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ARTICLE

# Remodelling of the superficial vascular network of skin flaps in rats, following a vasodilatory cream application, before elevation

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**ABSTRACT**

Flap necrosis on random pattern skin flaps continues to be a challenge. In this study, we evaluated whether topical application of a vasodilator substance (the prostaglandin $PGI_2$ analogue, Iloprost®, in cream form) as pre-treatment, would increase blood flow and improve flap viability. Wistar rats randomly allocated into four groups with 7 rats per group and two flaps 4cm × 2cm in the same rat i.e. 56 flaps were developed. Flaps on the wright side received pre-treatment with Cream in different drug concentrations, 2.5μgr/gr, 5 μgr/gr, and 10μgr/gr and 20 μgr/gr containing the active factor Iloprost®® ZK 36,374(M. W 360.5) prepared with white petrolatum as a base. Flaps on the left side received placebo cream (white petrolatum). After 10 days of flap pre-treatment, evaluation of blood flow by laser Doppler flowmetry (LDF) were recorded, then flap elevation and re suturing back were performed. After 7 days we estimated flap viability on digital imaging and the percentages of flap survival estimated. Means and standard deviations were used to describe blood flow measurements and survival percentages. The significance was set at 0.05 in all cases and the analysis were carried out with the use of the SPSS v23.0. Furthermore, we performed dynamic analyses of circulation using the radioisotope 99mTc which confirmed hyperaemia of the treated areas relative to that observed in control areas. These findings demonstrated that pretreatment of skin flaps with Iloprost® cream for 10 days prior to elevation increased blood flow in the flap and improved their overall survival rate.

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**KEYWORDS**
Random pattern skin flaps; rats; flap survival; iloprost; skin flap model; increase; cream

## Introduction

Random-pattern flaps are widely used in plastic surgery as an alternative to skin grafts and especially for covering facial defects, given that these flaps are aesthetically superior at defect coverage relative to skin grafts. Importantly, flaps show endogenous vascularity, which is responsible for flap viability, whereas grafts rely on diffusion until vascular restoration. However, skin flap necrosis continues to be a challenge in plastic surgery.

Despite advances in surgical techniques and flap design, random-pattern skin flaps continue to be associated with ischemia. Kerrigan [1] categorised the factors involved in flap-related peripheral necrosis (exogenous or endogenous), including systematic and local factors, such as malnutrition, hypotension, infection, external pressure, and thrombosis, with the only endogenous factor being arterial insufficiency. Therapeutic approaches to increasing flap viability include the use of pharmacological interventions administered either locally or *via* an enteral or parenteral route, with these factors demonstrating steady increases in the survival of random-pattern skin flaps in different experimental models [2].

Numerous studies have reported clinical applications of prostaglandin $I_2$ ($PGI_2$; prostacyclin). The $PGI_2$ analogue Iloprost® (Schering AG, Wedding, Berlin, Germany) is used therapeutically to address a variety of pathological conditions, including treatment of obstructive vascular disease, pulmonary hypertension, Raynaud's phenomenon, septic shock, and to enhance extracorporeal circulation. Experimental studies involving Iloprost®, report decreases in the extent of myocardial necrosis in rats [3], cytoprotective effects to neural tissue in rats [4], improved maintenance of liver grafts [4] and increased survival of colonic anastomoses [5]. Additionally, the $PGI_2$ Iloprost® has been used locally during microsurgery [6,7], with previous reports attempting to improve skin flap viability through direct application of either $PGI_2$ or other similar prostacyclin analogues [8], that resulted in all but one study [9] in increased flap survival. Moreover, Iloprost® has been administered in free flap surgery [10], as either monotherapy or an ingredient in washout solutions as prophylactic or therapeutic treatment for flap ischemia [11,12]. Topical administration of Iloprost® during the post-ischemic period showed some benefit, despite the inability of the medication to reach the peripheral end of the flap [13], However, there have been no studies focusing on the effect of topical application of Iloprost® in cream form prior to surgery as a prophylactic treatment.

Two goals of reconstructive surgery are in continuous conflict: one is ensuring tissue survival, and the other is the requirement for thin, flexible tissue capable of allowing an aesthetic contour. Surgery for aesthetic and functional reasons requires the use of thin flaps especially on the face (in example, forehead flaps for nasal reconstruction). Flap thinning might also be necessary in

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situations where the distal region of the flap needs to be manipulated to replace nasal mucosa or stretch beyond the midline of the forehead without jeopardising flap viability. Moreover, this application could be applied in face-lift procedures. Davies et al. [14] have described the usefulness of pharmacological manipulation and application of vasodilatory factors in patients with history of smoking to improve the survival of random-pattern skin flaps.

In the present study, we aimed to examine the ability of topical Iloprost® pre-treatment to remodel the cutaneous vascular network in order to increase the viability of thin, random-pattern skin flaps [15,16]. Furthermore, we tested the hypothesis that Iloprost® pre-treatment would allow for greater flap thinning by removing regions of subcutaneous tissue to increase the dependency of the flap from the –hopefully- dilated dermal vasculature. Traditional axial-pattern skin flaps show viability issues in their most distal part [17]; therefore, we determined whether Iloprost® pre-treatment could improve flap survival to reduce distal ischemic regions. We used a rat model to evaluate the efficacy of local application of Iloprost® in cream form for prolonged and sustainable increases in blood flow.

## Materials and methods

### Animal management

This was an experimental topical drug administration study. We acquired 35 male Wistar rats (220–250 g) from Theagenio Cancer Hospital (Thessaloniki, Greece). The experimental protocols were approved by the animal ethics committee veterinary service of the prefecture of Thessaloniki. Rats were kept in cages separately with *ad libitum* access to food (pellets) and water and under temperature-controlled conditions, with a 12-/12-h light/dark cycle. Each animal was weighed prior to surgery and on the day of sacrifice and identified according to a coloured ring on the tail.

### Pilot study and flap model determination

Preliminary pilot experiment was performed on five animals in order to standardize the flap model with regard to its vascularity.

### Experimental protocol

Rats were randomly separated into four groups (A–D; $n = 7$, 7, 7, and 7, thus 56 flaps were created). Animals were anaesthetised using Pentothal (25 mg/kgr injected intraperitoneally); followed by shaving the hair from the back and animal immobilisation into the prone position. Operations were performed under aseptic conditions. Following the procedures, animals were kept in separate cages in order to avoid cannibalism of skin flaps.

On the first 1st day of the experiment we initially drew an outline of the two flaps in a random pattern based caudally with reference to the iliac crest and 1 cm from the midline of the back (flaps were located at-least 2-cm apart). The surface area of each flap was 8 $cm^2$ (4 × 2 cm), with Laser Doppler Flowmetry (LDF) measurements taken at five points on each flap in order to determine blood circulation (Figure 1(A–C)). Iloprost® cream was topically applied on day 1, followed by o.i.d. treatment for 10 days. Iloprost drug comes in amp (0.1 mg/ml) that contains the active factor Iloprost® ZK 36,374(M.W. 360.5) and was obtained from

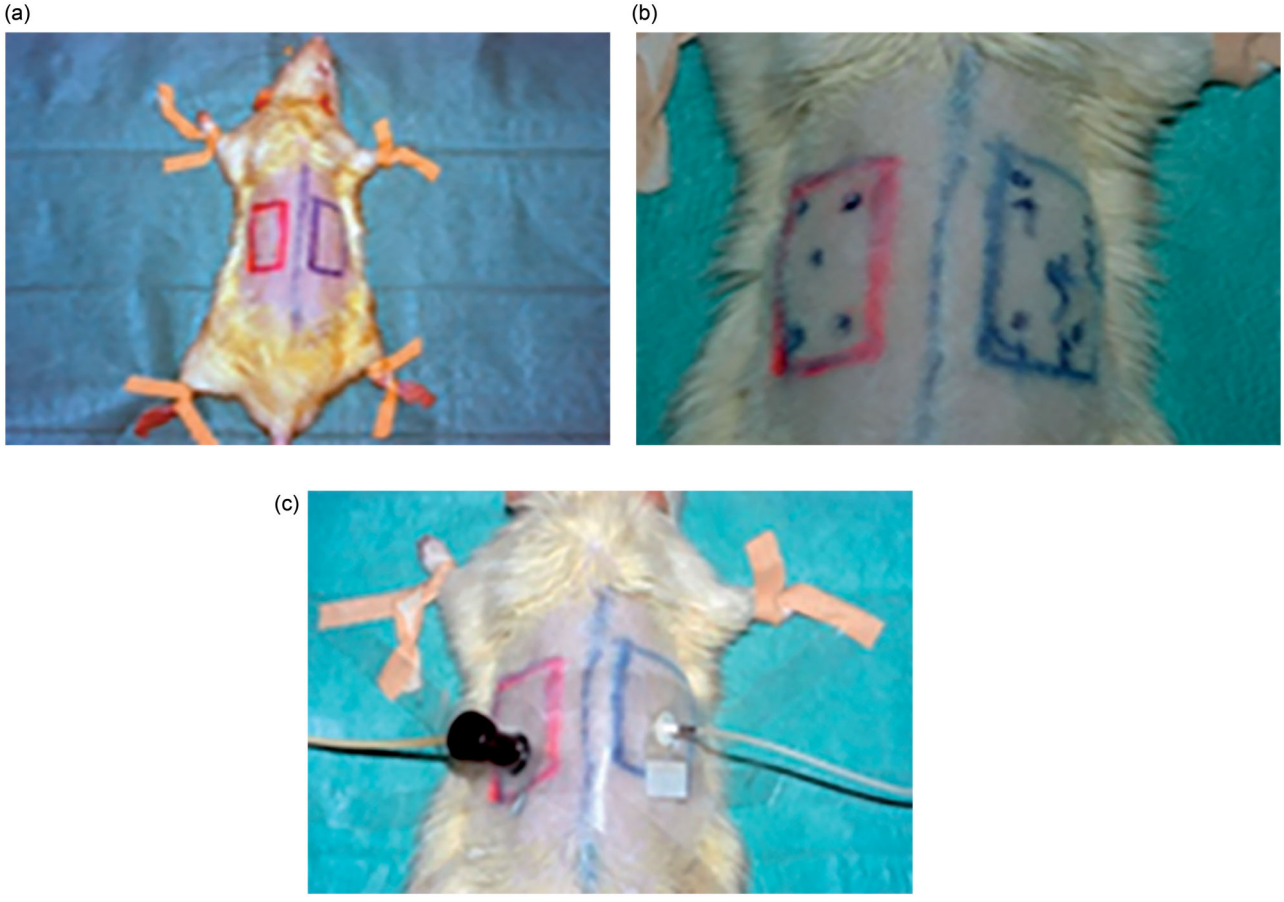


Figure 1. Flap Design (a b c). (a) Dimensions are 4 cm × 2 cm in rectangular shape and are based caudally. (b) The five points of the flaps where measurements were recorded. (c) Probes and mini-holders stabilised with double sided stick tape in place.

Sherring Germany for experimental use. Iloprost® cream form is not available commercially and thus cream preparation was done once at the begging of the experiment and afterwards we stored it in room temperature till the end of the experiment, since it is very stable in this cream form. This drug is fat dissolvent and has been tested in various galenic preparations: aqueous solution, hydrogel or fatty cream base, in which greater absorption has been reported. Therefore Iloprost® was prepared (0.1 mg/ml) in cream form containing the active ingredient Iloprost® mixed with white simple petrolatum as base, in different concentrations namely 2.5μgr/gr, 5 μgr/gr, 10μgr/gr and 20 μgr/gr. We applied this solution to the right side of each animal, thus creating the four pre-treated groups ($n = 7$ each). Care was taken to distribute cream evenly along the entire flap area creating a thin film; dosing was repeated o.i.d. on the flap skin area to the right side while placebo cream (white petrolatum alone) was applied to the other side (left), o.i.d. for 10 days. Blood circulation measurements were performed by laser Doppler flowmetry (LDF; PERIFLUX 4001; PERIMED, Stockholm, Sweden) using a system equipped with an L-type probe designed for measurements on the skin surface (Figure 2). The LDF device was used according to manufacturer instructions. The skin on each flap was cleaned with alcohol prior to placement of the probes at the measurement points [5] on each flap and attached using double-sided tape. PERISOFT software for Windows (v.2.0; PERIMED) was used to measure blood circulation in Perfusion Units (PU).

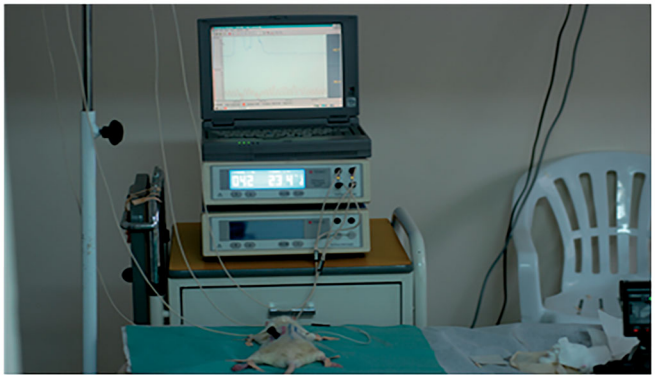

Figure 2. Laser Doppler flowmeter apparatus (LDF) connected to the computer and the probes placed and secured in place on the measured points of the flaps.

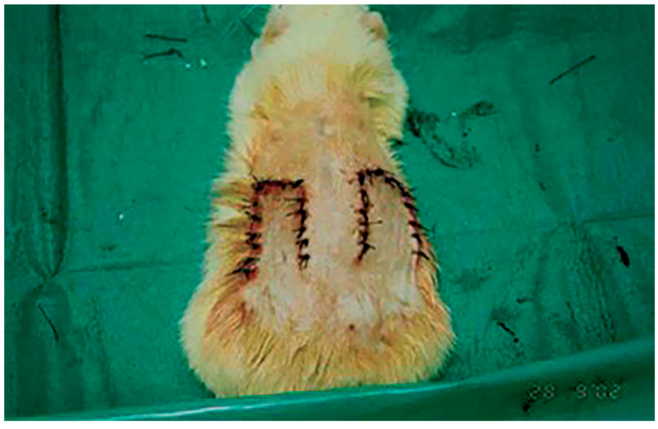

Figure 3. Flap undermined without panniculus carnosus (PCM) removal. On 7th post-operative day 100%. Survival.

### *Laser-Doppler measurement undermining-raising the flaps and stitching back*

Circulation of flaps has been measured with LDF on the 10th day after the cream application.

On preliminary experiment in five animals we raised two random patterns using the modified flaps Macfarlane technique. We rose on the rats' back two symmetrically rectangular flaps measuring 2 cm base and 4 cm long. I.e. developed the flap with greater dimensions than the proportion 1:1 for random pattern skin flaps but even when the flap proportions were 1:2 NO necrosis was observed (Figure 3).

On the study group flaps were undermined and raised in a superficial plane that excluded the main vessel that runs to the superficial muscle layer, panniculus carnosum muscle (PCM) and branches supply the flap base (panniculectomy) (Figure 4).

### *Measurements of flap viability*

On 16th day of the experiment animals were sacrificed on 7th post-surgery day animals were sacrificed with an overdose of pentobarbital injected into the heart (150 mg/kg) (Figure 5). Viable flap areas were traced through a transparency sheet which afterwards was scanned and digitised on the computer using special software image-pro plus Media cybernetics LP version 3.0.00 for windows 95/NT. Measurements of the flap total surface area, the necrotic and viable areas where recorded in mm [2,18].

(a) (b)

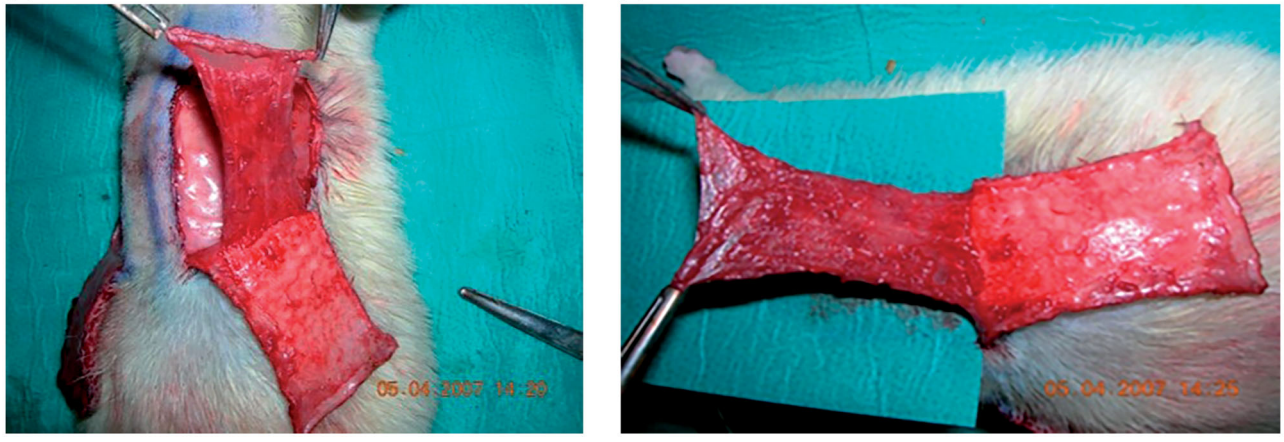

Figure 4. Both photos shows flaps were undermined and raised in a superficial subcuticular plane, thus precluding the main vessel that runs in the superficial muscular plane and his branches nourishing the base of the flap (Panicullectomy).

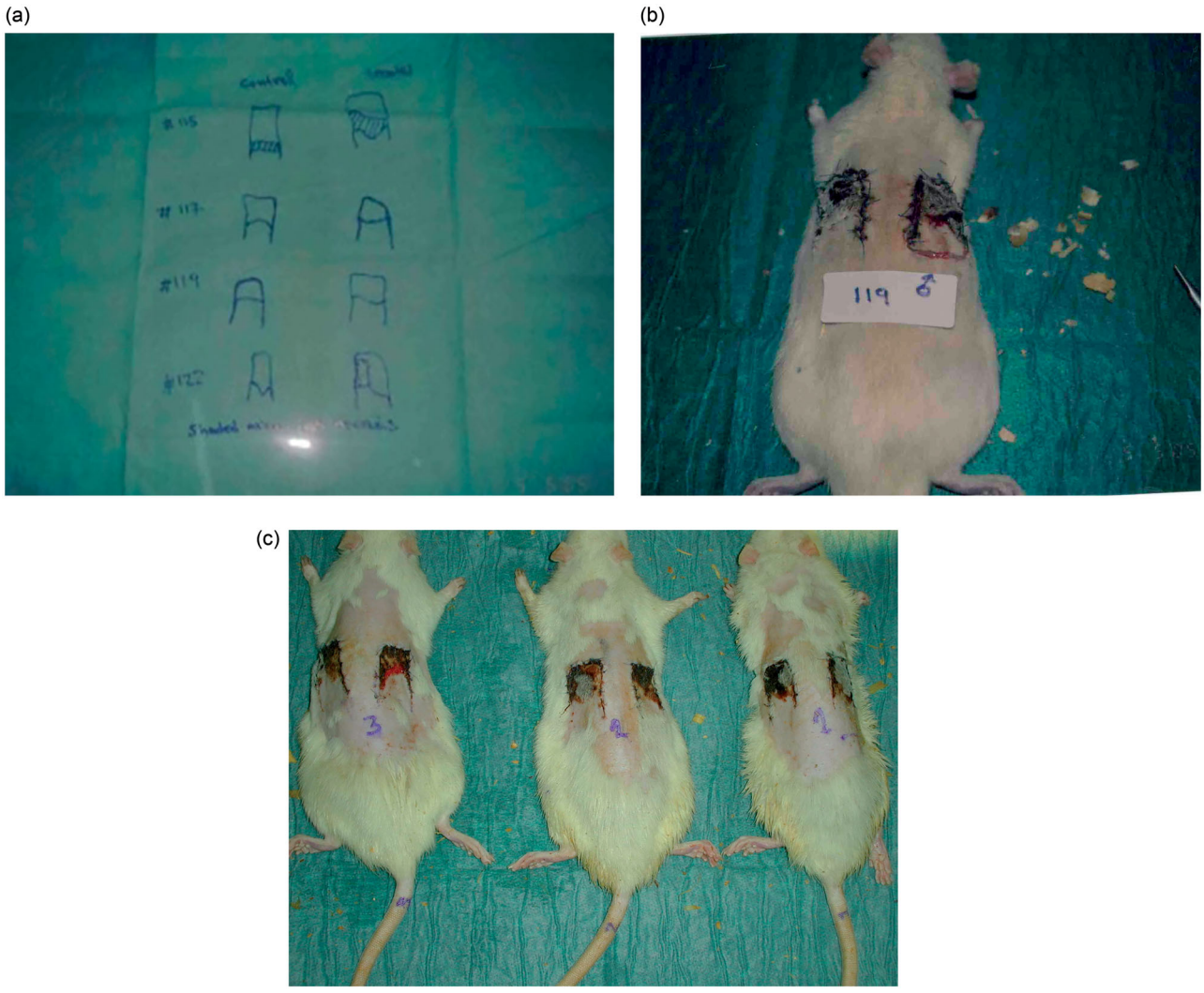


Figure 5. Measurement of flap viability. Trace in the transparent sheet of the viable and non-viable areas: (a) Transparency sheet, (b and c) Flap appearance on 17th protocol day when we did measurements of flap viability.

### *Radio isotopic circulation depiction*

On 10th day of protocol we tried to estimate the response (qualitative and quantitative) to pre-treatment using radioisotopes. More specifically comparative assessment of the two flaps was done after intravenous injection of 99mTc. Following catheterization of a dorsal tail vein with a 27-gauge needle we administered i.v 0.7 μCi/Kg $^{99m}$Tc followed by bolus administration of normal saline 0.5 ml. Dynamic simultaneous shot pictures have been taken lasting 2 s for 1 min (30 frames of 2 s 64 × 64 matrix size) in back projection. Then depiction of blood pool of the animals back in the same projection was created. The theoretical base of this study is that systematic changes should affect both hemi-halves equally and any difference in correspondence would be owing to the circulatory situation of the treated flap.

### *Collection for histology*

After we measured the flap viability and animal sacrifice we also performed histology on the group with 20μgr/gr treatment. Flaps were taken and fixed in paraffin. Sections were stained with Hematoxyline-Eosin (H&E stain).

### *Statistical analysis*

Means and standard deviations were used to describe blood flow measurements and survival percentages. Normality was assessed using the Kolomogorov Smirnov test. Differences between within-animal groups were assessed with the paired samples *t*-test, while differences among the different dosing groups were examined with Analysis of variance. The Bonferroni criterion was used to adjust for the multiple comparisons. The significance was set at 0.05 in all cases and the analysis were carried out with the use of the SPSS v23.0.

## Results

Preliminary pilot experiment: In five animals we created random pattern skin flaps 2 × 4 cm without removing the panniculus carnosus layer; flaps had 100% survival without any necrosis after 7 days. Whereas if panniculus carnosus layer is removed a reproducible percentage of flap necrosis is observed after 7 days with the survival rate being below 50%. This flap differs from the usual in that it is raised superficial to panniculus carnosus layer. The necrosis of the flap in this model is measurable and reproducible. Technically this is done using fine plastic surgery instruments and

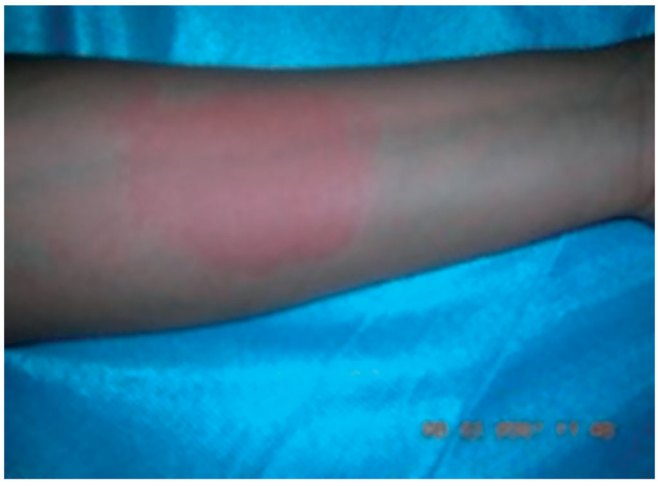

Figure 6. Reaction that have been noticed when the cream applied to human skin (upper arm) where appeared redness after 3 h and lasted for more than 72 h.

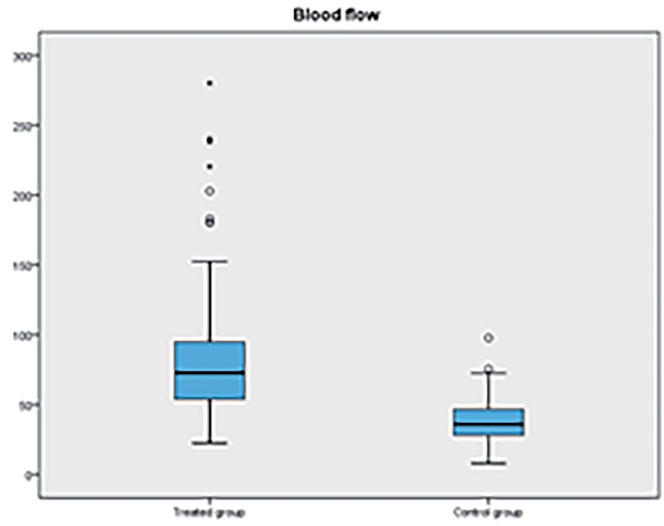


Figure 7. Blood flow measurements in the treated and control sides (in PU).

special attention is given not to include any tissue of panniculus carnosus. The flap is stitched back with interrupted 4-0 prolene sutures after cautious haemostasis.

### *Macroscopically*

During flap undermining process in the pre-treated flaps (Day 10, operation) we noticed longer and bigger haemorrhage from surgical edges in comparison to the left side control flaps. We did not notice any reaction in the rats' skin in contrast to the reaction that have previously noticed when the cream was applied to human skin (upper arm of a volunteer investigator, redness formed after 3 h which resolved in more than 72 h, Figure 6). Histology examination showed that temperature increase was owing to vasodilation and not to acute inflammatory reaction.

The total surface area at the end of the experiment appeared to be less owning to contracture and the necrotic area appeared more contractured than the survived area. The necrotic line was varied rather than straight. We did not notice any correlation between the cream application and local increase of hair growth.

### *Results on LDF measurements*

#### *Comparisons between the treated and the control side within animals*

Statistically significant differences were found between the treated and the control side when examined in total with a mean (s.d) value of 84.52 (47.70) PU for the treated side and 38.06 (14.78) PU for the control side in all dose groups (Figure 7). The difference was found to remain statistically significant for all groups defined by the different possible doses (20μgr/gr, 10μgr/gr, 5μgr/gr, 2.5μgr/gr) (Table 1).

#### *Comparisons between treated and control sides among all animals*

We compared the pooled flap flow measurements among all different dose groups, between the treated and the controls sides (Table 2). Statistically significant differences were found in the pooled flap flow measurements ofall different dose groups for each of the treated and the control side within each dose group respectively ($p < 0.001$.

Table 1. Differences in blood flow measurements in perfusion units (PU) in the treated and control side by dose group (Paired t-test).

| Dose Group | Side | Mean (PU) | Std. deviation | *p*-value |
|---|---|---|---|---|
| 20μgr/gr | Treated | 137.95 | 56.38 | <0.001 |
| | Control | 47.99 | 14.63 | |
| 10 μgr/gr | Treated | 67.47 | 19.53 | <0.001 |
| | Control | 30.48 | 6.44 | |
| 5 μgr/gr | Treated | 56.98 | 18.70 | <0.001 |
| | Control | 31.30 | 12.38 | |
| 2.5μgr/gr | Treated | 72.14 | 28.93 | <0.001 |
| | Control | 44.21 | 16.19 | |

As shown on Figure 7 regarding the treated group, and based on the multiple comparisons following based on the Bonferroni criterion, the 20μgr/gr dose is characterized by significantly higher blood flow measurements compared to all other three doses ($p < 0.001$ in all three cases), while the three lower ones do not differ statistically between them. Regarding the control group and based on the same statistical criteria the 20μgr/gr and the 2.5μgr/gr do not differ between them ($p = 0.667$) but they are higher in terms of blood flow measurements ($p < 0.001$ in all cases) compared to the 10μgr/gr and 5μgr/gr which do not differ between them ($p = 0.993$) (Figure 8). Still the differences observed were of a much smaller effect, not exceeding 18 *perfusion units (PU)* (Tables 1 and 2).

#### *Comparison of flap survival in the treated and the control groups (**Table 3**)*

Overall percentage of flap survived was 20% higher among all Iloprost – treated areas versus control areas (approximately 10% absolute increase, $p < 0.001$, Table 3, Figure 9). To strengthen the inference, examination of the total flap surface area (TSA) was found to have no statistically significant difference in the two groups ($p = 0.966$) (Table 3, Figure 9).

#### *Comparison of flap survival depending on dosage in the treated and the control group separately (**Table 4**)*

Flap survival rates were not significantly different among different doses on the treated or the control groups. Still, it must be noted that in the treated group the result is close to significance with a *p*- value of 0.058 (trend) therefore approaching significance, while in the control group the rates are clearly similar (Table 4). 20 and

Table 2. Comparisons of blood flow in perfusion units (PU) among different doses in the treated and control sides of all animals (ANOVA).

| Dose | Mean (PU) | Std. deviation | *p*-value |
|---|---|---|---|
| Blood flow measurements in the treated side of all animals | | | |
| 20μgr/gr | 137.95 | 56.38 | 0.001 |
| 10μgr/gr | 67.47 | 19.53 | |
| 5μgr/gr | 56.98 | 18.70 | |
| 2.5μgr/gr | 72.14 | 28.93 | |
| Blood flow measurements in the control side of all animals | | | |
| 20μgr/gr | 47.99 | 14.63 | 0.001 |
| 10μgr/gr | 30.48 | 6.44 | |
| 5μgr/gr | 31.30 | 12.38 | |
| 2.5μgr/gr | 44.21 | 16.19 | |

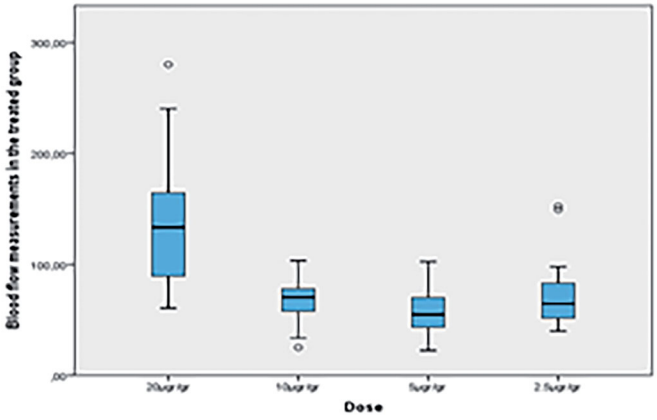


Figure 8. The 20μgr/gr dose is characterized by significantly higher blood flow measurements (PU) compared to all other three doses ($p < 0.001$ in all three cases), while the three lower ones do not differ statistically between them. Measurements from the treated side of animals only.

Table 3. Differences in percentage of total area survived (in $mm^2$) in flaps from the treated and control group.

| | Mean* | Std. Deviation | *p*-value |
|---|---|---|---|
| Percentage of Survived in the treated side | .6004 | .08369 | 0.001 |
| Percentage of Survived in the control side | .4915 | .12492 | |
| Total Surface Area in the treated side ($mm^2$) | 672.15 | 70.481 | 0.966 |
| Total Surface Area in the control side ($mm^2$) | 671.42 | 70.336 | |

*First two rows (%), last two rows ($mm^2$).

10 μgr/gr dose groups achieved a 60% flap area survival while the lower 5 and 2.5 μgr/gr doses achieved a 56% flap area survival in the experimental side. In the control side groups achieved less survival, approximately 50% also in line with the pilot study we performed (Table 4). Therefore a higher efficacy is reported for 10 μgr/gr dose but the 20 μgr/gr does not appear to confer incremental benefit. The same applies to the 2.5 μgr/gr dose which is not inferior to the 5 μgr/gr dose (Table 4).

### *Radio isotopic examination*

Estimation was visual and half-quantitative. Cumulative pictures from the dynamic study region of interest were placed (ROI region of interest) to the left and right of the middle line corresponding to the two flaps respectively. The total activity was measured in order to estimate the blood circulation. The mean values of hits per pixel were evaluated corrected for the substrate (background).

Quantitative evaluation of the hits on the treated animal. The mean hits per pixel to the treated flap (right) were calculated 159.9 cnts/pixel and to the control flap (left) of the animal 102.9 cnts/pixel (Figure 10(a)). Hyperaemia of the treated area is proved in comparison to the control area (RATIO treated: 1.42 and RATIO control: 0.75 (Figure 10(b)).

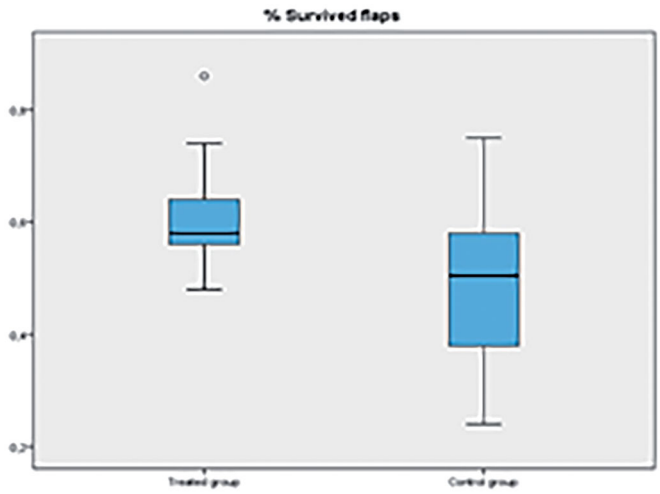


Figure 9. Percentage of total area survived in flaps from the treated and control sides of all dose groups.

Table 4. Differences in in percentage of total flap area survived in the treated and control sides across different dose groups.

| | Mean (%) | Std. Deviation | *p*-value* |
|---|---|---|---|
| Percentage of Survived in the treated group | | | |
| 20μgr | 0.6043 | 0.07254 | 0.058 |
| 10μgr | 0.6657 | 0.09271 | |
| 5μgr | 0.56 | 0.08206 | |
| 2.5μgr | 0.56 | 0.02236 | |
| Percentage of Survived in the control group | | | |
| 20μgr | 0.4743 | 0.18017 | 0.903 |
| 10μgr | 0.5086 | 0.12786 | |
| 5μgr | 0.51 | 0.08944 | |
| 2.5μgr | 0.466 | 0.0994 | |

*ANOVA.

### *Histology*

Histology did not show any particular evidence only indications of chronic inflammation, some vasodilation and the existence of vacuum cells suggesting some cream absorption (Figure 11).

## Discusion

Random-pattern skin flaps are still used in plastic surgery to cover defects following simple excision of skin tumours and for aesthetic reconstructive procedures. However, peripheral necrosis represents an issue that frequently results in flap morbidity, although there is disagreement regarding correlations between insufficient blood flow and skin flap necrosis. Kerrigan [1] attributed skin flap necrosis mainly to arterial insufficiency.

The beneficial effects of prostacyclins have been well documented, with both local and systemic application of prostaglandin $E_1$ ($PGE_1$) and $PGI_2$ showing positive effects on flap survival [9,19]. Additionally, experiments using both $PGE_1$ and $PGI_2$ consistently report improvements in skin flap survival and/or increased blood flow in the proximal region [9,20,21], with $PGI_2$ reportedly outperforming $PGE_1$ in these areas [22]. Previous studies described improvements in thigh-vein circulation following $PGI_2$ administration and that $PGI_2$ treatment decreased turgor pressure in the

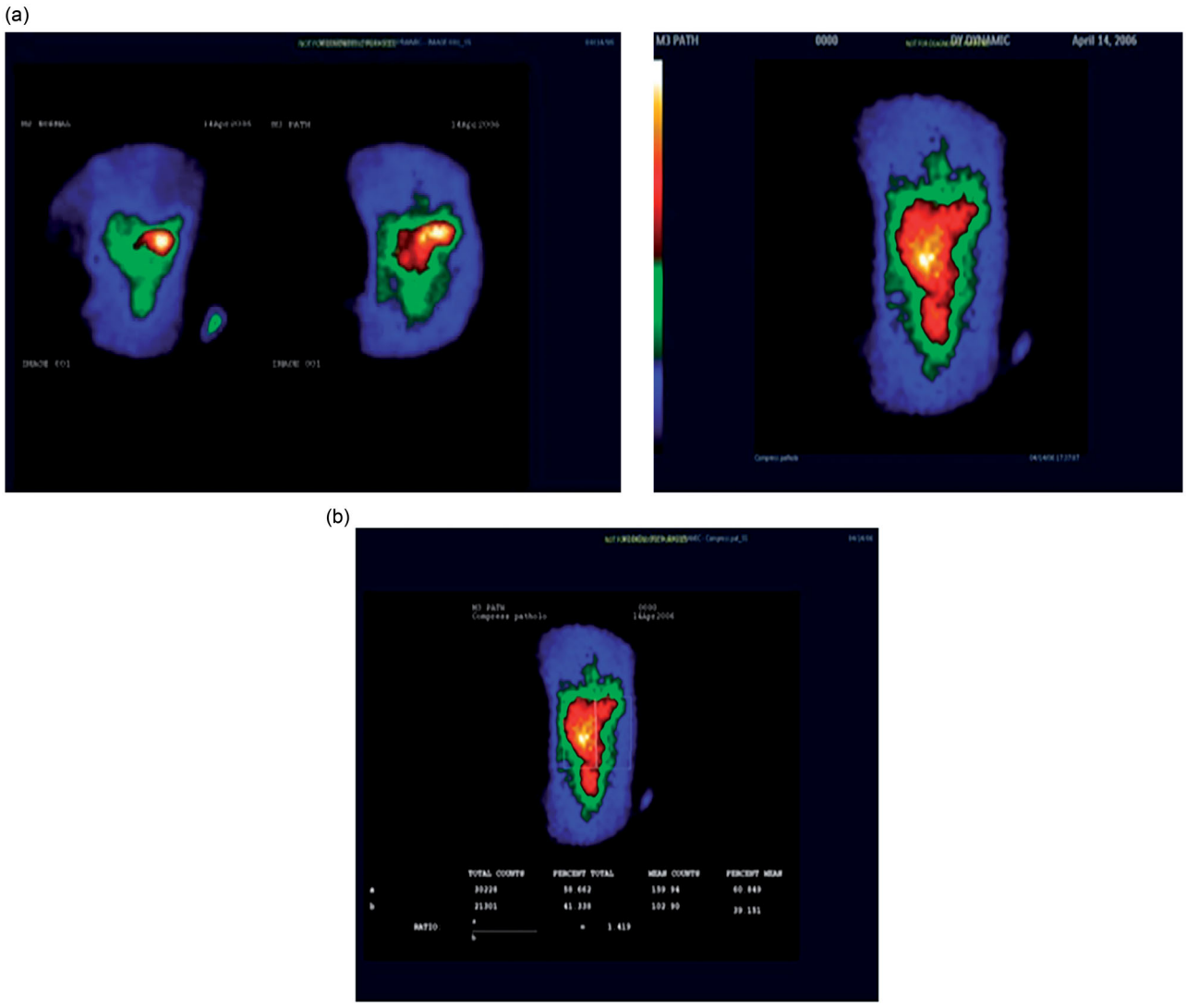


**Figure 10.** **(a)** Left: control, Right: treatment. **(b)** Pre-treated animal. Mean hits per pixel to the treated flap (right) were calculated 159.9 cnts/pixel and to the control flap (left) of the animal 102.9 cnts/pixel.

arterial wall, resulting in decreased interference of blood flow and clot formation [23,24]. Moreover, systemic administration of $PGI_2$ has also been associated increased flap survival rates [15]; however, these application have little therapeutic value due to the chemical instability and small half-life of $PGI_2$ (2–3 min at pH 7.5 and 37 °C). Furthermore, Emerson and Sykes [19] reported negative results associated with flap survival following local and intraperitoneal administration of $PGI_2$ prior to surgery, attributing this to the short half-life of the drug; however, a later experiment injecting $PGI_2$ at both the beginning of surgery and post-operation revealed an increase in flap survival.

A previous study reported that Iloprost® exhibits angiogenic properties and exerts favourable effects in wound healing. Intra-arterial injection of PGI2 into patients with advanced obstructive atherosclerosis resulted in symptom relief and promoted healing of ischemic ulcers [25], with the interpretation of the findings suggesting that in addition to decreased platelet adhesion and vasodilation, PGI2 might also promote the formation of new capillaries in ischemic areas.

Experimental studies in rats have confirmed beneficial effects for PGI2 and PGE2 [22] topical administration, significant reduction in distal necrosis of random skin flaps after intravenous infusion of L-arginine, iloprost, and L-arginine combined with iloprost [26] and intraperitoneal beraprost sodium immediately after the flap had been raised [17]. Another experimental study of free microvascular groin flaps in rats concluded that iloprost and cicaprost were effective in preventing venous occlusion induced failure [10]. Our results are in accordance with those already published. Erçöçen *et al* reported survival areas to be 59.05% ± 5.13%, and 50.40% ± 4.74%, in their control groups (saline and nicotine pre-treated respectively) whereas they were 67.89% ± 4.69%, and 62.45% ± 7.80%, for their PgI2-iloprost and PgE1-misoprostol treated groups respectively [26]. Their results support our pilot study flap survival rate of 50%. Our study adds the advantage of comparing the efficacy of Iloprost within the same animal.

An ideal drug for increasing flap survival must have vasodilatory and anticoagulant properties, as well as chemical stability under physiological conditions; Iloprost® is both a metabolically and chemically stable analogue. The literature findings suggest Iloprost® as a potentially viable drug for applications associated with increasing flap survival.

Additionally, Iloprost® administration reportedly increased the survival of an axial-pattern flap in a model of ischemia reperfusion [11].

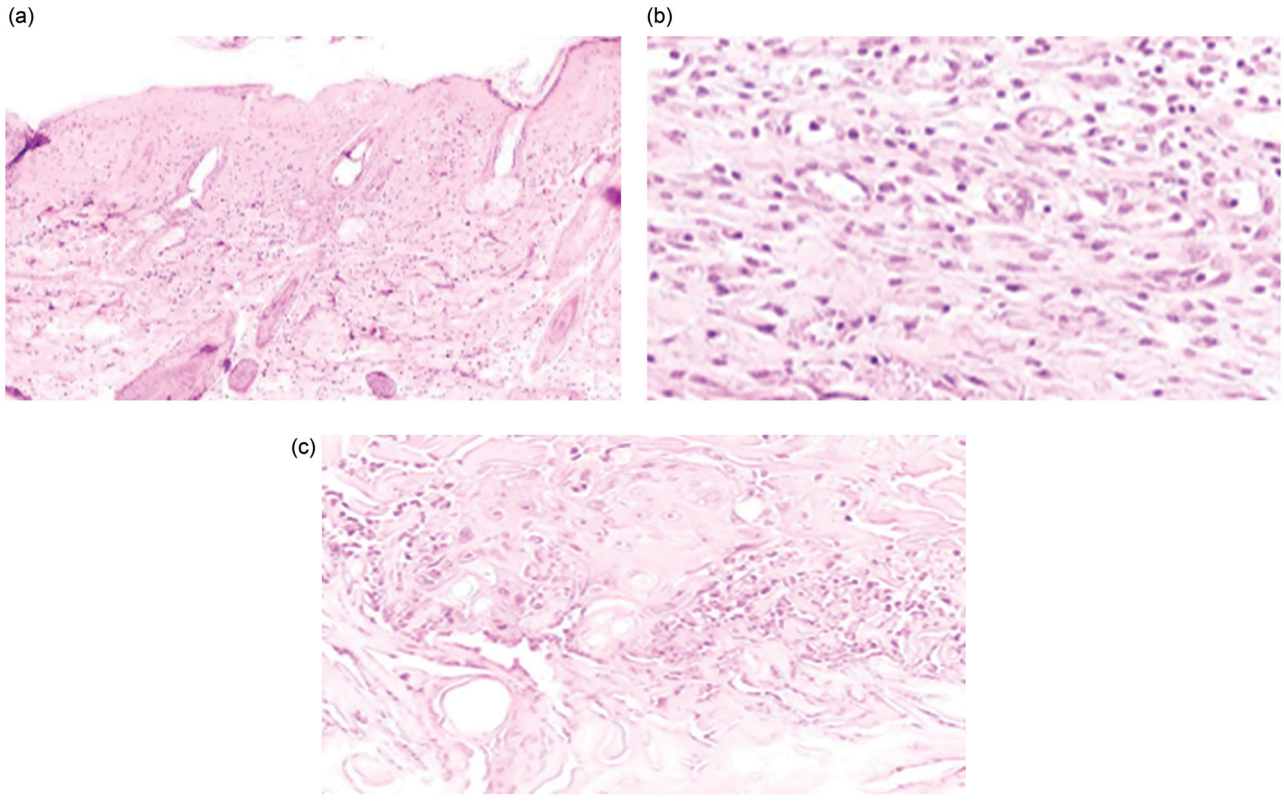


Figure 11. **(A)** Normal skin? **(B)** Pre-treated with Iloprost® cream flaps shows evidence of inflammation and vasodilation **(C)** Inflammatory evidence. Vacuum cells suggesting some cream absorption, epithelisation and inflammatory elements.

Therefore we investigated Iloprost® for some of these published beneficial effects in conjunction with the proven action of PGI2 and other prostanoids on flap protection from acute ischemia and thus it was considered to be a beneficial factor for increasing flap viability.

We selected local application because (a) systematically administered drugs are connected with some side effects [27] and (b) it is stable and feasible to administer. Additionally the hypotensive action of systemic administration of Iloprost® restricts its use on skin flap model in rats. Iloprost® has been reportedly used during operation and post operation while in the present study it has been used in topical cream form for 10 days before flap elevation as a preventive therapy. As Iloprost has been shown to also exert beneficial effects post-surgical therapy [11,12], a study comparing the prophylactic alone, versus the prophylactic and therapeutic (combined) versus the therapeutic alone could be of value to further elucidate the beneficial effect of Iloprost in angiogenesis and flap survival.

We selected white petrolatum as the base for cream preparation because the active ingredient is better dissolved in fatty base and this form has been reported to be better absorbed from human and rat skin. Penetration seems to happen from hair follicles; the keratin layer has been reported to act both as barrier and drug reservoir. Though the percentage rate of penetration and absorption from skin is not known for ZK 36,374, its long duration of action and following hyperemia denote slow release from some reservoir, probably the keratic layer as the life half time in plasma was reported to be roughly 15 min [28] (Schering AG, Wedding, Berlin, Germany). Iloprost® cream is not currently commercially available but it exhibits some benefit in our study and could be used as preventive and therapeutic in different pathological conditions, like flaps and skin grafts. Further research regarding the skin drug absorption is needed, while hyperemia (redness) which was observed when applied to the arm after only one application raises questions regarding the human skin tolerance and the inflammatory reaction. In experimental study in humans showed Iloprost administration was assocated with erythema and increase in body temperature [29].

A limitation of this study is the inability to maintain the applied cream as it was not possible to secure any dressing, owing to the high mobility of rat's skin.

We chose rats as they are widely used for flap survival studies and because are easy to manipulate. Standardized models of skin flaps in rats such as McFarlane and Finseth flaps imply one flap in every rat. One flap owing to blood flow fluctuations between animals can be problematic and big number of animals might be required in order to have statistically valid results. In this study we developed a –paired- model in which in one animal we designed two flaps. To the best of our knowledge this is the first study to present a two-flaps in a single animal model which has been pilot tested. Our model has (a) a predictable, reproducible rate of necrosis, (b) it is a random pattern skin flap because we remove the paniculus carnosum with its vessels and (c) in it, both control and treated flaps are in the same animal [28] Technical limitations of the model include: (a) total surface area is smaller at the end of the experiment owing to contracture, (b) necrotic area showed greater contracture compared to viable, and (c) the necrotic line varies in form complicating the distance measurement from the base of the flap to the necrotic area. A scale bar was not used during photography.

According to our results an increase in skin flap circulation and an increase in the survival rate of the Iloprost® pre-treated flaps happens to all animals and it appear to be dose dependent with better results associated with higher doses, as suggested by LDF and measurements of skin flap viability. 20 μgr/gr dose was associated with better blood flow (measured by laser Doppler) against

2.5, 5 and 10 μgr/gr doses of topical iloprost administration (Figure 8). This difference was not recorded for flap survival, thus 20 μgr/gr dose improved only blood flow, not flap necrosis rate.Although there are published studies reporting increased flap survival in random pattern flaps, using Iloprost® our results cannot compared with theirs because our flap model is different. Overall our results concur with those of other studies, reporting a beneficial action on flap survival for Iloprost®. More specifically, approximately 10% better flap surface survived following treatment when compared to the control group. The validity of the latter pooled result might be considered with caution as it is derived from comparing all the treated sides of all animals against all the control sides of all animals. Still treated surface was the same and 10% more survived in those treated.

10 μgr/gr and 2.5 μgr/gr dosing schemes might be more suitable for clinical trials.

It appears that our initial hypothesis that drugs targeting directly skin microcirculation and causing vasodilation may help increase random pattern skin flap survival is plausible. Though, rat's skin is different from human skin, Iloprost® might have therapeutic utility to increasing flap viability in the clinical setting.

In conclusion Iloprost® has a beneficial effect in skin flap survival in rats and the increased blood flow (as has been shown by LDF) may be the underlying mechanism of action.

## Disclosure statement

No potential conflict of interest was reported by the authors.

## References


[1] Kerrigan CL. Skin flap failure: pathophysiology. Plast Reconstr Surg. 1983;72(6):766–777.

[2] Aker JS, Mancoll J, Lewis B, et al. The effect of pentoxifylline on random-pattern skin-flap necrosis induced by nicotine treatment in the rat. Plast Reconstr Sur. 1997;100(1):66–71.

[3] Mueller B, Maass B, Krause W, et al. Limitation of myocardial unperfused area and necrotic zone 24 hours and 7 days after coronary artery ligation in rats by the stable prostacyclin analogue iloprost. Prostaglandins Leukot Med. 1986;21(3):331–340.

[4] Sanchez-Urdazpal L, Gores GJ, Ferguson DM, et al. Improved liver preservation with addition of iloprost to eurocollins and university of Wisconsin storage solutions. Transplantation. 1991;52(6):1105–1107.

[5] Arapoglou S, Kambaroudis A, Grivas I, et al. The effect of iloprost in the healing of colonic anastomosis in rats under chemotherapy with irinotecan. Chirurgia. 2017;112(6):705–713. Epub 2017/12/31.

[6] Crabb DJ, Niall M, Knight KR, et al. Topical use of prostacyclin in microvascular surgery. Br J Plast Surg. 1985;38(3):383–388.

[7] Iriz E, Erer D, Kula S, et al. Use of microsurgery and iloprost in the infantile arterial injuries. Circ J. 2007;71(4):554–558.

[8] Emerson DJ, Patel CB, Krishna BV, et al. The use of prostacyclin in preventing occlusion of microvascular anastomoses by platelet thrombus: an experimental study in rats. Br J Plast Surg. 1981;34(1):35–37.

[9] Forrest CR, Pang CY, Zhong AG, et al. Efficacy of intravenous infusion of prostacyclin (PGI2) or prostaglandin E1 (PGE1) in augmentation of skin flap blood flow and viability in the pig. Prostaglandins. 1991;41(6):537–558. Epub 1991/06/01.

[10] Frick A, Baumeister RG, Menger MD, et al. Secondary ischaemia in experimental free flaps-treatment by long acting prostacyclin analogues. Br J Plast Surg. 1999;52(5):392–398.

[11] Israeli D, Senderoff DM, Zhang WX, et al. Enhancement of fluorescein perfusion in experimental skin flaps following postischemic washout with iloprost, urokinase, verapamil, and university of Wisconsin solution. J Reconstr Microsurg. 1993;9(06):435–439.

[12] Tuncer FB, Durmus Kocaaslan FN, Yildirim A, et al. Ischemic preconditioning and iloprost reduces ischemia-reperfusion injury in jejunal flaps: an animal model. Plast Reconstr Surg. 2019;144(1):124–133. Epub 2019/06/28.

[13] Forman DL, Shah DK, Zhang WX, et al. Evaluation of a continuous systemic infusion of iloprost, a stable PGI-2 analog, on the survival of experimental skin flaps. J Reconstr Microsurg. 1995;11(5):339–344.

[14] Davies BW, Lewis RD, Pennington G. The impact of vasodilators on random-pattern skin flap survival in the rat following mainstream smoke exposure. Ann Plast Surg. 1998;40(6):630–636. Epub 1998/06/26.

[15] Li D, Luo J. [Experimental study of prostacyclin on the survival of random skin flaps. Zhonghua zheng xing shao shang wai ke za zhi = Zhonghua zheng xing shao shang waikf [ie waike] zazhi = Chin J Plast Surg Burns. 1997;13(5):349–351.

[16] Lu F, Mizuno H, Uysal CA, et al. Improved viability of random pattern skin flaps through the use of adipose-derived stem cells. Plast Reconstr Surg. 2008;121(1):50–58.

[17] Landecker A, Katayama ML, Mammana AK, Leitão RM, et al. Effects of retinoic and glycolic acids on neoangiogenesis and necrosis of axial dorsal skin flaps in rats. Aesthet Plast Surg. 2001;25(2):134–139. Epub 2001/05/15.

[18] Dölen UC, Sungur N, Koca G, et al. The vasodilator effect of a cream containing 10% menthol and 15% methyl salicylate on random-pattern skin flaps in rats. Arch Plast Surg. 2015;42(6):695–703.

[19] Emerson DJ, Sykes PJ. The effect of prostacyclin on experimental random pattern flaps in the rat. Br J Plast Surg. 1981;34(3):264–266.

[20] Ekerdt R, Luhm H, Töpert M. Blood supply can be increased in rabbit skin by a stable prostacyclin derivative without potentiation of plasma extravasation. Br J Dermatol. 1984;111(Suppl 27):144–146.

[21] Sasaki GH, Pang CY. Experimental evidence for involvement of prostaglandins in viability and acute skin flaps: effects on viability and mode of action. Plast Reconstr Surg. 1981;67(3):335–340. Epub 1981/03/01.

[22] Eskitascioglu T, Gunay GK. The effects of topical prostacyclin and prostaglandin E1 on flap survival after nicotine application in rats. Ann Plast Surg. 2005;55(2):202–206.

[23] Salemark L, Wieslander JB, Dougan P, et al. Adverse effects of topical prostacyclin application in microvascular surgery: an experimental study. J Reconstr Microsurg. 1991;7(1):27–30.

[24] Gross S, Tilly P, Hentsch D, et al. Vascular wall-produced prostaglandin E2 exacerbates arterial thrombosis and

atherothrombosis through platelet EP3 receptors. J Exp Med. 2007;204(2):311–320.

[25] Szczeklik A, Nizankowski R, Skawinski S, et al. Successful therapy of advanced arteriosclerosis obliterans with prostacyclin. Lancet. 1979;1(8126):1111–1114. Epub 1979/05/26.

[26] Riza Ercocen A, Apaydin I, Emiroglu M, et al. The effects of L-arginine and iloprost on the viability of random skin flaps in rats. Scand J Plast Recons Surg Hand Surg. 1998;32(1): 19–25

[27] Kecskér A, Blitstein-Willinger E. Pharmacological activity and local and systemic tolerance of topically applied iloprost. Arzneimittel-Forschung. 1993;43(4):450–454.

[28] Wollersheim H, Thien T. Hemodynamic effects of prostacyclin infusions in healthy volunteers. Angiology. 1988;39(3 Pt 1):227–233.

[29] Drury JK, Belch JJ, McGregor M, et al. The effect of a prostacyclin analogue (iloprost) on skin temperature. Prostaglandins Leukotrienes Med. 1985;19(3):301–303.