Transient hyperaemic response to assess vascular reactivity of skin: effect of topical anaesthesia

M. D. Wiles, E. Dickson and I. K. Moppett*

University Department of Anaesthesia, Nottingham University Hospitals NHS Trust, Queen’s Medical Centre Campus, Nottingham NG7 2UH, UK

*Corresponding author. E-mail: iain.moppett@nottingham.ac.uk

Background. Local anaesthetics affect blood vessels in various ways, depending upon the site of action and the drug used. There is controversy over the vascular effects of two widely used topical local anaesthetic agents, EMLA® and Ametop®. We used the transient hyperaemic response (THR) to the brief compression of the brachial artery to assess vascular reactivity of forearm skin after application of topical local anaesthesia.

Methods. Twenty healthy male volunteers were studied. Forearm blood flow-flux was measured using laser Doppler flowmetry, and the magnitude of the hyperaemic response to brachial artery occlusion for 20 s was recorded. Control cream, EMLA®, or Ametop® were applied and covered with an occlusive dressing for 60 min. Blood flow-flux and the hyperaemic response measurements were then repeated, with the laser Doppler probes sited over the areas of skin to which the local anaesthetic creams had been applied. Measurements were made at 60, 90, and 120 min after the application of the creams. The THR ratio (THRR) was calculated at each time point, defined as the net hyperaemic flow-flux divided by the baseline flow-flux.

Results. At 60 min, Ametop caused a significantly greater increase in blood flow and decrease in THRR over control than EMLA® [net increase of mean blood flow (SD) over control 95 (61) vs 2 (17) AU (P<0.001), net mean THRR decrease over control 1.33 (1.85) vs 0.34 (1.33) (P<0.02)].

Conclusions. The application of topical Ametop decreases microvascular tone and vasoreactivity of the forearm skin in healthy volunteers.

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The use of local anaesthesia for minor and major procedures is increasing. Some of the reasons put forward for its use are physiological with a possible improvement in blood flow. The evidence for the effects of local anaesthetics on blood flow and vessel reactivity is incomplete and contradictory, and the effects seen may depend on the drug used and the vessels studied. Tetracaine (Ametop®) and eutectic mixture of lidocaine and prilocaine (EMLA®) are commonly used topical agents that provide anaesthesia of the superficial and deep skin layers.

Conflicting effects upon skin blood flow-flux have been observed with EMLA. Various studies have shown no change or an increase or a decrease in blood flow.1–4 The limited numbers of studies with Ametop suggest that it has vasodilatory properties.

Vascular reactivity can be altered by a variety of disease states including hypertension,5 diabetes mellitus,6 and sepsis.7 Within the skin, vascular reactivity has been assessed using laser Doppler flowmetry and the hyperaemic response to a variety of stimuli including acetylcholine iontophoresis, heat, and ischaemia. Abnormalities in skin vascular reactivity have been shown to associate with abnormal behaviour of other more clinically significant vascular beds. The transient hyperaemic response (THR) to 20 s of arterial occlusion allows an easily repeatable, non-invasive assessment of vascular reactivity to be made.8 9

We hypothesized that Ametop, but not EMLA, would increase forearm skin blood flow-flux compared with placebo and reduce the hyperaemic response to transient ischaemia by an alteration in vascular tone.
Methods
Twenty healthy male volunteers, aged 20–32, were recruited with the following exclusion criteria: known systemic or vascular disease, including diabetes mellitus, hypertension, Raynaud’s phenomenon or systemic sclerosis; use of any medication in the preceding 14 days; smoking; and contra-indications to the use of either 4% tetracaine (Ametop, Smith & Nephew Healthcare Limited, Hull, UK) or an eutectic mixture of 2.5% lidocaine and 2.5% prilocaine (EMLA, AstraZeneca, London, UK). Volunteers were required to refrain from eating or ingesting caffeine for 2 h before the study. The Ethical Committee of Nottingham University Medical School approved the study and all volunteers gave written, informed consent.

The study took place in a quiet, warm room and began after a 15 min period of acclimatization. Each subject lay supine and was asked to remain quiet and still throughout the duration of the study. Both arms were abducted and supported by pillows at the level of the anterior axillary line.

Blood flow-flux was measured using a dual channel laser Doppler flowmeter (DRT4; Moor Instruments, Axminster, UK) with probes applied to the volar aspect of each forearm, taking care to avoid any superficial veins. Laser Doppler flowmetry has been validated as an accurate and reproducible measure of skin blood flow-flux.10 Flow-flux is calculated from the scatter of frequency shift of the reflected laser light and is measured in arbitrary units (AU). Owing to the nature of the blood flow in capillaries and connecting small blood vessels, and the effect of varying skin colour and structure, it is not appropriate to use absolute flow units. The use of these units would make it necessary to calibrate for the particular tissue type and site of measurement, which is impractical. To allow values to be assigned to a measurement, the monitor is calibrated using a standard flux signal, which is generated by the laser light scattered from polystyrene microspheres undergoing thermal (Brownian) motion. Flow-flux is a non-directional measure of total cutaneous blood flow in the microcirculation. This is in contrast to blood flow, which is directional and thus opposing flows can cancel out.

The output of the laser Doppler was continuously recorded by a laptop computer using custom software (DRT4WIN; Moor Instruments, Axminster, UK). The methodology of the THR test was as described in previous studies.8 9 11 To achieve brief arterial occlusion, the brachial artery was manually compressed for 20 s and then released. The THR test was only considered suitable for analysis if there was a sudden and maximal decrease in flow-flux at the onset of compression, and the flow-flux signal remained stable. This procedure was repeated three times at intervals of 120 s to allow the flow-flux signal to return to baseline between tests. The THR ratio (THRR) was calculated as the increase in flow-flux after occlusion release divided by the decrease in flow-flux on occlusion.

After recording control measurements on both arms, the skin areas on both arms that had been used for recording were marked with indelible ink. This has no effect on flow-flux measurements. EMLA cream and a control cream (Hospital Hand Cream, Pinewood Lab Ltd, Tipperary, UK) were applied to the measurement points on each subject’s left arm, whereas Ametop cream and the control cream were applied to the right arm. Cutaneous vascular reactivity is unaffected by the side of measurement.12 All the creams were covered with a transparent occlusive dressing (Tegaderm, 3M Health Care, MN, USA) according to normal clinical practice. After 60 min, the creams were removed and the laser Doppler probes reattached. Flow-flux was then measured and the THR test repeated three times at intervals of 120 s. Identical measurements were made at 90 and 120 min after the application of the creams.

Our primary hypothesis was that Ametop would cause a greater increase in flow-flux over control than EMLA, and a greater reduction in THRR at 60 min. Previous unpublished work in our institution has shown a subject coefficient of variation of around 12% for both THRR and flow-flux. We calculated that 20 subjects acting as their own control would be required to find a 25% reduction in THRR with a power of 0.8. Kolmogorov–Smirnov tests were used to test for the normality of data distribution. Statistical analyses were performed using SPSS version 15 software (SPSS, Chicago, IL, USA). Univariate analysis of variance (ANOVA), a post hoc Tukey’s honestly significant difference test, and a paired t-test were used to compare changes in blood flow-flux and THRR. P<0.05 was considered statistically significant.

Results
All the subjects developed a sensory block with EMLA and Ametop, as assessed by a diminished sensation to cold using ethyl chloride. Of 960 THR tests performed, five were considered unsuitable for analysis and were excluded. For these five time points, the mean of two, rather than three THR tests was used. No individual time points were lost due to these exclusions. Analysis of the data excluding the readings with only two rather than three tests made no material difference to the results. The room temperature and relative humidity remained constant during and between experimental days at 22°C and 35–40%, respectively.

Control values for THRR and baseline blood flow-flux were similar for EMLA, Ametop, and control creams.

The results for the two groups are given in Tables 1 and 2 and in Figures 1 and 2.

At 60 min, Ametop caused a significantly greater increase in blood flow and decrease in THRR over control.
than EMLA [net increase of mean blood flow (SD) over control 95 (61) vs 2 (17) AU (P,0.001); net mean THRR decrease over control 1.33 (1.85) vs 0.34 (1.33) (P,0.02)].

Ametop significantly increased skin blood-flow flux throughout the period of measurement, with a corresponding significant decrease in THRR. EMLA had no significant effect on skin blood flow-flux but significantly decreased THRR at 120 min post-application (60 min after removal), when control creams had returned to baseline.

There were no adverse events, such as skin reactions to any of the creams or occlusive dressings.

**Discussion**

We have shown that Ametop causes an increase in forearm skin microvascular flow compared with control cream after 60 min application, with an associated reduction in the THRR. These effects persist for up to 1 h after cream removal. EMLA caused no greater change in flow-flux than control cream, but does cause a reduction in THRR, smaller than that of Ametop, which persists up to 1 h after removal.

Unlike previous work,2 we demonstrated a small, but statistically significant, increase in blood flow after 60 min of control cream application. Forearm skin vessels are generally in a vasoconstricted state13 which may partly explain the lack of observed vasoconstriction. There was also a statistically significant reduction in THRR at 60 min with control, which would suggest that vascular reactivity is altered by topical creams *per se* separate from local anaesthetic activity.

We studied only healthy, non-smoking male volunteers to avoid the alterations in vascular reactivity seen with smoking,14 cardiovascular disease,5 and endogenous oestrogen and progesterone production.15

Our results for EMLA are consistent with some previous work1 3 4 16 and not others.3 17–19 Of note only one of these studies used a control cream,18 as the application of placebo creams may produce significant levels of skin vasoconstriction.2 16 We used 60 min as our primary end-point, as this is a clinically relevant time. It is possible that early or late effects were not seen as a consequence.

It is of note that basal skin blood flow for the EMLA and control creams differed. Small changes in probe positioning can affect recorded values without an overall change in microvascular function or blood flow. There is a recognized variation in blood flow-flux readings, both between individuals and between different sites within the same individual.

**Table 1** Skin blood flow-flux after the application of creams. Values are mean (SD). Time is measured in minutes after cream application, blood flow-flux is measured in arbitrary units. *P<0.001 vs baseline; **P<0.05 vs baseline; †P<0.001 vs paired control cream.

<table>
<thead>
<tr>
<th>Time</th>
<th>Ametop blood flow-flux</th>
<th>Control cream blood flow-flux</th>
<th>EMLA blood flow-flux</th>
<th>Control cream blood flow-flux</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>16.7 (3.9)</td>
<td>15.2 (3.7)</td>
<td>19.2 (5.6)</td>
<td>16.8 (7.1)</td>
</tr>
<tr>
<td>60</td>
<td>111.2 (65.2)*†</td>
<td>25.5 (14.4)*</td>
<td>21.1 (16.2)</td>
<td>23.7 (11.1)**</td>
</tr>
<tr>
<td>90</td>
<td>58.7 (34.0)*†</td>
<td>21.8 (16.2)</td>
<td>24.1 (17.3)</td>
<td>19.8 (15.7)</td>
</tr>
<tr>
<td>120</td>
<td>56.0 (61.1)*†</td>
<td>16.9 (5.8)</td>
<td>19.0 (7.2)</td>
<td>17.4 (10.2)</td>
</tr>
</tbody>
</table>

**Fig 1** Change in skin blood flow-flux (measured in AU) over time, after the application of Ametop, EMLA, and control creams. Values shown are mean, with standard errors of the mean.

**Table 2** Effect of Ametop and EMLA upon the THRR. Values are mean (SD). Time is measured in minutes after cream application. *P<0.001 vs control; **P<0.05 vs control.

<table>
<thead>
<tr>
<th>Time</th>
<th>Ametop THRR</th>
<th>Control cream THRR</th>
<th>EMLA THRR</th>
<th>Control cream THRR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>4.86 (1.86)</td>
<td>4.60 (1.82)</td>
<td>4.04 (1.76)</td>
<td>3.63 (1.35)</td>
</tr>
<tr>
<td>60</td>
<td>1.35 (0.67)*</td>
<td>2.41 (1.01)</td>
<td>3.17 (1.75)**</td>
<td>2.43 (1.28)</td>
</tr>
<tr>
<td>90</td>
<td>1.73 (0.63)*</td>
<td>3.26 (1.70)</td>
<td>3.05 (1.49)</td>
<td>3.45 (1.64)</td>
</tr>
<tr>
<td>120</td>
<td>2.17 (1.30)*</td>
<td>3.91 (1.71)</td>
<td>2.90 (1.12)**</td>
<td>3.62 (1.73)</td>
</tr>
</tbody>
</table>

**Fig 2** Change in THRR over time, after the application of Ametop, EMLA, and control creams. Values shown are mean, with standard errors of the mean.
This may be due to heterogeneity in skin blood flow, the localized nature of the flow signal (only 1 mm$^3$ is examined), and gross variability of microvasculature.

Little work has been published regarding the vascular effects of Ametop (4% tetracaine, Smith & Nephew, Hull, UK) and our results are consistent with these, suggesting that Ametop causes significant vasodilatation and reduction in vascular reactivity.\textsuperscript{20, 21}

The differences between EMLA and Ametop suggest that the effects seen are not purely a local anaesthetic class effect but that EMLA possessed vasoconstrictive properties which counteract the vasodilating effect of local anaesthesia. All subjects had anaesthetized skin on testing, yet the vasodilation with Ametop was much greater. It is possible that the effective dose of local anaesthetic used was different, but beyond demonstrating adequate anaesthesia there is no practical method of measuring delivered dose. Previous work investigating the effect of local anaesthetics on more prolonged ischaemia has assumed that all local anaesthetics have the same effect, and that local hyperaemia is mediated via a local sensory reflex.\textsuperscript{3} Our results suggest that this may not be true, and that EMLA does not inhibit the THR\textsuperscript{R} as much as control. Ametop on the other hand has significant vasodilatory effects coupled with inhibition of the hyperaemic response.

There could be a variety of physiological mechanisms responsible for the differing vasoactive properties of the two creams. Non-steroidal anti-inflammatory drugs decrease skin microvascular tone by blocking prostacyclin production\textsuperscript{11} and this is unaffected by EMLA-induced topical anaesthesia.\textsuperscript{3} Calcitonin gene-related peptide (CGRP) and substance P are potent vasodilators that may be released in response to sensory C fibre stimulation. EMLA-induced topical skin anaesthesia did not diminish the increase in cutaneous blood flow induced by the intradermal injection of CGRP.\textsuperscript{3} It is possible that Ametop may have effects upon either of these pathways, independent of its local anaesthetic action, and could form the basis of future experimental work.

In summary, we have shown that in healthy volunteers, Ametop causes a greater degree of vasodilatation than EMLA or control creams and this is associated with a reduction in skin vascular reactivity. This would suggest that the effects seen are not purely due to local anaesthesia but may relate to the properties of Ametop itself.

References