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Research Papers

In-vitro release of bupivacaine from injectable lipid formulations investigated by a single drop technique – relation to duration of action in-vivo

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Abstract

The aim of this study was to develop an in-vitro release method suitable for injectable slow-release lipid formulations of local anaesthetics (or other drugs). We also aimed that the results of the in-vitro measurements should have a clear relationship to duration of action in-vivo. Six formulations of bupivacaine base in medium-chain triglyceride–glyceryl dilaurate mixtures were developed. A new apparatus was constructed for determination of their in-vitro release profiles. A bulbous glass tube was fixed inside a standard glass bottle, which was then filled with release medium. A stirring magnet was enclosed in the perforated polypropylene cylinder holding the glass tube. The stirring created a continuous, rotating downward flow of medium inside the tube, which kept the lipid phase, introduced by means of a syringe, suspended as a single, free drop. Release profiles were obtained by sampling of the release medium for up to 72 h and analysis by gas–liquid chromatography. The duration of action in-vivo of the respective formulations was tested by the hot-plate method in rats. The release profiles of bupivacaine in-vitro were mono-exponential for four formulations and bi-exponential for the other two. There was a positive correlation between the proportion of glyceryl dilaurate in the formulation and the slow half-life of release of bupivacaine. All formulations showed prolonged duration of action in-vivo, median values within the range 4.5–12 h, as compared with a 2-h effect of bupivacaine hydrochloride solution. A comparison of in-vitro release curves and durations of action in-vivo suggested that to maintain nerve blockade in-vivo the formulations must release bupivacaine at a rate of approximately 350 μg h^{-1} under the in-vitro conditions. To conclude, we designed and tested a novel apparatus for measuring release of a local anaesthetic (or other drug) from a fluid or semi-solid formulation in-vitro. Release rates obtained in-vitro by means of this technique may be used to guide the development of formulations with suitable durations of action in-vivo. The apparatus is, however, as yet a prototype. Rigorous evaluation of performance should be carried out on devices built to specific standards according to their intended application.

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Introduction

Peripheral nerve blockade with a local anaesthetic provides excellent pain relief but its clinical utility (e.g. for the treatment of postoperative pain) is sometimes limited by the short duration of effect. It is therefore of great clinical interest to develop a local anaesthetic that is effective for days instead of hours (Duncan & Wildsmith 1995; Kuzma et al 1997; Renck & Wallin 1996). A number of slow-release formulations have been developed and promising pre-clinical results have been

Table 1 Compositions and consistencies (at room temperature) of the six bupivacaine–lipid formulations.

Formulation	Bupivacaine (% w/w)	Glyceryl dilaurate (% w/w)	Triglyceride (% w/w)	Consistency
A	4.2	0	95.8	Clear fluid
B	5.5	40	54.5	Semi-solid, suspension
C	7.0	46.5	46.5	Semi-solid, suspension
D	7.0	0	93	Fluid, suspension
E	7.0	14	79	Semi-solid, suspension
F	14	13	73	Semi-solid, suspension

reported. Various approaches have been explored, such as inclusion of the local anaesthetic into liposomes, neutral lipids or synthetic or natural polymers.

In the development of such formulations the release profile of local anaesthetic is normally investigated in-vitro, and the duration of action is then tested in animals. None of the reported in-vitro release methods were specifically designed for testing lipid formulations, neither do they seem particularly suitable for the purpose. Liposphere and liposomal preparations have been enclosed in dialysis tubing, which is then immersed in release medium (Hersh et al 1992; Grant et al 1994; Masters & Domb 1998). Solid preparations have simply been incubated with medium (Masters et al 1993a, b; Curley et al 1996) or also enclosed in dialysis bags after suspension in saline solution (Kohane et al 2000). Lidocaine (lignocaine)-containing gels have been investigated in apparatuses in which release takes place from the formulation in a compartment covered with a cellulose membrane into a large volume of stirred buffer solution (i.e. the US Pharmacopoeia rotating paddle apparatus) (Dyhre et al 2001) or a similar device (Paavola et al 1995).

In addition, there has been no clear relation between in-vitro and in-vivo results in most studies (Hersh et al 1992; Grant et al 1994; Curley et al 1996; Masters & Domb 1998; Kohane et al 2000; Dyhre et al 2001). When bupivacaine hydrochloride was incorporated into biodegradable polymer matrix formulations (Masters et al 1993a, b), the duration of in-vivo effects was generally similar to the total time of release of bupivacaine in-vitro, although the in-vitro data were not used to predict differences in in-vivo duration between different formulations. In a series of injectable gels containing lidocaine, Paavola et al (1995) found a correlation between cumulative release in-vitro and cumulative in-vivo effect over time. Prolongation of action of lidocaine was, however, modest and not predicted by the in-vitro data. Rational development of slow-release formulations would be greatly facilitated by the development of methods for in-vitro release testing that yield data which can be directly related to in-vivo performance.

The aim of this study was to develop an in-vitro release method suitable for injectable lipid formulations of local anaesthetics (or other drugs). We also aimed that the results of the in-vitro measurements should have a clear relationship to duration of action in-vivo. This would allow a rational selection of candidates for in-vivo testing and thus reduce the number of animal experiments. For this purpose six different lipid formulations of bupivacaine base were developed and tested in a novel in-vitro apparatus as well as by a standard in-vivo method in rats.

Materials and Methods

Materials

Bupivacaine base was a gift from Camurus AB (Lund, Sweden). A medium-chain triglyceride mixture of European Pharmacopoeia quality was procured by Apoteket AB (Sweden). According to specifications in the Pharmacopoeia, the fatty acid moieties of the mixture are $\leqslant 2\%$ caproic, 50–80% caprylic, 20–50% capric, $\leqslant 3\%$ lauric and $\leqslant 1\%$ myristic acid. Glyceryl dilaurate was supplied by Danisco Ingredients A/S (Brabrand, Denmark). Chemicals for analysis and for the preparation of phosphate-buffered saline solution (PBS) were of analytical grade.

Formulations

Six lipid formulations of bupivacaine base were prepared (Table 1). The duration of action in-vivo was modulated in two ways, by changing the percentage of active substance and by changing the viscosity of the preparation. The medium-chain triglyceride mixture was an oily liquid at room temperature (21°C). Glyceryl dilaurate is solid at room temperature, at which it also has

low solubility in the triglyceride mixture. It could therefore be used as a stiffener to increase viscosity. All formulations were prepared by mixing the components in a glass container and melting to a homogenous, transparent liquid, at a maximum temperature of 90°C. After cooling to room temperature, all formulations could be injected through a 29-Gauge needle. Formulations with high viscosity were filled directly into the barrel of the syringe, after removal of the plunger.

In-vitro release apparatus and experiments

The prototype release apparatus is depicted in Figure 1. The principle of the device was to keep the lipid phase suspended as a single, free drop in the release medium. The sample, which must have a lower density than the medium, was thus held in a continuously rotating downward flow of solution inside the bulbous glass tube. The flow was controlled by the stirring rate of the magnet. The glass tube was made from a 15-mL volumetric pipette of borosilicate glass, held in place by the perforated polypropylene cylinder enclosing the stirring magnet. The outer bottle was of standard type Schott Duran glass, of either 500 or 1000 mL nominal volume, sealed with a screw cap. Since the bottle was filled practically to the rim, formulations containing $\leqslant 7\%$ of bupivacaine base (i.e. formulations A–E) were in fact tested in 550 mL of release medium, while formulation F, containing 14% of bupivacaine base, was tested in 1100 mL of medium. The release medium (PBS) contained Na^+ 198 mM, K^+ 19 mM, phosphate 100 mM and Cl^- 36 mM, pH 7.4. The surrounding water bath was thermostatted to 37 ± 0.5°C.

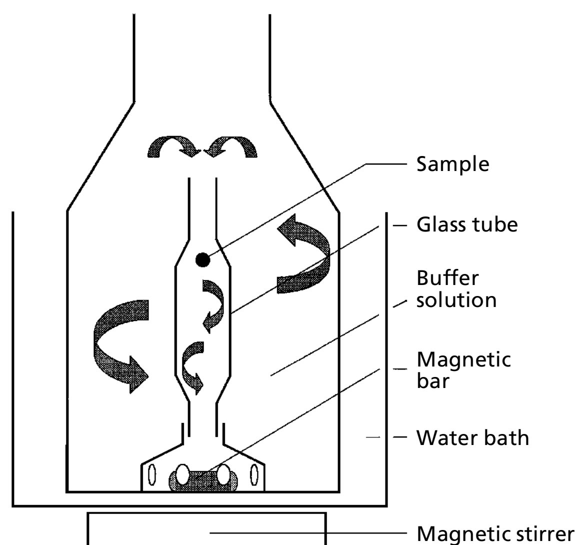

Figure 1 A schematic representation of the novel release apparatus.

By means of a 1-mL syringe with a square-cut needle, a 0.1-mL sample of formulation A, D, E or F was introduced into the bulbous glass tube to form a fluid or semi-fluid drop. Application of a sample was, as far as possible, performed in the same way as injection into the rat (see below). The precise weight of sample was determined by weighing the syringe before and after extrusion of the formulation. Due to their high viscosity, formulations B and C could not be shaped to a drop by direct injection into the glass tube. They were therefore melted by heating to 90°C and moulded to 0.1-mL spheres, which were weighed before application. In these cases, the bulbous glass tube had been cut horizontally at the middle and the pieces were re-attached by means of silicone-based glue after introduction of the semi-solid sample. Samples of release medium, 1.5–3.0 mL, were drawn at 0.25, 0.5, 1, 2, 3, 4 and 5 h from the start of the experiment and thereafter at appropriate times, depending on the formulation. Total sampling time varied between 23 and 72 h. Care was taken to keep the vortex undisturbed, since otherwise the lipid drop may disintegrate or become attached to the inner wall of the glass tube. The withdrawn sample volume was replaced with PBS.

Solubility and adsorption of bupivacine base

The solubility of bupivacaine in the release medium was measured by suspending 100 mg of bupivacaine base in 10 mL of PBS. The suspension was incubated at 37°C and vortex-mixed 4 times daily. Samples of the supernatant were taken after 24 and 48 h and analysed after centrifugation and filtration through a 0.22-μm filter. For each sampling time, samples were assayed in duplicate.

Adsorption was investigated by filling the apparatus with a release medium containing 1.4 $\mu g\ mL^{-1}$ of bupivacaine base. The medium was analysed (duplicate samples) at 3, 7 and 24 h after filling and the concentration compared with that of the original solution.

Assay of bupivacaine

Bupivacaine concentrations in the release medium were determined by gas–liquid chromatography, by a modification of a previously described method for lidocaine (Dyhre et al 2001). To a 0.10–0.50-mL volume of sample were added blank human plasma (0.2 mL) and

mepivacaine (100 μL of a 10-μg-mL^{-1} solution) as internal standard. The sample was then made alkaline with 0.20 mL of 0.5 M NaOH solution and extracted with 5 mL of n-hexane containing 0.08% of triethylamine. The mixture was centrifuged for 10 min at 1200 *g*. The organic phase was collected and, after addition of 25 μL of 0.1 M acetic acid in diethyl ether, evaporated to dryness. The residue was dissolved in 25 μL of ethanol and 1 μL was injected on a gas chromatograph (Varian 3800, Palo Alto, CA) equipped with a nitrogen-selective detector. The column was packed with OV-17 and the oven temperature was 265°C. The limit of detection was approximately 10 ng mL^{-1}. The within-day coefficient of variation (CV) was 3.0% at 0.1 μg (amount in the sample), 2.7% at 1.0 μg and 2.4% at 10 μg (n = 8).

Calculation of rate of release in-vitro

Two concentration curves of bupivacaine in release medium were obtained for each formulation. The amount (A_{rel}) of bupivacaine released at each sampling time was calculated, taking into account the accumulated loss by sampling. Since the sample weight of formulation introduced into the apparatus varied slightly between experiments, the A_{rel} data were adjusted to correspond to a nominal sample weight of 95 mg, and the data from the two experiments were then pooled. Mono- and bi-exponential release functions were then fitted to the A_{rel}-versus-time data by means of the SCIENTIST software (MicroMath, Salt Lake City, UT):

$$A_{rel} = A_{final} - \sum_{i=1}^{n} A_i \times e^{-k_i \times t} \quad (1)$$

In this expression A_{final} is the final amount of bupivacaine released after equilibration between the formulation and the medium, n is 1 or 2, A_i is a pre-exponential coefficient, k_i is a first-order rate constant of release and t is time. Half-lives of release were calculated as $\ln(2)/k_i$. The choice between a mono- and bi-exponential fit was made according to the SCIENTIST Model Selection Criterion, a modification of the Akaike Information Criterion. In addition, when the data did not support the bi-exponential function the calculated confidence interval of A_2 often included zero.

If a bi-exponential function was found to give the best description of the data, the percent contribution of each phase to the exponential curve was calculated as:

$$Contribution_i\,(\%) = 100 \times \frac{A_i/k_i}{A_1/k_1 + A_2/k_2} \quad (2)$$

The net rate of release (R_{rel}) of bupivacaine (in μg h^{-1}) was then calculated, as a function of time, as the first derivative of the amount-versus-time curve:

$$R_{rel} = \frac{dA_{rel}}{dt} = \sum_{i=1}^{n} A_i \times k_i \times e^{-k_i \times t} \quad (3)$$

Animals and housing

The experimental protocol was approved by the Ethics Committee on Animal Experimentation at Lund University. Adult male Sprague–Dawley rats (Møllegaard Breeding & Research Centre, Ejby, Denmark) were kept four to a cage with food and water freely available. They were maintained on a 12-h light–dark cycle in a temperature-controlled environment and allowed a four-day habituation period before the experiments.

Nerve block

The procedures have been described previously (Masters et al 1993b; Dyhre et al 2001). The rats were anaesthetized briefly with halothane, 2–3% in oxygen, by face mask. The sciatic nerve on one side was surgically exposed. Under magnification 0.1 mL of the test formulation was injected directly beneath the clear fascia surrounding the nerve but outside the perineurium proximal to the sciatic trifurcation using a 29-Gauge hypodermic needle. The wound was closed with four sutures and halothane administration ended. The procedure took approximately 10 min.

The rats were accustomed to the test situation on the day before the experiments. Each experiment included testing of sensory and motor effects before and at suitable times (see below) after injection of the test formulation. To test the sensory block by the pain withdrawal reflex we used a small hot-plate, thermostatted to 52°C. Restraining the rat by hand grip we tested the blocked leg as well as the opposite leg alternately five times. Since the block did not comprise the motor nerves to the hip muscles all rats were able to withdraw the tested paw in response to pain. A cut-off time of 10 s was applied to avoid tissue damage. Sensory block was defined as no withdrawal reaction within the 10 s, at any of the five occasions. The duration of sensory block was given as the time from injection of the test formulation to the first occurrence of at least one withdrawal response within the 10 s. The duration of motor block was given as the time from injection of the test formulation to regained ability to walk and grip normally with the toes.

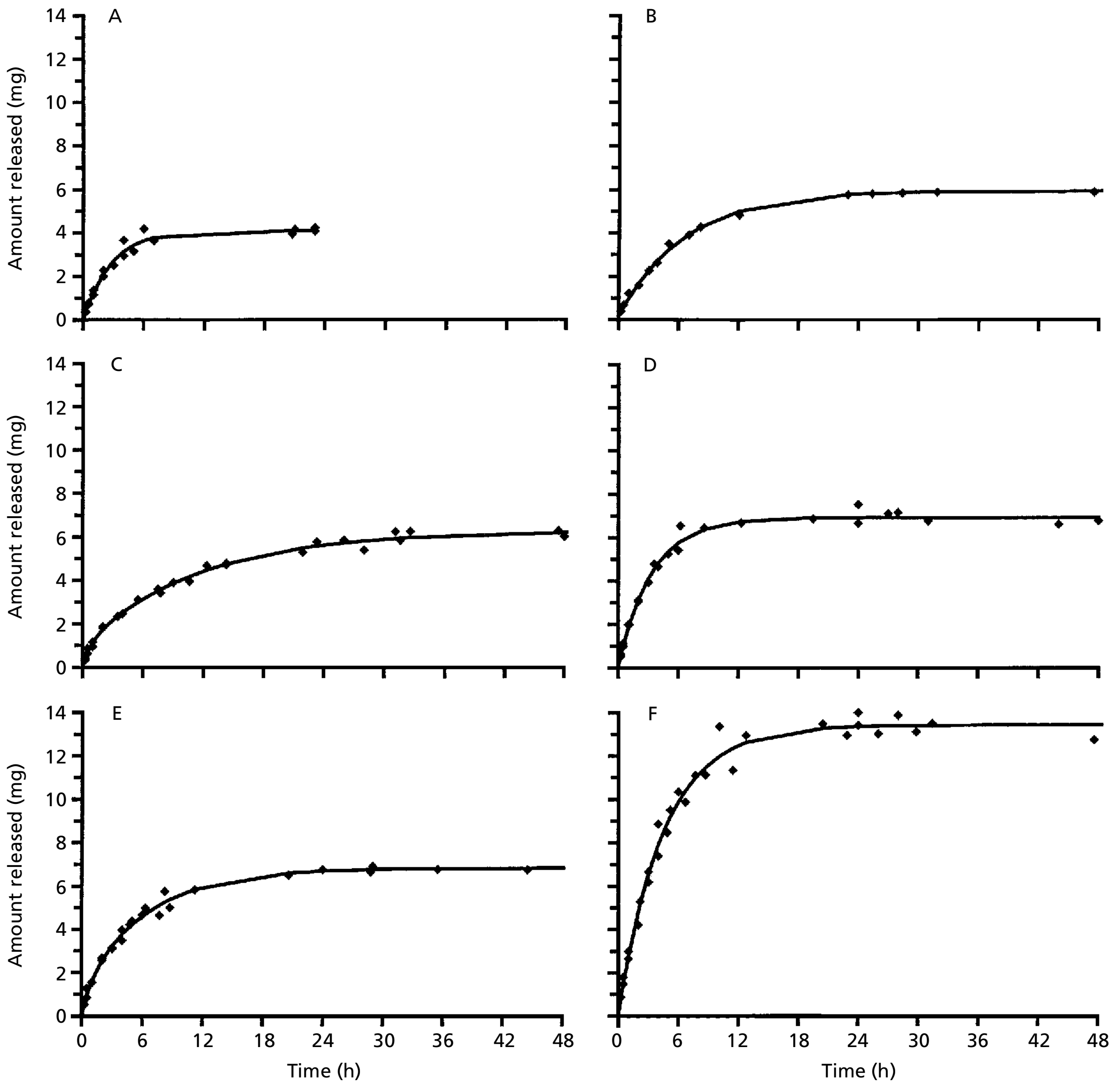

Figure 2 In-vitro release of bupivacaine from lipid formulations A–F. Measured data of amount released (concentration × volume of release medium) and fitted exponential functions. Data are shown up to 48 h, although the sampling continued for up to 72 h for formulation B and 57 h for formulation C. Correlations (r^2) between predicted and measured values were in the range 0.994–0.999 in the six data fits.

The preparations were first tested in pilot experiments to give an indication of approximate duration of drug action. A total of 15 pilot rats were used. The definitive tests were performed in two sessions. In the first, 24 rats were randomised to receive either bupivacaine hydrochloride solution, 5 mg mL^{-1}, or formulation A, B or C. Nerve blocks were tested every 10 min for up to 4 h after drug administration and then, if necessary, every half hour up to 18 h. In the second session, 18 rats were randomised to receive formulation D, E or F. Nerve blocks were tested every 30 min up to 10 h and then, if necessary, hourly up to 24 h (authors LS and HD working in relays). For each rat, observation was terminated after two successive negative results.

In-vitro–in-vivo comparison

To relate the in-vitro and in-vivo results, the net rate of release of bupivacaine from each formulation was calculated (by equation 3) at the time points (n = 6)

corresponding to the duration of sensory nerve block induced by that formulation.

Statistics

The relationship between proportion of glyceryl dilaurate in the formulation and the slow half-life of release of bupivacaine in-vitro was investigated by linear regression. The statistical significance of variation between preparations, as regards duration of action in-vivo and rate of in-vitro release at offset of sensory block, was investigated by means of the Kruskal–Wallis test.

Results

Solubility and adsorption of bupivacaine

The concentration of bupivacaine in the PBS solution was 332 μg mL^{-1} after 24 h and 347 μg mL^{-1} after 48 h of incubation and mixing at 37°C. The difference of less than 5% indicates that steady state had been reached by 48 h. In the adsorption experiment, the concentrations of bupivacaine were 1.3, 1.4 and 1.3 μg mL^{-1} after 3, 7 and 24 h, respectively (i.e. no detectable decrease was found compared with the measured initial concentration of 1.4 μg mL^{-1}).

In-vitro release experiments

The in-vitro release of bupivacaine from the formulations is shown in Figure 2. The final concentration of bupivacaine in the release medium ranged between 7.5 and 12.2 μg mL^{-1}. The curves of A_{rel} versus time were mono-exponential for formulations A, B, D and F and bi-exponential for formulations C and E. The contributions of the rapid (high k_i) and slow (low k_i) phase, respectively, to the bi-exponential curves were 1.3% and 98.7% for formulation C and 2.5% and 97.5% for formulation E. There was a positive correlation between the proportion of glyceryl dilaurate in the formulation and the slow half-life of release of bupivacaine as illustrated in Figure 3. The calculated release-rate curves of bupivacaine are shown in Figure 4.

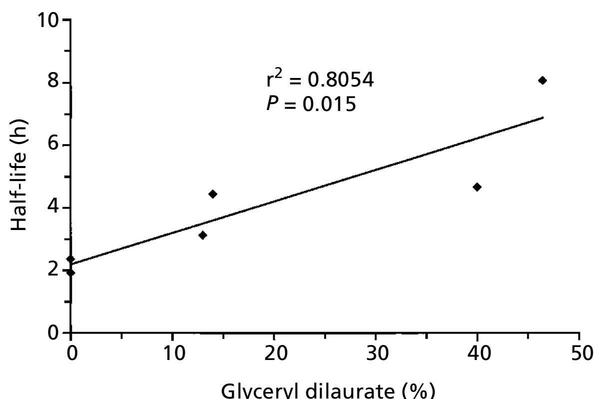

Figure 3 The half-life of release of bupivacaine in-vitro as a function of proportion of glyceryl dilaurate in the formulation.

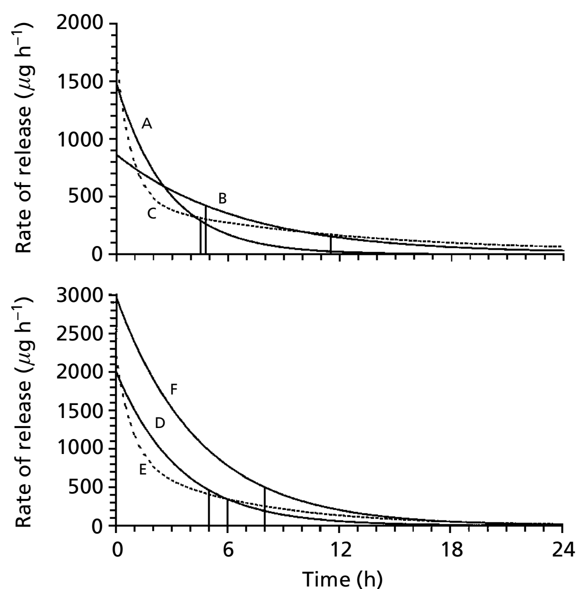

Figure 4 Calculated release profiles of bupivacaine from formulations A–C (top) and D–F (bottom). Please note the difference in y-axis ranges. The vertical lines indicate the median duration of sensory block in-vivo, in the order A, B, C (top) and D, E, F (bottom).

In-vivo studies – nerve block

Sciatic nerve blocks were successfully induced by all formulations. All rats showed signs of complete motor and sensor block within 20 min of termination of halothane administration. Bilateral testing of the pain withdrawal reflex before injection, as well as testing of the control side after injection, induced a brisk flexion response within 1–3 s after placing the paw on the hot plate. No prolongation of time to reflex withdrawal was observed on the control side during the course of individual experiments.

The duration of nerve blocks induced by bupivacaine base in lipid formulation, as compared with results

Table 2 Duration of sensory and motor nerve block induced by 0.1 mL of the bupivacaine–lipid formulations or by 0.1 mL of bupivacaine hydrochloride solution, 5 mg mL^{-1}, as well as body weights and number of rats in the groups.

Formulation	Duration of block		Body weight (g)	n
	Sensory block (h)	Motor block (h)		
Bupivacaine hydrochloride	2.0 (1.5–2.5)	1.8 (1.5–2.3)	287 (274–292)	6
A	4.5 (3.0–7.0)	4.0 (3.0–5.0)	288 (272–296)	6
B	4.8 (2.5–7.5)	4.0 (2.5–7.5)	282 (272–288)	6
C	11.5 (4.5–16.0)	11.0 (4.5–16.0)	280 (274–298)	6
D	5.0 (4.0–6.0)	5.0 (4.0–6.0)	284 (273–289)	5
E	6.0 (5.0–7.0)	6.5 (5.0–9.0)	282 (271–294)	6
F	8.0 (6.0–20.0)	9.0 (7.0–22.0)	282 (276–290)	6

Data are presented as median (and range).

obtained with bupivacaine hydrochloride solution, 5 mg mL^{-1}, are given in Table 2. All formulations of bupivacaine base in lipid vehicles showed prolonged duration of block compared with bupivacaine hydrochloride; sensory and motor blocks were of very similar duration. The overall difference between preparations (including bupivacaine hydrochloride) was significant ($P < 0.0001$ by the Kruskal–Wallis test) for both types of block. One rat was excluded from group D, because of possible nerve injury during the operating procedure. After 48 h of observation, the rat still showed both motor and sensory block.

In-vitro–in-vivo comparison

The in-vitro release rates of bupivacaine at the times corresponding to the offset of sensory block in the rat are shown in Table 3. The Kruskal–Wallis test of between-preparation variation gave $P = 0.06$, chiefly due to the lower values estimated for preparation C. The median value for all preparations, ignoring this trend, was 349 μg h^{-1} (interquartile range 286–462 μg h^{-1}).

Table 3 Release rates in-vitro at times corresponding to cessation of sensory block in-vivo.

Formulation	Release rates (μg h^{-1})
A	291 (118–499)
B	423 (281–592)
C	170 (115–315)
D	462 (344–619)
E	349 (298–410)
F	501 (35–781)

Data are presented as median (and range).

Discussion

Many different methods exist for testing drug dissolution or release in-vitro. The purpose of the test may be only to document the properties of a pharmaceutical formulation, and predicting pharmacokinetics or effects in-vivo may be diffi cult. As regards injectable drug formulations, one of the few successful attempts was reported by Gido et al (1994), who were able to predict plasma concentrations of doxepin in dogs after intramuscular injection of slow-release formulations by combining in-vitro release profiles with in-vivo disposition functions.

In preparation for the in-vivo study on doxepin formulations, Gido et al (1993) compared in-vitro release profiles obtained by two different methods. In one apparatus a dialysis membrane separated the formulation from the release medium and in the other the formulation was coated on glass beads and in direct contact with the medium. They concluded that release in the membrane system was slow and even incomplete in comparison to the non-membrane system, and they also pointed out that the membrane has no physiological equivalent after parenteral administration of formulation. The influence of the membrane is shown even more clearly by other studies. The release of bupivacaine from lipid–protein–sugar particles was evaluated by enclosing a suspension in an 8000 molecular weight cut-off dialysis bag and dialysing against PBS (Kohane et al 2000). However, when a plain bupivacaine solution was enclosed in the same way it took more than 5 h to obtain

100% release. In similar experiments by others (Hersh et al 1992; Grant et al 1994; Masters & Domb 1998), quantitative diffusion of bupivacaine from a plain solution took around 2–3 h. Thus, with this method even a saline solution to some extent appeared to behave as a slow-release preparation. Finally, Dyhre et al (2001) incorporated 20% lidocaine base in a lipid vehicle. This formulation was tested in a US Pharmacopoeia (23rd Edn) apparatus in which it was contained in a cell under a cellulose membrane with a cut-off at molecular weight 6000–8000. Only 50% of the lidocaine was released within 48 h. In comparison, sensory blockade in-vivo lasted for just 3.5 h.

Membranes can sometimes be avoided in flow-through devices, of which several types have been described (Langenbucher et al 1989; Wennergren et al 1989), provided that the preparation is somehow held in place. Gido et al (1993) studied the release of doxepin from lipid vehicles in a flow-through system, comparing phosphate buffer with and without addition of human plasma (1:3 volume ratio) as release media. In their apparatus, 20 glass globules were coated with formulation in various ways; in one case a hollow half sphere was filled with 2–3 additional drops of formulation. As the formulation was coated on glass globules, the surface area exposed to the release medium must have been very large in relation to the total volume of the formulation. In addition the flow of release medium was very slow, only 7 mL h^{-1}. Release rates were found to depend both on the method of sample application and on the nature of the medium. It is therefore not clear under what conditions the biologically most relevant release profile is obtained.

Both membranes and holding devices for the formulation can be avoided by the use of a single-drop technique. The strengths of this technique are that the entire surface of the formulation is exposed to the medium, that the diffusion area is relatively constant and that a drop, being approximately spherical during moderate movement relative to the surrounding medium, has a well defined volume–surface relationship. Mensing & Schügerl (1968) studied the release of radioisotope-labelled compounds from single drops suspended in a continuous, strictly laminar flow. To hold the drop stable, a flow-through cell with a large diameter in relation to the drop size had to be used. The apparatus was a technically advanced system of pump, flow meter, storage tanks and detectors. The total volume of release medium and the inner surface area of the apparatus must therefore have been considerable. This is a disadvantage if one wants to measure released compound in the medium itself, due to high dilution and risk of adsorption to the various surfaces. Another single-drop technique, that of drops falling or rising in an instrumented chromatography column, has been used successfully for the calculation of mass transfer across aqueous–organic interfaces (Brodin & Ågren 1971; Nahringbauer & Larsson 1983). However, this technique permits contact between drop and medium only for some seconds and is therefore not suitable for the much longer release experiments.

Consequently, a new release apparatus was designed. As the flow-through cell is placed directly in the storage vessel, no pump, tubing or external tank is needed. This minimises the total surface area of the apparatus and thus the potential for adsorption of drug. Experimentally, no adsorption could be found at the low bupivacaine concentration of 1.4 μg mL^{-1}. To maintain sink conditions, the maximum concentration of bupivacaine base in the release medium must never exceed 10% of its total solubility, and this can be assured by the choice of a bottle of suitable size. The concentration of bupivacaine during the release experiments never exceeded 3.7% of the separately determined solubility.

As for the tested formulations, all except formulation A are heterogeneous systems (i.e. suspensions) since both bupivacaine and glyceryl dilaurate are only partially soluble in the triglyceride. Heterogenous systems can be expected to produce release profiles with several exponential phases. This was, however, observed only for formulations C and E, and the contributions of the rapid phases were quite small. It thus seems pertinent to discuss only the slow phases of the curves. For these, a positive correlation between half-life of release and percentage of glyceryl dilaurate was found. Increasing the concentration of glyceryl dilaurate resulted in increased viscosity of the formulation. The partitioning of bupivacaine between solidified glyceryl dilaurate and the fluid phase in these formulations was not possible to investigate. Bupivacaine may be incorporated into suspended glyceryl dilaurate that acts as a coating and therefore contributes to the decrease in rate of release.

Comparison of in-vitro and in-vivo findings suggests that to maintain nerve blockade in-vivo, the formulations must release bupivacaine at a rate of approximately 350 μg h^{-1} under the in-vitro conditions. The inter-individual variance shown in Table 3 can be explained both by differences in clearance of bupivacaine from the injection site and in the final shape and proximity to the nerve of the injected formulation. Despite this expected biological variation, the numbers may serve as a guide in the development of further formulations. Only those that show such rates of release of bupivacaine over a suitable time would be candidates for testing in-vivo.

Conclusion

We designed and tested a novel apparatus for measuring release of a local anaesthetic (or other drug) from a fluid or semi-solid formulation in-vitro. Release rates obtained in-vitro by means of this technique may be used to guide the development of formulations with suitable durations of action in-vivo. The apparatus is, however, as yet a prototype. Rigorous evaluation of performance should be carried out on devices built to specific standards according to their intended application.

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