ml of 40% barium sulfate suspension (Radyobarit Suspension®; Bracco Österreich GmbH, Vienna, Austria) and 10 ml of 10% gelatin were mixed inside 100 cc serum saline solution with heating. The mixed solution was injected from aorta cannula into the vascular system. Afterward, the taps of both cannulas were closed to prevent leakage. At the end of the procedure, whole bodies of the

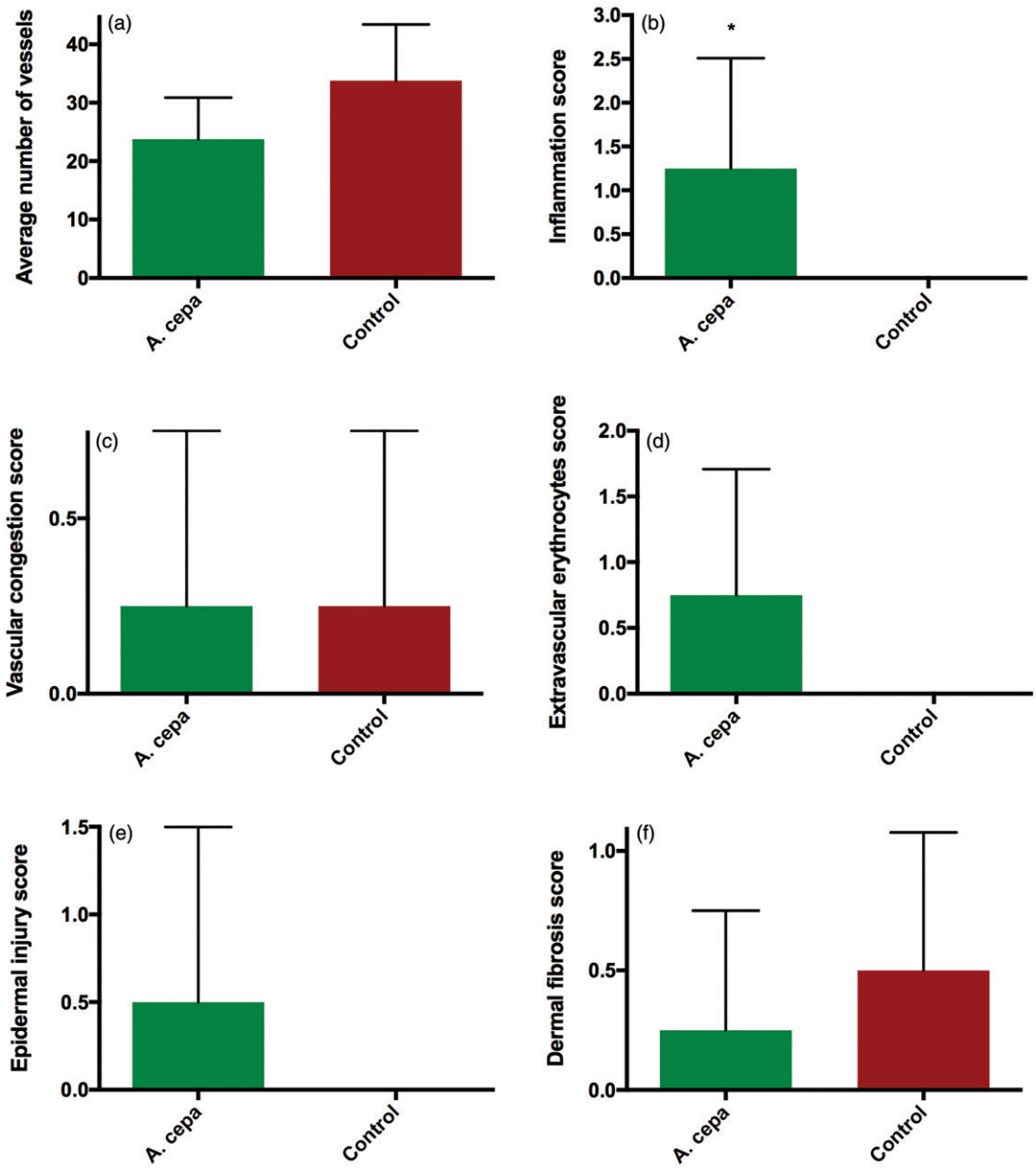

Figure 4. Mean number of (a) capillary formations and histological parameters ((b) inflammation, (c) vascular congestion, (d) extravascular erythrocytes, (e) epidermal injury and (f) dermal fibrosis) calculated for each group ($^{*}p<.05$).

animals were wrapped in plastic bags and kept in a freezer for overnight at −18 °C. The following day, the bodies were removed from the freezer, defrosted at room temperature and the dorsal flaps were separated from the body. The detached flap tissue was placed in +4 °C refrigerator for one more night. The next day the, remaining fascia and muscles were removed from the flap and the flap was stabilized on a cartoon board to prevent folding. High-resolution mammography machine (GE Health care, Chicago, IL) was used for the imaging of the flaps [11].

The fixation of the tissue samples for histological examination was performed with 10% formaldehyde. The fixed tissues were embedded in paraffin. Following microtome sectioning, the specimens were stained with hematoxylin and eosin for evaluation under light microscopy. The number of vessel formations in the dermal layer of the dorsal skin was evaluated by the same pathologist under high magnification. Histological parameters including inflammation, vascular congestion, extravascular erythrocytes, epidermal injury and dermal fibrosis were evaluated. These parameters were scaled from 0 to 3 (0: none 1: low positive 2: moderate positive and 3: high positive).

The mean percentage of necrotic flap area over the whole flap 7 d following the flap elevation and histological results for were compared using the Mann–Whitney, unpaired *t*-test and Dunn's multiple comparison tests. Prism 7 software version 7.00 for Windows was used for statistical analysis (GraphPad Software, La Jolla, CA).

Results

Flap necrosis area percentage results

The mean of necrotic flap area was 25.06 and 50.6% in the *A. cepa* and control groups, respectively. Mean of necrotic flap area

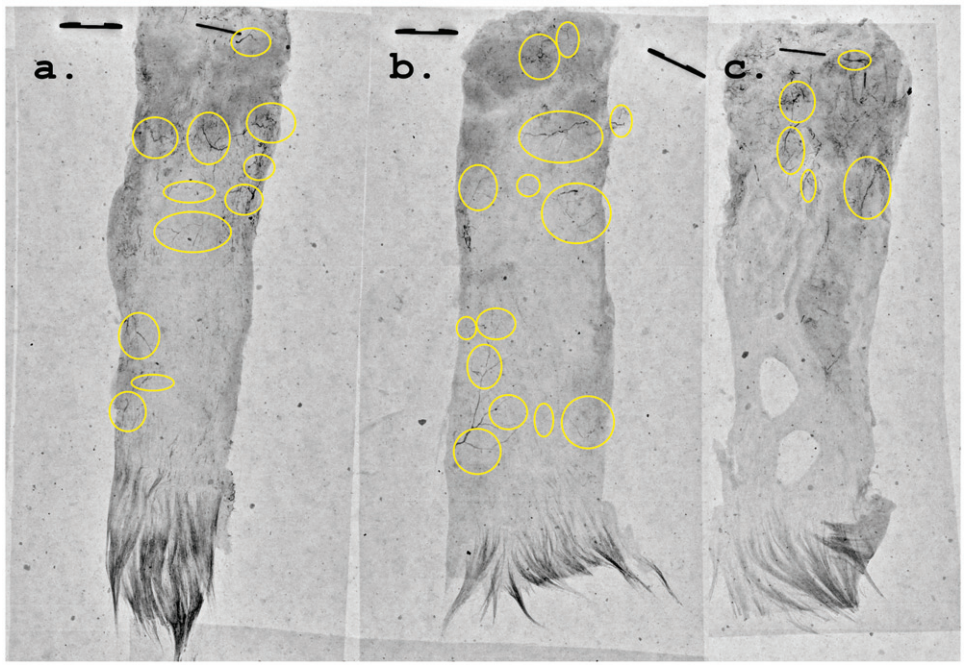

Figure 5. Microangiography results. (a and b) *Allium cepa* group and (c) Control group (Capillary formations are indicated with circles).

in each group was compared using the Mann–Whitney test. Statistical significant was present between the groups (p=.0079) (Figure 2).

Evaluation of histological results

Mean number of capillary formations counted was 23.75 ± 0.7 and 33.75 ± 9 in experiment and control groups, respectively (p =.7457). Mean of the inflammation score was 1.25 ± 1.2 and 0 in experiment and control groups, respectively (p=.0471). Mean of the vascular congestion score was 0.25 ± 0.5 and 0.25 ± 0.5 in experiment and control groups, respectively (p=.500). Mean of the epidermal injury score was 0.5 ± 0.1 and 0 in experiment and control groups, respectively (p =.178). Mean of the dermal fibrosis score was 0.25 ± 0.5 and 0.5 ± 0.5 in experiment and control groups, respectively (p =.2685) (Figures 3 and 4).

Evaluation of angiography images

The images from high-resolution mammography machine were enlarged in the computer and vessel formations identified. Capillary formations were marked in selected images from each group. In the *A. cepa* group more noticeable vascular structures were found when compared with the control group (Figure 5).

Discussion

It has been shown in a previous study that *A. cepa* peel extract can have a positive effect on endothelial function in overweight and obese individuals [12]. We believe that this might be related to the enhanced capillary formation and increased microcirculation. In another study, Naseri et al. have shown the vasodilatation effect of *A. cepa*. This effect of *A. cepa* has been related to quercetin, antioxidant activity and inhibition vascular smooth muscle cells [8]. In our study *A. cepa* extract was shown to have a beneficial effect on flap survival and more prominent vessels were identified in the angiographic images of the *A. cepa* group. Despite this, there was no significant difference between the number of capillary formations in the *A. cepa* and the control group. These findings are consistent with previous studies which have shown that *A. cepa* has vasorelaxant effects.

Quercetin is a polyphenol molecule which is found in many vegetables. It has been shown to have antioxidant properties [4,13]. It has also been shown to estrogenic properties which might have effects on circulation. It has been previously shown that antioxidants can improve flap survival [14,15]. We believe that the local effect of quercetin might have a positive effect on ischemic flap injury by binding the oxygen radicals but more detailed studies are required to investigate this specific effect.

Anti-inflammatory effects of *A. cepa* have been shown in previous studies. Thiosulfinates have been related to these effects. Allicin is one of the well-known thiosulfinates which is an active ingredient of garlic. In onions, thiopropanal S-oxide, thiosulfonates, bis-sulfines, sulfides and cepaenes are the major sulfur-containing compounds instead of allicin. There are some studies showing that these sulfur compounds can inhibit cyclooxygenase (COX) and lipoxygenase activities [16,17]. These, as a result, could be responsible for the anti-inflammatory effects. Contrary to the previous studies in our study, we found that inflammatory findings were more prominent in the *A. cepa* group. Despite the increase in inflammation, vascular congestion scores were similar between the groups. In some of the earlier studies on flap survival, increased inflammation in the absence of infection has been associated with increased flap survival [18,19]. In addition, immunomodulatory effects of onion and garlic have been shown in previous studies. These effects have been related to an increase in IL2 and IL4 production which can alter T cell function [20]. These changes could alter the healing and inflammatory processes during flap recovery.

A. cepa injections were performed from two points on the flap. It is important to note that the inflammation in the experiment group could be related to the injection itself. Small PPD needles were used for the injection to minimize this problem. But despite this, it was not possible to completely eliminate this problem. This is one of the shortcomings of this study.

We think that further investigation is necessary to better clarify the mechanisms behind the effect of the *A. cepa* solution on flap survival and microcirculation. These results could be valuable as they could be applied to other fields of medicine related to flap surgery, microcirculation and ischemic injury.

Disclosure statement

No potential conflict of interest was reported by the authors.

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