International Journal of Pharmaceutics, 51 (1989) 183–187
Elsevier

IJP 01731

Non-invasive monitoring of percutaneous local anaesthesia using laser–Doppler velocimetry

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(Received 11 August 1988)
(Modified version received 4 October 1988)
(Accepted 11 October 1988)

Key words: Percutaneous local anesthesia; Amethocaine; Laser–Doppler velocimetry

Summary

The dermal absorption of amethocaine from percutaneous local anaesthetic preparations has been monitored in a panel of 10 volunteers using laser–Doppler velocimetry (LDV). LDV is a non-invasive technique which responds to increased perfusion in the cutaneous microcirculation. The study was fully randomised and double-blinded. A standard regimen involving a 30 min application time for the preparations was followed in all cases. Statistical analysis of the LDV results was made using one-way analysis of variance and the Newman–Keuls multiple-range test. A significant increase in peak blood cell flux resulted from application of the formulations, correlating with an increase in the drug concentration. In all cases the increased perfusion was significantly different from placebo. The time at which the peak flux occurred was in good agreement with the previously determined mean onset times for anaesthesia with these preparations. However, the area under the perfusion curve was not a reliable indicator of the duration of anaesthesia. The transient erythema occasionally observed with amethocaine percutaneous anaesthetic preparations was confirmed as a pharmacological property of the drug rather than a slight side-effect of the formulation.

Introduction

The advantages of a topical anaesthetic preparation which would render painless such procedures as venepuncture and catheterisation are now becoming widely recognized in paediatric practice. The ideal percutaneous anaesthetic formulation should provide rapid, deep and relatively long-lasting anaesthesia of both the skin surface and underlying tissues (Monash, 1957; Lubens and Sanker, 1964; Adriani and Dalilli, 1971; Hallen et al., 1984), and should contain the minimum concentration of local anaesthetic agent consistent with producing the desired clinical effect (Woolfson et al., 1988).

Currently, only one percutaneous anaesthetic preparation is commercially available. This preparation consists of a eutectic mixture of lignocaine and prilocaine. Effective dermal anaesthesia is produced but with the disadvantages of a long onset time and a short duration of effect (Ehrenstrom-Reiz et al., 1983). Recent studies (McCafferty et al., 1988a; Woolfson et al., 1988) have demonstrated that amethocaine is a suitable drug on which to base a percutaneous local

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anaesthetic formulation. Profound dermal anaesthesia can be achieved with a significantly reduced onset time and enhanced duration of effect (McCafferty et al., 1988b; Small et al., 1988).

In studying candidate percutaneous anaesthetic formulations one particular difficulty encountered is the inevitably subjective nature of pain assessment, particularly in children. In order to determine the minimum concentration of anaesthetic agent required for effective dermal anaesthesia a non-subjective method of concentration–response analysis would clearly be advantageous. Laser–Doppler velocimetry (LDV) is a non-invasive method of monitoring blood perfusion through the cutaneous circulation (Holloway, 1983). It has been used to follow the percutaneous absorption of topically applied vasoactive compounds, notably potent vasodilators such as nicotinic acid esters (Guy et al., 1983, 1986). Both vasodilatation and anaesthesia are due to a drug–receptor interaction. Many local anaesthetics, including amethocaine, are known vasodilators (Martindale, 1982). LDV is used in the present study as a non-subjective indicator of the effect of drug concentration on the percutaneous absorption of amethocaine. Results may be compared with the subjective pin-prick concentration–response analysis previously reported (Woolfson et al., 1988).

Materials and Methods

Chemicals and formulations

Amethocaine base U.S.P. was obtained from Ward Blenkinsop, Cheshire, U.K. Formulations containing, respectively, 2%, 4% and 6% m/V amethocaine were prepared as previously described (Woolfson et al., 1988) and dispensed in 5 g lacquered tubes. A placebo formulation containing 4% m/V Avicel (microcrystalline cellulose) in an identical base to the active formulations was also prepared.

Laser–Doppler velocimetry

LDV measurements were made using a Periflux PF2 laser–Doppler flowmeter (Perimed, Stockholm, Sweden) in conjunction with a standard PF 108 probe and plastic holder. The laser was a 2 mW helium–neon laser operating at 632.8 nm. The probe holder was attached to the test site by means of a double-sided adhesive ring (3M, St. Paul, U.S.A.). All measurements were made in a thermostatically controlled ($\pm 1°C$), quiet environment with the probe coincident with the centre of the test site. Volunteers were seated with the test arm on a flat surface below heart level. Basal perfusion measurements were established over a 15 min period prior to each test.

Volunteer studies

A panel of 10 healthy adult subjects (6 male, age range 21–35 years) was used in the study. Each subject gave written informed consent and the study was approved by the local ethical committee. Each subject received all formulations including a placebo. Formulations were allocated to subjects on a random basis and the study was double-blinded. Both left and right forearms (ventral surface at or just below the anterior cubital fossa) were used and a minimum period of 7 days was maintained between successive trials on previously treated individuals.

Each formulation (0.5 g) was applied to the test site under a dressing (Op-Site I.V., Smith and Nephew, Hull, U.K.) for 30 min. The preparation was then removed, the site wiped clean and the LDV probe holder placed directly over the site. LDV measurements were then made at 5 min intervals for 1 h, following which anaesthesia was finally assessed by the pin-prick method (McCafferty et al., 1988a).

Statistical analysis

Results from each subject were analysed both in terms of the recorded peak flux value and the area under the perfusion (flux/time) curve (Fig. 1). Statistical analysis was made using single factor analysis of variance (ANOVA) with repeated measurements (Winer, 1971). Specific comparisons of formulations were made using the Newman–Keuls multiple range test (Zar, 1974).

Results and Discussion

The use of LDV for the non-invasive monitoring of blood perfusion through the cutaneous mi-

crocirculation is now well-established (Holloway, 1983). The LDV probe uses a low-power laser light. When placed against the skin this illuminating light undergoes partial reflection, and consequently a Doppler frequency shift, upon striking blood cells traversing the illuminated volume of skin. The actual quantity measured is blood cell flux, defined as the product of the number of red blood cells moving in the measured volume and the mean cell velocity. It is thus the velocity of cutaneous blood vessel perfusion which dictates the magnitude of the output signal obtained by LDV.

The topical application of a vasodilator such as methyl nicotinate results in an increased blood cell velocity (Guy et al., 1986) and an enhanced LDV signal (flux). Amethocaine and many other local anaesthetics are vasodilators, though their action in this respect is much less potent than the nicotinates. Nevertheless, it had been observed during use of the amethocaine percutaneous anaesthetic formulation that some subjects exhibited a slight, transient erythema following removal of the preparation. Use of LDV was therefore appropriate not only as a means of following the percutaneous absorption of amethocaine but also in confirming that this occasional slight erythema was a pharmacological effect of the drug rather than a side-effect of the formulation.

In the present study a placebo and 3 active formulations were used. All formulations were identical except for their amethocaine concentrations, respectively 0%, 2%, 4% and 6% m/V. Previous studies have demonstrated that formulations containing 4% or 6% m/V amethocaine produced effective percutaneous anaesthesia when assessed subjectively by the pin-prick method (McCafferty et al., 1988b; Woolfson et al., 1988). An application time of 30 min is required. The preparation is then completely removed from the skin, allowing the LDV probe to be positioned over the treated area. Fig. 1 shows typical responses to the 4 preparations by an individual subject. The perfusion curves (flux vs time) demonstrate a marked increase in blood flow through the cutaneous microcirculation following treatment with the various amethocaine formulations. A peak response is noted about 10 min after removal of the preparation and positioning of the probe, i.e. a total response time of approximately 40 min. This corresponds to the mean onset times for anaesthesia found in earlier subject studies (McCafferty et al., 1988b; Woolfson et al., 1988). Interestingly, the peak responses obtained correlate well with the anaesthetic concentrations, the maximum response being due to the preparation containing 6% m/V amethocaine.

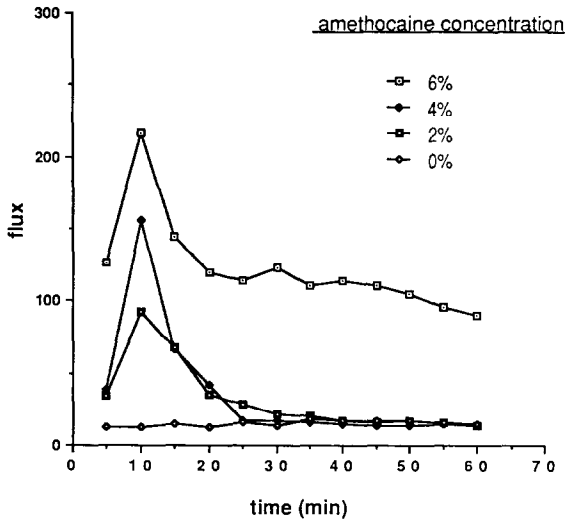

Fig. 1. Typical LDV perfusion curves following treatment of a subject with percutaneous anaesthetic formulations (2%, 4% and 6% m/V amethocaine) and placebo. Flux measurements are in arbitrary units.

The responses to placebo did not increase significantly throughout this study. This was typical for all subjects, the values being similar to the basal responses established at the start of each test. The overall mean response to placebo was 14.06 ± 1.23 ($\pm$S.D.) (arbitrary units), consistent with basal values. It was therefore unnecessary to normalise individual responses with respect to the basal values.

In LDV studies of cutaneous blood flow the results may be quantified either in terms of peak flux or the area under the perfusion curve (AUC). These are, respectively, measures of the magnitude and duration of the effect. AUC can readily be calculated by the trapezoidal rule (Gibaldi and Perrier, 1982). The experimental protocol was such that all subjects received all treatments. Therefore,

TABLE 1

LDV monitoring of percutaneous local anaesthetic formulations

	Anaesthetic conc. % m/V				Significance of difference
	0	2	4	6	
Mean peak flux [a]	16	59	92	132	$P < 0.01$
Mean area under curve [a]	833	1499	1692	3027	$P < 0.01$

[a] Arbitrary units.

statistical analysis was by one-way analysis of variance with repeated measures (Winer, 1971). Table 1 gives the results of ANOVA when both the peak response and AUC were used as quantitative measures. In both cases the formulations were significantly different ($P < 0.01$). Therefore, a more detailed intercomparison of formulations was made using the Newman–Keuls multiple range test (Zar, 1974). From Table 2 it can be seen that the intercomparison of individual formulations was also in terms of both peak flux and AUC. For the former, all amethocaine preparations were significantly different ($P < 0.05$) from placebo. All other comparisons were also significantly different ($P < 0.05$) with the peak flux increasing with increasing amethocaine (% m/V) concentration, i.e. $6 > 4 > 2$. These results are essentially similar to the previously reported subjective study (Woolfson et al., 1988). However, a less well-defined result was obtained when AUC was used as a measure of the response (Table 2). Although the 6% m/V preparation was significantly different from all other preparations, the other active preparations were not significantly different from placebo, or from each other.

TABLE 2

Intercomparison of formulations (Newman – Keuls multiple-range test)

Comparison (in terms of anaesthetic conc. % mV)	Response	Significance of difference
6 vs 0	AUC	$P < 0.05$
6 vs 2		$P < 0.05$
6 vs 4		$P < 0.05$
4 vs 0		$P > 0.05$
4 vs 2		$P > 0.05$
2 vs 0		$P > 0.05$
6 vs 0	Peak flux	$P < 0.05$
6 vs 2		$P < 0.05$
6 vs 4		$P < 0.05$
4 vs 0		$P < 0.05$
4 vs 2		$P < 0.05$
2 vs 0		$P < 0.05$

Since the peak flux value on the perfusion curve is a measure of the magnitude of the response to the vasodilatory effect of amethocaine, it is perhaps not surprising that this measure appears to follow well the percutaneous absorption of the drug as adjudged subjectively (Woolfson et al., 1988). Peak fluxes occurred at mean total times following application of the preparations of 41 ± 5.6 min (6% m/V amethocaine) and 43 ± 6.3 min (4% m/V amethocaine), typical mean onset times for anaesthesia in adult subjects with these preparations (McCafferty et al., 1988b; Woolfson et al., 1988).

In this study subjects were asked to make a final subjective assessment of anaesthesia by the pin-prick method. All subjects were still fully anaesthetised 90 min after the initial application with both the 6% and 4% m/V preparations. However, the flux and, therefore, AUC values were in decline at this time, in many cases reaching basal levels again. Therefore, it is not surprising that use of AUC as a measure fails to differentiate adequately between the preparations. Clearly, although AUC is a measure of the duration of the drug effect on the peripheral microcirculation this latter factor is related only to the onset time for anaesthesia and not its duration. Duration of anaesthesia is dependent on the extent of drug retention in the stratum corneum and the rate of steady-state release to the underlying nociceptors, together with the inherent potency of

the drug. Both these factors are in turn largely dictated by the lipophilicity of the anaesthetic agent (McCafferty et al., 1988b).

This objective LDV study has demonstrated that the blood cell flux in the cutaneous microcirculation is significantly increased by percutaneous anaesthetic preparations containing amethocaine, and that this increase is indicative of the percutaneous absorption of the drug. Considering that vasodilatation is a saturable process and that the dose–response curves may not be linear (Guy et al., 1983, 1986), the results obtained by LDV nevertheless correlate well with an earlier subjective study which demonstrated that a preparation containing 4% m/V amethocaine most nearly met the requirements for an ideal percutaneous anaesthetic preparation. Further, the increase in flux shows clearly that the transient erythema sometimes observed with these preparations should be regarded as a pharmacological effect of the drug rather than a slight side-effect of the formulations.

Acknowledgements

The authors gratefully acknowledge the loan of the laser–Doppler flowmeter by Professor J. Hadgraft of the Welsh School of Pharmacy.

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