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MIXED ARTERIO-VEINUS INSUFFICIENCY IN RANDOM SKIN FLAPS IN THE RAT: IS THE APPLICATION OF MEDICINAL LEECHES BENEFICIAL?

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Schlaudraff K-U, Bezzola T, Pepper M*, Montandon D, Pittet B. Mixed arterio-venous
insufficiency in a random dorsal skin flap model in the rat: Is the application of medicinal leeches
beneficial?

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ABSTRACT

Background: Leeches are commonly used in reconstructive surgery for the treatment of venous congestion in flaps. Documented mechanisms of action are: 1) injection of the anticoagulant hirudin; 2) active suction of blood; and 3) passive oozing of the bite wound. Even though the benefits of leeches in venous congestion are widely accepted, little is known about their effects in mixed arterio-venous insufficiency.

Methods: 30 Wistar rats were randomized into 3 groups (n=10 each) and 9x3cm ischemic random skin flaps were elevated on the rat dorsum. Group 1 served as controls. In group 2, one leech was applied to the distal part of the flap and in group 3, three leeches were applied, one at a time, at 8 hour intervals. Postoperatively, flap survival and perfusion were quantitated by daily planimetry and Laser-Doppler imaging.

Results: The application of a single leech on postoperative day 7 did not lead to any statistically significant changes in total flap survival or tissue perfusion. Triple leech application, however, caused a significant decrease in flap survival of 6.6% at day 7 (3x leech vs. control: $45.8 \pm 8.5\%$ vs. $52.4 \pm 8.5\%$, respectively) ($p < 0.01$) and a decline in flap perfusion of 13% (3x leech vs. control: $71.3 \pm 16.6\%$ vs. $84.3 \pm 9.3\%$, respectively) ($p < 0.01$).

Conclusion: The data demonstrate that the application of leeches in the setting of mixed arterio-venous insufficiency can be hazardous to flap viability. Pure venous congestion with an adequate arterial supply remains the only indication for controlled leech application in the clinical setting.

Keywords: ischemia, venous congestion, *Hirudo medicinalis*, arterio-venous insufficiency, bloodletting, skin/blood supply, skin/pathology

INTRODUCTION

“Random” skin flaps are frequently used to reconstruct tissue defects of small or medium size (1, 2) e.g., after trauma or tumor surgery (3, 4). They are characterized by the absence of a vascular pedicle and tissue perfusion relies entirely on a randomly distributed vascular network. Signs of “randomization” may also be observed in peripheral areas of “axial” flaps whenever their design exceeds the defined limitations of the underlying arteriosome/venosome and therefore includes tissue that is not directly perfused by the flap’s pedicle vessels. For this reason, a less-than-optimal design of “random” as well as “axial” flaps may lead to peripheral arterio-venous insufficiency and finally to partial flap necrosis that may jeopardize the success of the reconstructive procedure. Unlike pure “axial” flaps in which flap failure is usually due to problems with the vascular pedicle, arterio-venous insufficiency in “random” or randomized “axial” flaps is the result of progressive microcirculatory disturbances. These are caused either by an initial decrease in arterial inflow and subsequent venous stasis, or by a primary increase in venous outflow resistance that progressively alters the arterial hemodynamics. Initially, venous congestion causes an increase in interstitial pressure, edema, as well as hemoconcentration, and a secondary slowing of the capillary blood flow. The subsequent microthrombosis then results in arterial insufficiency and may lead finally to flap ischemia or even necrosis (5-8).

In the quest for alternative salvage procedures for imminent flap failure, one of the oldest medical practices has been re-discovered (9-11) – the letting of blood, achieved by the application of leeches (12-14). In historical reports, leeches were used for their superb decongestant properties (15, 16) and were empirically applied whenever surgeons faced pronounced venous insufficiency (17-19). The most commonly used leech is *Hirudo medicinalis*, a freshwater hermaphrodite, which was chosen primarily for its ability to inflict the deepest bite and to cause the most prolonged period of post-bite blood oozing (20). The therapeutic benefit of leeches is a combination of 1) the injection of a salivary cocktail of anticoagulants into the dermis that stimulates and perpetuates local blood flow (21, 22), 2) the mechanical effect of active suction of blood from the flap with a negative pressure of up to 1/10 atm (23, 24), and 3) the passive oozing of the bite wound after leech detachment (20, 25). The application of leeches thus allows the evacuation of venous blood from engorged flaps and results in a temporary restoration of the capillary blood flow as well as improved tissue viability (26, 27). Thus far, the application of leeches has only been described in the clinical setting of severe venous congestion (8, 28) or the complete absence of venous drainage in “axial” flaps (20, 29), replanted ears (30, 31) or digits (32, 33). However, the pathophysiology of mixed arterio-venous

insufficiency in failing “random” flaps, as well as the effects of the application of leeches in this setting remain poorly understood.

In the present study in the rat, we investigated whether the application of leeches could increase the total survival of arterio-venous insufficient random dorsal skin flaps. We hypothesized that the mechanical suction of blood by the leech may not only reduce venous congestion but also promote arterial inflow in the ischemic tissue and that the anticoagulants of the leeches’ saliva may inhibit microthrombosis in the ischemic part of the flap, thereby maintaining local microcirculation.

METHODS

Animals

The present study was approved by the ethics committee of the University of Geneva as well as the local veterinary authorities in accordance with Swiss guidelines. Male Wistar rats weighing 250-300g were housed individually at room temperature with a 12-h day/night cycle and water and food ad libitum. A total of 30 animals were randomly assigned to control or study groups of 10 rats each and elevation of a random dorsal skin flap was performed as described below: group 1 served as controls (*no leech*); in group 2, we performed a single leech application at 5min postoperatively (*single leech*); and group 3 underwent a leech application at 5min, 8h and 16h after flap elevation (*triple leech*).

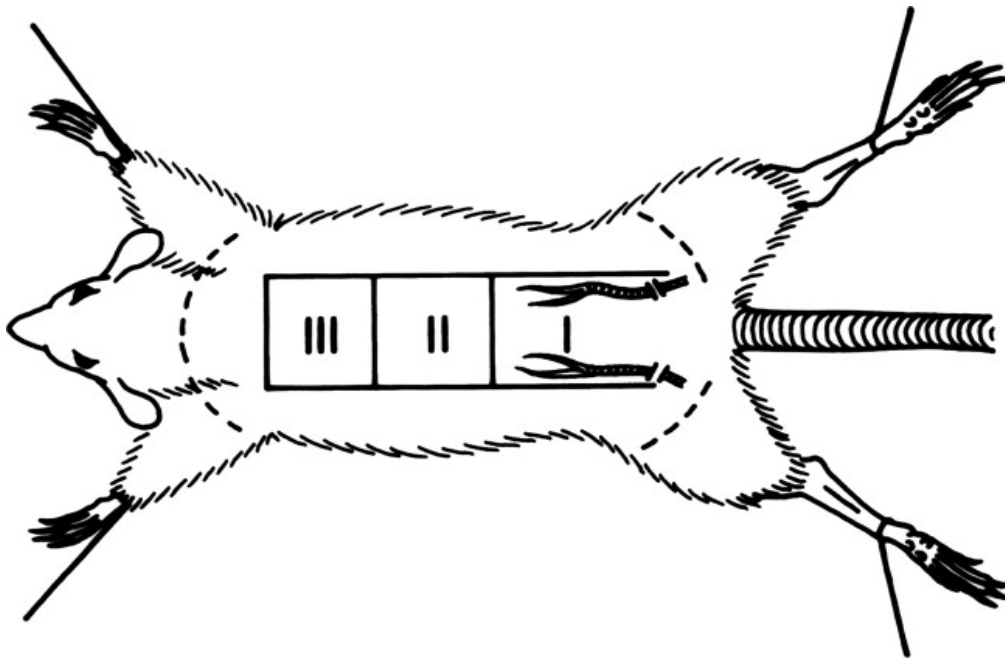


Figure 1: Schematic representation of a random dorsal skin flap in the rat (9x3cm) divided into proximal (I), intermediate (II) and distal (III) areas. Note that during flap elevation both sacral arteries were sectioned (36).

Flap elevation

For surgical procedures, rats were anesthetized by non-assisted inhalation of 2% isoflurane (Abbott AG, Baar, Switzerland) and rectal temperature was maintained at 34°C. Prior to surgery, the entire rat dorsum was shaved and chemically depilated. The skin was thoroughly washed with soap and water and rinsed with non-chlorinated water to remove any remnants of depilating cream or repelling odors. Random dorsal skin flaps were elevated using the model initially described by McFarlane (34) and later modified by Rinsch et al (35). We outlined caudally-based, 9x3cm large skin/panniculum carnosum flaps on the back of the rats and systematically sectioned both sacral arteries. The flaps were immediately sutured back onto their original anatomic site. For analysis, the flap area was divided into three distinct zones of equal size reflecting the clinical aspect of complete flap survival in the proximal area I, a mixed pattern of flap survival and partial necrosis in the intermediate area II, and full thickness necrosis in the distal area III (figure 1) (36).

Leech application

Fasting leeches were provided by the pharmacy of the University Hospitals of Geneva in a cloth-covered container and stored in a solution of distilled water and Hirudo Salt (Leeches USA) in the dark at 7°C. Before use, the leeches' initial weight was noted and immediately after surgical flap elevation, leeches were allowed to attach in the center of the distal part of the flap (area III) and to feed at will while the rats were still anesthetized. After spontaneous release, leeches were weighed again and total weight gain was converted to ml of blood (1g of weight gain equals 1ml of blood) (37). In group 2, one leech was allowed to feed in the distal part of the flap and in group 3, a triple application of one leech at a time was performed at 8h intervals. The rats in group 1 (control group) were not treated with leeches. All rats were anesthetized for either the leech application or the sham session and then put back into their cages for recovery.

Macroscopic and planimetric analysis

Macroscopic and planimetric analysis was carried out daily during a two-week period. The rats' dorsum was photographed with a Nikon Coolpix 4500. For planimetry, the margins of the flap and the area of necrosis were traced on transparent acetate sheets. Both surfaces were quantitated with a polar planimeter and flap survival was measured by subtracting the area of necrosis from the total flap surface. Results were expressed as percentage values and represent the average of three measurements.

Laser-Doppler flowmetry

During a two-week period, daily skin perfusion analysis was carried out using a percutaneous Laser-Doppler perfusion imager (Derma PIM II, Lisca AB, Linköping, Sweden). The Laser-Doppler unit scanned the entire surface of the skin flap and the surrounding tissue of the rat's back. Cutaneous blood flow was expressed in arbitrary perfusion units (PU) and encoded in a color-graded image. Quantification was carried out with Patchtest-Wizard 1.2 software (Lisca AB) and regions of interest were outlined according to the three predefined areas of the flap (36). As a reference value, blood flow in normally perfused skin was measured in two regions localized laterally to the flap. Blood flow at areas I, II and III was expressed as the percentage of mean perfusion of normal skin. Final values represent the average values of three measurements.

Statistical methods

Statistical analysis of the data was performed using Fisher's ANOVA computed on GraphPad's Instat 3.0 software after a standard normality test. All values are expressed as mean \pm sd. The significance level was established at $p < 0.05$.

Results

Leech application:

After elevation of the random dorsal skin flap, leech attachment was obtained without difficulty in all animals of experimental groups 2 and 3. Feeding activity was normal until spontaneous release after engorgement and feeding time averaged 72 ± 22 min ($n=40$). Average weight gained by the feeding leech was 0.91 ± 0.17 g and mean oozing time from each leech bite averaged 188 ± 52 min. None of the rats were exsanguinated by leeching nor did we encounter any problems of scratching/biting the fresh bite sites. No signs of adverse reactions due to the leech application were observed.

Macroscopic analysis

After random dorsal skin flap elevation, distal perfusion was initially maintained, as indicated by continuous arterial bleeding from the borders of the flap. Within the first 12h postoperatively, we noted a purple discoloration, an increase in tissue edema and cessation of peripheral bleeding of the distal flap area III – i.e. signs of progressive arterio-venous insufficiency.

At baseline, venous congestion/edema was similar in all three study groups. After a single leech application tissue edema in the distal part of the flap was visibly reduced when compared to the control animals. However, in both the control and the single leech group, development of tissue

necrosis started simultaneously at postoperative day 2 with superficial epidermolysis in the most distal part of the flap that progressively extended more proximally. Full thickness necrosis, indicated by the appearance of a black scab, was fully established in area III at postoperative day 4.

After the triple leech application, tissue edema was visibly less when compared to the control and single leech group and flaps showed only a little purple discoloration. Progressive arterial insufficiency due to an overly pronounced withdrawal of blood was rapidly indicated by dark red and black spots in the distal area III – indicative of early epidermolysis. We also observed the precocious development of flap necrosis that was already present when the last leech was applied (at 16h postoperatively) and located at the site of the leech bites. Full thickness necrosis in the distal area III was already fully established at postoperative day 2 ($p < 0.01$) (figure 2).

Flap survival

At postoperative day 7, planimetric analysis in the control group showed an average survival rate of $52.4 \pm 8.5\%$ of the total flap surface. The application of a single leech (group 2) did not lead to any statistically significant changes: at day 7, total flap survival was $52.7 \pm 6.4\%$ in the single leech group vs. $52.4 \pm 8.5\%$ in the control group. However, triple leech application led to a decrease in total flap survival by 6.6% ($p < 0.01$): at postoperative day 7, flap survival in this group was $45.8 \pm 8.5\%$ vs. $52.4 \pm 8.5\%$ in the control group (figure 3).

Laser-Doppler flowmetry

At day 2 after elevation of the random skin flap in control animals, Laser-Doppler measurements showed a flap perfusion of $93.2 \pm 16.5\%$ of normal cutaneous values in the proximal area I and of $57.2 \pm 10.3\%$ of normal skin perfusion in area II. After a single leech application (group 2) Laser-Doppler flowmetry revealed a significant increase in blood flow in the proximal area I of 9% (1x leech vs. control: $102.2 \pm 13.2\%$ vs. $93.2 \pm 16.5\%$, respectively) and in area II of 15.2% (1x leech vs. control: $72.4 \pm 9.1\%$ vs. $57.2 \pm 10.3\%$, respectively) ($p < 0.01$). After the triple leech application, flap perfusion remained unchanged in area I (3x leech vs. control: $92.7 \pm 16.7\%$ vs. $93.2 \pm 16.5\%$, respectively) and was increased by 2.3% in the intermediate area II (3x leech vs. control: $59.5 \pm 8.5\%$ vs. $57.2 \pm 10.3\%$, respectively), but values did not reach statistical significance ($p < 0.064$). Measurements in area III, the distal part of the flap, were not considered since the leeches' bitemarks and the early superficial epidermolysis impaired reliable perfusion analysis. However, immediately after flap elevation, Laser Doppler flowmetry showed a blood perfusion of $48.1 \pm 4.5\%$ of normal cutaneous values (control, area III, 1h postoperative).

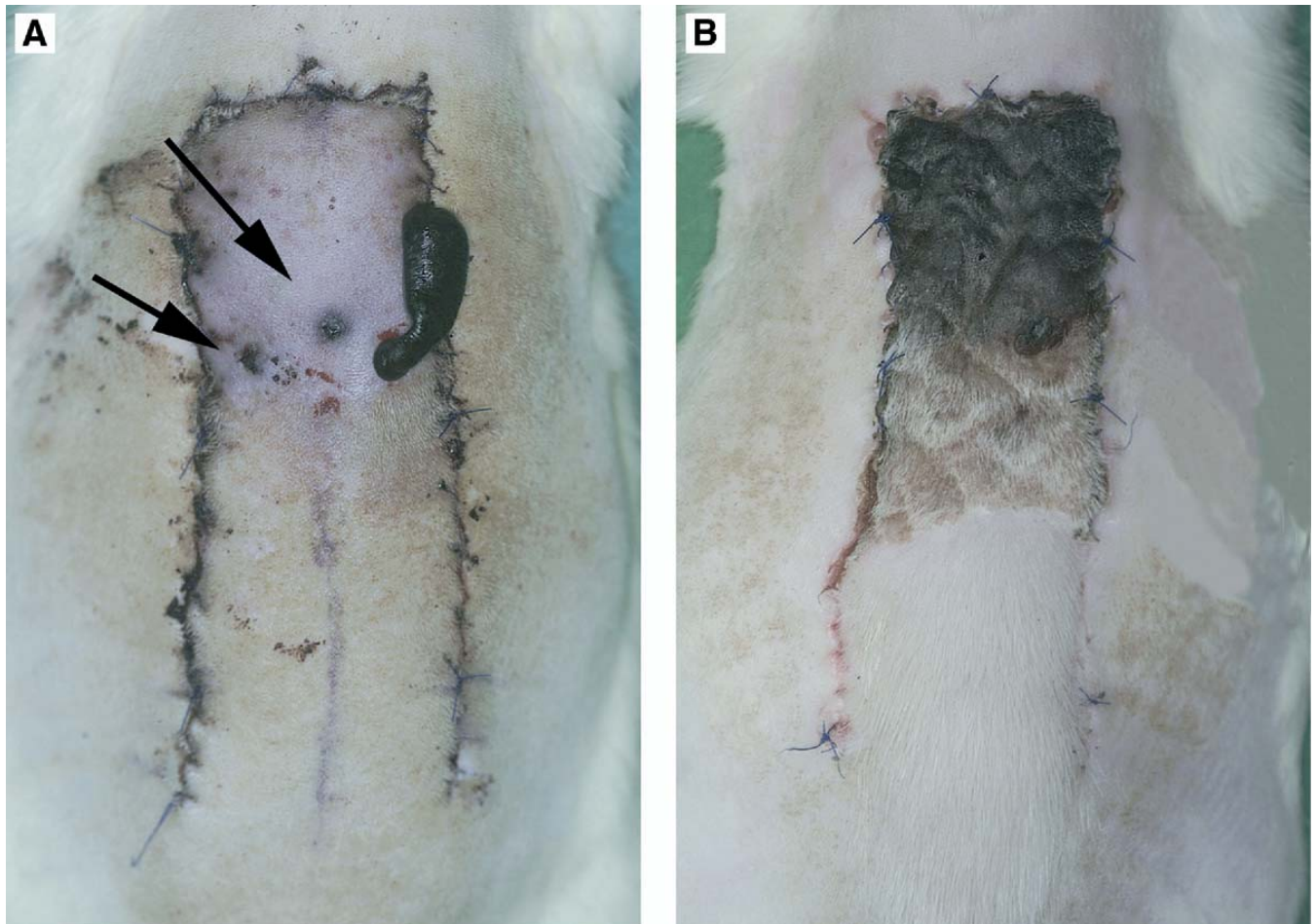


Figure 2: Distal flap necrosis after triple leech application in a random dorsal skin flap at 48h postoperatively (right). At 16h postoperatively (left), note the presence of a dark purple color and tissue edema in the distal area III as well as precocious skin necrosis at the site of the leech bite (arrows).

At day 7 after elevation of the random skin flap in the control animals, we observed a flap perfusion of $111.1 \pm 16\%$ of normal cutaneous values in the proximal area I and of $84.3 \pm 9.3\%$ of normal skin perfusion in area II. After a single leech application (group 2), Laser-Doppler flowmetry revealed a significant increase in proximal blood flow of 15.6% at postoperative day 7 (1x leech vs. control: $126.7 \pm 14.9\%$ vs. $111.1 \pm 16\%$, respectively) ($p < 0.01$). In the intermediate area II, we also observed a moderate increase in flap perfusion of 6.9% (1x leech vs. control: $91.2 \pm 14\%$ vs. $84.3 \pm 9.3\%$, respectively) but values did not reach statistical significance ($p < 0.058$). The triple leech application however, significantly reduced cutaneous perfusion: at postoperative day 7, blood flow in the proximal area I was diminished by 16.1% (3x leech vs. control: $95 \pm 19.4\%$ vs. $111.1 \pm 16\%$, respectively) and in the intermediate area II it was decreased by 13% (3x leech vs. control: $71.3 \pm 16.6\%$ vs. $84.3 \pm 9.3\%$, respectively) ($p < 0.01$) (figure 5).

DISCUSSION

Today, it is commonly accepted that the application of leeches is of great benefit in “axial” flaps suffering from venous congestion when normal arterial inflow is maintained. By ingesting blood volumes of 20-60ml each (9, 27, 38), leeches provide efficient venous decompression, and thereby maintain the capillary microcirculation until the process of neovascularization has established new venous connections for drainage (39). This has been confirmed by clinical reports of the successful use of leeches for the salvage of pedicled (28, 40, 41) and free “axial” flaps (38, 42, 43), as well as in severe venous congestion of replanted digits (32, 33) and ears (26, 31). However, the effects of the application of leeches in “random” or “randomized axial” flaps suffering from mixed arterio-venous insufficiency have not yet been investigated. Given the probably predominant role of the venous component in mixed arterio-venous insufficiency, we hypothesized that the use of leeches may be an effective means of alleviating venous congestion, restoring minimal perfusion of the capillary bed and thereby increasing flap survival.

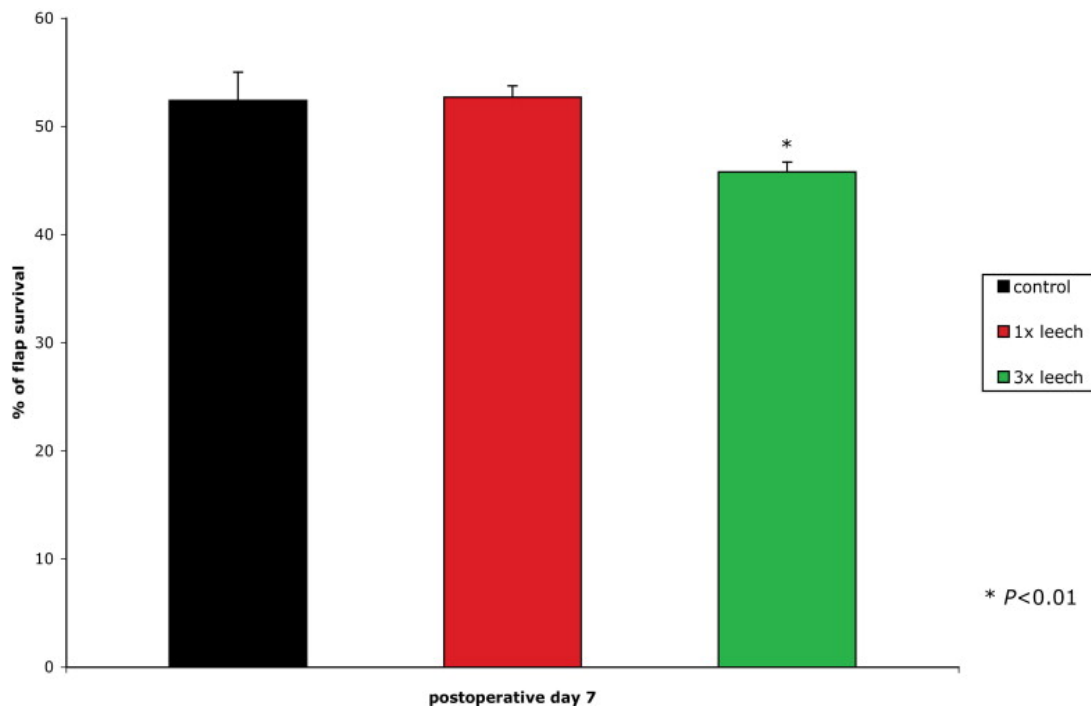


Figure 3: Total flap survival after single and triple application of leeches compared to control animals. Note the significant decrease in flap survival in the triple leech group at postoperative day 7 ($p < 0.01$).

Earlier experimental studies in a 3x6cm 'axial' epigastric skin flap in the rat showed a direct correlation of the severity and duration of venous congestion and of the level of arterial perfusion to the volume of the leeches' blood meal and the total ingestion time (44, 45): a prolonged venous congestion of 5-6h already causes slowing of the arterial inflow –a secondary arterial insufficiency – and results in a significantly smaller blood meal as well as an increased ingestion time (46, 47). Our animal model resulted in pronounced mixed arterio-venous insufficiency indicated by the presence of severe venous congestion as well as a reduced but maintained arterial blood flow (control, area III, 1h postoperative: $48.1 \pm 4.5\%$). Even though we did not encounter any problems of leech attachment, which are indicative of complete arterial insufficiency (48), the measured average blood meal of $0.91 \pm 0.17\text{ml}$ is also clearly inferior to previously reported values and the ingestion time of $72 \pm 22\text{min}$ is considerably prolonged. In the present study, we observed that the single leech application induces a transitory beneficial effect on blood flow in the random dorsal skin flap: at postoperative day 2, we measured a significant increase in blood perfusion in the intermediate area II of 15.2% ($p < 0.01$). This increase, however, progressively diminished to 6.9% at postoperative day 7 and values did not reach the level of significance ($p < 0.058$). Knowing that improved flap viability can only be achieved by maintaining an increase in perfusion in the critically ischemic intermediate area II, total flap survival at postoperative day 7 consequently remained unchanged. These results differ from the findings by Cottler et al. who observed a significant improvement in both flap survival and blood flow at postoperative day 7 (47) and by Lee et al who confirmed an increase in flap perfusion of 17% as well as an improved flap survival of 35% (46). However, both studies investigated the effects of single leech application in an "axial" flap with a temporarily clamped venous pedicle but a maintained arterial inflow and not in a "random" flap suffering from pronounced changes in both arterial and venous blood flow.

Triple leech application resulted in pronounced deleterious effects: at postoperative day 2, flap perfusion in the intermediate area II was increased by a mere 2.3% and values did not reach statistical significance ($p < 0.064$). At postoperative day 7, however, we measured a statistically significant decrease in blood flow in the intermediate area II of 13% , reflected by a reduction in flap viability of 6.6% ($p < 0.01$). Clinically, this deleterious hemodynamic effect of triple leech application was visible as the precocious development of distal flap necrosis, starting at the site

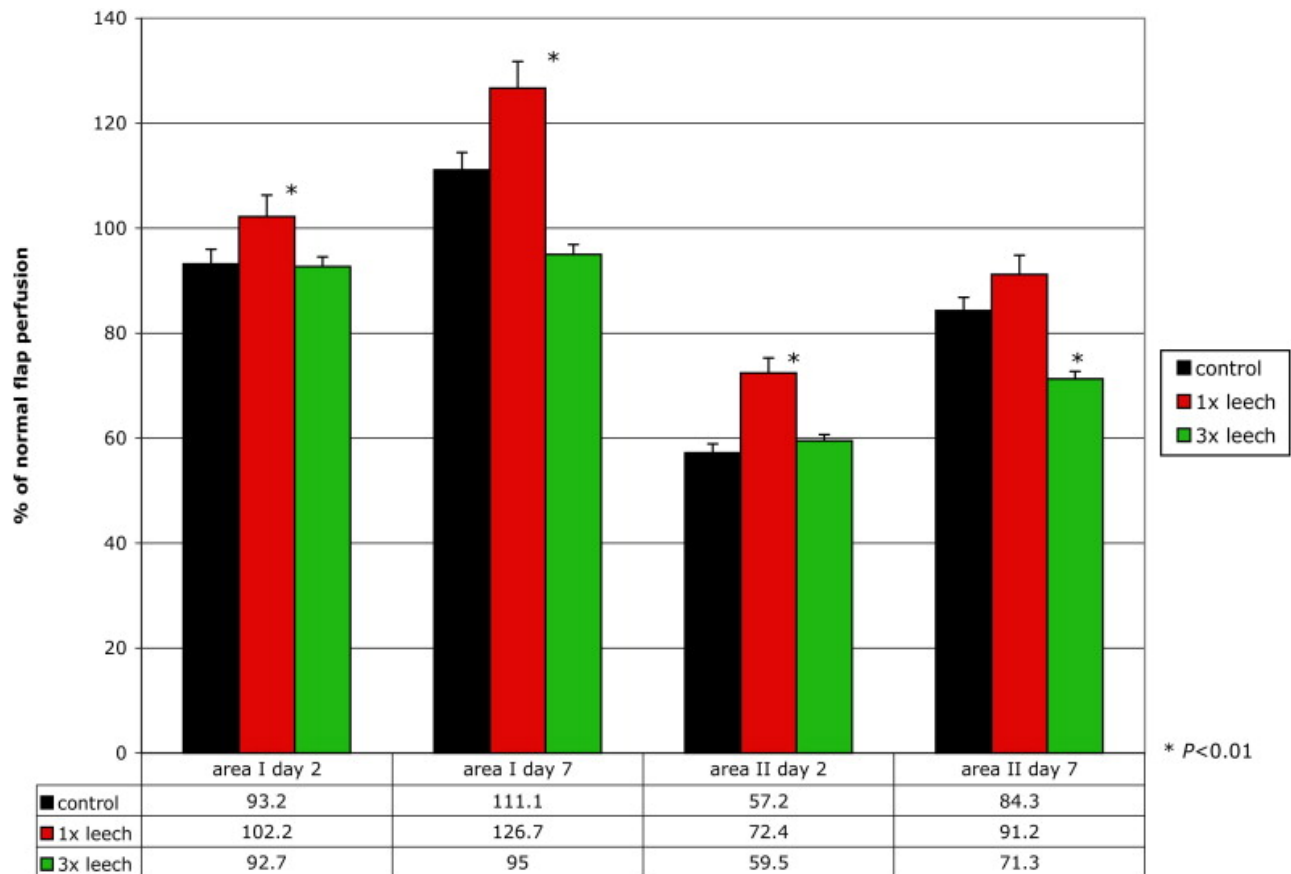


Figure 4: Laser-Doppler flowmetry after single and triple application of leeches and in control animals. In the single leech group, we observed a transitory increase in flap perfusion in intermediate area II at day 2 ($p < 0.01$) that, however, could not be confirmed on day 7. In the triple leech group, note the absence of any beneficial effects in area II at postoperative day 2 and, especially, the significant decrease in blood flow at postoperative day 7 ($p < 0.01$).

of the leech bite and complete by postoperative day 2, two days earlier than in the control and single leech group (figure 2). This thus far unknown phenomenon may be explained by a reverse effect of triple leech application: instead of restoring the balance between arterial inflow and venous drainage by alleviating venous congestion (27), triple leech application appears to cause a pronounced withdrawal of both venous and arterial blood. This results in a significant decrease in arterial perfusion in the surroundings of the leech bite, further enhancing local ischemia and finally causing tissue necrosis in random skin flaps.

We conclude that: 1) the use of leeches in mixed arterio-venous insufficiency results in deleterious effects on local blood perfusion and reduces total survival of ischemic random skin flaps in the rat; 2) a thorough clinical evaluation of the nature of the vascular insufficiency is mandatory before the use of leeches and needs to exclude obvious arterial but also mixed

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arterio-venous insufficiency; 3) the frequency and number of leeches applied as well as the duration of application have to be adapted to the degree of venous insufficiency and the size of the flap; and 4) pure venous congestion with maintenance of arterial supply remains the only indication for controlled leech application in the clinical setting.

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