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Controlled release injectable liposomal gel of ibuprofen for epidural analgesia

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Abstract

The epidural administration is used commonly in the treatment of pain. Nonsteroidal anti-inflammatory drugs, especially ibuprofen, would have potential in epidural use. Like many epidurally useful drugs it, however, has a short duration of action, which is a limiting factor. To improve epidural pain treatment, a long-acting, single-dose gel injection is being developed. In the present study, the possibility of using liposomal systems to control the release and dural permeation of ibuprofen was investigated in vitro. Liposomal solutions of ibuprofen·Na (20 mg/ml) were prepared by high-pressure homogenization from egg phosphatidylcholine. The liposomal gel consisted of poloxamer 407 and the liposomal solution. No signs in the ^{1}H-NMR spectroscopy of line broadenings or chemical shifts were observed. The liposomal formulations were reproducible and stable. Ibuprofen release in phosphate buffer, pH 7.4, at 37°C from the liposomal solution and the liposomal gel were prolonged significantly compared with their respective solution and gel controls. The liposomal gel controlled ibuprofen release and dural permeation in vitro and showed a permeation pattern favourable for maintaining constant drug levels. The liposomal poloxamer gel represents a new formulation approach to increase the local epidural availability of ibuprofen. It appeared to be a promising injectable controlled-release drug delivery system.

Keywords: Controlled release; Dural permeation; Ibuprofen·Na; Injectable; Liposome; Poloxamer 407 gel

1. Introduction

Prolonged release formulations are important in parenteral administration. In epidural analgesia, reduction of the frequency of injections and localization of drug molecules at the site of injection would be a great advantage allowing administration of drugs without continuous catheterization and its associated problems. A long-acting, single-dose epidural formulation would improve clinical treatment of various pain states. Therefore, an injectable epidural gel is being developed (Paavola et al., 1998b).

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The use of nonsteroidal anti-inflammatory drugs (NSAIDs) for spinal pain treatment is a new area of analgesic therapy (Drower et al., 1987; Malmberg and Yaksh, 1992). Because the mechanism of action of NSAIDs is not through receptors the risks of spinal opioids, such as development of tolerance, respiratory depression, urinary problems, itching and motor nerve block are prevented. Especially ibuprofen can produce significant analgesia after epidural administration (Wang et al., 1994), but its duration of action is fairly short.

Poloxamer 407 is a biocompatible polymer widely used in medical and pharmaceutical purposes and because of its low toxicity and weak immunogenic properties suitable for parenteral drug delivery (Schmolka, 1972; Johnston and Miller, 1985; Miller et al., 1997). Previously we have demonstrated that poloxamer 407 gel (25%) controls the release and dural permeation of ibuprofen and lidocaine (Paavola et al., 1998a). Poloxamer 407 possesses a reverse-phase thermal gelation property, so that poloxamer gel is an injectable liquid when cold (5–10°C) and forms a gel in situ at a physiological temperature. After the epidural injection of 2% ibuprofen·Na containing poloxamer 407 gel (25%) controlled analgesia with reduced systemic absorption of ibuprofen was achieved in animals (Paavola et al., 1998b). The duration of analgesia, however, was shorter than expected. Therefore, in order to prolong the analgesic effect the epidural availability of ibuprofen should be increased.

Liposomes are suitable carriers of hydrophilic and hydrophobic drugs and can be used to increase drug activity and reduce toxicity (e.g. Gregoriadis, 1973; D'Silva and Notari, 1982; Poste, 1983; Boogaerts et al., 1993). Liposomes of appropriate composition may be suitable carriers of therapeutic agents also into the central nervous system (Adams et al., 1977; Isackson et al. 1995), and they provide a promising system for drug delivery in epidural administration (Mashimo et al., 1992). Liposomes, however, are cleared easily by the reticuloendothelial system (Poste, 1983). When liposomes are used as controlled-release epidural systems, the aim should be to have these vehicles remain at the site of injection.

The aim of the present investigation was to study the possibility of using colloidal carriers, liposomes, in controlling ibuprofen release properties of the injectable 25% poloxamer 407 gel studied previously. For this purpose, ibuprofen·Na liposomes were prepared by high-pressure homogenization. The liposomal solution and liposomal poloxamer gel formulations were characterized in vitro. Although the fate of drugs given epidurally in general is complicated, the amount of drug transferred through the dural membrane and entering the spinal nerve tissue determines the intensity and the duration of analgesia. The efficacy of these liposomal systems for use in epidural analgesia was assessed in vitro through dural membranes of pigs.

2. Materials and methods

2.1. Materials

Liposomes were made from egg phosphatidylcholine and α-tocopherol (Sigma, St. Louis, MO). Stearylamine and dicetylphosphate (Sigma, St. Louis, MO) were used as negatively and positively charged lipids. All other chemicals used in liposome analyses were of reagent grade. The gel-forming polymer used was polyoxyethylene–polyoxypropylene copolymer, poloxamer 407 (Lutrol F-127; BASF, USA). The drug was the sodium salt of ibuprofen (ibuprofen·Na), which was kindly donated by Knoll Pharmaceuticals of BASF (UK).

2.2. Preparation of liposomal solution and liposomal gels

Phosphatidylcholine (20 mg/ml) was dissolved in ethanol. To prevent oxidation, α-tocopherol (0.02 mg/ml) was added in the suspension. Stearylamine (20 μmol) and dicetylphosphate (20 μmol) were used to adjust the charge. The respective solutions were concentrated by evaporating ethanol under vacuum using a rotary evaporator to yield a thin lipid film. The film was kept under vacuum in a lyophilizator overnight to remove any traces of solvent. Subsequently, the film was

hydrated with 2% ibuprofen·Na–saline solution (0.15 M) using vortex-mixing to remove all lipids from the side of the flask. The pH of the dispersion formed was adjusted to 7.4 with HCl, and it was homogenized by a high-pressure homogenizer (EmulsiFlex-B3, Avestin, Canada) to form the liposomes. The working pressure of the homogenizer was kept at 160 MPa, and the batch size was 2.5 ml. The liposomal solution was finally extruded through two stacked polycarbonate filters, pore size of 200 nm, with an extruder (Liposo-Fast-Basic, Avestin, Canada) to stabilize the liposomal solutions before use. Nitrogen was bubbled through the liposomal solution.

The liposomal poloxamer gel was prepared by adding the required amount of poloxamer 407 (25% w/w) in small quantities to the cold 2% ibuprofen·Na containing liposomal solution (5°C), and letting it slowly dissolve under gentle stirring with a magnetic bar.

2.3. Characterization of liposomal formulations

The size distribution of the liposomes was determined by photon correlation spectroscopy (PCS) using laser light scattering (Malvern Instruments, series 7032, UK) at an angle of 90° (10 min, 25°C). The zeta potential (surface charge) of the liposomes was determined after measurement of the electrophoretic mobility (20 s, 25°C) by means of a laser doppler anemometer (Zeta Seizer, Malvern Instruments, UK). To assess the possible lipid loss in the preparation of liposomes the lipid content was identified by phosphorus analysis (Bartlett, 1959).

The ibuprofen content of liposomes was measured by high performance liquid chromatography (HPLC) after the liposomally entrapped ibuprofen (pellet) was separated from the non-entrapped ibuprofen (supernatant) using an ultracentrifuge (100 000 × g, 180 min) (Beckman Inc., Palo Alto, Canada). The remaining liposome pellet was washed three times with saline, and dissolved in methanol. The non-encapsulated drug concentration in the supernatant of the final wash was less than 0.1%. The ibuprofen content of the liposomal formulations (uniformity of batches) was analyzed after dissolution and dilution of samples (0.2 g) in methanol ($n = 3$). The HPLC method, a slightly modified version of the method described by Avgerinos and Hutt (1986), was used at a wavelength of 222 nm. A reversed-phase C_{18}-column (μBondapak 10 μm, 125 Å, 3.9 × 300 mm^2) (Waters Ltd., Milford, USA) combined with a guard column (μBondapak) was used at ambient temperature. The isocratic mobile phase was acetonitrile:0.1 M sodium acetate (35:65), with pH adjusted to 6.2 using glacial acetic acid. The flow rate was 2 ml/min. The standard curve of known ibuprofen concentrations in methanol was linear over a concentration range of 0.05–30 μg/ml ($r > 0.999$). The intra-assay variability was 2% or less, and the detection limit was 0.05 μg/ml.

2.4. ^{1}H-NMR measurements

The molecular stability of ibuprofen with phosphatidylcholine and poloxamer was determined. Therefore, ^{1}H-NMR spectras of the control solution of ibuprofen, liposomal ibuprofen solution, ibuprofen in poloxamer, and liposomal ibuprofen solution in poloxamer were measured at 30°C using a Varian Unity 500 NMR spectrometer (Varian Associate, USA). The measurements were performed directly after preparation and after 2-week storage at 4–5°C. The signals were referenced to the residual deuterium oxide signal (4.70 ppm). Phosphatidylcholine liposomes were made in deuterium oxide or ibuprofen·Na containing deuterium oxide solution. The concentration of ibuprofen·Na, phosphatidylcholine and poloxamer in deuterium oxide was 20 mg/ml.

2.5. Drug release experiments

The two-compartment in vitro method (Paavola et al., 1995) was used to evaluate ibuprofen release from the formulations. The formulation sample (0.5 g) was introduced into a small donor compartment separated by an inert cellulose membrane (Spectrapore, mwco 12000–14000, Thomas Scientific, USA) from the large acceptor compartment, a phosphate buffer, pH 7.4, (450 ml) at 37°C. The effective diffusion area in the donor compartment was 1.13 cm^2. The acceptor solution was stirred with a magnetic bar at 350

rpm. The system was connected to a flow-through spectrophotometer (Ultrospec II, LKB Biochrom Ltd., UK) via a peristaltic pump (Watson-Marlow 503S, Smith and Nephew, UK). Samples were withdrawn from the acceptor solution, and the absorbances in 10-mm flow-through cells were measured automatically at regular intervals at a wavelength of 221 nm by a computer running TDS software (LKB Biochrom Ltd., UK). The absorbances were converted to the amount of ibuprofen released using calibration curves based on the standard solutions of ibuprofen in a phosphate buffer, pH 7.4, over a concentration range of 1–60 μg/ml ($r > 0.999$).

2.6. Permeation experiments

The permeation of ibuprofen through the dural membrane from the liposomal solution and liposomal gel, along with the control ibuprofen·Na solution (20 mg/ml), was evaluated in the two-compartment system described above. The formulation sample was introduced into the donor compartment, which was separated from the acceptor compartment by a porcine lumbar dural membrane. The dural membranes were obtained from pigs that were killed with an overdose of intravenous anaesthetic as part of the organ transplantation research programme of Helsinki University Central Hospital. The experiment was approved by the Ethics Committee on Animal Care and Use of the University of Helsinki. Immediately post mortem pieces of dura mater, with the arachnoidal membrane attached, were excised from the animals and put into cold physiological saline solution. The membranes were washed with phosphate buffer, pH 7.4, cut into appropriate specimens and mounted on the openings of the donor tubes. The dural membranes were used for the experiment within 5 h after excision. The thickness of the lumbar dural membranes could be considered constant as each study time all the formulations were investigated using membrane specimens from one animal.

2.7. Data analysis

Because of the different structure of the formulations, ibuprofen release and permeation mechanisms between formulations are different. In order to facilitate the comparison between the release and the permeation patterns of ibuprofen from different formulations, first-order exponential curves of cumulative ibuprofen release and permeation against time were calculated, $f(t)$, and the release and permeation rates of ibuprofen were obtained from the differentiated first-order equation, $g(t)$. The equations were as follows:

$$f(t) = a \cdot (1 - e^{-bt}) \rightarrow g(t) = c \cdot e^{-bt} \quad (1)$$

where a is the theoretical maximum of the cumulative amount released, b is the critical constant describing release stability, t is time and c is the intrinsic release constant at $t = 0$.

Differences between the liposomal formulations and the controls were compared and assessed by Student's paired t-test. Differences were considered statistically significant at the $P < 0.05$ level. Pairwise Pearson analysis was used to compare the correspondence of the release and permeation patterns.

3. Results

3.1. Properties of liposomes

High-pressure homogenization was chosen for manufacturing process of liposomes to produce reproducible liposome batches and uniform liposomes of small size. The efficacy of unilammellar liposomes made by high-pressure homogenization in entrapping ibuprofen was quite low (5%) (Table 1). The zeta-potential of the neutral phosphatidylcholine liposomes containing ibuprofen was slightly negative, and stearylamine increased the potential toward the positive. The mean diameter of the neutral liposomes was 90 nm, while charged lipids somewhat increased the diameter (Table 1). Based on Bartlett analysis no significant lipid loss during preparation occurred.

3.2. Stability

In the ^{1}H-NMR the approach was to follow the

Table 1
Average size, zeta-potential, and encapsulation efficacy of ibuprofen·Na (20 mg/ml) containing neutral phosphatidylcholine liposomes, and these liposomes with stearylamine (positive charge) or dicetylphosphate (negative charge) ($n = 3$)[a]

Liposomes	Size (nm ± S.D.)	Zeta-potential (mV ± S.D.)	Encapsulation efficiency (% ± S.D.)
Neutral	90 ± 20	−16.8 ± 3.6	5.2 ± 0.5
Positive charge	144 ± 38	0.4 ± 0.24.0 ± 0.6	
Negative charge	140 ± 35	−15.3 ± 2.4	4.2 ± 0.5

[a] Polydispersity index was <0.3. The refraction index used was 1.50.

chemical shifts of the identifiable resonance from ibuprofen in the presence of different components. The ^{1}H-NMR spectras of the ibuprofen solution, the liposomal ibuprofen solution, ibuprofen in poloxamer, and the liposomal ibuprofen solution in poloxamer were depicted and compared directly after preparation and after 2-week storage. Ibuprofen did not cause any chemical shift of the peaks in liposomes, poloxamer or in the liposome–poloxamer system nor were signs of line broadenings observed.

3.3. In vitro release

Significant prolongation of ibuprofen release was achieved with the liposomal solution ($P < 0.001$) and the liposomal gel ($P < 0.001$) in comparison with the respective control solution and gel (Fig. 1). The deviation (mean ± S.D.) in the release of ibuprofen was minimal, indicating that consistent liposomal batches were produced by the homogenization technique. The cumulative amounts of ibuprofen released in 24 h from the liposomal gel was 42% compared with 58% released from the control gel and 75 and 99% from the liposomal solution and the control solution, respectively. Ibuprofen release from the liposomal gel and the control gel was steady and slow, but the rate of ibuprofen release from the liposomal solution and the control solution decreased as a function of time (Fig. 2).

3.4. In vitro dural permeation

The liposomal solution significantly ($P < 0.001$) prolonged ibuprofen permeation compared with the control solution (Fig. 3). The cumulative amounts of ibuprofen permeated in 24 h were 72 and 88%, respectively. Compared with the control gel (38%) the cumulative permeation of ibuprofen was significantly ($P < 0.001$) faster from the liposomal gel (57%). The delay (lag-time) at the beginning of the permeation was 1 and 2 h for the liposomal gel and the control gel, respectively. The permeation of ibuprofen from the liposomal gel and the control gel occurred at a constant rate, whereas the overall permeation of ibuprofen from the liposomal solution and from the control solution decreased as a function of time (Fig. 4).

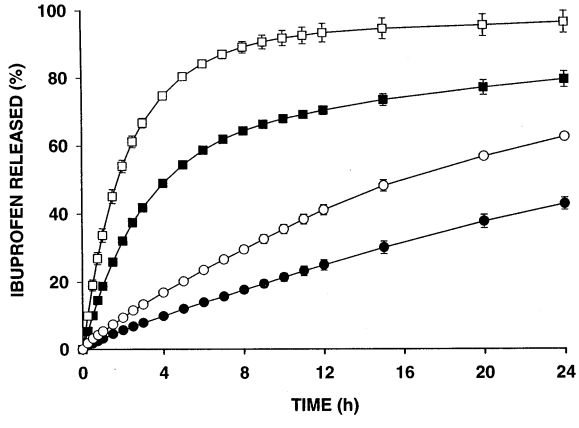

Fig. 1. Cumulative release of ibuprofen from ibuprofen·Na (20 mg/ml) containing solution, liposomal solutions with neutral and charged lipids, and liposomal gel into phosphate buffer, pH 7.4, at 37°C. Means ± S.D. ($n = 6$) are presented. Symbols: □, solution; ■, liposomal solution; ○, poloxamer gel; ●, liposomal poloxamer gel.

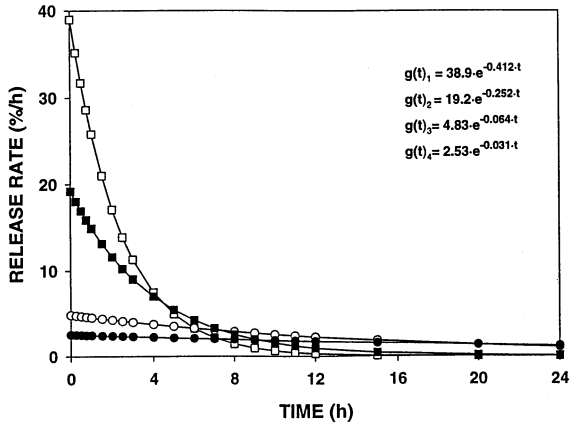

Fig. 2. The curves and equations, $g(t)$, for release rates of ibuprofen from different formulations in an hour (%/h). Symbols: □, solution (g_1); ■, liposomal solution (g_2); ○, poloxamer gel (g_3); ●, liposomal poloxamer gel (g_4).

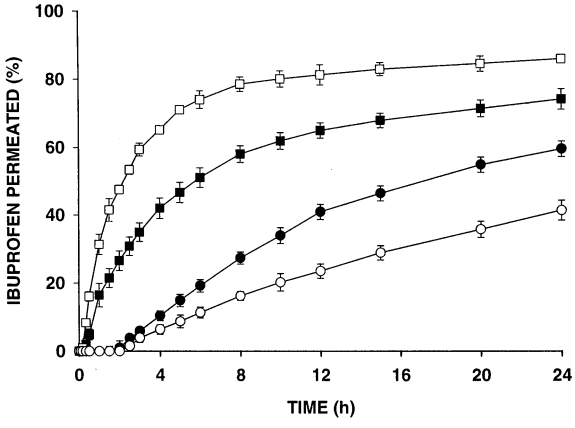

Fig. 3. Permeation of ibuprofen from ibuprofen·Na (20 mg/ml) containing solution, liposomal solution, and liposomal gel through porcine lumbar dural membrane into phosphate buffer, pH 7.4, at 37°C. Means ± S.E.M. ($n = 6$) are presented. Symbols: □, solution; ■, liposomal solution; ○, poloxamer gel; ●, liposomal poloxamer gel.

4. Discussion

4.1. Properties of the liposomal ibuprofen systems

The liposomal solution significantly prolonged ibuprofen release compared with the control solution, although entrapment of ibuprofen in liposomes made by high-pressure homogenization was relatively low at 5%. Many technological aspects and quality control criterions, like simplicity of the method validation and uniformity of formed liposomes, however, support the use of high-pressure homogenization for preparation of injectable liposomes (Brandl et al., 1993). Ibuprofen is highly ionized at pH 7.4 ($pK_a = 4.6$). Being a monovalent ion it forms an electrical double layer which, as detected in zeta-potential measurements, caused a weak net negative charge on the surface of the liposomal lipid bilayer. The surface potential of liposomes results from unequal adsorption of anions and cations that interact with the lipid bilayer but can be effected by the groups with fixed charged lipids (Winiski et al., 1986). High amounts of charged lipids cause strong surface charges and effect the biodistribution of liposomes, but small amounts ($> 10\%$) can be used to modify the lipid bilayer structures (Poste, 1983; Law et al., 1987). The spacing between phospholipid molecules and the entrapment of an ionized drug were expected to be changed by incorporation of charge-inducing lipids. Although the size of the liposomes increased, the entrapment of ibuprofen could not be changed by the charge-inducing agents studied. Therefore, ibuprofen liposomes made without charged lipids were used for further studies.

Stabilization of drugs in liposomes is dependent on the degree of association of drug molecules

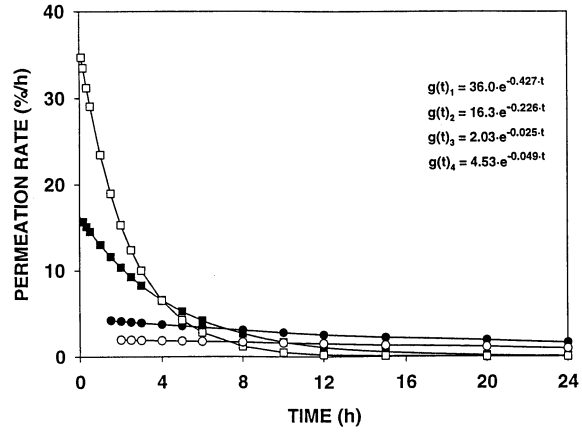

Fig. 4. The curves and equations, $g(t)$, for permeation rates of ibuprofen from different formulations in an hour (%/h). Symbols: □, solution (g_1); ■, liposomal solution (g_2); ○, poloxamer gel (g_3); ●, liposomal poloxamer gel (g_4).

with phospholipid bilayers (Grit et al., 1993). The stability of liposomes has been enhanced by micro-encapsulation (Yeung and Nixon, 1988) and by coating the liposomes with different polymers (Dong and Rogers, 1993; Moghimi et al., 1991). Based on the present NMR results, binding of ibuprofen with lipids seems to be non-specific, and the drug entrapment more of a physical type in which only weak electrostatic and hydrophobic interactions are effective. No signs of hydrolytic degradation products or oxidation were detected by NMR-spectroscopy after 2 weeks storage of the lipid systems studied. The components of the formulations were compatible and stable in this respect.

4.2. Liposomal gel and effects on ibuprofen release

Poloxamer 407 gel is composed of cubically orientated spheroidal micellar subunits (Schmolka, 1972), and the structures can stabilize liposomal systems by preventing liposome aggregation. On the other hand, liposomes may function as another drug release controlling barrier in the poloxamer gel. It is important to note that since the liposomal poloxamer gels were liquids at temperatures below 10°C and gels at 37°C, the thermogelling property of poloxamer was maintained despite the liposomes.

The liposomal poloxamer gel significantly prolonged ibuprofen release compared with the liposomal solution and the control gel. The release of amphiphilic ibuprofen from the liposomal gel is a combination of the release of free, surface-bound and encapsulated drug through the micellar network channel structures of the gel. The hydrophobic polyoxypropylene groups of poloxamer have a tendency to adsorb at hydrophobic surfaces, while the hydrophilic polyoxyethylene groups of poloxamer protrude out from the surface (Wesemeyer et al., 1993). It has been suggested that the polyoxypropylene group of poloxamer possibly penetrates the lipid bilayer of fluid egg phosphatidylcholine liposomes, and could affect the retention of hydrophilic drugs (Jamshaid et al., 1988). The release profile and amounts of ibuprofen were, however, not changed after 2 weeks of storage. The liposomal formulations were opalescent, and no sign of turbidity was observed in visual or stereomicroscopic examination.

4.3. Effects of liposomal formulations on ibuprofen permeation

Ibuprofen permeation through the dural membrane was significantly prolonged by the liposomal solution compared with the control solution. The dura mater with the attached arachnoid mater resembles a complex sieve-like structure consisting mainly of collagen and elastin fibers and only of a very thin mesothelial cell layer of arachnoid mater, the membrane thus not being a typical cellular lipid barrier (Moore et al., 1982; McEllistrem et al., 1993). In the penetration of drugs through dural membrane drug molecules are first sieved through dura mater and after that transported through arachnoid mater transcellularily or through the small paracellular pores of arachnoid.

Liposomes are flexible lipid vesicles, and interactions between liposomes and biological membranes commonly occur (Poste, 1983). The uneven, sieve-like structure of the dural membrane and flexibility of liposome surface promote mechanical adherence of liposomes on the dural membrane and thus increase possibility for interactions. The changes in ibuprofen permeation profiles compared with the release profiles would reflect that interactions affect the permeation. However, the shapes of the permeation and release patterns of the liposomal gel were based on Pearson analysis correspondent ($r > 0.99$; $P < 0.001$), which reflects the uniformity of release and permeation kinetics.

The release and the dural permeation of ibuprofen from the liposomal poloxamer gel were constant, and in this respect the formulation fulfilled the theoretical demand for an ideal controlled-release epidural formulation. The liposomes mixed in the poloxamer gel structure interestingly increased ibuprofen permeation through dural membrane as compared with the plain poloxamer gel. Although the permeation and release patterns of the liposomal gel were fairly similar ($r > 0.97$,

$P < 0.001$), ibuprofen permeation was significantly ($P < 0.05$) faster compared with the release. Poloxamer forms a soft-surfaced but compact implant-like system that is well localized at the injection site (Johnston and Miller, 1985; Paavola et al., 1998b). The micellar polyoxyethylene-polyoxypropylene structure of poloxamer makes its adsorption on biological membranes possible (Wesemeyer et al., 1993; Miller et al., 1997). Therefore, poloxamer gel also most likely retains liposomes at the dural interface. Thus the contact of the liposomes with the dural membrane is improved, and controlled and targeted permeation of ibuprofen is achieved. Furthermore, the elimination of liposomes in vivo by uptake into the reticuloendothelial system can be prolonged. This is because the hydrophilic nonimmunogenic surface properties of poloxamer gel (Miller et al., 1997; Johnston and Miller, 1985) cover the liposomes and decrease adhesion of phagocytic cells on the liposomes (Lee et al., 1996). Based on these results the liposomal gel can be expected to produce constant drug levels in vivo at the site of epidural injection. It seems that poloxamer micelle structures in a combination with liposomal membrane structures interact with the dural membrane interface, although not changing the permeation pattern, however, facilitating ibuprofen permeation deeper into and through the dural membrane structures. Thus, liposomes of the poloxamer gel enhance dural permeation of ibuprofen in vitro which is a very important obsevation when considering in vivo epidural administration of ibuprofen.

5. Conclusions

The liposomal solution and the liposomal poloxamer gel prolonged the in vitro release of ibuprofen in comparison with the respective control formulations. The liposomal ibuprofen formulations were reproducible and stable, and in this respect pharmaceutically acceptable. The liposomal gel markedly increased dural permeation of ibuprofen compared with the gel control showing a constant permeation rate. The gel can target ibuprofen release on the surface of the dural membrane at the injection site, while liposomes further efficiently control the permeation of ibuprofen. The liposomal gel represents a promising new alternative to increase the epidural availability and spinal uptake of ibuprofen after epidural administration, and may possibly be considered also for other injectable applications. The evaluation of toxicology and biodistribution of these novel liposomal ibuprofen formulations is justified.

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