



Research paper

Controlled release ibuprofen-ploxamer gel for epidural use – A pharmacokinetic study using microdialysis in pigs

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ARTICLE INFO

Article history:

Received 23 June 2016

Revised 3 September 2016

Accepted in revised form 6 September 2016

Available online 09 September 2016

Keywords:

Controlled release

Epidural

Intrathecal

Ibuprofen-Na

Injectable

Microdialysis

Poloxamer 407 gel

ABSTRACT

In order to avoid the risks of sideeffects of epidural local anesthetics and opioids, the use of nonsteroidal anti-inflammatory drugs (NSAIDs) epidurally would be an interesting option of analgesic therapy. The fairly short duration of action of spinally administered NSAIDs, e.g., ibuprofen, may be prolonged by using controlled release poloxamer gel formulation. Using a microdialysis technique we studied the epidural and intrathecal pharmacokinetics of ibuprofen after its epidural administration as a poloxamer 407 formulation or a solution formulation. In addition, plasma ibuprofen concentrations were analyzed from central venous blood samples. Ibuprofen concentrations in the epidural space were significantly higher and longer lasting after the epidural gel injection compared with the epidural solution injection. The epidural AUC of ibuprofen was over threefold greater after epidural ibuprofen gel injection compared with the ibuprofen solution injection ($p < 0.001$). The systemic absorption of ibuprofen from 25% poloxamer 407 gel was very low. The *in situ* forming poloxamer gel acted as a reservoir allowing targeted ibuprofen release at the epidural injection site and restricted ibuprofen molecules to a smaller spinal area. Ibuprofen diffusion from the epidural space to the intrathecal space was steady and prolonged. These results demonstrate that the use of epidurally injectable poloxamer gel can increase and prolong ibuprofen delivery from epidural space to the CSF enhancing thus ibuprofen entry into the central neuroaxis for spinal analgesia. Further toxicological and dose-finding studies are justified.

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1. Introduction

Different systems to control epidural drug release have been studied during the last years, including the use of lipid solutions [1], cyclodextrin [2], liposomes [3], microparticles [4,5] and emulsified solutions [6]. The results have been to some extent conflicting [7], and only bupivacaine liposomes have been studied epidurally in humans [8,9]. So far, to our knowledge, for general clinical epidural use there is only one slow-release liposome product of morphine available on market (DepoDur[®]) [10]. One bupivacaine liposome formulation is on market for regional pain treatment (Exparel[®]), but it is not indicated for epidural or intrathecal use [11].

Intravenously administered non-steroidal anti-inflammatory drugs (NSAIDs) can penetrate into the CSF [12,13] and some have been administered intrathecally in pain patients [14]. The possibil-

ity of using indomethacine instead of corticosteroids epidurally has been explored in patients with postlaminectomy syndrome [15]. The spinal analgesic and anti-inflammatory effects of nonsteroidal anti-inflammatory drugs (NSAIDs) seem to be related to their ability to inhibit cyclo-oxygenase enzyme and the synthesis of prostaglandins [16,17], thus blocking the excessive sensitivity to pain induced by the activation of the spinal glutamate and substance P receptors [18,19]. Since NSAIDs act through an enzymatic system instead of receptors the risks of spinal opioids, such as tolerance, respiratory depression, urinary problems, motor blockade and dependence [20] are unlikely to develop in epidural use of NSAIDs. Ibuprofen is known as a potent oral NSAID, but has been found to be able to act also on the spinal cord reducing pain even more efficiently after intrathecal administration than after systemic administration [18]. Ibuprofen can produce significant analgesia after epidural administration [21], but the biological half-life of ibuprofen is fairly short ($t_{1/2} \sim 2$ h).

In epidural analgesia and anesthesia it would be important that the application of drug molecules is localized near the epidural injection site and spot-targeted drug delivery through the spinal

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membranes into the cerebrospinal fluid (CSF) could be achieved. Poloxamer is a biocompatible polymer widely used for medical and pharmaceutical purposes. Different poloxamer grades have been under study for ocular, nasal, topical, rectal, vaginal and uterine use, and are also used in oral preparations. Because of poloxamer's low toxicity and weak immunogenic properties it has been considered suitable also for parenteral drug delivery [22–26]. The reverse phase thermal gelation of the 25% (w/w) poloxamer gel makes it an easily injectable liquid when cold (5–10 °C) and it forms a highly viscous gel *in situ* at a physiological body temperature.

We have previously shown controlled release of ibuprofen through porcine dura mater *in vitro* from an ibuprofen-poloxamer 407 gel [27]. *In vivo* lumbar epidural injection of ibuprofen containing poloxamer 407 gel to pigs resulted in analgesia to pinching and reduced systemic absorption of ibuprofen [28]. Epidurally administered ibuprofen does not cause motor blockade [19,28], and in this respect ibuprofen can also be regarded as a promising drug for post-operative spinal pain treatment.

The fate of epidurally administered ibuprofen is not clear, and details of the spinal area kinetics after controlled release epidural poloxamer gel injection are needed. Our hypothesis is that ibuprofen concentration at the epidural injection site can probably be maintained by poloxamer gel and ibuprofen could be “transferred” into intrathecal space in adequate amounts and for a prolonged length of time to produce analgesia at the spinal cord level. The knowledge of ibuprofen epidural pharmacokinetics would be critical for optimizing ibuprofen spinal dose.

The present *in vivo* study in pigs was designed to characterize and compare the distribution of ibuprofen in the spinal area after an epidural bolus injection of ibuprofen in poloxamer gel or in saline. Ibuprofen concentrations in epidural and intrathecal space as well as in plasma were investigated using microdialysis techniques which allowed continuous sampling from the spinal spaces.

2. Materials and methods

2.1. Drug formulation

The poloxamer gel was prepared as previously using the cold preparation technique [29]. An appropriate amount (25% w/w) of poloxamer 407 ((HO(C₂H₄O)₁₀₁(C₃H₆O)₅₆(C₂H₄O)₁₀₁H, i.e., polyoxyethylene - polyoxypropylene block co-polymer) (Lutrol F-127, BASF, USA) was added into a cold solution (5 ± 1 °C) while maintaining constant agitation with a magnetic stirrer. The gel was stored at 4 °C until a clear solution was formed. Ibuprofen·Na (C₁₃H₁₇NaO₂, i.e., sodium 2-[4-(2-methylpropyl) phenyl] propionate) (Knoll Pharm., United Kingdom) (2% corresponding to 1.6% ibuprofen) was added in the gel and the pH was adjusted carefully to 7.4 with HCl solution. The pH was measured with a pH meter (Mettler, Halstend, UK) while the gel was in the liquid state at 4 °C. Ibuprofen·Na-solution (2%) was used as the control formulation.

The viscosity measurement was a quality control method to assess the behavior of the gel at body temperature. The apparent viscosity value of the 2% ibuprofen·Na containing 25% poloxamer gel was after 10 min rotation at 37 °C 67.1 ± 1.8 Pa·s (Brookfield digital rheometer DV-III, spindle RV7, Brookfield RVT laboratories, Stoughton, USA). The temperature of the gel samples was adjusted to 37 °C using a thermostated water jacket.

2.2. Microdialysis experiment

2.2.1. Animals

The animal study was approved by the University of Washington's Institutional Animal Care and Use Committee

(Seattle, Washington). The guidelines of American Association for Laboratory Animal Care (NIH Publications) for the use of experimental animals were followed throughout the experiments. The animals used were healthy farm-raised pigs (n = 12) weighing 11–14 kg. Before the experiment the animals were on *ad libitum* diet and they were housed in a 12 h light-dark cycled rooms. The animals were euthanized after the experiment.

The animals were randomly divided into two main study groups: ibuprofen solution (n = 6) and ibuprofen poloxamer gel (n = 6) groups. The animals were anesthetized by mask inhalation with halothane (1–2%) and nitrous oxide (70%) in oxygen. The muscle functions were controlled by an intramuscular succinylcholine injection (100 mg). The orotracheal tube was placed and anesthesia was maintained with isoflurane (1–2%) in balance oxygen. Animals were mechanically ventilated to maintain end-tidal CO₂ between 36 and 40 mmHg. A Datex (Helsinki, Finland) model F-W8-28-01 airway gas analyzer was used to measure end-tidal CO₂ and isoflurane concentrations. The body temperature of the anesthetized animal was maintained at 37–38 °C by a heat-lamp controlled by a rectal thermometer system. The right femoral artery of the animal was cannulated for blood sampling. Blood pressure and heart rate were measured by pressure transducer. The transducers used were Hospira Transpac IV Monitoring Kit (Hospira Inc., Lake Forest, IL, USA). Saline solution infusion was given as a maintenance fluid through the cannulated right femoral vein and was administered at the rate of 4 ml/kg/h using volume controlled pump (Abbot/Shaw Lifecare Pump Model 4, Abbot Laboratories, Alameda, CA, USA).

2.2.2. Implantation of microdialysis probes and drug administration

The microdialysis technique used was a slightly modified version of the method previously used in this laboratory for local anesthetic analysis [30,31]. Using microsurgical technique, the vertebral laminae were exposed posteriorly between T11 and L5. The epidural probe was inserted through the *ligamentum flavum* between L1 and L2 and was directed cephalad in the posterior epidural space. The intrathecal probe was directed caudal in the subarachnoid space. The probes were secured to be in place by cyanoacrylate glue. The microdialysis probes were made from cellulose dialysis fibers (Spectrum Medical Industries, Inc., Houston, Texas) with a 215 μm inside diameter, a 235 μm outside diameter, and a molecular weight cutoff of 6000 Daltons. The probes were perfused with bicarbonate buffered mock cerebral spinal fluid (NaCl 140 mEq, NaHCO₃ 25 mEq, KCl 2.9 mEq, MgCl₂ 0.4 mEq, urea 3.5 mEq, glucose 4.0 mEq, CaCl₂ 2.0 mEq; pH = 7.38–7.42; 292–298 mOsm) through which 5% CO₂/95% air gas was bubbled. The microdialysis probes were connected to the cellulose tubing and syringe pump. The syringe pump (Harvard Apparatus Inc., model 22, Holliston, MA) was used to pump mock CSF through the dialysis probes at the rate of 10 μl/min.

The 2% ibuprofen·Na containing poloxamer gel or 2% ibuprofen·Na solution was administered as a single dose epidural injection into the 5th lumbar interspace through a single G20 epidural catheter (B Braun Medical, Bethlehem, PA) connected to a syringe pump (Harvard apparatus, USA) using a speed of 2 ml/min. The formulations were injected cold (4–8 °C) when the gel was in the liquid state.

At the end of the experiments Richardson's blue was injected into the epidural space via the epidural catheter. The spinal cord and epidural catheter were removed. The intrathecal catheter was removed and the spinal cord was longitudinally sectioned to ascertain that the spinal membranes had remained intact, excluding the catheter insertion holes, and the tissue samples were examined under a dissecting microscope.

2.3. Pharmacokinetic experimentation

2.3.1. Epidural and intrathecal ibuprofen concentration assay

Dialysis samples from epidural and intrathecal spaces were collected at 0, 5, 10, 15, 20, 25, 30, 40, 50, 60 min and then every 20 min until 240 min after the ibuprofen gel or ibuprofen solution injection. The samples were stored at -20°C until analysis.

Ibuprofen concentrations of the epidural and intrathecal dialysate and plasma samples were determined by HPLC at a wavelength of 222 nm using a modified version of a method described by Minkler and Hoppel [32]. A supelcosil LC-18DB column (Supelco, 5 μm , 125 \AA , 1.6×1500 mm) (Waters Ltd., USA-Milford) was used at ambient temperature. The isocratic mobile phase was acetonitrile: deionized water (40:60 v/v), and the pH was adjusted to 6.2 with phosphoric acid. The flow rate was 2 ml/min. The accuracy and reproducibility of the method were assessed according to recommendations [33]. The coefficient of variation of intra-assay variability was 3% or less. The detection limit was 0.05 $\mu\text{g/ml}$.

2.3.2. Plasma ibuprofen concentration assay

Blood samples were collected into EDTA tubes from the cannulated right brachial vein 0, 5, 10, 20, 30, 60, 90, 120, 150, 180 and 240 min after the injection. Plasma was separated by centrifugation at 3000 rpm for 10 min and stored in plastic tubes at -20°C until analysis.

Ibuprofen plasma concentrations were determined using high performance liquid chromatography (HPLC) at a wavelength of 222 nm using a slightly modified version of a method described by Averginos and Hutt [34]. A reversed-phase C_{18} column ($\mu\text{Bondapak}$ 10 μm , 125 \AA , 3.9×300 mm) (Waters Ltd., USA-Milford) combined with a guard column ($\mu\text{Bondapak}$) was used at ambient temperature. The isocratic mobile phase was acetonitrile: 0.1 M sodium acetate (35:65 v/v), and the pH was adjusted to 6.2 with glacial acetic acid. The flow rate was 2 ml/min. The accuracy of the method was tested according to recommendations [33]. The reproducibility of the method was investigated by the repeated analysis ($n = 6$) of plasma samples spiked with ibuprofen at concentrations of 0.1 and 20 $\mu\text{g/ml}$. The coefficient of variation of intra-assay variability was 2% or less. The detection limit was 0.05 $\mu\text{g/ml}$.

2.3.3. Pharmacokinetic parameters and statistics

The pharmacokinetic parameters assessed from the epidural and intrathecal microdialysis samples and the plasma samples were the maximum epidural dialysate, intrathecal dialysate and plasma concentration (C_{max}), as well as the time of peak epidural dialysate, intrathecal dialysate and plasma concentration (t_{max}), the area under the concentration-time curve from time zero to infinity ($\text{AUC}_{0-\infty}$), the area under the concentration-time curve

from time zero to the last measured point (AUC_{0-t}), the apparent elimination half-life ($t_{1/2}$), and the mean residence time (MRT). The analyses were performed with PK Solution 2.0 software (Summit Research Services, Montrose, Colorado, USA). The measured C_{max} and t_{max} values were used as such. The AUC and MRT values were calculated according to the trapezoidal method without logarithmic transformation.

The results are expressed as means \pm SEM. Statistical analysis was performed. Intergroup comparisons for all data were performed using analysis of variance (ANOVA). t_{max} values have been analyzed by Mann-Whitney U test. For all analyses, differences were considered statistically significant at a value of $p < 0.05$.

3. Results

3.1. Epidural space pharmacokinetics

The ibuprofen concentrations after the poloxamer gel injection were much lower but lasted much longer as compared with the solution injection (Fig. 1). The epidural AUC values were significantly higher after epidural gel injection of ibuprofen compared with the corresponding solution injection (Table 1). The maximum epidural concentration of ibuprofen (1189 $\mu\text{g/ml}$) was reached 5 min after the epidural injection of ibuprofen solution, while the C_{max} (614 $\mu\text{g/ml}$) after the gel injection was reached in 34 min. The MRT and $t_{1/2}$ values were significantly ($p < 0.001$) longer after the gel injection than after the solution injection.

3.2. Intrathecal space pharmacokinetics

The difference in the intrathecal concentrations of ibuprofen after the epidural gel injection compared with epidural solution

Table 1

Epidural pharmacokinetic parameters after epidural injection of 40 mg dose of ibuprofen poloxamer gel ($n = 6$) or ibuprofen solution ($n = 6$). Means \pm SEM are presented.

Parameter	Gel	Solution
$\text{AUC}_{0-\infty}$ ($\mu\text{g/ml}\cdot\text{min}^{-1}$)	12572 \pm 7860***	3588 \pm 711
AUC_{0-t} ($\mu\text{g/ml}\cdot\text{min}^{-1}$)	9832 \pm 5612**	3530 \pm 707
C_{max} ($\mu\text{g/ml}$) ^a	614 \pm 271**	1189 \pm 286
t_{max} (min) ^a	34.1 \pm 18.5**	5.1 \pm 0.1
MRT (min)	209 \pm 43***	38.2 \pm 7.4
$t_{1/2}$ (min)	132 \pm 78***	31.2 \pm 6.1

Significant difference * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ compared with epidural solution.

^a The C_{max} and t_{max} are apparent values.

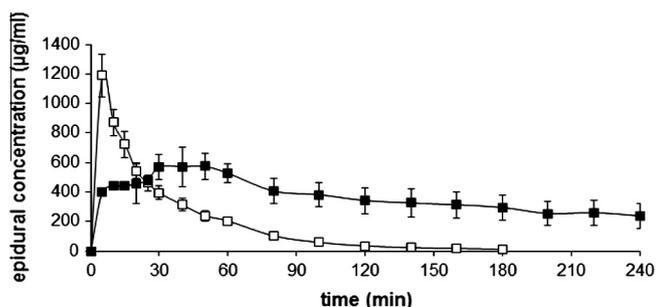


Fig. 1. Mean epidural dialysis concentrations of ibuprofen in pigs after epidural injection of 40 mg ibuprofen in poloxamer gel or solution. Means \pm SEM are presented. Symbols: ■ gel ($n = 6$), □ solution ($n = 6$).

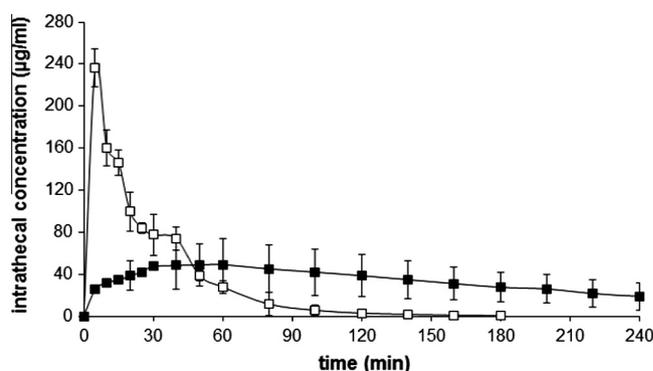


Fig. 2. Mean intrathecal dialysis concentrations of ibuprofen in pigs after epidural injection of 40 mg ibuprofen in poloxamer gel or solution. Means \pm SEM are presented. Symbols: ■ gel ($n = 6$), □ solution ($n = 6$).

injection is depicted in Fig. 2. After epidural gel injection intrathecal C_{max} (45 $\mu\text{g/ml}$) was reached in 55 min while after solution injection C_{max} (238 $\mu\text{g/ml}$) occurred after 11 min (Table 2). The MRT of ibuprofen increased significantly ($p < 0.001$) after epidural injection of the gel (131 min) as compared with the solution (43 min). The elimination $t_{1/2}$ time for ibuprofen was also significantly ($p < 0.001$) longer after the gel injection compared with the solution.

3.3. Systemic absorption

The epidural injection of ibuprofen gel prolonged the absorption of ibuprofen into blood compared with the epidural solution injection of ibuprofen (Fig. 3). The maximum plasma ibuprofen concentration (C_{max} 2.3 $\mu\text{g/ml}$) after the epidural gel injection was reached at about 1 h, while after solution injection C_{max} (9.8 $\mu\text{g/ml}$) was reached already 35 min earlier (Table 3). The MRT and $t_{1/2}$ values were significantly larger after the gel injection compared with the solution. Furthermore, the AUC values were significantly smaller ($p < 0.001$) after epidural gel injection

Table 2

Intrathecal pharmacokinetic parameters after epidural injection of 40 mg dose of ibuprofen poloxamer gel ($n = 6$) or ibuprofen solution ($n = 6$). Means \pm SEM are presented.

Parameter	Gel	Solution
$AUC_{0-\infty}$ ($\mu\text{g/ml}\cdot\text{min}^{-1}$)	3850 \pm 2766***	647 \pm 514
AUC_{0-t} ($\mu\text{g/ml}\cdot\text{min}^{-1}$)	3516 \pm 2644***	645 \pm 512
C_{max} ($\mu\text{g/ml}$) ^a	45.9 \pm 17.7**	238 \pm 208
t_{max} (min) ^a	55.0 \pm 5**	11.1 \pm 5.8
MRT (min)	131 \pm 33***	43.8 \pm 24.7
$t_{1/2}$ (min)	86.8 \pm 51**	31.1 \pm 7.4

Significant difference * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ compared with epidural solution.

^a The C_{max} and t_{max} are apparent values.

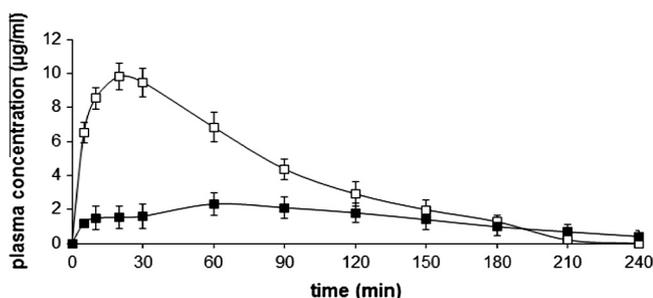


Fig. 3. Mean plasma concentrations of ibuprofen in pigs after epidural injection of 40 mg ibuprofen in poloxamer gel or solution. Means \pm SEM are presented. Symbols: ■ gel ($n = 6$), □ solution ($n = 6$).

Table 3

Plasma pharmacokinetic parameters after epidural injection of 40 mg dose of ibuprofen poloxamer gel ($n = 6$) or ibuprofen solution ($n = 6$). Means \pm SEM are presented.

Parameter	Gel	Solution
$AUC_{0-\infty}$ ($\mu\text{g/ml}\cdot\text{min}^{-1}$)	622 \pm 426***	1121 \pm 382
AUC_{0-t} ($\mu\text{g/ml}\cdot\text{min}^{-1}$)	604 \pm 284**	942 \pm 368
C_{max} ($\mu\text{g/ml}$) ^a	2.34 \pm 1.15**	9.8 \pm 2.3
t_{max} (min) ^a	58.2 \pm 14.7**	24.2 \pm 4.8
MRT (min)	45.9 \pm 10.4***	31.0 \pm 31
$t_{1/2}$ (min)	23.0 \pm 9.1***	19.1 \pm 34.9

Significant difference * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ compared with epidural solution.

^a The C_{max} and t_{max} are apparent values.

(604 $\mu\text{g/ml}\cdot\text{min}^{-1}$) compared with the epidural solution injection (942 $\mu\text{g/ml}\cdot\text{min}^{-1}$).

4. Discussion

4.1. Epidural and intrathecal concentrations

An important finding was that the intrathecal AUC of ibuprofen was significantly larger after epidural poloxamer gel injection compared with the epidural solution injection (Tables 1 and 2). The ibuprofen concentrations in the epidural and intrathecal spaces remained at a steady-state level in the poloxamer gel group for a long time (Figs. 1 and 2). The cold 25% poloxamer gel injected into the epidural space rapidly forms a soft but compact 2–3 cm wide structure of poloxamer gel at and around the epidural injection site [28]. Ibuprofen is an amphiphilic molecule containing both hydrophilic and hydrophobic regions [35,36], and it forms micelles in solutions [37]. The micelle forming capacity of ibuprofen in solutions probably decreases the surface tension at the dural membrane interface thus speeding up ibuprofen molecule diffusion. Interestingly the epidural C_{max} of ibuprofen after ibuprofen solution administration was lower than expected based on the dose administered. Due to ibuprofen's lipophilic nature, part of the dose may have rapidly distributed into epidural tissues, and into epidural fat in particular. The lipophilicity of the epidurally administered drug may have a significant impact on the spinal kinetics through uptake in epidural tissues, in particular epidural fat [38]. The more lipophilic the local anesthetics the more drug has been shown to be absorbed in epidural fat [39–41]. Theoretical lipophilicity of ibuprofen (logP 4.0) is near to that of the commonly used epidural anesthetic bupivacaine (logP 3.4) and analgesic sufentanil (logP 3.5). The pK_a of ibuprofen is low, i.e., 3.5 [42], which suggests strong ionization at physiological pH. Therefore, ibuprofen in the epidural space fairly poorly penetrates membranes, unlike more lipophilic drugs, e.g. local anesthetics and morphine [38]. On the other hand, because of a carboxyl group in the molecular structure, ibuprofen is able to form hydrogen bonds with various epidural tissue structural components, which also can lead to a reduced amount of ibuprofen in the epidural space. The epidural AUC of ibuprofen was, however, over threefold larger after epidural ibuprofen gel injection compared with the ibuprofen solution injection ($p < 0.001$) suggesting that the bioavailability of ibuprofen in the CSF was improved by 25% poloxamer gel.

Based on the rate parameter results (MRT, t_{max} and C_{max}) of epidural and intrathecal samples ibuprofen diffused steadily from the gel in the epidural space through the spinal membranes into the intrathecal space and CSF. It remains to be shown whether such intrathecal concentrations are able to relieve pain associated with e.g. surgery. In the rat paw formalin test Malmberg and Yaksh [43] found that a spinal analgesic effect could be achieved with about only a one hundredth part of an effective systemic dose of variety of analgesics, including ibuprofen.

4.2. Systemic absorption after epidural injection

Rapid systemic absorption or extensive spread in the CSF of drug molecules after epidural administration, is the most common reason of unwanted adverse-effects of epidurally used drugs. The physicochemical properties of diffusing molecules are important in determining the permeation of molecules through spinal meninges and subsequent distribution into blood, CSF and spinal tissues [41]. As seen in the present study, injection of ibuprofen solution resulted in initially very high epidural and intrathecal concentrations (Figs. 1 and 2). This was followed by a relatively rapid concentration decline, and a concomitant rise in ibuprofen

plasma concentrations (Fig. 3). Poloxamer gel reduced and slowed the systemic absorption of ibuprofen. Although plasma levels of ibuprofen after epidural gel administration were very low, and clearly lower than that ($7 \mu\text{g/ml}$) was found to reduce pain in man [44], constant systemic absorption into blood circulation from spinal area was observed for 3–4 h. The vascular network in the epidural space and of the meninges are potential sites for any drug to be cleared into the systemic circulation before they reach the spinal cord [45].

4.3. Poloxamer gel effects on epidural ibuprofen kinetics

Drug movement through the spinal meninges is diffusion, and in *in vitro* studies evidence of active transport or facilitated diffusion of drug molecules has not been seen [46]. After injection of the ibuprofen solution, fast diffusion through the epidural membrane seems to correlate with fast redistribution into the plasma and decreased efficacy at the spinal site. Based on the present study, it can be concluded, that poloxamer gel can improve utilization and therapeutic index of epidurally administered ibuprofen. The gel can permit more accurate targeting of drug molecules to the specific spinal cord segments responsible for pain because it prevents wide spread of drug molecules within the epidural space, which occurs with injected solutions. The amount of ibuprofen entering the intrathecal space was sixfold higher after ibuprofen gel compared with solution injection (Tables 1 and 2), and the escape from epidural space to plasma was significantly reduced (Table 3). This is in line with and complements our previous *in vitro* *dura mater* permeability findings in which ibuprofen permeability through porcine *dura mater* membrane after solution injection was fivefold higher compared to that after poloxamer gel; apparent permeability coefficients being $35.5 \times 10^{-6} \text{ cm/s}$ and $7.3 \times 10^{-6} \text{ cm/s}$, respectively [27]. *In vitro* permeability of ibuprofen through *dura mater* in case of solution was in the beginning linear as a function of time and continued to be linear as a function of square root of time from 60 min forward. In contrast, the cumulative amount of ibuprofen permeated *in vitro* through *dura mater* membrane from the gel as a function of time was linear [27]. However, *in vivo* the tight junctions of *arachnoid mater* seem to form a strong functional barrier to diffusion in the meninges which thus can decrease the *in vitro* observed permeation [46,47].

The innermost structure of poloxamer gel is composed of cubically orientated micellar subunits where the inner part is hydrophobic polypropylene oxide and outer polyethylene oxide part hydrophilic [29,48]. Polyethylene oxide-polypropylene oxide polymers have been found to increase drug concentration levels in brain tissues by producing drug accumulation in selected organs and even overcome blood-brain-barriers intrinsic drug resistance [49,50]. The intrathecal dialysis concentrations show that 25% poloxamer gel, in addition to being a diffusion targeting mechanical gel matrix, could act as an ibuprofen absorption increasing system on the epidural membrane interface.

Although the fate of drugs given epidurally is complicated, the amount of drug transferred through the dural membrane from the epidural space and entering the intrathecal space determines the anesthetic effect [45,51]. Epidural and intrathecal elimination of ibuprofen after epidural gel administration (Figs. 1 and 2) was linear as a function of time, while the elimination after the solution was more of first order. The poloxamer gel was able to reduce peak concentrations of ibuprofen and increase its total exposure over time. Based on our results 25% poloxamer gel could ensure a constant ibuprofen concentration in the spinal nervous tissue and a prolonged duration of analgesic action, i.e. pain-suppressing activity. While the redistribution of ibuprofen from epidural space to blood circulation is minimized by the gel, ibuprofen entry into the central neuroaxis will be enhanced.

4.4. Safety of poloxamer

Poloxamer is compatible with many drugs and forms *in situ* a controlled release system increasing residence time of the drug at the injection site. Many gels have limitations in terms of strength, e.g. pore size of collagen gels is often too large to control diffusion [52,53]. The properties of poloxamer gel still seem very promising for injectable controlled release use, and therefore meaningful to be studied further. The aqueous systems of poloxamers 407 are smart, temperature stimuli-responsive chemically and physically reducible and stable systems [26,54,55]. Poloxamer 407 is generally classified as an inactive molecule and based on the available data it can be considered as non-toxic and biocompatible [56]. In rabbits, only very high intraperitoneal doses (140 mg/kg) of some poloxamer types have resulted in lipid metabolism alterations [57].

4.5. Microdialysis and epidural space kinetics

Epidural and intrathecal pharmacokinetics of drugs is a complex area of kinetics. Microdialysis techniques are sensitive methods with which also distribution of drugs in the spinal area can be studied [30,58–61]. Structurally the epidural space is the outermost part of the spinal canal, which is formed by the surrounding vertebrae outside the *dura mater* of the spinal meninges. *Dura mater* and *arachnoid mater* are attached to each other and enclose the *subarachnoid space*, i.e., the intrathecal space, the cerebrospinal fluid, and the spinal cord (Fig. 4). The drug molecules penetrate the dural and arachnoid membranes, in order to get access to the nerve roots and the spinal cord in the subarachnoid space. Four main mechanisms in drug delivery from epidural space are competing, i.e., diffusion through the spinal meninges, systemic absorption, distribution into the epidural fat and diffusion through dural nerve root cuffs [47,62,63]. Of these the amount of drugs diffusing across the spinal meninges is the most important factor affecting drug delivery into the spinal cord nerve tissue and thus determining the analgesic effect after epidural administration. Microdialysis makes it possible to measure ibuprofen concentrations in the fluid-free epidural space and the intrathecal space without altering the dynamics of CSF.

4.6. The limitations of describing epidural drug behavior and spinal drug delivery with microdialysis

It is not ethical or possible to study spinal distribution of drugs *in vivo* in humans during formulation development. Microdialysis allows continuous sampling of spinal region after epidural ibuprofen administration and the true pharmacokinetics of the epidural space can be evaluated. There are some minor differences in spinal

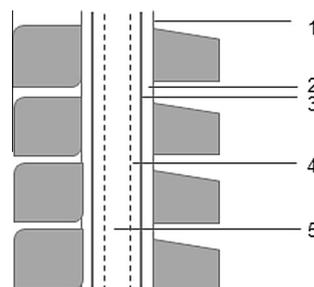


Fig. 4. Schematic cross-sectional illustration of the spinal canal inside the vertebral canal of the spine. 1. *Ligamentum flavum*, 2. Epidural space, 3. *Dura mater* with attached *arachnoid mater*, 4. Intrathecal space, i.e., subarachnoid space with cerebrospinal fluid (CSF), 5. The spinal cord and CSF.

anatomy between pigs and humans, e.g., in caudal extension of the spinal cord. However, the background literature data of pigs and their use in central nervous system studies indicate that regarding anatomy, physiology and pharmacology pigs very closely resemble those of humans [64,65]. The possible ibuprofen absorption in the epidural fat remained as an uncontrollable variable in this study design, but because of minuscule epidural fat amount in the young animals ibuprofen absorption into plasma could be considered as the most prominent epidural bioavailability reducing factor. The microdialysis method has been used successfully previously for other commonly epidurally used drugs such as bupivacaine and opioids [30,31]. It is a reproducible and applicable method.

5. Conclusions

The present microdialysis study indicated that the relative bioavailability of ibuprofen in the CSF was increased after epidural injection of the 25% poloxamer 407 gel. The poloxamer gel permitted accurate and effective targeting of ibuprofen to specific spinal cord segments. It prevented a wide spread of the drug within the epidural space and allowed controlled drug delivery from the epidural space into the intrathecal space. It also seemed to diminish the redistribution of ibuprofen to extraspinal sites, including plasma. In addition to being a diffusion targeting mechanical gel matrix, poloxamer gel seemed act as an ibuprofen epidural permeation enhancing system.

Acknowledgments

The grants from the Academy of Finland and Alfred Kordelin Foundation (Finland) for the study of A.P. at the University of Washington (Seattle, USA) are greatly acknowledged. The authors wish to thank Prof. Janne Backman (University of Helsinki) for the statistical review of the data. The authors declare that there are no conflict of interests in this study.

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