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Ultralong Peripheral Nerve Block by Lidocaine:Prilocaine 1:1 Mixture in a Lipid Depot Formulation

Comparison of In Vitro, In Vivo, *and Effect Kinetics*

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Background: **The aim of this study was to develop stable and easily injectable lipid depot preparations of local anesthetics in which the drug concentration can be varied according to desired duration of action.**

Methods: **The formulations contained a 2.0, 5.0, 10, 20, 40, 60, 80, or 100% eutectic mixture of lidocaine and prilocaine base in medium-chain triglyceride. Duration of sciatic nerve block and local neurotoxicity was investigated in rats with 2.0% lidocaine: prilocaine HCl solution and 99.5% ethanol as controls. The rate of release of local anesthetic from the site of administration and the possibility to predict *in vivo* depot characteristics from *in vitro* release data were investigated for the 20 and 60% formulations.**

Results: **The duration of sensory sciatic block was prolonged 3 times with the 20% formulation and approximately 180 times with the 60% formulation, in comparison with the 2% aqueous solution. With the 80 and 100% formulations, all animals still showed nerve block after 2 weeks. The *in vivo* release of local anesthetic could be approximately predicted from *in vitro* data for the 20% but not for the 60% formulation. The formulations of 60% or greater and ethanol showed neurotoxic effects.**

Conclusions: **The pharmaceutical properties of these formulations compare favorably with those of other depot preparations. The high-percentage ones showed the longest duration of action yet reported for sciatic nerve block in rats. The possibility of using a high-concentration local anesthetic depot formulation as an alternative to ethanol or phenol for long-term nerve blocks in chronic pain merits further investigation.**

PERIPHERAL nerve blockade with a local anesthetic provides excellent pain relief, but its clinical utility for the treatment of acute or postoperative pain is sometimes limited by a short duration of effect. In cancer pain, a more or less irreversible nerve blockade may be desirable. It is therefore of great clinical interest to develop a local anesthetic that would be effective for days or even weeks instead of hours.[1–3] Accordingly, a huge amount of work has been put into preclinical development of depot formulations of local anesthetics.[4–28] These 25 references deal with peripheral nerve blockade evaluated in animal models and exclude investigations on spinal or topical analgesia. Despite at least two decades of published work, only small phase I studies in humans and no full-scale clinical trials have as yet been reported.[3,29,30]

The apparent problems in bringing these preparations to clinical trials may be due to their various shortcomings. The most common formulation approach seems to be inclusion of local anesthetic into liposomes or lipospheres.[6–8,11,14,17,18,21,24–27,30] However, it seems that only low concentrations of local anesthetic, typically 0.5–2% or 10–12% at the outmost,[18,27] can be included in these preparations. In addition, their physical stability may be poor and their shelf life very short. Other common depot formulations are microspheres or microparticles loaded with local anesthetic.[4,5,12,15,16,19,20,28,29,31–33] These may contain high percentages of active drug and show very prolonged release. However, they must be injected as a suspension, and residues of the matrix may remain at the injection site long after the dissipation of effect. The latter problem may also be encountered with liposome[14] or hydrogel[13] formulations. Polymer matrix pellets[9,10] can be prepared with a high concentration of local anesthetic but must be surgically implanted. Finally, prolonged release of local anesthetics can be obtained from solutions or suspensions in natural lipids.[22,23,34] These preparations may, however, have high viscosity or other properties that make filling of syringes and later injection difficult or impossible. In addition, the solubility of local anesthetic in the lipid may be low, *e.g.*, around 40 mg/ml of bupivacaine in a medium-chain triglyceride.[23]

The aim of this study was to develop a physically stable and easily injectable depot preparation of local anesthetics in which the concentration of active compound(s) can be varied between 0 and 100%. This, in turn, would permit modulation of the duration of effect to achieve a good balance between effect and risk of systemic toxicity. The already well-known eutectic mixture of local anesthetics (EMLA; developed at AstraZeneca Inc., Södertälje, Sweden), which consists of a 1:1 mixture of lidocaine and prilocaine base,[35,36] proved to be freely soluble in lipid vehicle to give one-phase formulations with low viscosity at any concentration. Therefore, preparations containing from 2 to 100% of this mixture were

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screened for duration of sciatic nerve block in rats. In addition, local neurotoxicity was evaluated by light microscopy examination of the sciatic nerve 2 weeks after administration of the preparations. Two of the lipid formulations were chosen for further study on the putative relations between drug release rates *in vitro* and *in vivo* and between *in vivo* drug concentration and duration of sensory block.

Materials and Methods

Materials

Prilocaine base was produced by Synthelec AB (Lund, Sweden). Lidocaine base and medium-chain triglyceride (MCT) were of European Pharmacopoeia quality and supplied by Apoteket AB (Stockholm, Sweden). According to specifications in the Pharmacopoeia, the fatty acid moieties of MCT are 2% or less caproic, 50–80% caprylic, 20–50% capric, 3% or less lauric, and 1% or less myristic acid. Prilocaine and lidocaine hydrochlorides (HCl) were obtained from AstraZeneca Inc. Chemicals for chromatographic analysis and for the preparation of phosphate-buffered saline solution were of analytical grade.

Preparations

Six lipid formulations were prepared, containing 2.0, 5.0, 20, 40, 60, or 80% (wt:wt) of lidocaine:prilocaine (1:1, by weight) in MCT. They were made by mixing the components in glass vials and equilibrated with gentle agitation for at least 12 h at room temperature (21°C). In addition, pure vehicle (MCT) and 100% lidocaine:prilocaine were prepared for *in vivo* testing. All these formulations were oily liquids at room temperature, which could be filled into a 1-ml syringe and injected through a 29-gauge needle. In addition, saline solutions of lidocaine:prilocaine HCl were prepared at 0.40 and 2.0% strength (corresponding to 2.0 + 2.0 and 10 + 10 mg/ml of respective base). Furthermore, sterile 99.5% ethanol was used as an active control in one experiment.

Animals

The experimental protocol was approved by the Ethics Committee on Animal Experimentation at Lund University, with the condition that the experiments be terminated by 72 h. Adult male Sprague-Dawley rats (Møllegaard Breeding and Research Centre, Ejby, Denmark) were kept four to a cage with food and water available *ad libitum*. They were maintained on a 12-h light–dark cycle in a temperature-controlled environment and were allowed a 4-day habituation period before the experiments.

Sciatic Nerve Block

Duration of nerve block after administration of a total of 10 preparations was investigated in three separate randomized experiments. The first session formed a "proof-of-concept" study in which first 18 rats (weight, 286–336 g) were randomly assigned to treatment with lipid vehicle (MCT), 2% lidocaine:prilocaine HCl, or 2% lidocaine:prilocaine in MCT, and then (on the same day) another 18 rats (290–316 g) were assigned to treatment with 5 or 20% lidocaine:prilocaine in MCT or with 100% lidocaine:prilocaine. The second session was a concentration–duration study in which 24 rats (288–338 g) were treated with 20, 40, 60, or 80% lidocaine:prilocaine in MCT. Separate approval for a third session was then obtained from the Ethics Committee, with permission to continue the study for 14 days. This session was also a randomized study in which 24 rats (246–278 g) were subjected to nerve blocks with 60 or 80% lidocaine:prilocaine in MCT, 100% lidocaine:prilocaine, or 99.5% ethanol.

The syringes containing the preparations were randomized and labeled (see Statistical Analysis section) by S. B., who did not otherwise take part in these experiments. The experimenters (L. S. and H. D.) were blind in respect to the lidocaine:prilocaine formulations given (these were not physically distinguishable), whereas injection of aqueous solution or ethanol could not be effectively blinded in comparison with the other preparations.

The procedures have been described previously.[10,22,23] The animals were anesthetized briefly with halothane, 2–3% in oxygen, by facemask. The sciatic nerve on one side was surgically exposed. Under magnification, 0.10 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. A small hotplate, thermostat-adjusted to 52° ± 1°C, was used to test the sensory block by the thermal nocifensive response. During restraint of the rat by hand grip, the blocked leg and the opposite leg were tested alternately in duplicate. Because the block did not comprise the motor nerves to the hip muscles, all animals were able to withdraw the tested paw in response to pain. A cutoff time of 10 s was applied to avoid tissue damage. Full sensory block was defined as no withdrawal reaction within these 10 s, whereas partial block was defined as a withdrawal latency of 4–10 s. The duration of sensory block is given as the time from injection of the test preparation to the last occurrence of full and partial block, respectively. Full motor block was defined as no dorsiflexion ability, the rat walking with curled toes, whereas partial block was defined as normal dorsiflexion but inability to spread the toes. The duration of motor block is given as the time from injection to the last

observation of full and partial block, respectively. The animals were monitored for self-mutilation (autotomy)[37] of the anesthetized paw.

In sessions 1 and 2, the rats were tested for full sensory and partial motor block starting 10 min after drug administration and then every 10 min until 20 min after dissipation of effects. In those cases where nerve blocks lasted for more than 5 h, observation was continued as appropriate, with the aim that the intervals should not exceed 10% of the total time elapsed after drug administration. Maximum allowed time was 72 h. In session 3, after initial observation for up to 6 h, the test intervals (starting from the time of administration) were every 8 h for 3 days, every 12 h for another week, and then every day until termination at 14 days.

Based on the outcome of the nerve block experiments (as detailed in the Results section, Duration of Local Anesthetic Action), two formulations underwent further studies. These were 20 and 60% lidocaine:prilocaine in MCT, *i.e.*, the lowest concentration formulation to give a clear depot effect and the highest concentration formulation for which the median duration of full sensory block could be determined.

In Vitro *Release*

In vitro release of lidocaine and prilocaine was determined by means of the previously described single-drop technique.[23] In brief, a 0.10-ml sample of formulation was suspended as a free drop in the rotating downward flow of release medium (phosphate-buffered saline solution) in the glass tube of the release apparatus. Samples of medium, 2.5 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 times varied between 25 and 28 h. Release studies should be performed under "sink conditions," meaning in established practice that the concentration of drug in the release medium should never exceed one tenth of the saturation concentration (*i.e.*, solubility) of the drug. Therefore, the solubility of lidocaine and prilocaine together in the release medium was investigated by preparation of a 0.275 + 0.275-mg/ml solution (corresponding to 10 times the final concentration in the 60% lidocaine:prilocaine experiment). Two separate solutions were prepared, kept at 37°C, and examined visually at 24 and 48 h and by microscopy at 48 h.

Lidocaine and prilocaine concentrations in the release medium were determined by high-performance liquid chromatography. The mobile phase consisted of acetonitrile–0.05 M phosphate buffer, pH 7.4 (1:1 v/v). One hundred microliters of sample (diluted as needed in mobile phase) was direct injected on a LiChrosorb RP-18 250 × 4.0-mm, 7-μm particle size column (ChromTech AB, Hägersten, Sweden). Column flow was 1.0 ml/min delivered by a Spectra Series P100 high-performance liquid chromatography pump (Thermoquest, San Jose, CA), and lidocaine and prilocaine were detected at 230 nm using a Spectra Series UV 100 spectrometric detector (Thermoquest). The within-day coefficients of variation were 3.2% (lidocaine) and 2.1% (prilocaine) at 2 μg/ml and 2.5% (lidocaine) and 2.1% (prilocaine) at 25 μg/ml (n = 12 in all cases).

Duplicate experiments were performed so that two concentration curves each of lidocaine and prilocaine in release medium were obtained for each formulation. The amount (A_{rel}) of lidocaine and prilocaine released at each sampling time was calculated as previously described,[23] taking into account the accumulated loss by sampling. Because the amount 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. Monoexponential and biexponential release functions were then fitted to the A_{rel}-*versus*-time data by means of SCIENTIST software (MicroMath, Salt Lake City, UT):

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

In this expression, A_{final} is the final amount of lidocaine or prilocaine released after equilibration between the formulation and the medium, n is 1 or 2, A_i is the preexponential 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 monoexponential and a biexponential 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 biexponential function, the calculated confidence interval of A_2 often included zero.

Pharmacokinetic Studies

A total of 20 rats (weight, 268–344 g) were randomly divided into four groups (see Statistical Analysis section). Under brief halothane anesthesia, PE 50 catheters (dead space 0.1 ml) were inserted into a common carotid artery and tunneled subcutaneously. The first group of rats also had a PE 50 catheter inserted into the internal jugular vein. This group (n = 6) received 10-min (1.5-ml) intravenous infusions of 2.0 + 2.0 mg/ml lidocaine:prilocaine HCl by means of a syringe pump (Sage Instrument M.351; Orion, Houston, TX). The other three groups received 0.10 ml of 2% lidocaine:prilocaine HCl (n = 4) or 20% (n = 4) or 60% (n = 6) lidocaine:prilocaine in MCT by the technique described above for sciatic nerve block. Blood samples were obtained from the arterial catheter at 2, 5, 9, 12, and 15 min after the start of the intravenous infusion; at 2, 5, and 10 min after injection of 2% lidocaine:prilocaine HCl; or at 5 and 10 min after administration of the lipid formulations. Thereafter, samples were taken from all animals at 20, 30, 45, 60, 90, 120, and 180 min and then as appropriate for up to

4 (or 4.5) h after the intravenous infusion, 3.5 h after 2% lidocaine:prilocaine HCl, and 24–32 h after the lipid formulations. A blood volume of 0.2 ml was first withdrawn, which, after the sampling, was reinjected followed by 0.2–0.4 ml heparin, 0.5 U/ml in saline. Total sampling volumes up to and including the penultimate sample were 2.8–2.9 ml. After the last sample, 0.5 ml, the animal was immediately killed by carbon dioxide asphyxiation.

Lidocaine and prilocaine blood concentrations were determined by gas–liquid chromatography by a modification of a previously described method.[22] In brief, blank, hemolyzed (frozen and thawed) human blood, 0.2 ml, was added to 0.05–0.50 ml of the hemolyzed rat blood sample, and etidocaine was added as an internal standard. The sample was then alkalinized with 200 μl NaOH solution, 0.5 M, and extracted with 5 ml n-hexane containing 0.08% triethylamine. The mixture was centrifuged for 10 min at 1,900*g*. The organic phase was collected and, after addition of 25 μl acetic acid, 0.1 M, in diethyl ether, evaporated to dryness. The residue was dissolved in 15 μl ethanol, and 1 μl was injected on a gas chromatograph (Varian CP 3800; Palo Alto, CA) equipped with a nitrogen selective detector. The column was packed with OV-17, and the oven temperature was 245°C. The within-day coefficients of variation at 20, 200, 1,000 and 10,000 ng/ml were 4.8, 9.8, 6.6, and 13% (lidocaine) and 5.5, 8.4, 5.0, and 11% (prilocaine), respectively (n = 8 in all cases).

Compartmental models were fitted to the arterial blood concentration data by means of NONMEM version V (NONMEM Project Group, San Francisco, CA). Two- and three-compartment models were evaluated in the case of intravenous infusion, and one- and two-compartment models were evaluated for the other treatments. For each group of rats, the pharmacokinetic parameters were estimated in the population mode, and individual estimates were obtained using the POSTHOC option. The fits were evaluated by means of the objective function value and by inspection of the residuals. The fitted parameters were the volume of distribution (V) of the central compartment and all intercompartmental ("micro") rate constants as well as those of absorption (k_a) and elimination (k). Secondary parameters, *i.e.*, area under the curve, clearance, volume of distribution at steady state, and mean residence time, were calculated by standard equations.[38] In addition, the unit disposition functions (UDFs) of lidocaine and prilocaine were calculated from the fitted functions after intravenous infusions. The UDF describes the disposition of 1 dose unit (in this case 1 mg) of drug after instantaneous input into the circulation. It thus has the form of an ordinary biexponential or triexponential blood concentration–*versus*–time function.

The rates of release of lidocaine and prilocaine from the injection site into the circulation after administration of 2% lidocaine:prilocaine HCl or the two lipid formulations were estimated by numerical deconvolution.[39,40] The response (or output) functions were the blood concentration data after administration of the preparations and the weighting (or disposition) functions were the UDFs calculated from the intravenous infusion data. The MS DOS–based software[40] was provided by Davide Verotta, Ph.D. (Associate Professor, Department of Biopharmaceutical Sciences, University of California, San Francisco, California).

In addition, blood concentration curves of lidocaine and prilocaine after administration of the two lipid formulations were predicted by convolution.[41] The MS DOS–based software was provided by Peter Veng-Pedersen, Ph.D. (Professor, Division of Pharmaceutics, College of Pharmacy, The University of Iowa, Iowa City, Iowa). The input functions were the fitted exponential functions from the *in vitro* experiments. The weighting functions were the fitted exponential functions (population mean) from the administration of the 2% lidocaine:prilocaine HCl preparation. The predicted curves were compared to the actual blood concentrations found in the *in vivo* experiments.

Histopathology

In a final group of rats (weight, 282–336 g), local toxicity was investigated after administration of various preparations as described under Sciatic Nerve Block. Nerve blocks were thus induced with 2.0, 10, 20, 60, or 80% lidocaine:prilocaine in MCT and also with the control physiologic saline solution, pure MCT, 2.0% lidocaine:prilocaine HCl in saline solution, or 99.5% ethanol (0.10 ml and n = 5 for all preparations). After 14 days, all treated sciatic nerves, plus five from untreated hind legs, were dissected out. They were placed on small strips of cardboard and fixed in buffered (pH 7.4) 4% formaldehyde solution. Each nerve specimen was given a number according to a table of randomization, and they were all sent in this blinded form to Scantox Inc. (Ejby, Denmark) for processing and evaluation.

The nerve samples were investigated by light microscopy for axonal swelling and neuronal degeneration after staining with hematoxylin and eosin as well as for demyelization and myelin degeneration after Klüwer staining. Inflammation was evaluated by characterization of leukocyte infiltration. Pathologic changes were graded as 0 = not observable, 1 = minimal, 2 = slight, 3 = moderate, and 4 = marked.

Statistical Analysis

The treatments in the sciatic nerve block experiments were randomized in blocks of three (session 1) or four (sessions 2 and 3), and group sizes were always n = 6. The prefilled syringes were labeled with consecutive numbers, which after the administration were transferred to the rats. A similar block randomization was used in the pharmacokinetic study, which was performed in four separate sessions.

Observed differences in duration of effect were tested for statistical significance by the one-tailed Mann–Whit-

Table 1. Duration of Sensory and Motor Nerve Block Induced by 0.10 ml of the Respective Preparations in Three Experimental Sessions

	Duration of:	
Preparation	Full sensory block	Partial motor block
Session 1		
MCT vehicle	None	None
2% L:P HCl solution	30 (20, 48) min	55 (43, 60) min
2% L:P in MCT	20 (0, 30) min	50 (0, 50) min
5% L:P in MCT	30 (20, 48) min	45 (33, 65) min
20% L:P in MCT	90 (70, 100) min	100 (70, 130) min
100% L:P	72 (66, 72) h*	72 (72, 72) h*
Session 2		
20% L:P in MCT	80 (70, 100) min	100 (80, 130) min
40% L:P in MCT	150 (123, 223) min	44 (11, 59) h
60% L:P in MCT	65 (22, 72) h*	72 (28, 72) h*
80% L:P in MCT	72 (64, 72) h*	72 (72, 72) h*
Session 3		
60% L:P in MCT	89 (28, 224) h	336 (336, 336) h*
80% L:P in MCT	≥326 (227, 336) h*	336 (336, 336) h*
100% L:P	291 (142, 331) h	336 (336, 336) h*
99.5% Ethanol	133 (23, 183) h	336 (336, 336) h*

Data are presented as median (25th, 75th percentiles).

* 72 h (sessions 1 and 2) and 336 h (14 days; session 3) = censored values corresponding to the end of the experiment.

L:P = lidocaine:prilocaine 1:1 mixture; MCT = medium-chain triglyceride.

ney U test for unpaired data. However, many differences were obvious without statistical tests.

Results

Duration of Local Anesthetic Action

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 hotplate. No prolongation of time to reflex withdrawal was observed on the control side during the course of individual experiments. Full sensory and motor blocks were apparent at the first observation time (10 min) in all animals that had received preparations containing local anesthetics. The durations of full sensory and partial motor block, respectively, after administration of the various preparations are shown in table 1. In session 1, the 2 and 5% lidocaine:prilocaine in MCT formulations did not differ significantly in duration from the aqueous solution. The 20% lidocaine:prilocaine in MCT formulation showed a clearly prolonged effect ($P < 0.01$ for sensory block), whereas that of 100% lidocaine:prilocaine in most animals lasted for the whole 72-h period. There was also very good reproducibility in the repeated testing of 20% lidocaine:prilocaine in MCT in sessions 1 and 2. In the latter experiment, there was a clear and marked increase in duration of sensory block between the 40 and 60% formulations. After dissipation of the local anesthetic effects, all animals seemed to behave normally.

The long duration of action of 60, 80, and 100% lidocaine:prilocaine was confirmed in session 3. At 14 days, one rat in the 60% group, three in the 80% group, and two in the 100% group still had full sensory blocks. As can be seen in figure 1, the effects generally dissipated in the order full motor block—full sensory block—partial sensory block—partial motor block. The full sensory block due to 80% lidocaine:prilocaine in MCT was significantly longer than that elicited by the 60% formulation or by 99.5% ethanol ($P < 0.05$ in both compari-

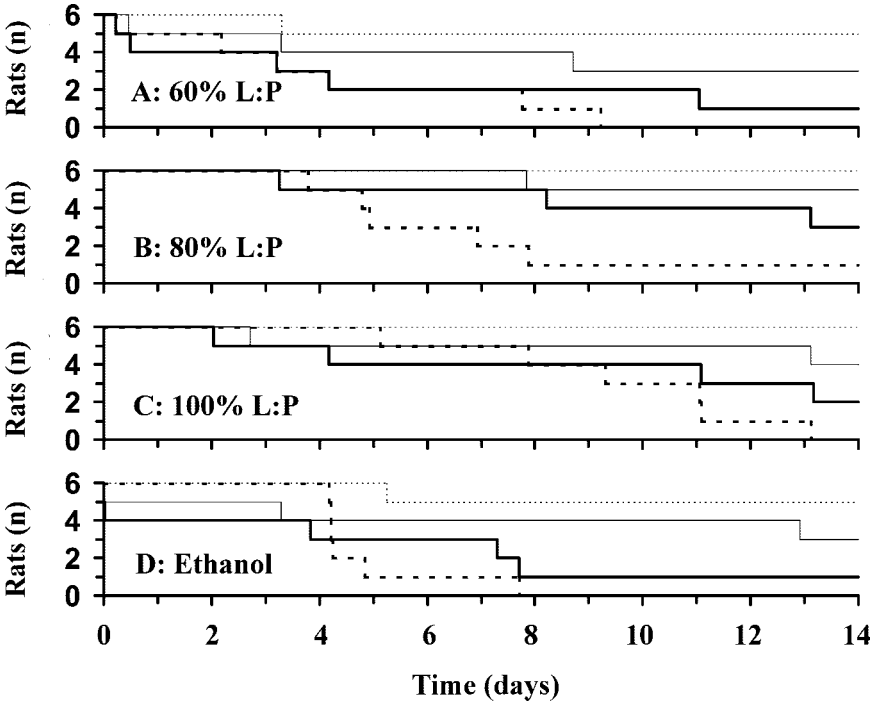

Fig. 1. Number of rats, as a function of time, showing sensory and motor blocks after treatment with the 60, 80, or 100% lidocaine:prilocaine (L:P) lipid formulation or with 99.5% ethanol, respectively (*A–D*). *Bold solid lines* = full sensory block; *thin solid lines* = partial sensory block; *bold dashed lines* = full motor block; *thin dashed lines* = partial motor block.

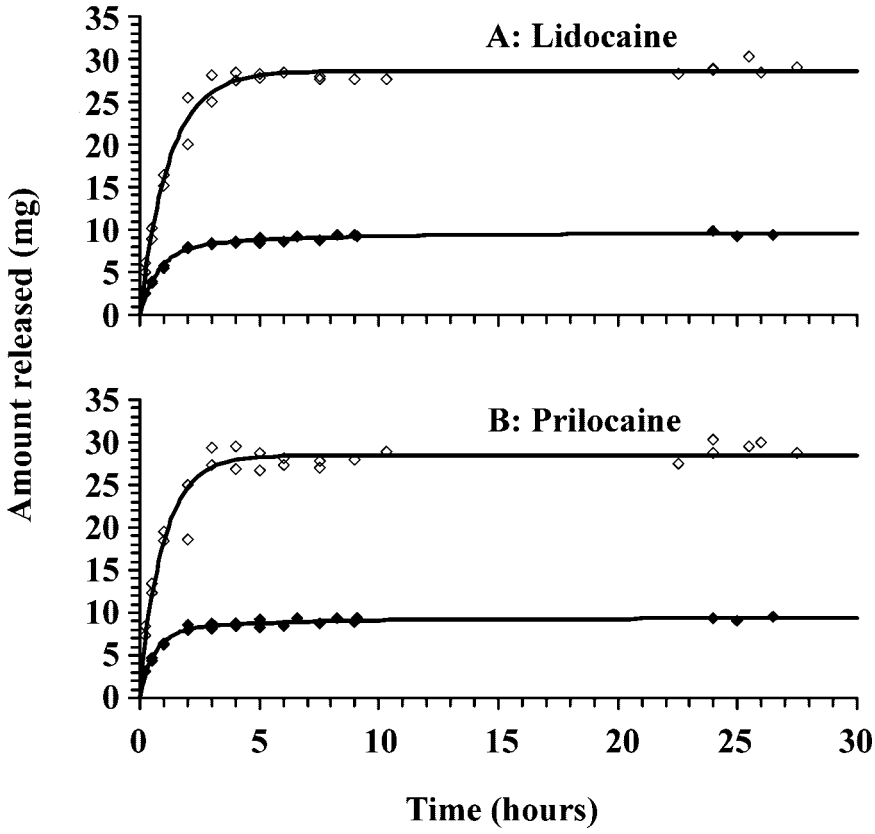

Fig. 2. Cumulative *in vitro* release profiles of lidocaine (*A*) and prilocaine (*B*) from the 20% (*closed symbols*) and 60% (*open symbols*) lipid formulations.

sons). The effect of ethanol was variable. In two animals, only very transient full sensory blocks (< 1 h) were obtained, whereas one rat still had a complete block at 14 days. All animals, however, had full motor blocks for at least 4 days. Despite the long local anesthesia, there were no signs of self-mutilation in any group.

In Vitro *Release Profiles*

In vitro release profiles are shown in figure 2. The release profiles of both lidocaine and prilocaine from the 20% formulation were biexponential. The fitted half-lives of release were 32 min and 4.0 h (lidocaine) and 25 min and 3.3 h (prilocaine). The 60% formulation, on the other hand, disintegrated during the experiment because the release of the local anesthetics entailed loss of 60% of the mass of the formulation. The fitted curves were monoexponential, with half-lives of "release" of 51 min (lidocaine) and 43 min (prilocaine), respectively. The concentrations of lidocaine and prilocaine in the release medium never exceeded 27 + 27 μg/ml. No precipitation could be observed in the 10-times-more-concentrated solutions that were prepared for comparison. Sink conditions were consequently maintained.

Blood Concentrations and Pharmacokinetics of Lidocaine and Prilocaine

The concentration curves of lidocaine and prilocaine after intravenous infusion and administration of 2% lidocaine:prilocaine HCl are shown in figure 3. Lidocaine and prilocaine were detectable for 2.5–4.5 h after the intravenous infusion and for 3.5 h after nerve block with the 2% solution. After intravenous administration, a three-compartment model was needed to describe the disposition of lidocaine, whereas a two-compartment model was adequate for prilocaine. Pharmacokinetic parameter values calculated from the intravenous data are given in tables 2 and 3. For the nerve block administration of 2% lidocaine: prilocaine HCl, one-compartment models applied, and parameter values are shown in table 3.

The concentration curves of lidocaine and prilocaine after administration of the lipid formulations are shown in figure 4. Also in these experiments, the two local anesthetics were detectable in the circulation over the whole sampling periods. The paucity of data at the late sampling times was due to problems with the catheters. Although the concentration data for the 20% formulation may suggest two-compartment pharmacokinetics, the objective function values favored one-compartment models. The C_{max}, T_{max}, and apparent terminal $t_{½}$ of the two drugs are shown in table 3. The mean terminal half-lives were fourfold to ninefold longer than after intravenous or extravascular administration of aqueous solution and obviously reflect slow release into the circulation from the site of administration. Dose-adjusted area-under-the-curve values were not significantly different between the treatment groups, except that the cal-

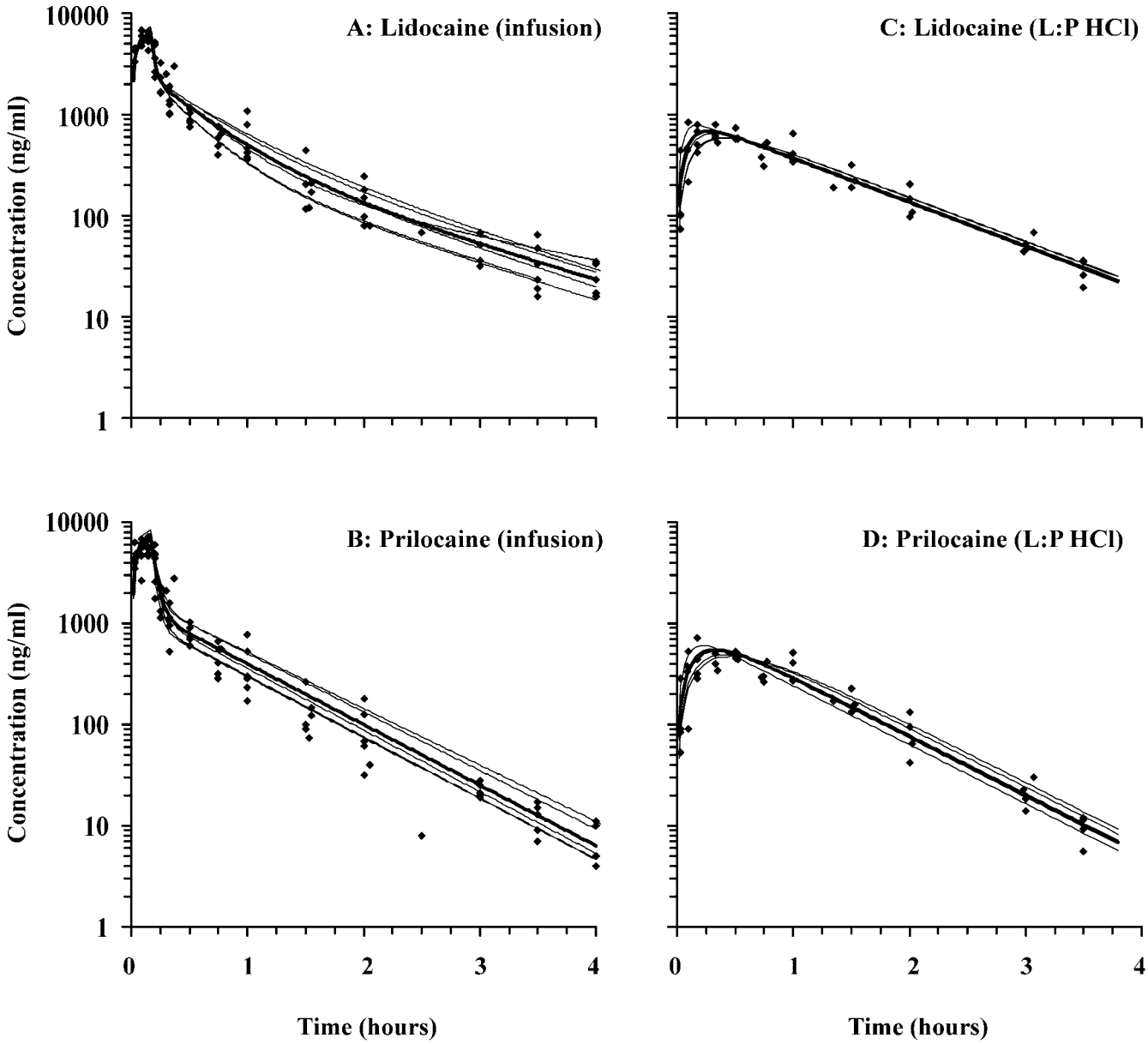

Fig. 3. Arterial blood concentration curves of lidocaine and prilocaine after intravenous infusion of the mixture to six rats (*A* and *B*) and after peripheral nerve block with the 2% lidocaine:prilocaine (L:P) HCl solution in four rats (*C* and *D*). *Bold curves* = population mean fit; *thin curves* = disposition according to POSTHOC individual estimates.

culated value for lidocaine unaccountably was higher ($P < 0.01$) after 2% lidocaine:prilocaine HCl than after intravenous infusion.

In Vivo *Release from the Site of Administration*

In vivo release profiles of lidocaine and prilocaine obtained by deconvolution of the population mean curves are shown in figure 5. Calculations for the individual rats gave the estimated times for 50% release from the injection site given in table 3. There was a clear difference between the aqueous solution and the lipid formulations. Time for 50% release was less than one elimination $t_{½}$ after administration of the aqueous solution. As regards the lipid formulations, there was good agreement between calculated times for 50% release and the fitted terminal half-lives of the local anesthetics.

Prediction of In Vivo *from* In Vitro *Release Functions*

The concentration curves of lidocaine and prilocaine predicted by convolution of the *in vitro* profile with the *in vivo* weighting function are also shown in figure 4. Prediction of *in vivo* from *in vitro* release profiles was reasonably successful for the 20% formulation, although early blood concentrations were overpredicted and late concentrations underpredicted. The biexponential *in vitro* release profile was not reproduced *in vivo*. Instead, the *in vivo* half-lives of release corresponded well

Table 2. Basic Pharmacokinetic Parameters, and Zero-time Intercepts and Hybrid Rate Constants of the Unit Disposition Function, of Lidocaine and Prilocaine after Simultaneous Intravenous Administration to Six Rats

Parameter	Lidocaine	Prilocaine
Clearance, ml/min	23 (21–24)	26 (22–31)
V_{dss}, l	0.73 (0.56–0.83)	0.60 (0.47–0.69)
MRT, min	27 (19–33)	18 (14–24)
UDF		
A, ng/ml	8,287 (8,225–8,353)	7,119 (6,039–8,820)
B, ng/ml	724 (598–837)	457 (363–550)
C, ng/ml	163 (88–257)	—
α, min^{-1}	0.61 (0.47–0.69)	0.37 (0.33–0.46)
β, min^{-1}	0.040 (0.035–0.051)	0.023 (0.021–0.023)
γ, min^{-1}	0.013 (0.009–0.014)	—

Population mean values and ranges of individual estimates are shown.

α, β, and γ = hybrid rate constants of the UDF; A, B, and C = zero-time intercepts of the UDF; MRT = mean residence time; UDF = unit disposition function; V_{dss} = volume of distribution at steady state.

to only the terminal *in vitro* half-lives (lidocaine 3.6 *vs.* 4.0 h and prilocaine 3.2 *versus* 3.3 h, respectively). In contrast, the *in vitro* $t_{½}$ of "release" (or rather disintegration) of the 60% formulation did not predict the *in vivo* characteristics.

Histopathology

The histopathologic findings are summarized in table 4. Moderate to marked neurotoxicity was elicited by the 60 and 80% formulations and (in one animal) by 99.5% ethanol. The toxicity was apparent as axonal swelling and neuronal degeneration accompanied by demyelization and myelin degeneration. In addition, diffuse inflammation affecting both the epineural tissue and the neuronal tissue could be observed, again with moderate to marked severity only after treatment with long-acting preparations. The infiltrating cells were mainly macrophages, lymphocytes, fibroblasts/fibrocytes, and, in most of the grade 4 specimens, occasional giant cells.

Discussion

The goal to develop an easily injectable formulation in which the concentration of local anesthetics can be varied at will was attained by using the lidocaine:prilocaine eutectic mixture as the active component (in contrast, the solubility of pure lidocaine and prilocaine base in MCT was < 30% wt:wt for both drugs). The formulations are physically stable at room temperature and can be frozen and thawed without phase separation. Chromatographic assay of a 20 and a 60% formulation that had stood for 1 yr at room temperature indicated a decrease of 5% or less in the concentrations of local anesthetics. In addition, the preparations can be drawn into a syringe and then injected through a 29-gauge needle (which is thinner than the needles that would be used clinically). The lidocaine:prilocaine mixture has previously been incorporated, at 5% concentration, in a polyethylene oxide–polypropylene oxide–polyethylene oxide block copolymer.[42] This preparation (Oraqix; Dentsply Ltd., Weybridge, England) is intended for application in periodontal pockets, gives rapid release of local anesthetics, and shows a 15- to 20-min effect duration.[43] There is consequently not much similarity between this and our preparations.

The effect of 20% lidocaine:prilocaine in MCT was prolonged approximately three times in comparison with the 2% aqueous HCl solution. The 2% solution corresponds to the most concentrated lidocaine solution for peripheral nerve blocks in clinical use. The mean C_{max} values of the local anesthetics in blood were only approximately twice as high after the 20% formulation despite the 10-fold higher dose. The terminal half-lives and the calculated times for 50% release into the circulation attested to the depot characteristics of this formulation. The findings are similar to results obtained in the same animal model when 20% lidocaine base in a polar lipid formulation was compared with 2% lidocaine HCl

Table 3. Pharmacokinetic Parameter Values Relevant for Comparison of the Preparations

Preparation	Intravenous Infusion	2% L:P HCl Solution	20% L:P in MCT	60% L:P in MCT
Lidocaine				
Dose, mg	3.1 (2.9–3.1)	1.0 (1.0–1.0)	10 (10–10)	30 (30–30)
AUC/dose, h/l	0.74 (0.69–0.80)	0.89 (0.89–0.92)	0.75 (0.55–1.18)	0.68 (0.45–0.94)
C_{max}, ng/ml	6,480 (6,037–7,357)	680 (585–775)	1,301 (924–1,968)	2,550 (1,707–3,386)
T_{max}, min	10 (10–10)	16 (10–25)	35 (16–67)	42 (38–46)
Terminal $t_{½}$, min	53 (49–80)	42 (42–42)	213 (171–232)	304 (271–341)
50% release, min	NA	21 (15–36)	161 (96–142)	231 (123–301)
Prilocaine				
Dose, mg	3.1 (2.9–3.1)	1.0 (1.0–1.0)	10 (10–10)	30 (30–30)
AUC/dose, h/l	0.65 (0.53–0.76)	0.63 (0.60–0.65)	0.67 (0.53–1.01)	0.53 (0.33–0.78)
C_{max}, ng/ml	7,035 (6,549–8,389)	546 (471–599)	1,330 (1,111–2,088)	2,301 (1,553–3,622)
T_{max}, min	10 (10–10)	20 (13–28)	30 (13–50)	32 (30–32)
Terminal $t_{½}$, min	30 (30–32)	31 (31–31)	189 (147–186)	265 (205–261)
50% release, min	NA	21 (18–31)	147 (63–98)	206 (101–222)

Population mean values and ranges of individual estimates are shown.

50% release = time for 50% release from the site of administration into the blood circulation; AUC = area under the curve; C_{max} = maximal blood concentration; L:P = lidocaine:prilocaine 1:1 mixture; MCT = medium-chain triglyceride; NA = not applicable; $t_{½}$ = half-life; T_{max} = time of maximal blood concentration.

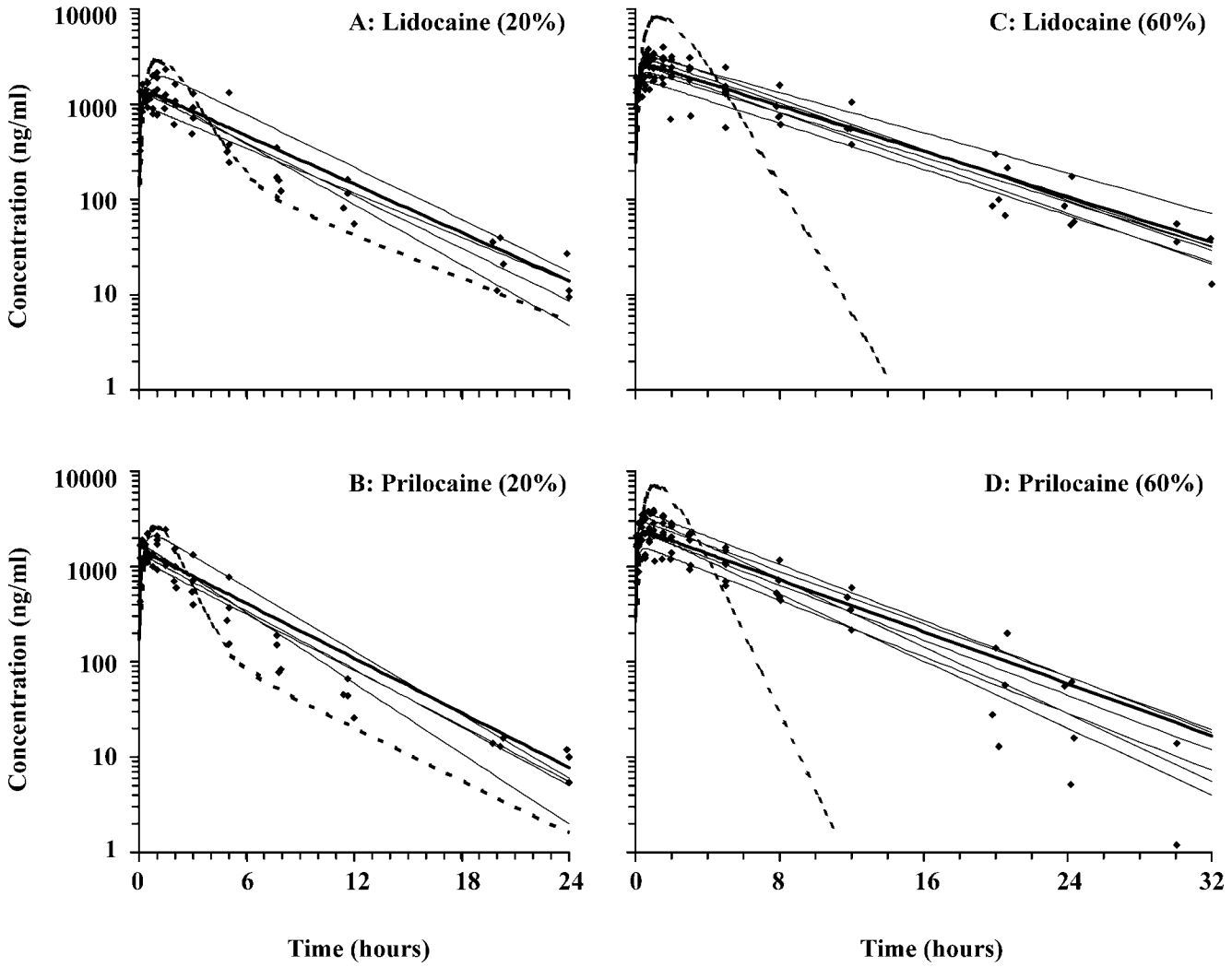

Fig. 4. Arterial blood concentration curves of lidocaine and prilocaine after peripheral nerve block with 20% (*A* and *B*; four rats) or 60% (*C* and *D*; six rats) lidocaine:prilocaine in lipid formulation. *Bold curves* = population mean fit; *thin curves* = disposition according to POSTHOC individual estimates; *dashed curves* = predictions from *in vitro* release profiles.

solution.[22] Also in this case, the depot formulation showed a three-times-longer median duration of sensory block.

The sharp increase in duration of effect at higher percentages of lidocaine:prilocaine in MCT was unexpected and not predicted either by the *in vitro* dissolution or by the *in vivo* pharmacokinetics. Comparison of the median duration of sensory block with the estimated $t_{½}$ of release from the site of administration revealed some striking differences between the preparations. The effect of the 2% lidocaine:prilocaine HCl solution dissipated when approximately 40% of the local anesthetics, or approximately 0.8 mg in total, remained at the site of administration. That of 20% lidocaine:prilocaine in MCT dissipated in less than one half-life of release, when as much as 75% (15 mg) of the local anesthetics would remain at the site. The 60% formulation was the most concentrated one for which the median duration of full sensory blockade could be determined within the 14-day time limit. The 89-h median duration of this formulation corresponds to approximately 18 times the fitted terminal $t_{½}$ of lidocaine and 20 times that of prilocaine. At this time, only traces of local anesthetic, less than 0.001% of the dose, would theoretically remain at the site (even if there should be a longer, undetected terminal $t_{½}$ of release, there would still be a marked difference between the 60% and the other two preparations). Therefore, the modes of action of the low- and high-percentage formulations seemed to be different. Apparently, the low-concentration preparations gave normal, reversible nerve blocks, whereas higher concentrations of local anesthetics induced effects that persisted long after their disappearance from the site.

The durations of action achieved for the high-percentage formulations are longer than for any other depot preparation of local anesthetics yet described. The longest duration of sensory block so far reported in comparable experiments is 4–5 days of total block (according to our definition of no pain reaction within 10 s) or 5–8 days of partial block.[9,10,15,16] This was achieved either by

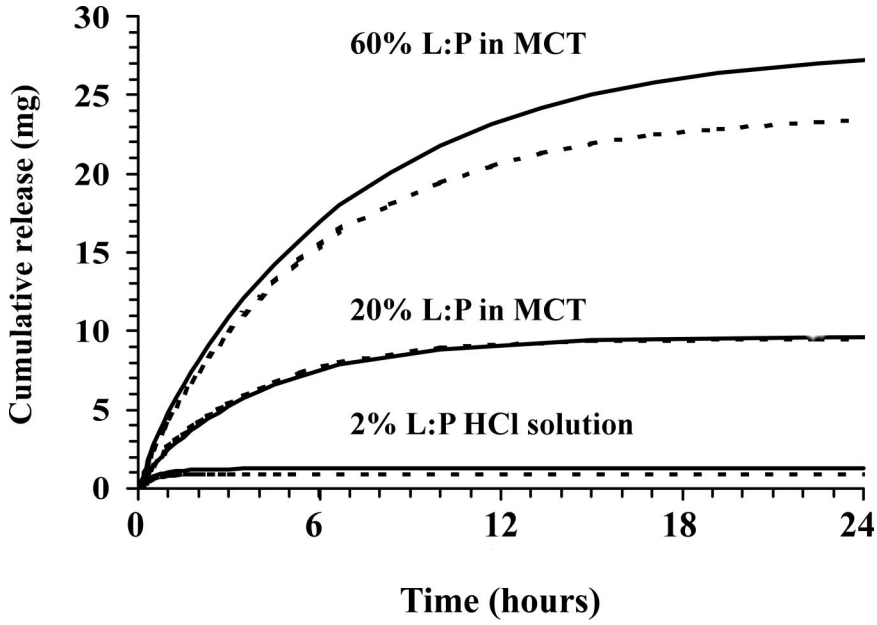

Fig. 5. Calculated release profiles of local anesthetics from the site of administration into the circulation. L:P = lidocaine:prilocaine 1:1 mixture; MCT = medium-chain triglyceride. *Solid curves* = lidocaine; *dashed curves* = prilocaine.

surgical implantation of three 100- to 125-mg polymer matrix pellets containing 20% bupivacaine HCl[9,10] or by injection of 0.6 ml of suspension of microspheres containing 75% bupivacaine[15,16] close to the sciatic nerve in rats. These volumes are up to six times larger than the 0.10 ml needed with our formulations. The need for implantation instead of injection limits the possible clinical use of polymer matrix pellets. In addition, the matrix remained in place long after dissipation of the effect.[10] The microspheres[15,16] were suspended in injection fluids containing carboxymethylcellulose and Tween 80, and the suspensions had to be vortexed immediately before injection. Microspheres, or residues of them, may also remain for considerable times at the site of administration.[31–33] The advantages of using easily injectable liquids containing minor amounts of neutral lipid as the only matrix are obvious.

The histopathologic findings confirmed the conclusion based on the comparison of duration of action and rate of release from the site of administration, *i.e.*, that the low- and high-concentration formulations had different modes of action. The latter ones were clearly neurotoxic. Therefore, these would not be clinically useful for ordinary local anesthesia. However, they could be a possible alternative to ethanol or phenol for long-term blocks of nerve transmission in chronic pain, because this use entails the deliberate application of a neurotoxic agent. Our experiments in rats resemble alcohol neurolysis of the sciatic[44,45] or the tibial[46] nerve in patients with intractable leg spasms. Here, the aim is to obtain a sustained motor block. In our experiments, the 80 or 100% lidocaine:prilocaine formulations seemed superior to 99.5% ethanol in this respect (fig. 1). The main indication for neurolysis is, however, cancer pain of the abdominal viscera, in particular pain due to pancreatic cancer.[47–50] The aim of this treatment is to destroy afferent nerves in the celiac plexus, which consists of two large ganglia lying on either side of the abdominal aorta.

Table 4. Histopathologic Findings on Sciatic Nerve Samples Collected 2 Weeks after Perineural Treatment with Various Preparations

	Grade of Toxicity		
Preparation	Axonal Swelling/Neuronal Degeneration	Demyelization/Myelin Degeneration	Inflammation
Untreated control side	0, 0, 0, 0, 0	0, 0, 0, 0, 0	0, 0, 0, 0, 0
0.9% NaCl solution	0, 0, 0, 0, 0	0, 0, 0, 0, 0	0, 0, 0, 0, 0
2% L:P HCl solution	0, 0, 0, 0, 0	0, 0, 0, 0, 0	0, 0, 0, 0, 0
MCT vehicle	0, 0, 0, 0, 0	0, 0, 0, 0, 0	0, 0, 0, 0, 0
2% L:P in MCT	0, 0, 0, 0, 0	0, 0, 0, 0, 0	0, 0, 1, 0, 0
10% L:P in MCT	0, 0, 0, 0, 0	0, 0, 0, 0, 0	1, 0, 1, 1, 0
20% L:P in MCT	1, 1, 0, 0, 0	1, 1, 0, 0, 0	2, 0, 0, 0, 2
60% L:P in MCT	4, 4, 4, 4, 0	4, 4, 4, 4, 0	4, 4, 4, 4, 0
80% L:P in MCT	4, 4, 4, 4, 4	4, 4, 4, 4, 4	4, 4, 4, 4, 4
99.5% Ethanol	0, 3, 0, 0, 2	0, 3, 0, 0, 2	0, 3, 0, 0, 2

Individual scores (0–4; see text) are shown. n = 5/group.

L:P = lidocaine:prilocaine 1:1 mixture; MCT = medium-chain triglyceride.

The neurolysis is normally performed by injection of 50–100% ethanol either during surgery[47] or percutaneously under radiologic guidance. Typically, 20 ml ethanol may be injected in the region of each ganglion, and the result of the treatment is critically dependent on the spread of the ethanol in the celiac area.[50] Use of a viscous local anesthetic lipid formulation instead of a free-flowing liquid could permit more precisely guided injections, with more predictable results and avoidance of adverse effects on surrounding tissues. The large variability in both nerve blocks (fig. 1) and neurotoxicity (table 4) in our ethanol treatment group illustrate the problems to apply the liquid to the best effect (although the 60% formulation seems to have missed its mark in one animal, too). The long-term efficacy of a lidocaine: prilocaine formulation in comparison with ethanol or phenol, however, must be ascertained in further studies.

In this study, the primary evaluation of effect duration (as reported in table 1) is based on the data on full sensory block and partial motor block, which represents full therapeutic effect with minimal side effect. For the long-acting preparations, both full and partial blocks are shown (fig. 1). Partial motor blocks tended to outlast both full and partial sensory blocks. This may be a general problem with very slow release depot preparations, because during the slow terminal decline of local drug concentration, less sensitive unmyelinated C (pain) fibers may regain their function faster than the more sensitive myelinated A (motor) fibers.[20,28] The clinical importance of this drawback depends on the sensory *versus* motor fiber composition of the nerve that one wishes to block.

The pharmacokinetics of lidocaine after intravenous infusion were in general agreement with literature data.[51,52] An early study on prilocaine in rats[53] showed a disposition rather similar to that of lidocaine, but no standard pharmacokinetic parameter values were reported. The clearance values of the two highly metabolized local anesthetics were also reasonable in comparison to the total hepatic blood flow in rats (approximately 25 ml/min), which is another check of the validity of the data.[54] Intravenous administration and calculation of the UDF allowed the possibility of examining the rate of release of the local anesthetics from the injection site into the circulation, from the 2% lidocaine:prilocaine HCl solution as well as from the lipid formulations. As is apparent from figure 3 and table 2, interindividual variance in the disposition of lidocaine and prilocaine after intravenous infusion was modest. Therefore, the population mean UDF, or weighting function, could be applied to calculations for the whole group of animals.

Using, in turn, the exponential blood concentration function from the nerve block with aqueous solution as the weighting function, an attempt could be made to predict *in vivo* characteristics of the 20 and 60% depot formulations from the *in vitro* data. Few successful predictions of this type have so far been reported for parenteral drugs. The *in vivo* release of the antidepressant doxepin from three different microparticle suspensions, after intramuscular injection, was accurately predicted in dogs.[55] Good correspondence was also found between *in vitro* and *in vivo* release of bupivacaine from a solution in coconut and castor oil.[34] However, the solution was injected subcutaneously in the neck of the rats and not applied to produce a nerve block. Our 20% formulation showed an initial rapid release *in vitro*, which was not reproduced *in vivo*; however, it was clear that the terminal rate of release observed *in vitro* also governed the release *in vivo*. For the 60% formulation, it was not possible to obtain a true release profile *in vitro*.

In conclusion, we prepared the first injectable lipid depot formulation of local anesthetic in which the percentage of active compounds can be varied between 0 and 100%. The pharmaceutical properties of these mixtures compare favorably with those of other depot preparations. The high percentage formulations showed the longest duration of action yet reported for sciatic nerve block in rats. A comparison between duration of nerve block and rate of release of local anesthetic from the site of administration indicated that the modes of action of the low- and high-concentration formulations were different. A histopathologic examination confirmed this conclusion, showing that the high-percentage formulations were neurotoxic. The *in vivo* behavior of the 20% local anesthetic formulation but not that of the 60% formulation could approximately be predicted by *in vitro* release data. The possibility of using a high-concentration local anesthetic depot formulation as an alternative to ethanol or phenol for long-term nerve blocks in chronic pain merits further investigation.

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