INTRADERMAL LIGNOCAINE INJECTIONS INCREASE BLOOD CELL FLUX BUT NOT FLAP SURVIVAL IN RATS

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Abstract. In four groups of six rats a random dorsal flap was raised, and 5, 20, or 200 mg/ml lignocaine or 0.9% sodium chloride, were injected intradermally. Cutaneous laser Doppler blood cell flux was measured at 12 time points over 130 minutes. In six other groups 5, 20, 100, 200, or 400 mg/ml lignocaine concentrations, or sodium chloride were tested. Blood cell flux was measured at the time that the flap was raised and 30 minutes after the injections. The area of the flap that survived was measured on day 10. Raising of the flaps resulted in a significant reduction in blood cell flux, which was followed by a significant increase at 10 minutes (p < 0.05) after the injections with all lignocaine concentrations tested. Injections of 5, 20 and 100 mg/ml lignocaine elicited a significant increase (p < 0.05) in blood cell flux compared with baseline values. There was no reduction in blood cell flux values at any concentration of lignocaine tested. Compared with sodium chloride, injections of lignocaine had no significant effect on flap survival. We conclude that, despite an increase in blood cell flux, lignocaine had no effect on flap survival in this model.

Key words: intradermal lignocaine, blood cell flux, flap survival, experimental flap.

Prolonged vasoconstriction is a problem in microvascular and flap surgery and can lead to pronounced reduction in circulation and an inability to predict success or failure of a flap. Many drugs have been used in attempts to improve circulation and the survival of flaps in experimental studies (1, 3, 5, 9, 10, 14). Animal studies in vitro (1) and in vivo (9), and human studies (8), have shown that lignocaine has a biphasic action on arteries; vasoconstriction dominates at low concentrations and vasodilatation at high ones.

As local anaesthetics are often used when local flaps are raised, and lignocaine has been used as a topical vasodilatating agent during microsurgical operations, we have studied the effect of lignocaine on blood cell flux and flap survival in rats at concentrations relevant in clinical practice.

We have investigated, the effect of intradermal injections of lignocaine on blood cell flux and survival of ischaemic tissue in a partially devascularised musculocutaneous flap model in rats (11, 13).

MATERIALS AND METHODS

Two sets of experiments were carried out. In the first set, four groups of six male albino rats (Sprague-Dawley, body weight 150–200 g) were used. The rats were anaesthetised with chloral hydrate (0.4 g/kg, intraperitoneally, Kebo, Sweden) and their backs were shaved. A random dorsal pattern flap, 2 cm wide and 7 cm long, was designed according to a standard procedure (11, 13) based on a line between the caudal parts of the scapulas.

The flap was raised from the deep fascia of the muscles and included the superficial fascia, panniculus carnosus, subcutaneous tissue, and skin (11, 13). A blind pharmacological study was then carried out by intradermal injection of 0.2 ml lignocaine (Xylocaine, Astra, Sweden) at six points on the internal surface of the flap at concentrations of 5 mg/ml, 20 mg/ml, and 200 mg/ml, or of 0.9% sodium chloride (Fig. 1). We used this technique because it is easier to give a reproducible intradermal injection from the inside than from the outside of the flap. Each group of animals was tested with one concentration of lignocaine.

In both experiments, relative changes of the cutaneous blood cell flux in the flaps was measured by a laser Doppler flux meter (Periflux PF2B laser-Doppler fluxmeter, Perimed AB, Järfalla, Sweden) at predetermined points 1 cm apart, from the base.
of the flap towards the tip (Fig. 1). Discrete values of the laser Doppler flowmetry data were selected at each site. The settings were: gain 10, bandwidth 12 kHz, time constant 0.2 seconds. Values of the laser Doppler flux meter are presented in arbitrary units (PU, mV) and expressed as mean with 95% confidence for each group.

In the first preliminary experiment the laser Doppler probe was held by hand and the readings were taken with the flap resutured to its anatomical bed. The readings were taken when the signal was stable and no movement artefact was present. Blood cell flux was measured at 12 time points over 130 minutes (before cutting the flap, after cutting the flap, after injection and re-suturing the flap, and at 5, 10, 15, 20, 30, 45, 60, 90 and 120 minutes after the injection).

In the second definite set based on the results of the preliminary study, six groups of 14 to 22 male albino rats (Sprague-Dawley, body weight 150–200 g) were used. The variation in the number of animals in each group is because during the evaluation of the results some experiments had to be excluded for technical reasons and the corresponding groups had to be supplemented with new animals. To be able to maintain the blind fashion of the study, more groups had to be increased and this resulted in the differences in size of the groups.

The animals were prepared and the dorsal flaps raised as in the previous groups. A masked pharmacological study was then carried out in which intradermal injections of 0.2 ml lignocaine were given at six points from the internal surface at concentrations of 5 mg/ml, 20 mg/ml, 100 mg/ml, 200 mg/ml, and 400 mg/ml, or of 0.9% sodium chloride (Fig. 1). Cutaneous blood cell flux in the flaps was measured by a laser Doppler flux meter. During the measurements, the flaps were mounted in a frame and the laser Doppler probe was maintained in position by a standard plastic holder (7). The blood cell flux was measured immediately after the flaps had been raised and again 30 minutes after the injections. The flaps were then restored with a continuous running suture and the animals were kept in individual cages.

The survival area of the flap was inspected again for comparison with the previous values. For the statistical analysis, the flap survival area was assessed on day 12 after fixation for two days because the demarcation of the necrotic tissue was then clearer than on day 10. No significant differences in the estimated survival area were found, however, between days 10 and 12. The survival area was expressed as a percentage of the total flap area and measured by weighing pieces of paper (standardised paper, 0.8 g/cm²) representing the surviving and necrotic areas of the flaps. Values are expressed as mean (SD).

Student’s t test (paired for data on laser Doppler flux values and unpaired for data on flap survival) was used to assess the significance of differences. Probabilities of less than 0.05 were considered significant.

**RESULTS**

In the preliminary set of experiments, cutting and raising the flaps resulted in significantly reduced blood cell flux at all points compared with the pre-cutting values (at each point in all flaps the reduction ranged from 40% to 52% of the original blood cell flux values, p < 0.05).

Intradermal injections of sodium chloride caused no significant difference in blood cell flux compared with baseline (preinjection) values at any point 10 minutes after the injections.

All groups showed significant increases of blood cell flux 10 minutes after the injections of lignocaine compared with sodium chloride injections (at each point in all flaps treated with lignocaine, the blood cell flux values were 10% to 25% higher than in the sodium chloride group, p < 0.05). The blood cell flux values 30 minutes after the injections were constant and stable. Thirty minutes was, therefore, chosen as a suitable time for blood cell flux measurements in the second study.

In the second group of experiments, the sodium chloride injections did not significantly change blood cell flux at any point on the flaps (Fig. 2a). Injections of 5 mg/ml, 20 mg/ml, and 100 mg/ml lignocaine solutions elicited significant increases (p < 0.05) in blood cell flux (Figs. 2b, c, and d, respectively). The response to the 20 mg/ml and 100 mg/ml concentrations was, however, significant only at the distal and proximal parts of the flaps, respectively (Figs. 2c and d).

The increased blood cell flux seen after
Effects of lignocaine on blood cell flux and flap survival

Fig. 2. Effects of (a) sodium chloride 0.9% (n = 22), lignocaine (b) 5 mg/ml (n = 21), (c) 20 mg/ml (n = 15), (d) 100 mg/ml (n = 14), (e) 200 mg/ml (n = 15), and (f) 400 mg/ml (n = 20) on blood cell flux in the dorsal musculocutaneous flap in rats. The figure shows comparisons of blood flux in the flaps before (---) and after (-----) intradermal injections. Measurements of blood cell flux are shown at different locations (mm from base). The laser Doppler flowmetry values are expressed in arbitrary units (PU, mV). Values are given as mean with 95% confidence; * = p < 0.05; ** = p < 0.01; *** = p < 0.005.

Table I. Comparison of the mean (SD) flap survival in the different treatment groups

<table>
<thead>
<tr>
<th>Lignocaine concentrations</th>
<th>Control (n = 20)</th>
<th>0.5% (n = 22)</th>
<th>2% (n = 15)</th>
<th>10% (n = 14)</th>
<th>20% (n = 15)</th>
<th>40% (n = 18)</th>
</tr>
</thead>
<tbody>
<tr>
<td>% Flap survival</td>
<td>35.2 (17)</td>
<td>35.2 (17.4)</td>
<td>32.1 (17.2)</td>
<td>39.7 (12.8)</td>
<td>35.5 (20.2)</td>
<td>33.9 (15.2)</td>
</tr>
</tbody>
</table>
200 mg/ml and 400 mg/ml was, however, not significant. No concentration of lignocaine reduced blood cell flux. The different concentrations of lignocaine had no significant effect on flap survival compared with sodium chloride injections (Table I).

DISCUSSION

Lignocaine is a local anaesthetic that acts as a stabiliser of the cell membrane and so may prevent vasoconstriction by action on nerves, or smooth muscles, or both (4). Topical applications of 12 mg/ml, 16 mg/ml, and 20 mg/ml concentrations of lignocaine have been shown to reverse experimental vasospasm in the tail arteries of rats (4). Intradermal injections in healthy human volunteers resulted in vasoconstriction with 0.125 mg/ml, 0.5 mg/ml, and 1 mg/ml concentrations, and vasodilatation with 2 mg/ml concentrations, of lignocaine (2).

In our study no decrease in the blood cell flux as measured by laser Doppler was found at any of the tested lignocaine concentrations. As previous reports have shown a biphasic vascular effect of lignocaine in vitro (1, 8) and in vivo (9), with vasoconstriction at low concentrations and vasodilation at higher ones, the lack of vasoconstriction in our study could be the result of differences in sensitivity and anatomy between species. Furthermore, in the present study raising the flap resulted in decreased blood cell flux, indicating that a degree of vasoconstriction was present in the control group. This phenomenon may have masked the vasoconstriction caused by the lignocaine.

Vasoconstriction may have occurred at concentrations lower than those tested, but in this study only clinically relevant concentrations of lignocaine were tested.

As sodium chloride injections did not change the blood cell flux values, it seems that the volume of the injections does not affect blood cell flux. The mere introduction of an intradermal injection needle in unanesthetised humans has been reported to increase cutaneous blood flow (6). Injections of saline have also increased blood flow, but we could not confirm this. In the study by Holloway (7) the blood flow was measured at the injection site and the observation period was shorter. Because of differences in the design of the experiments the results cannot be compared directly, and the influence of the injection needle itself on blood cell flux cannot be ruled out; it seems unlikely, however, that such an influence could be of major importance 30 minutes after withdrawal of the needle in an anaesthetised rat.

In the extended study, blood cell flux increased at all the concentrations of lignocaine, but only at 5 mg/ml was the increase significant at all the tested points. The increase was significant at the tip and the base of the flaps with 20 mg/ml and 100 mg/ml, concentrations of lignocaine, respectively. The lack of vasodilatation at high concentrations in our study, compared to that observed during topical application (9), may be explained by differences in sensitivity and distribution of the drug after raising the flaps. Furthermore, the laser Doppler technique measures blood cell flux and records only part of the flow through the arteriovenous shunt (15), and therefore the lack of information about blood volume may also contribute to the disparity between the observed increase in blood cell flux and the lack of increased flap survival after single injections of lignocaine. We chose the single injection procedure because it is the most commonly used method in clinical practice, and single injections of other drugs or treatments have previously been shown to affect flap survival (12). The effect of repeated injections of lignocaine will have to be studied further.

We conclude that, in this model, injection of lignocaine produced no discernible vasoconstriction; only blood cell flux increased. Low concentrations were the most effective in producing vasodilatation. Despite the increase in blood cell flux, flap survival was not affected by single injections of lignocaine.

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Effects of lignocuine on blood cell flux und flup survival

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