Vasoactive properties of lignocaine administered by iontophoresis in human skin

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ABSTRACT

The vasoactivity of lignocaine has an important influence on its clinical efficacy and systemic vascular absorption. The aim of this study was to evaluate its vasoactive properties when administered by the non-invasive technique of iontophoresis. We used laser Doppler imaging to measure the forearm skin blood flow responses of seven healthy young males to iontophoretic delivery of two preparations of 20 g/l of lignocaine hydrochloride, one containing the preservatives methylparaben and propylparaben and one without. The subjects were blind to the order of drug administration, and we assessed analgesia at the sites using a pinprick test. Delivery of both preparations of (positively charged) lignocaine under the anode caused demonstrable analgesia, but no change in skin blood flow. An increase in perfusion was measured, however, when the preservative-containing preparation was administered under the cathode. There was little or no response to the solution without preservatives, although the difference in response between the two preparations was not statistically significant ($P = 0.063$). Although there were no vasoactive effects of lignocaine at the relatively low dose used in the present study, our results suggest that the preservatives methylparaben and propylparaben are the most likely cause of the vasodilatation that we observed under the cathode, and may therefore have a significant influence on the vasoactivity of this preparation when administered by injection. Both are negatively charged in solution and have been reported to possess vasodilator properties. It might be worth considering the use of alternative, non-vasoactive preservatives in local anaesthetic preparations, or avoiding the use of additives altogether, when this is feasible.

INTRODUCTION

Lignocaine is the most widely used of all local anaesthetics. For infiltration, it is usually injected in concentrations of 0.5–1% (w/v) and its effects last between 1 and 2 h [1]. This anaesthesia can be prolonged by co-injecting the vasoconstrictor adrenaline, thereby delaying washout of the drug from its site of action [2], as well as limiting its systemic vascular absorption and potentially reducing blood loss from incisions in highly vascular areas.

It follows that the vasoactive properties of lignocaine (and of other local anaesthetics) are important in determining the duration of its anaesthetic activity. In common with most other amide anaesthetics, evidence suggests that lignocaine is a vasodilator at clinical doses, but a vasoconstrictor at lower concentrations [3]. Studies in human skin have reported increases in cutaneous blood

Key words: methylparaben, propylparaben, skin blood flow.
Abbreviations: AUC, area under the response curve with respect to baseline flow; POB, peak flow for each response divided by baseline.
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flow after injection of lignocaine [4], although injection introduces an additional source of vascular stimulation for which investigators attempt to control by comparison with a similar injection of saline.

The aim of this study was to assess the cutaneous vasoactive properties of lignocaine administered using iontophoresis, a non-invasive method of drug delivery that transfers charged particles across the skin by applying a direct electric current. Commercial iontophoresis devices safely deliver therapeutically effective doses of lignocaine [5], but a lower current and appropriately designed electrodes are necessary for research purposes to prevent non-specific electrical effects on the circulation [6] and to enable measurement at the site of administration.

In solution, lignocaine dissociates to form a positively charged ion and should therefore be administered under the anode [7]; however, in preliminary experiments, we also observed blood flow changes when the cathode was used, suggesting that the anaesthetic solution contains some additional, negatively charged, vasoactive constituent, which we hypothesized could be one or both of the added preservatives. (In addition, some investigators have reported the use of the cathode to deliver lignocaine by iontophoresis [8].) We therefore investigated this phenomenon more fully by applying both an anodal and cathodal current to distinguish the influence of different constituents of the anaesthetic solution.

**METHODS**

We recruited seven male volunteers from students and staff of Ninewells Hospital, Dundee, to take part in the study, which was carried out in accordance with the Declaration of Helsinki (2000) of the World Medical Association, and had been approved by the Tayside Committee on Medical Research Ethics. All subjects were healthy non-smokers aged between 20 and 35 years, and each gave written, informed consent to participate. They were not taking any concomitant medication and had no history of cardiovascular disease, asthma or sensitivity to local anaesthetics. The experiments were conducted in the early afternoon, in a laboratory at an environmental temperature of 22 °C, and the subjects were seated with their arms supported at heart level.

**Iontophoretic drug delivery**

We conducted our experiments using two preparations of lignocaine hydrochloride solution in water (20 g/l): one containing the preservatives methylparaben and propylparaben (Phoenix Pharmaceuticals Ltd, Gloucester, U.K.), and one containing no additives (Tayside Pharmaceuticals, Dundee, U.K.). The subjects were blind as to which preparation was being tested at any time. Lignocaine was delivered to the skin using the non-invasive technique of iontophoresis, in which charged ions of the drug are driven across the epidermis by applying a direct electric current [7].

We prepared a measurement site on the volar surface of the forearm by removing surface keratinocytes with adhesive tape and cleaning the area with an alcohol swab. Each iontophoresis chamber (Moor Instruments Ltd, Axminster, Devon, U.K.) consists of a Perspex ring of internal diameter 20 mm with a wire electrode running around its inner surface. Two chambers were fixed to the skin with adhesive tape. They were positioned adjacent to one another and each filled with 2.5 ml of test solution and covered with a glass microscope slide. The purpose of the cover was to prevent planar reflection and movement artefact from the surface of the liquid, both of which can affect the laser Doppler signal. The positive lead of a current source was connected to one electrode and the negative lead to the other, so that the two were in series (Figure 1).

When an electrical potential difference is established, ions of the drug migrate across the skin, and the dose delivered is therefore a product of the magnitude and duration of current. We used a current of 0.24 mA, which is the highest the iontophoresis controller is calibrated to deliver to the electrodes that we used, so as to avoid causing non-specific electrical effects on blood flow, or discomfort from a high-current density. The current was applied for 23 min, which resulted in a total delivered charge of 331.2 mC. In pilot studies, we determined that a longer duration of delivery did not produce any further vasodilatation, or indeed stimulate change at an initially unresponsive site. This procedure was performed for both solutions of lignocaine that were under investigation (i.e. with and without preservatives) in a random order, on separate occasions at least 2 days apart.

**Laser Doppler perfusion imaging**

We measured cutaneous microvascular perfusion at the delivery sites using laser Doppler imaging (moorLDI; Moor Instruments Ltd). A 2-mW helium–neon laser scans the surface of the skin, and light back-scattered from moving erythrocytes is shifted in frequency by an amount proportional to their velocity, according to the Doppler principle. These Doppler shifts are collected and processed by the instrument [9]. For each scan, the computer builds up a colour-coded image representing skin perfusion in two dimensions. This relative measure of volume flow is called the laser Doppler flux and is expressed in arbitrary perfusion units. We have used this technique in previous studies of local anaesthetic vasoactivity [10].

The laser head was positioned 50 cm from the measurement site with a hood mirror deflecting the beam on to the skin surface. The scan region, encompassing both iontophoresis chambers (Figure 1), was 4 × 8 cm, translating to an area of 70 × 140 pixels on-screen; each scan lasted for
Analysis

The recorded images were analysed using dedicated image-processing software (Moor Instruments Ltd). For each time-point, we calculated the median laser Doppler flux in the area of skin enclosed by the chamber, so building up a response–time curve. We then evaluated the total area under each response curve, with respect to baseline flow, as an index of the total response, and also the peak flow for each response divided by baseline (POB).

The Shapiro–Wilk test showed that neither of these variables was distributed normally. We therefore used Wilcoxon signed-ranks tests to distinguish the statistical significance of differences in the area under the response curve with respect to baseline flow (AUC) and POB between each blood flow response that was measured. We tested the differences between each of the anaesthetic solutions, and between the use of an anodal or cathodal current. As two comparisons were performed on each variable, we used Bonferroni correction in our interpretation of the results, so that statistical significance was acknowledged if the probability of a type-1 error was less than 2.5% (i.e. $P < 0.025$).

RESULTS

Lignocaine solution with preservatives

When the lignocaine solution containing the preservatives was administered under the cathode, we measured minimal change in blood flow over the initial 5–10 min of iontophoresis, followed by a gradual increase in flow which reached a plateau shortly towards the end of the delivery period (Figure 2).

In contrast, no response was detected when this solution was delivered under the anode (Figure 2). The difference in responses between the electrodes was statistically significant ($P < 0.018$ for both AUC and POB, Wilcoxon signed-rank test), and these results are presented in Table 1.

### Table 1  Skin blood flow responses to iontophoresis of 20 g/l lignocaine hydrochloride, administered in solutions both with and without preservatives, and under the anode and cathode

<table>
<thead>
<tr>
<th></th>
<th>With preservatives</th>
<th>Without preservatives</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Anode</td>
<td>Cathode</td>
</tr>
<tr>
<td><strong>AUC</strong></td>
<td>15.3 (318.3)</td>
<td>1636.5 (860.3)</td>
</tr>
<tr>
<td><strong>POB</strong></td>
<td>1.03 (0.39)</td>
<td>4.16 (2.97)</td>
</tr>
</tbody>
</table>

Assessment of analgesia

Analgesia was assessed at the end of the experiment, immediately after the solution and electrodes had been removed, by applying a 27-gauge disposable dental needle (Bayer Dental, Japan) repeatedly and with moderate pressure to the surface of the skin over the treated area. Each volunteer was asked to confirm the presence or absence of pin-prick sensation.
DISCUSSION

The results of this pilot study demonstrate that lignocaine, at the fairly low dose that we delivered, has probably no vasoactive effect, but that some other constituent of the anaesthetic solution, most likely one or both of the preservatives, does have vasodilatory action.

When administered alone by iontophoresis, lignocaine caused no change in skin blood flow, indicating that, at the dose delivered in the present study, this anaesthetic has no vascular effect. The results of previous investigations suggest that higher concentrations of lignocaine (10–20 g/l) cause vasodilatation, whereas lower doses have either no effect or else cause vasoconstriction [3,4,11]. The method of drug delivery we used means that it is not possible to compare our results directly with those of studies in which lignocaine was administered by intradermal injection; however, by using a pin-prick test, we found that all subjects experienced a degree of analgesia, although it was described as ‘patchy’. (Although subjective, this method did give an indication of whether anaesthetic had in fact entered the skin after each delivery.) Our results therefore suggest that we delivered a lower dose of lignocaine, and the lack of a blood flow response may then agree with this previous work.

Iontophoresis is a valuable research tool because it allows us to deliver drugs to the skin without the trauma associated with intradermal injection. Any resulting increases in blood flow are therefore due entirely to the compound being tested, as long as the current is not high enough to cause electrical effects; however, with the protocol we used in the present study, we delivered a fairly small dose of lignocaine. We administered a total of 331.2 mC over a period of 23 min, whereas commercial iontophoresis devices typically deliver seven times this dose in half the time. A larger dose can be administered by increasing either the iontophoretic current or its delivery duration, but increasing the former much beyond the 0.24 mA that we used would introduce non-specific electrical effects. We performed some experiments using a 1 mA current, but found that this current caused an immediate flare of erythema in and around the administration site, indicating a non-specific, possibly histamine-mediated response. Therapeutic iontophoresis devices typically use a current of 2–4 mA, which would also result in an unacceptably high-current density with our wire electrodes; however, there remains the possibility that our smaller dose of lignocaine did not penetrate deep enough into the dermis to affect blood flow. The blood flow responses that we measured tended to plateau towards the end of the 23-min delivery period, and pilot studies showed that a longer duration produced no further effect, suggesting that a balance had been established between drug delivery and washout.

When we administered the anaesthetic solution under the cathode, we measured demonstrable vasodilatation in response, but only when the solution containing preservatives was used. Although the difference between the solutions appears fairly clear from Figures 2 and 3, it was not statistically significant; however, this may be due to the small number of subjects that we used in our pilot study. The chemical structures of methylparaben and propylparaben suggest that they would both dissociate to form negatively charged ions in solution, and may therefore enter the skin with the iontophoretic current at the cathode. Positively charged lignocaine should not have been delivered in this situation and, indeed, no
analgesia was reported. This implicates one or both of these preservatives as a cause of the increase in blood flow that we observed, and both are reported vasodilators.

Methylparaben is responsible for 72% of the colonic hyperaemia caused by the corticosteroid enema preparation Cortenema, according to a study in dogs [12]. Both preservatives are cerebral non-endothelium-dependent vasodilators in dogs [13], although these effects were not seen in vivo in humans [14]. In addition, methylparaben has been implicated in skin hypersensitivity reactions to local anaesthetics [15].

If additional vasoactive constituents are present in a lignocaine (or any other anaesthetic) preparation, it is likely they would have a significant impact on that drug’s vascular effects when delivered by injection. From a scientific point of view, this would complicate the analysis of an anaesthetic’s inherent vasoactivity, although previous studies do not report whether or not their preparations contained preservatives [3,4,11]. From a clinical point of view, vasocnstriction is preferable in order to promote slow washout of the drug, to limit systemic vascular absorption and to reduce blood loss, and so the addition of a vasodilator is undesirable. It might therefore be better to avoid the use of preservatives, where this is feasible, or to find alternative, non-vasoactive preservatives for use with local anaesthetics. This might then reduce the dose of adrenaline needed in clinical practice.

Are there any other possible causes of the vasodilatation we observed? Although non-specific effects of the iontophoretic current on vascular tone are possible [6], we attempted to limit these by using as low a current as possible to achieve drug delivery. In our experience, 0.24 mA is not high enough to cause non-specific vasodilatation, and it did not have this effect under the anode when the solution without preservatives was used. For the same reason, the negatively charged chloride ions in the solution are also unlikely to be a cause of the observed vasodilatation. (Two subjects did show small responses under the cathode with the preservative-free solution, with the consequence that the difference between the anodal and cathodal responses was close to statistically significant at the 2.5% level, i.e. $P = 0.028$. The rapid onset for these subjects suggests that this was perhaps due, in part, to non-specific vasodilatation rather than any drug effect.)

In conclusion, we have found that one or both of the preservatives methylparaben and propylparaben used in lignocaine preparations may have significant vasodilator activity in the skin. This could have an important consequence on the anaesthetic’s efficacy and duration of action. It might therefore be worth considering the use of alternative, non-vasoactive preservatives or avoiding the use of additives when this is feasible.

Although iontophoresis has proven to be an effective clinical tool for delivering lignocaine non-invasively [5], we were only able to deliver a fairly low dose without inducing non-specific vascular effects. It therefore does not seem to be as valuable a technique for assessing the vasoactivity of this drug (and possibly other local anaesthetics) as it has been in testing other vasodilators [16], although in this study iontophoretic administration did enable us to distinguish between the different constituents of the lignocaine solution. In future, we plan to investigate the mechanisms of vasoactivity of lignocaine (and of the preservatives directly) for a range of doses delivered by injection.

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