Vasoactive characteristics of bupivacaine and levobupivacaine with and without adjuvant epinephrine in peripheral human skin

D. J. Newton1*, G. A. McLeod2, F. Khan1 and J. J. F. Belch1

1Vascular Diseases Research Unit, The Institute of Cardiovascular Research and 2University Department of Anaesthesia, Ninewells Hospital and Medical School, Dundee DD1 9SY, UK

*Corresponding author. E-mail: d.j.newton@dundee.ac.uk

Background. Epinephrine is added to local anaesthetic preparations to prolong their action and reduce their systemic absorption. Bupivacaine and levobupivacaine cause vasodilatation at clinical doses, but lower doses appear to cause vasoconstriction. The aim of this study was to characterize fully the vasoactive effects of these anaesthetics, using an objective measure of blood flow, and to assess the influence of adding epinephrine.

Methods. Laser Doppler imaging was used to measure the forearm skin blood flow responses to intradermal injection of eight doses of bupivacaine and levobupivacaine in 10 healthy male volunteers. The doses tested ranged from 0.008% to 0.75%, and the five highest doses were administered both with and without adjuvant epinephrine 2.5 μg ml⁻¹.

Results. The cumulative responses to the lower subclinical concentrations (0.008–0.0625%) of both anaesthetics were smaller than or similar to that produced by saline alone, indicating a net vasoconstrictive effect. Higher doses caused net vasodilatation, and the levobupivacaine responses were generally lower than the corresponding bupivacaine responses (P=0.022). Epinephrine 2.5 μg ml⁻¹ significantly reduced the responses to clinical doses of both drugs (P<0.001), producing net vasoconstriction.

Conclusions. Bupivacaine and levobupivacaine have a biphasic vascular effect when injected intradermally, with subclinical doses causing net vasoconstriction. The addition of epinephrine 2.5 μg ml⁻¹ decreases these responses markedly.


Keywords: anaesthetics local, bupivacaine; anaesthetics local, levobupivacaine; analgesic techniques, infiltration; sympathetic nervous system, epinephrine

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The vascular properties of local anaesthetics help to determine the duration of their therapeutic activity and the extent to which they are absorbed systemically. The vasoconstrictor epinephrine is commonly added to local anaesthetic preparations in order to prolong their action and reduce their systemic absorption and risk of toxicity. Mixtures of local anaesthetic and epinephrine are used for infiltration anaesthesia of skin incision sites for laparoscopic surgery and to provide postoperative pain relief after vaginal hysterectomy and inguinal hernia repair.

In common with other amide anaesthetics, bupivacaine causes vasodilatation at clinical doses when administered by intradermal injection. Lower doses appear to cause vasoconstriction, although to date this has only been observed in isolated smooth muscle and animal models, and by subjective assessment of skin colour. Similar findings for levobupivacaine indicate that this (S)-isomer of bupivacaine may also have a biphasic effect on the cutaneous microcirculation.

The aim of the present study was to characterize fully the vasoactive effects of bupivacaine and levobupivacaine using a range of subclinical and clinical doses, with or without the addition of epinephrine, in the peripheral skin of healthy humans. Laser Doppler imaging was used to provide an objective measure of skin blood flow.

Methods

We recruited 10 healthy male participants into this study. The study was approved by the Tayside Committee on Medical Research Ethics and was conducted according to
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the principles outlined in the Declaration of Helsinki. The participants were all non-smokers, aged 18–35 years, with no history of cardiovascular disease, asthma or hypersensitivity to amide local anaesthetics. Each gave written informed consent to participation in the study. The experiments were conducted in a laboratory at an environmental temperature of 22°C, and the participants were seated with their arms supported at heart level. Our previous studies with local anaesthetics have demonstrated that 10 subjects are sufficient to show significant differences between doses using these investigations.5 9

Injection sites, approximately 3 cm apart, were marked on the volar surface of both forearms, and the skin in this area was cleaned with an alcohol swab. Using a 27 G needle, we injected 0.1 ml of 0.9% physiological saline and eight concentrations of bupivacaine (Astra Zeneca, Luton, UK) and levobupivacaine (Abbott Laboratories, Kent, UK) intradermally at these sites over the course of two visits. The sites of injection were randomized over both arms and both visits, and were unknown to the participant and investigator. The visits were at least a week apart. The concentrations tested were 0.008%, 0.016%, 0.031%, 0.0625%, 0.125%, 0.25%, 0.5% and 0.75% of both anaesthetics, and the injections were made up in saline. At each visit, the injections were given consecutively over a period of ~1 min. Pilot experiments demonstrated that a 3 cm separation between injection sites was sufficient to prevent the responses overlapping.

These experiments were repeated with 2.5 μg ml⁻¹ of epinephrine added to the five highest doses (0.0625%, 0.125%, 0.25%, 0.5% and 0.75%) of both drugs. This concentration of epinephrine was chosen because we demonstrated recently that it is as effective a vasoconstrictor as a concentration of 5 μg ml⁻¹ when injected intradermally alone or in combination with bupivacaine or levobupivacaine.9

We measured the microvascular skin blood flow at the delivery sites using laser Doppler imaging (moolLDI, Moor Instruments Ltd, Axminster, UK). This instrument works by scanning a 2 mW helium–neon laser across the surface of the skin. Light backscattered 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, which produces a colour-coded image representing a relative measure of skin perfusion (laser Doppler flux) in two dimensions.10 We have used this technique in previous studies of local anaesthetic vasoactivity,5 11 12 and the imager was calibrated monthly using a flux standard to ensure signal stability and measurement reproducibility.

The laser head was positioned 50 cm above the measurement site, with a hood mirror deflecting the beam onto the skin surface. The scan region, encompassing all four or five injection sites on each arm, was 10.5×5 cm. This translated to an area of 100×100 pixels onscreen after the resolution had been reduced to reduce the scan time to 1 min. We recorded images before and at 2, 10, 20, 40 and 60 min after the injections, and each arm was scanned in turn.

We analysed the images using dedicated image-processing software (Moor Instruments Ltd, Axminster, UK). A measure of blood flow at each site was obtained by calculating the median laser Doppler flux within a circular region (radius 1 cm) centred on the point of injection. For an index of the total cumulative response over the first 20 min, we calculated the area under the response curve (AUC), in arbitrary units, with respect to the response to saline alone. All AUC results reported are divided by 1000.

The Shapiro–Wilks test revealed that all data were distributed normally. Differences between the two anaesthetics, and between the doses administered, were tested by univariate analysis of variance.

Results

Injection of all concentrations of both bupivacaine and levobupivacaine caused a rapid increase in skin blood flow which, in most cases, had fallen back to baseline levels after 40 min (Figs 1 and 2). The blood flow changes were dose dependent, with higher doses of both anaesthetics producing increasing cumulative responses (AUC with respect to saline response) over the first 20 min of measurement (P<0.001 for both bupivacaine and levobupivacaine) (Fig. 3). The levobupivacaine responses were consistently lower than the corresponding bupivacaine responses (P=0.022) (Fig. 3). Bupivacaine 0.75% caused the most vasodilatation, and this response was still relatively high even after 60 min (Fig. 1).

Over the first 20 min of measurement, the cumulative responses to the lower concentrations (0.008%, 0.016% and 0.031%) of both anaesthetics were smaller than that produced by saline alone, indicating a net vasoconstrictive effect (Fig. 3); higher doses caused net vasodilatation.

Adding epinephrine 2.5 μg ml⁻¹ to the five highest doses of the local anaesthetic preparations reduced the responses to both bupivacaine (Fig. 4) and levobupivacaine (Fig. 5). These responses were all below the response to saline, indicating a net vasoconstrictive effect (Fig. 6), and the cumulative responses with respect to saline were significantly lower than those without epinephrine (P<0.001 for both anaesthetics).

Discussion

For the first time, the vasoactive properties in human skin of the amide-type local anaesthetic bupivacaine and its (S)-isomer levobupivacaine have been characterized across a range of clinical and subclinical concentrations. Clinical concentrations (0.125–0.75%) of both drugs caused dose-dependent vasodilatation which, in most cases, had subsided after ~40 min. In contrast, the lower subclinical concentrations (0.008–0.031%) caused less vasodilatation than saline alone, while 0.0625% had little effect beyond that observed...
Fig 1 Skin blood flow responses (in arbitrary perfusion units [PU]) to intradermal injection of eight concentrations of bupivacaine and saline.

Fig 2 Skin blood flow responses (in PU) to intradermal injection of eight concentrations of levobupivacaine and saline.

Fig 3 Cumulative responses over 20 min (expressed as AUC with respect to saline response, in arbitrary units) to intradermal injection of eight concentrations of bupivacaine and levobupivacaine.
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**Fig 4** Skin blood flow responses (in PU) to intradermal injection of five concentrations of bupivacaine plus epinephrine 2.5 μg ml⁻¹.

**Fig 5** Skin blood flow responses (in PU) to intradermal injection of five concentrations of levobupivacaine plus epinephrine 2.5 μg ml⁻¹.

**Fig 6** Cumulative responses over 20 min (expressed as AUC with respect to saline response, in arbitrary units) to intradermal injection of five concentrations of bupivacaine and levobupivacaine plus epinephrine 2.5 μg ml⁻¹.
for saline (i.e. the vasodilation caused by the injection trauma). Adding epinephrine $2.5 \, \mu g \, ml^{-1} \ (1:400 \, 000)$ to the five highest concentrations of both anaesthetics caused vasoconstriction in all cases, as noted previously.\(^9\)

These biphasic characteristics have been noted before, although only \textit{in vitro} or by subjective assessment. Using an isolated smooth muscle preparation from the umbilical artery, Tuvemo and Willdeck-Lund\(^6\) found that lower concentrations (5–25 $\mu g \, ml^{-1}$) of bupivacaine caused dose-dependent constriction, while higher concentrations (125–250 $\mu g \, ml^{-1}$) caused relaxation. Similarly, Johns and colleagues\(^7\) noted constriction in the rat cremaster muscle arteriole with 0.1–100 $\mu g \, ml^{-1}$ bupivacaine, with higher doses showing the tendency to dilate.\(^7\) However, the \textit{in vitro} nature of these experiments means these findings cannot be compared directly with ours, although the trend is the same.

\textit{In vivo}, Aps and Reynolds\(^1\) showed by visual inspection of skin colour that 0.25–0.5% bupivacaine produced vasodilatation, while there was either no change or else skin pallor in response to 0.125% bupivacaine. Studies using a more objective measurement technique (such as laser Doppler flowmetry or imaging) have reported dose-dependent vasodilatation to intradermal bupivacaine,\(^5,13,14\) but they have not looked at subclinical concentrations. Similar results have also been obtained with levobupivacaine,\(^5,8\) although this isomer tends to cause less dilatation and more constriction.\(^15\)

Biphasic vasoactivity has also been reported after intradermal injection of other amide-type local anaesthetics. At concentrations $<1\%$, lidocaine appears to cause cutaneous vasoconstriction on visual inspection of skin colour,\(^4,16\) while laser Doppler studies have revealed dose-dependent constriction in response to concentrations of ropivacaine $<1\%$.\(^13,17\) These effects may be associated with tissue calcium. Åberg and colleagues\(^18,19\) suggested that the vasoconstrictor effect at low concentrations is caused by mobilization of $Ca^{2+}$ in smooth muscle, while at high concentrations smooth muscle is relaxed by stabilization of the membrane surrounding $Ca^{2+}$ stores, thereby inhibiting its release.

As reported previously,\(^5\) we found that levobupivacaine had greater vasoconstrictive properties than bupivacaine, even though levobupivacaine contains 11% more molecules, because it is expressed as the base rather than the hydrochloride.\(^20\)

We looked at the vascular effects of these two drugs when used for infiltration anaesthesia in peripheral skin, and therefore we cannot extrapolate to other vascular beds such as the peripheral nerves or epidural space. In addition, we have not looked at the effects of anaesthetic or epinephrine dose on the duration of block. Pinprick tests to assess the depth and duration of analgesia were not practical here because they would have disturbed the blood flow that we were measuring. We also did not measure whether there was any effect on the blood levels of the anaesthetics. Kopacz and colleagues\(^21\) have shown that levels of levobupivacaine are reduced when epinephrine $2.5$ or $5 \, \mu g \, ml^{-1}$ is added to the anaesthetic mixture for spinal surgery. Studies of infiltration anaesthesia in this setting are still required.

We made up the local anaesthetic solutions in physiological saline, and we also used saline to control for the mechanical effects of injection (i.e. skin penetration and tissue compression). We have shown previously, using non-invasive iontophoretic delivery, that $0.5\%$ sodium chloride has no vasodilatory effects in human skin.\(^22\)

In summary, we have used the objective method of laser Doppler imaging to show that intradermal injection of subclinical concentrations of bupivacaine and levobupivacaine cause less vasodilatation than saline alone, suggesting a dose-dependent vasoconstrictive effect of both drugs. These findings confirm those of previous studies using \textit{in vitro} models or more subjective, less accurate measurement techniques. Levobupivacaine was the more vasoconstrictive of the two drugs.

We have also shown that adding epinephrine $2.5 \, \mu g \, ml^{-1}$ has a marked vasoconstrictive effect on the responses to both bupivacaine and levobupivacaine (in a previous study, we found that a larger dose of epinephrine had no additional effect\(^9\)). Despite the introduction of new safer local anaesthetics such as levobupivacaine and ropivacaine, toxicity is still a problem,\(^23\) with reports of convulsions with levobupivacaine\(^24\) and ropivacaine,\(^25\) and cardiac arrest caused by frank overdose with ropivacaine.\(^26\) Our findings suggest that, in a clinical setting, adding epinephrine to these local anaesthetics has the potential to decrease the risk of systemic toxicity and to increase the margin of safety when giving large doses for infiltration anaesthesia.

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