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Effect of Systemic Antioxidant Allopurinol Therapy on Skin Flap Survival

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Abstract

Background: This study was undertaken to evaluate whether steady state concentrations of allopurinol can improve ischemia in canine skin flaps.

Methods: Twenty dogs underwent one skin flap surgery with a 2-week interval. The first procedure was performed according to the standard protocols. The second phase was started by a 1-week pretreatment with allopurinol. Length of the necrotic zone was measured and recorded daily. At each phase, flaps were removed and sent for histopathological study after 1-week observation.

Results: Mean length of the necrotic zone in allopurinol treated skin flaps has been significantly less than normal flaps over all 7 days of observation (p-value<0.0001). Histopathology study showed less inflammation and more normal tissue structure in the allopurinol treated skin flaps.

Conclusions: Findings obtained from our study demonstrate that systemic administration of allopurinol significantly improves skin flap survival and may have significant clinical implications.

Keywords: Allopurinol; Antioxidant; Skin necrosis; Reperfusion injury; Flaps; Tissue ischemia.

Introduction

Surgeons rely on skin flaps to reconstruct tissue defects resulting from trauma, ablative surgery or congenital malformation. However, prolonged tissue ischemia in setting of trauma or prolonged procedures can lead to tissue necrosis and eventual demise of the skin flap [1, 2]. Despite remarkable progress in plastic surgery over the previous two decades, partial flap necrosis has been reported in 7-20% of free flaps and 20-33% of pedicled flaps [3-9].

Ischemia-reperfusion (IR) injury is one of the most important causes of flap damage. IR injury likely results in a complex pathway involving neutrophil infiltration and production of superoxide free radicals which lead to irreversible tissue damage, need for reoperations, prolonged hospitalization and increased medical costs [10-14].

Xanthine oxidase (XO) plays an important role in the pathogenesis of IR injury. There is a significant up-regulation of XO system during ischemia of skin flaps [15, 16]. XO is a major source of reactive oxygen species (ROS) which triggers release of several inflammatory mediators [17, 18]. Allopurinol is an antioxidant drug, and inhibits XO. Hence, systemic administration of allopurinol reduces ROS formation and may improve survival rate of skin flaps [19, 20]. However, effectiveness of allopurinol in improving skin flap survival is still controversial [21, 22].

Given our understanding of IR and the ongoing controversies about the role of Allopurinol in the XO pathway, we set out to design a study to further elucidate the steady state concentration level of allopurinol required to improve skin flap survival in dogs.

Materials and Methods

This clinical trial was performed at veterinary research clinic of the Isfahan University Medical Centre. The study received IRB approval by the University ethics committee (391136) and adhered to the strict guidelines of the Helsinki Declaration.

Twenty male, healthy dogs aged between 3 to 4 years and mean weight of 5.4 kg (range 4-6 kg) were included in the present study. All dogs had physical examination prior to the study. They had been completely vaccinated, and had received a complete course of anti-parasite medication. All dogs were kept in separate cages during the study and were fed with normal diet.

The subjects were divided into two groups of ten (Group A and B). All dogs underwent one skin flap surgery by a single blinded surgeon (a total number of 20 skin flaps). Group A (control group) procedure was performed under the standard conditions with no additional medication. Group B procedure was performed similar to the first one, but all dogs were pre-treated with unlabeled use of oral...
allopurinol from one week before the surgery until 48 hours after
skin flap surgery.

**Group A skin flap surgery**

After 12 hours of fasting, the surgical site (the back of the dogs)
was shaved. Dogs were anaesthetized with ketamine (75 mg/kg of
body weight) and a cuffed endotracheal tube was inserted into the
trachea. Then an island adipofascial cutaneous flap, 4×24 cm, was
created in one side of the spinal column extending down to the
fascia. Afterwards, the flap was sutured in place immediately using
simple interrupted Nylon 1-0 sutures [Figure 1].

**Figure 1:** Schematic view of the surgical site and sutures’ sites.

After the operation dogs were treated with appropriate dose of
analgesics. In addition, a daily dose of 30 mg/kg Cefazolin was
commenced intravenously 60 minutes prior and every day after
surgery. All dogs were visited on a daily basis by a veterinarian and
the surgeon to check the general condition and skin flap status. At
the end of this one-week observation flaps were removed. After
removal, flap site was closed with simple continuous suture of
nylon-01.

**Group B skin flap surgery**

All ten dogs in second group received allopurinol (MD-Allopur-
urinol, 100 mg, Mehr Darou, Tehran, Iran, daily dose of 50 mg/kg
of body weight) through the nasogastric tube for 9 days (7 days prior
to surgery, 2 days during the observation). Skin flap surgery was
performed one week after commencing allopurinol. Otherwise, the
second operation was completely similar to the first group one.

**Measurements**

All flaps were assessed daily regarding the size of the necrotic area.
Surface of the necrotic area was measured by the surgeon in-vivo,
using a single standard ruler with the accuracy of 1/10 centimeter. All
measurements were recorded in cm². Length of the necrotic zone was
defined as the longest distance between the necrotic edge and the
survived area. Care was taken to ensure maintenance of a straight line
during the measurement.

**Pathologic study of the skin flaps**

One week after each skin flap surgery, the skin flaps were removed,
fixed in 10% formalin and sent for histopathological study. After
hematoxylin and eosin (H&E) and toluidine blue staining, all samples
were investigated by a single pathologist who was unaware of the type
of treatment. Samples were studied regarding the number of
neutrophils, fibroblasts, mast cells and other inflammatory cells, using
a light microscope.

**Statistical analysis**

Data was analyzed by SPSS 16.5 software. Independent-t test was
used to compare mean length of necrotic zone between standard flaps
and allopurinol treated flaps.

**Results**

Comparison of two types of skin flaps showed that mean total area
of the necrotic zone in allopurinol treated skin flaps (27.6 Cm²) have
been significantly less than necrotic area in normal flaps (78 Cm²)
over all 7 days of observation [Figure 2]. Dogs in the control group
presented an average of 81.25% of necrosis in the flap total area; those
which received allopurinol presented an average of 28.75% of necrosis.
Table 1 illustrates the changes in length of necrotic zone for both
groups during observation time. Differences in area of necrotic zones
increased proportionally with observation time (P < 0.05). [Figure 3
and 4] represent skin flaps treated with allopurinol 3 and 7 days after
skin flap surgery whereas [Figure 5 and 6] show control group without
allopurinol treatment 3 and 7 days after skin flap surgery.

**Figure 2:** Illustration of changes in the mean area of necrotic zone in both
groups. Data are presented as mean area of necrotic zone in cm² in different
days.
Table 1: Comparison of mean length of necrotic zone between two types of flaps in different days. Data are presented as mean (±SD) length of the necrotic zone, in cm, during the observation time. N = number of cases; SD = standard deviation.

<table>
<thead>
<tr>
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<th>Mean length of the necrotic zone (cm)</th>
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<tbody>
<tr>
<td></td>
<td>Day 1</td>
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<tr>
<td>Normal skin flaps (n=10)</td>
<td>5.9±0.6</td>
</tr>
<tr>
<td>Allopurinol treated skin flaps (n=10)</td>
<td>2.4±0.3</td>
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<tr>
<td>p-value</td>
<td>&lt;0.0001</td>
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</tbody>
</table>

Normal skin flaps findings of different zones

1. Necrotic zone findings: Coagulative necrosis with large amounts of vessel thrombosis can be detected. A few neutrophils and fibrotic tissue in margins were seen [Figure 7a].

2. Transitional zone findings: Destruction of the epidermis in most of the regions. In superficial dermis there was infiltration of inflammatory cells - mostly neutrophils - between collagen bundles. Existing microabscess. More severe inflammation was found in the subcutis fat in form of non-septal panniculitis. Fat necrosis with intensive infiltration of inflammatory cells - neutrophils, macrophage and lymphoplasma cells could be detected. Numerous dilated vessels with evidence of thrombosis in some of them were present [Figure 7b].

3. Intact zone findings: Moderate infiltration of inflammatory cells - macrophage and plasma cells - in the subcutaneous fat and deep dermis. There was focal fat necrosis. Furthermore, existing extravasation of red blood cells and vasodilatation [Figure 7c].

Allopurinol treated skin flaps findings of different zones

1. Necrotic zone findings: Shedding of the superficial epidermis. Intensive infiltration of inflammatory cells - neutrophils and macrophage - around the muscle bundles with focal microabscess and dilated vessels and thrombosis in some of them [Figure 8a,b].

2. Transitional zone findings: Focal shedding of the epidermis accompanied by mild infiltration of neutrophils. Obvious red blood cell extravasation in superficial dermis. Severe panniculitis, fat necrosis, infiltration of neutrophils and dilated vessels with some thrombosis were seen in the deep dermis [Figure 8c].

3. Intact zone findings: Completely normal structure of the skin tissue [Figure 8d].
grafts to tolerate longer periods of ischemia and found it a useful method to improve skin flap survival. A previous study performed by Tamir et al. confirms our findings by a different study design in rats. They investigated effects of allopurinol on survival of island skin grafts under prolonged period of ischemia, and consequently, by preventing tissue damage [28].

Picard-Ami et al. reported different levels of XO activity in rats, pigs and humans [36]. Therefore, in contrast to the above mentioned studies, they studied effects of allopurinol on skin flaps of pigs and found it ineffective [22]. This finding shows that although allopurinol can improve skin flap survival by reducing ROS levels, this effect is completely dependent on the level of XO activity in the target organ.

Even though critical flap monitoring is within 72 hours postoperatively [37], we did monitor the flaps over 1 week. Nevertheless, our study lacks an extended observation period and thus no statement can be made regarding long-term survival. The present experiment had some critical differences with aforementioned studies. Instead of rats which are most commonly used to investigate this subject, we used dogs. Accordingly, flaps dimensions can be dissected similar to possible human correlates and there is no size limitation like rats, also we chose to focus on dogs because we wanted to show the difference in a setting where the surface area of flaps raised would be similar to clinical scenarios in humans and the dog model was the closest animal that would be able to provide. More importantly, in contrast to the previous studies in which allopurinol was administrated a few hours before [22] or even after the procedure [28], in this study dogs were pre-treated with allopurinol for 1 week prior to the surgery which helped us to reach a steady plasma concentration of the drug.

In summary, findings obtained from our study demonstrated that systemic administration of allopurinol significantly improved skin flap survival and had a protective effect against flap necrosis in dogs. Clinical impressions were confirmed by histopathological examination.

Although results of this method regarding administration of allopurinol are promising, further studies such as comparison of XO activity of dogs with humans are required prior to apply it on humans. Furthermore, studies with even smaller dimensioned flaps and longer timelines should be planned. It would be of utmost interest to find clinical patients who are managed on Allopurinol for other medical conditions and see whether they respond better to flap surgery using in vivo vascular studies.

Acknowledgments

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References
