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Biopharmaceutics of intrathecal baclofen-loaded microparticles in a goat model

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Abstract

The goal of this study was to develop a goat model allowing reliable pharmacokinetic (PK) studies of intrathecal baclofen (ITB) sustained release dosage forms using an implanted silicone catheter. ITB PK parameters (clearance, volume of distribution) following intrathecal bolus injection were determined for doses ranging from 100 to 560 μg and a comparison to human data was made. Baclofen-loaded microparticles were then implanted in the intrathecal space of goats and the resulting baclofen levels were determined during 28 days. Finally, PK parameters were used to predict cerebrospinal fluid (CSF) baclofen rates from in vitro release profiles of baclofen-loaded microspheres.

The catheter was well tolerated and did not interfere with behavioral testings. Baclofen CSF clearance (mean = 8.59 ± 2.43 ml/h) and volume of distribution (21.06 ± 13.32 ml) were not significantly affected by the increase of the dose ($p > 0.05$). In vivo, the baclofen levels in CSF were stabilized at 200 μg/l after a period of 3 days. The predictive value of the in vitro release studies was good since the theoretical levels ranged between 128 and 257 μg/l. In conclusion, a large animal model was developed and allowed the biopharmaceutic evaluation of baclofen microparticles injected via intrathecal route.

Keywords: Drug delivery; Baclofen; Pharmacokinetics; Goat; Microspheres; Sustained release dosage form

1. Introduction

Since almost 20 years, intrathecal baclofen (ITB) chronic injection, using implanted pumps and catheters, remains the reference conservative surgical

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treatment of severe spasticity from medullar and cerebral origin (Penn and Kroin, 1985, 1987; Lazorthes et al., 1990; Albright et al., 1991, 1993; Coffey et al., 1993; Meythaler et al., 2001). Spastic hypertonia consecutive to spinal cord injury (approximately 250,000 persons afflicted worldwide) was severe enough to have warranted treatment in 46% of patients in an epidemiological multicentric study (Maynard et al., 1990). Spasticity is present in more than half of the 750,000 people suffering from cerebral palsy (Albright et al., 1993). From 1984 to end of 2002, ITB was evaluated in more than 650 patients in 76 published studies (Emery, 2003). Other therapeutic applications of ITB, such as central deafferentation pain treatment (Taira et al., 1995; Becker et al., 2000), generalized dystonia (Albright et al., 2001) or Stiff-man syndrome (Stayer et al., 1997) have been investigated in the last 10 years. All of these present and future applications of chronic intrathecal baclofen infusion make this drug a very valuable tool in pain or neuromuscular disorder treatments. Unfortunately, chronic intrathecal injection has some drawbacks: it is an expensive technique (Postma et al., 1999) thus limiting the number of treated patients; moreover, the risk of infection or catheter dysfunction can be significant (Levin and Sperling, 1995; Coffey and Burchiel, 2002; Emery, 2003). In consequence, it would be interesting to develop a baclofen sustained release dosage form displaying an enhanced security profile associated with economical benefits. Over all the pharmaceutical dosage forms compatible with intrathecal injection, it appears that only biodegradable microparticles or implants are able to provide a sustained release of a drug longer than a couple of weeks (Lagarce and Benoit, 2004). Baclofen-loaded microspheres, designed for intrathecal drug delivery, have been recently studied by our group (Menei et al., 1998; Cruaud et al., 1999; Lagarce, 2004). The preparation and tolerance of baclofen poly(lactide-co-glycolide) (PLGA) microspheres have been described and the sustained pharmacological activity over a rabbit model based on electromyographical measurements has been assessed (Lagarce et al., 2005).

The evaluation of performance and security of these microparticles in a large animal model was then needed. Indeed, large animal models should be preferred over small rodents to perform pharmacokinetic studies after spinal drug delivery (Yaksh and Malkmus, 1999) because they are often claimed as more predictable of human data. In fact, the morphology of the intrathecal space is a determinant parameter with respect to the fate of a drug injected intrathecally. Sheep (Castro and Eisenach, 1989; Eisenach et al., 1994; Payne et al., 1996; Ochs et al., 1998), pigs (Ummenhofer et al., 2000; Bernards et al., 2003), dogs (Drenger et al., 1993; Sabbe et al., 1993; Yaksh et al., 1997) and non-human primates (Dhiri et al., 1987; Heideman et al., 1993; Egorin et al., 2002) have been used for pharmacokinetic or toxicologic studies of intrathecally injected drugs. European adult goats (*Capra hircus* var. saanen) were used for opioids pharmacokinetic studies (Andersen et al., 1986; Larsen et al., 1986). Goats fulfil the main criteria proposed by Yaksh and Malkmus (1999) to consider large animal species for preclinical studies of spinal drug delivery: their husbandry is well understood and their behavior is adapted to spinal studies, moreover the regulatory requirements and costs are compatible with large scale preclinical studies. To our knowledge, no pharmacokinetic data related to pharmacokinetics of intrathecal baclofen in large animal models is described in the literature.

The purposes of the current investigations were thus: (1) to develop a goat model for pharmacokinetic studies of sustained release baclofen dosage forms, (2) to evaluate intrathecally injected baclofen microparticles using this model and (3) finally to compare these results with theoretical data modelized from in vitro release assays.

2. Materials and methods

2.1. Animals

Goats were handled and cared for in accordance with the European directive no. 86/609. Animals, given free access to water and hay, were housed separately but in a way they could keep a visual and auditory contact at each other. The experimental protocol was carried out in compliance with French regulations and with local ethical committee guidelines for animal research. Fifteen adult Saanen goats (50–75 kg) were used for this study, in accordance with protocols approved by the local ethic committee of the University of Nantes, France. IT catheters were implanted in 11 animals, 3 other animals were used for pharmacokinetic study after IV bolus injection and one animal was sham

operated. Before surgery, animal health was checked by a veterinarian.

2.2. Drugs

For the anesthesia, subcutaneous morphine hydrochloride (Aguettant, Lyon, France), intravenous ketamine 1% (Clorketam®, Vetoquinol, Lure, France), xylazine 2% (Rompun®, Bayer, Puteaux, France), Halothane® (Belamont, Paris, France), and intrathecal lidocaïne 1% (Xylocaïne®, Astra, Rueil-Malmaison, France) were used. During the first 4 days after surgery, marbofloxacine 10% (Marbocyl®, Vetoquinol, Lure, France) was injected intramuscularly to prevent infections. Baclofen concentrated sterile solution 2 mg/ml (Lioresal®, Novartis, Rueil-Malmaison, France) was diluted in sterile 0.9% saline to solutions containing 50, 100, 140, 200, and 280 μg/ml baclofen. The dilution procedure was performed in aseptic conditions under laminar sterile flow. For euthanasia 20% pentobarbital sodium (Dolethal®, Vetoquinol, Lure, France) was used.

2.3. Catheterization material

Silicone catheters were kindly given by Medtronic, Inc. (Boulogne-Billancourt, France) within intrathecal sets (model Indura®, ref. 8709) containing a 15G Tuohy needle, a catheter guide and silicone anchors. A Luer connection was made from a sterile needle: the tip of a 21G needle was cut with a file, the remaining part i.e. the female Luer connection and 1.5 cm of the filed needle was cleaned and decontaminated with 70% alcohol.

2.4. Surgical procedure

The goat was weighed and a 20 cm × 30 cm area was shaved on its back from the sacrum to the third lumbar vertebrae. Diluted morphine (0.1 mg/kg) was injected subcutaneously. After 10 min, a catheter was inserted in the jugular vein and a mixture of 2 mg/kg ketamine with 0.1 mg/kg xylazine was injected. The animal was then intubated with an endotracheal tube (size 8.0) using a laryngoscope. After inflation of the cuff, the tube was connected to a Halothane®/Oxygen source. A gastric tube was introduced to avoid entry of rumen juice in the trachea. The goat was laid on its left side; the shaved area was decontaminated with alcohol and Povidone iodine 10%. The legs of the animal were tied together to ensure the rachis was in convex position. A 1 cm skin incision was made on the space between L4 and L5. A Tuohy needle containing the lumen guard was inserted slowly, perpendicularly to the spinal cord. Then, 1 ml lidocaïne was injected directly through the Tuohy needle, into the intrathecal space. After 5 min, the catheter was inserted slowly through the lumen of the needle and thus guided into the IT space along 12 cm. A catheter length of 15 cm was left outside the animal. The CSF flowed directly at the outer tip of the catheter indicating its correct position. The exact location of the catheter was checked under radiographic control. In any doubt, 10 ml of contrast agent (Iopamiron® 370, Schering SA, Lys-lez-Lannoy, France) were injected via the catheter and a second radiographic control was performed. Finally, the Tuohy needle was withdrawn leaving the catheter in place, the Luer customized connection was adapted at the outer tip and a stopper was screwed. The catheter was then sutured to the skin at different spots. A rectangular sheet of Elastoplaste® (Smith & Nephew, Le Mans, France) was sutured to the skin with non-biodegradable sutures to protect the catheter on the back of the animal. A window made in the Elastoplaste® allowed access to the outer tip of the catheter. A prophylactic antibiotic treatment was then injected intramuscularly each day during 4 days after surgery at the dose of 2 mg/kg marbofloxacine 10%. The entire procedure varied from 30 to 90 min.

2.5. Catheter tolerance

The tolerance of the catheter was assessed by comparing the behavior of the goat before and after implantation. The ability to walk and run on a 100 m distance was checked. The gait was observed in order to detect possible motor or coordination deficits. The posture and general behavior were observed regularly by personal accustomed to goat husbandry. Any sign of pain or disturbance was sought and noted when observed.

In order to check the tissue reaction after catheter implantation, five animals were used for histological studies: one animal was sham operated (needle insertion in the subarachnoid space without catheter implantation) and sacrificed after 24 h, another one was sacrificed at day 1, one at day 6 and two others at day 28 after catheter implantation. Euthanasia was

performed by a veterinarian with intravenous injection of 30 ml 20% pentobarbital sodium. Extemporary, spines were harvested and fixed in 10% formalin during 1 week. The spines were then stained with hematoxylin pholcodin safran and observed under optical microscopy.

2.6. Baclofen microparticles

The preparation and characterization of the microparticles used in this study was precisely described and discussed elsewhere (Lagarce et al., 2005). Briefly, microspheres were prepared by the emulsion extraction methods, using ethyl acetate as the dispersed solvent. Baclofen crystals were dispersed in an organic phase containing the 85/15 PLGA polymer. An emulsion was made under vigorous stirring between this organic phase and a 5% (w/w) poly(vinyl-alcohol) aqueous solution. The microparticles were obtained after the extraction of ethyl acetate by a large amount of water. One batch of microparticles previously prepared and characterized was used for all animal experiments described in this study. Drug loading was 10.9% (w/w) and mean volumetric size was 30.05 ± 1.76 μm. The release of the drug, performed with a continuous flow system (Lagarce et al., 2005), was sustained during 174 days in vitro with a burst effect of 16% after the first 24 h and a mean release of 530 ng of baclofen per day and per mg of microspheres from the third day to the end of the release period as shown in Fig. 1.

2.7. Pharmacokinetic and behavior studies

In order to determine the reference parameters of baclofen pharmacokinetics after intrathecal or intravenous injection in goats, two studies were performed: in three goats after IT bolus injection (five doses of 100, 200, 280, 400 and 560 μg in duplicate) and in three other goats after IV bolus injection (one dose of 10 mg in triplicate).

2.7.1. Spinal pharmacokinetic studies

Spinal pharmacokinetic studies have been initiated 24 h after the implantation of the catheter. Firstly, 400 μl reference CSF were sampled. The aspect of the CSF was checked and 100 μl were kept for analysis. Secondly, a 0.22 μm filter (Minisart, Sartorius) was mounted on the Luer connection of the catheter. Then, 2 ml of baclofen solution were injected in 1 min, according to the concentration of the solutions checked by LC/MSMS after dilution; the injected doses were 100, 200, 280, 400 and 560 μg. The same goat received different doses with a minimum 2 days interval between injections. Finally, the remaining 300 μl CSF sampled

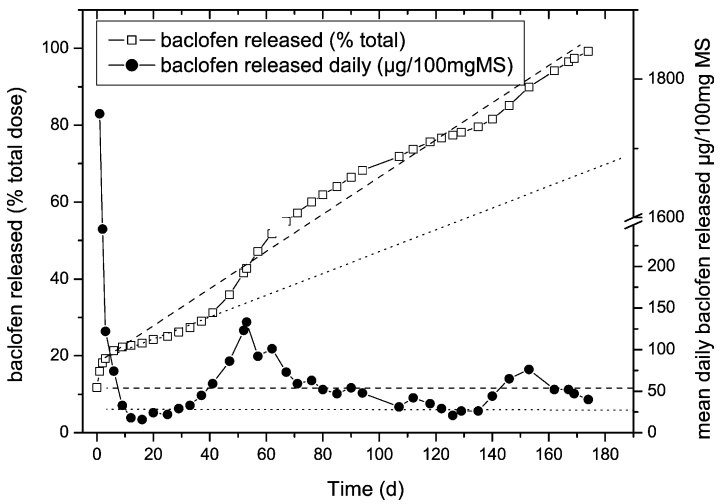

Fig. 1. Cumulated release (open symbol) and daily release (close symbol) after in vitro release of baclofen from 10.9% loaded microspheres in a continuous flow apparatus. Dash lines and dot lines represent linearization between days 3 and 174 ($a = 0.53$ μg/mg/day, $r^2 = 0.98$) or between days 3 and 28 ($a = 0.26$ μg/mg/day, $r^2 = 0.96$), respectively.

previous to baclofen injection were injected to flush the dead volume of the catheter which was between 60 and 80 μl. The filter was taken off and CSF was sampled at regular times: 15, 30, 45, 60, 120, 180, 240, 300, 360, 420 and 1440 min. The sampling procedure consisted in rejecting the first 150 μl sampled and keeping the last 200 μl for analysis, thus 350 μl were sampled for each time point. The samples were kept at −20 °C prior to analysis. During the whole study the behavior of the goat was checked. The animal gait was observed, the time when the animal begun to remain seated was noted. In that latter case, help to stand the animal was given every 10 min. If, after stimulation and help, the animal was unable to stand alone, hind limb paralysis status was mentioned.

2.7.2. Pharmacokinetic parameters after IV injection

To determine the reference pharmacokinetic parameters after intravenous injections, three goats received 10 mg baclofen IV and blood samples were taken during 24 h at 15, 30, 60, 120, 180, 240, 300, 600, 720 and 1440 min. The blood samples were centrifuged and plasmas were kept at −20 °C prior to analysis.

2.7.3. Pharmacokinetic parameter determination

The pharmacokinetic parameters were determined with Kinetica 4.2 software (InnaPhase Corp, Philadelphia, PA, USA) using non-compartmental analysis: area under plasma or CSF concentration versus time curve (AUC) was determined from trapezoidal rule, then clearance (CL) was determined from the ratio between baclofen injected dose and AUC; mean retention time (MRT) was determined from the ratio of area under the first moment versus time curve (AUMC) and AUC. The apparent volume of distribution of baclofen in the intrathecal space (V_d) was determined by the ratio of (dose × MRT)/AUC. For all calculations, AUC total was considered, i.e., the sum of AUC determined using all data (AUC_{last}) and the extrapolated AUC (AUC_{extra}). AUC_{extra} was calculated from the elimination rate constant (L_z) and the last observed concentration (C_{last}): $AUC_{extra} = C_{last}/L_z$, and was always inferior to 5% of AUC_{last}. L_z was calculated by linearization over the three last data point for each experiment assuming a first-order elimination from 600 min to ∞.

2.7.4. Follow-up after microparticles intrathecal implantation

After the determination of the PK parameters following intrathecal or intravenous injection of baclofen solutions, three other goats received 100 mg of 10.9% baclofen-loaded microparticles. Intrathecal implantation of microspheres was performed during the catheter insertion surgical procedure. Microparticles were dispersed in 2 ml of 1% carboxymethyl cellulose and implanted in the intrathecal space, using the Tuohy needle just before the catheter insertion step. CSF was sampled as previously described, at day 1, 2, 3, 6, 9, 13, 17, 22 and 28, and the behavior of the animals was carefully observed during the duration of the experiment.

2.8. Baclofen determination

Baclofen concentration was determined in the CSF by electrospray tandem mass spectrometry. Analysis was performed on a triple quadrupole Quattro Micro mass spectrometer (Waters, St-Quentin-en-Yvelines, France) equipped with an atmospheric ionization source via an ionspray interface. Baclofen was separated on a X-Terra MS C8 5 μm 100 mm × 2.1 mm column (Waters, St-Quentin-en-Yvelines, France). The mobile phase was a mixture of water (60%) and acetonitrile (40%). Solvent flow was set at 250 μl/min and 20 μl sample was injected. To increase specificity, the multiple reaction monitoring (MRM) mode was used: the detection of baclofen was focused on the daughter ion (151.2 amu) coming from the fragmentation in the collision cell of the parent protonated molecular ion of baclofen (214.1 amu). Before analysis, the CSF samples were diluted ten times to limit the perturbation of the salts during the evaporation process. The plasma samples were purified with a solid phase extraction procedure previously described (Flardh and Jacobson, 1999). After a four-time dilution, 50 μl of a 20 μg/l internal standard (KM 08205, Maybrige, Cornwall, UK) solution was added prior to purification on the C18 column (Bond Elut C18 1CC, Varian, Courtaboeuf, France). The purified plasma samples were then treated like the CSF samples but against a specific dosage curve. The limit of quantification corresponding to a peak to noise ratio of 10 associated with an accuracy over 15% was 1 μg/l for CSF samples and 4 μg/l for plasma samples.

2.9. Determination of in vivo theoretical values from the in vitro release study

The determination of the reference PK parameters following intrathecal bolus injection of baclofen made it possible to calculate the theoretical values of the in vivo baclofen rate in the intrathecal space from in vitro release results. Considering that microspheres behaved as a perfusion pump delivering baclofen continuously after the burst effect period, the baclofen CSF concentrations can be calculated from the release rates observed in vitro.

$$C = \frac{K_0}{C_L}(1 - e^{-K_e t})$$

C represents the concentration of baclofen in the CSF, K_0 represents the mean input of baclofen in the intrathecal space, and can be estimated to 0.53 or 0.26 μg/mg/day if one considers linearization over the whole in vitro release profile or only over the plateau between days 3 and 28 as shown in Fig. 1 (Lagarce et al., 2005); C_L represents the mean clearance of baclofen from CSF obtained after the in vivo bolus study and K_e, the constant of elimination of baclofen from CSF, can be calculated from the ratio of the observed clearance and the volume of distribution of baclofen in the CSF.

3. Results and discussion

3.1. Development of the animal model

3.1.1. Catheter tolerance and functionality

The tolerance of the implanted catheter was good: no modification of animal behavior was observed in absence of baclofen. The catheter allowed injection and multiple sampling on the same animal, making intra animal comparison possible. The catheter had a maximum duration of functionality of 28 days. Injections and sampling were performed by a single person, on awake, non-anesthetized animal. For pharmacokinetic studies, the catheter allowed eight sampling time points over 7 h in the same day. The histological studies showed that after 1 day, the aspect of the spinal cord was comparable to the sham-operated animal with polynuclear cells present along the needle pathway and a slight infiltration of macrophages in the dura mater (Fig. 2A). A limited tissue reaction after 6 days and 28 days was detected: dura mater and pia mater had a normal aspect, but a focal thickening of the arachnoid appeared along the catheter subarachnoidian pathway with presence of macrophages (Fig. 2B). No inflammatory granuloma was observed, and the catheter implantation did not lead to arachnoitidis. The limited tissue reaction induced by the implanted catheter was not expected to modify the fate of baclofen injected in the IT space.

3.1.2. Behavioral and pharmacokinetic study after bolus intrathecal injection of baclofen

After injection of ITB, the animal behavior was observed. Signs of baclofen activity were displayed within the whole range of baclofen doses injected intrathecally. The first clinical signs of baclofen activity appeared after 30 min to 1 h: the hind quarter was less tonic and a modification of gait appeared. After 1.5–2 h, the first signs of drowsiness were displayed (from the ITB dose of 400 μg): the goat remained seated. Between 4 and 12 h after injection, the animal became unable to stand up and a reversible paralysis of the hind limbs appeared (for doses of 560 μg). The toxicological profile of lumbar intrathecal baclofen in the goat is similar to the human's concerning the nature of the pharmacological effects observed (hypotonia, drowsiness and paralysis) and their schedules. The first signs of baclofen activity appeared 30–60 min after injection as it was previously observed in rabbits (Kroin et al., 1984; Lagarce et al., 2005) and humans (Penn and Kroin, 1987) and lasted for 12–48 h within the dose range tested. The modification of gait and posture are the two observable clinical signs appearing first and lasting until total recovery. Unconsciousness was not observed within the dose range tested. In fact, the goal of our study was not to assess the whole spectrum of toxicological effects of baclofen in the goat. This is why the study was stopped after the dose of 560 μg injected IT, leading to a hind limb paralysis of 12 h. During the period of paralysis care was taken to give the goat an easy access to food and water, but the animal slowed down its alimentation.

After bolus injection of increased doses of baclofen in the intrathecal space a two-phase decrease of baclofen concentration in CSF was observed (Fig. 3). This data suggested a bi exponential decay, a bi-compartmental model fitted well the data but it was not used for the present study because it needs to be

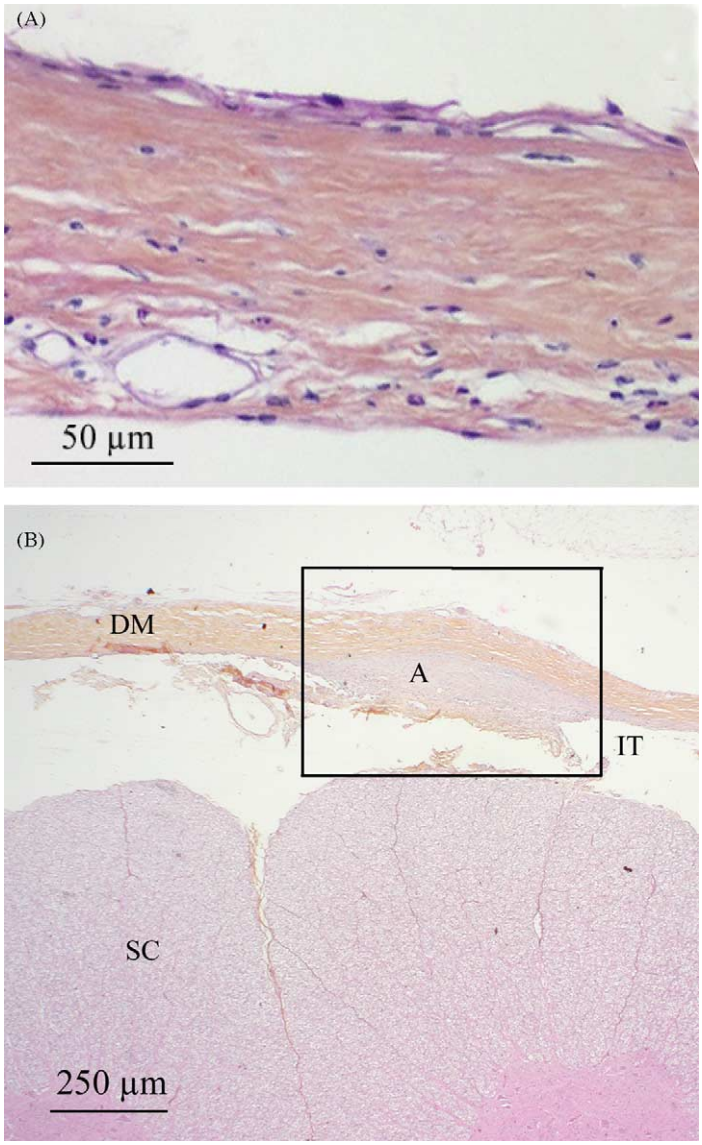

Fig. 2. (A) Infiltration of the dura mater by macrophages observed 24 h after catheter implantation. (B) Histological aspect of the spinal cord and the meninges 28 days after a silicone catheter implantation in the subarachnoid space of a goat. In the square area the thickening of the arachnoid along the catheter route is visible. DM: dura mater, A: arachnoid, IT: intrathecal space, SC: spinal cord.

validated with inclusion of more experimental points in the terminal elimination phase (from 6 to 24 h). The analysis of the obtained CSF concentrations was thus made using non-compartmental modeling: Kinetica 4.2 software allowed us to determine CL, V_d and MRT parameters (Table 1). All the observed concentrations were found above the limit of quantification in CSF by LC/MSMS, but at 24 h the concentrations were in the LOQ range for 100 and 200 µg intrathecal baclofen dose. The pharmacokinetic parameters, calculated using non-compartmental analysis, after 10 mg baclofen IV bolus injection in three goats are also displayed in Table 1. Spearman's rank correlation coefficient showed that no significant linear relationship

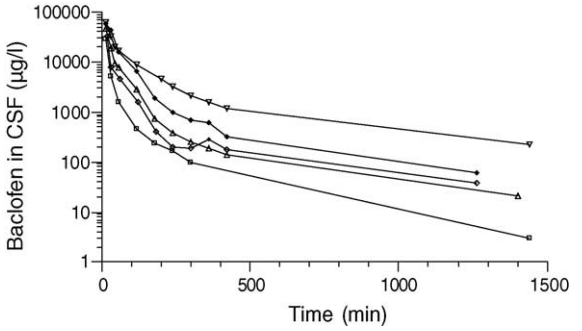

Fig. 3. Baclofen CSF concentration after bolus injection of five different doses ((□) 100 μg, (◊) 200 μg, (△) 280 μg, (♦) 400 μg, (▽) 560 μg of baclofen). Every dose was tested in duplicate, only one dataset is represented for each dose to avoid confusion between data. The limit of quantification of baclofen by LC/MSMS in the CSF was 1 μg/l.

($p > 0.05$) was found between C_L or MRT or V_d and the dose in CSF. The fact that C_L appeared not significantly changed with the dose suggests a constant ratio between dose and AUC i.e. a linear pharmacokinetic process in the dose range tested. This relationship between AUC and the dose was also found in humans after a continuous intrathecal infusion of baclofen: baclofen CSF concentration after reaching the steady state was found proportional to the outflow of perfusion (Müller et al., 1988). The fact that AUC in CSF can be easily connected to the intrathecal dose is important for the usefulness of the model.

3.1.3. Comparison with human data

The relevance of the animal model was checked by comparing the similarity of baclofen elimination between goat and human from CSF and blood (Table 2). The terminal half-lives determined after 10 mg baclofen IV injection in three goats (266 min ± 78) by linearization on the three last data points were similar to the half-lives observed in healthy humans which were between 217 and 400 min (Kochak et al., 1985; Wuis et al., 1985, 1989).

The terminal half-life was not determined in CSF because the last sampling points were close to the LOQ for the two lowest doses of intrathecal baclofen. In this case, the error on linearization may become significant. CSF clearance of baclofen was rather constant for intrathecal doses up to 560 μg. The mean clearance was 8.59 ml/h which was inferior to what has previously been reported in humans (Table 2) (Kroin and Penn, 1991; Sallerin-Caute et al., 1991) for doses between 50 and 137.6 μg. The differences may be due to physiologic particularities: the goat lumbar CSF volume is in fact inferior to what is currently reported in human's (30 versus 160 ml) as is CSF production in goat (0.16 ml/min) versus man (0.35 ml/min) (Artru, 1999). But it is also possible to attribute these discrepancies to the sampling procedure. In fact, in our study the sampled CSF volume was small (350 μl) for each time point, the baclofen extracted from the intrathecal space was thus minimized. This is consistent with a reduced observed clearance of the drug. Moreover,

Table 1
Elimination pharmacokinetic parameters from the cerebrospinal fluid and the blood following IT or IV baclofen injection in goats

Dose (μg)	Route	Sampling	C_L (ml/h)	MRT (h)	V_d (ml)
10000	IV	Jugular	1230 ± 24	4.61 ± 0.96	6147 ± 2300
100	IT	L3	13.68	3.52	48.22
100	IT	L3	8.76	2.77	24.62
200	IT	L3	9.20	1.41	13.04
200	IT	L3	9.23	2.55	23.63
280	IT	L3	6.47	0.88	5.69
280	IT	L3	5.64	1.23	6.94
400	IT	L3	6.36	1.47	9.45
400	IT	L3	9.72	2.08	20.56
560	IT	L3	6.54	3.53	23.3
560	IT	L3	10.26	3.41	35.2
Mean ± S.D.	IT		8.59 ± 2.43	2.28 ± 1.01	21.06 ± 13.32
R (Spearman)	IT		0.30 ($p = 0.39$)	0.21 ($p = 0.55$)	0.02 ($p = 0.95$)

R = Spearman's rank correlation coefficient.

Table 2
Pharmacokinetics parameters determined after intrathecal injection of baclofen in humans (according to the literature) and in goats (present study)

n	Last time point (h)	Dose range (μg)	C_L range (ml/h)	C_L mean (ml/h)	V_d range (ml)	V_d mean (ml)	Reference
4	6–15	75–137.6	13–88	36.2	52–157	119	Sallerin-Caute et al. (1991)
7	3–4	50–100	34.2–77.5	39.2	27.1–169	85.6	Kroin and Penn (1991)
10	24	100–560	5.64–13.68	8.59	5.7–48.2	21.1	Present study

the clearance was calculated by dividing the injected dose by the observed (or extrapolated) AUC. In our study, CSF was sampled during 24 h which was longer in comparison to the reference studies performed in humans (Table 2). This increased the AUC and thus contributed to reduce the calculated clearance. The ratio between baclofen clearance in plasma and in CSF is 143 in the goat (Table 1), this ratio was found in the range of 200–500 in humans (Sallerin and Lazorthes, 2003). The comparison of the pharmacokinetic parameters of our model with human data enlighten some differences (C_L, V_d) but the overall process of baclofen elimination from CSF and from plasma seem very comparable (CLcsf/CLplasma, half life in plasma) which contributes to the relevancy of the model.

3.2. Utilization of the model

3.2.1. Tolerance and baclofen rates in CSF after microsphere implantation

Microparticles (100 mg) containing 10.9% (w/w) baclofen were implanted in the intrathecal space of three goats. The baclofen concentration in CSF was rather constant (around 200 μg/l) after the burst effect lasting the first 3 days (Fig. 4). The ratio between the highest observed concentration and the concentration after equilibration in CSF was 8. The high baclofen CSF concentration were correlated with toxic effect of baclofen. The goats were paralyzed during 4–5 days due to an exaggerated release of baclofen, no period of unconsciousness was observed. After this period the animal behavior returned to normal; no modification of gait and posture was observed after day 6 in the three goats. The comparison of the baclofen concentration in the CSF with human data after pump implantation is difficult because the result is very dependent on the sampling procedure. Indeed, the distance between sampling locus and the injection point has an impact on the observed concentration because of the poor diffusion of baclofen in the CSF: the mean ratio between lumbar to cisternal baclofen in CSF after constant infusion was found to be 4/1 (Kroin and Penn, 1991). In the same study, the observed cisternal concentration of baclofen ranged from 39 to 410 μg/l for daily doses of 100–600 μg. The lumbar concentration ranged from 130 to 1240 μg/l. In our study, the sampling was performed 12 cm from the injection site of the microparticles and the observed concentration after steady state was 200 μg/l. Thus the obtained baclofen levels in CSF were in the therapeutic range. Anyway, as it is not the case with implants, the drug level can be easily adjusted by lowering or increasing the amount of implanted particles. However, this adjustment cannot be modified after injection, which can be done with the electronic implanted pumps; this drawback may limit the application of the microparticles to the cases where baclofen levels have not to be monitored continuously

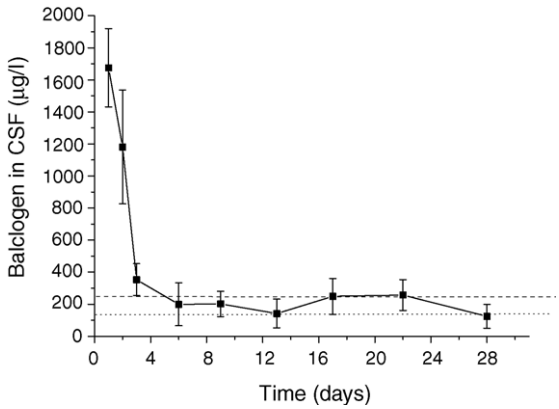

Fig. 4. Baclofen concentration in CSF during 28 days after intrathecal implantation of 100 mg of 10.9% baclofen loaded microparticles in three goats. The theoretical concentrations awaited after in vitro experiments (see text for calculation details) are represented in dot line (linearization over 28 days) and dash line (linearization over 174 days).

with precision i.e. where the best autonomy of the patient is wanted.

3.2.2. Comparison of in vivo data with theoretical data derived from in vitro experiments

In order to verify that the in vitro release rate was predictive of the in vivo level of baclofen in CSF, the in vivo theoretical concentration of baclofen was calculated from the in vitro data and compared to the in vivo level of baclofen in the CSF of the goats.

The determination of mean C_L and V_d after baclofen intrathecal injection allowed us to calculate the theoretical concentration of baclofen in CSF after equilibrium of the system using the following equation:

$$C = \frac{K_0}{C_L}(1 - e^{-K_e t})$$

It was assumed that the microparticles behaved as an infusion pump delivering baclofen continuously with a constant rate over 174 days. If one consider the period from days 3 to 174, the mean baclofen release rate observed in vitro is $K_{0_1} = 2.21$ μg/h ($r^2 = 0.98$) for 100 mg implanted microparticles, this mean release rate falls down to $K_{0_2} = 1.10$ μg/h ($r^2 = 0.96$) if one consider the period between days 3 and 28 (Fig. 1). Three days after the steady state $e^{-K_e t}$ is approaching 0 and C tends to K_0/CL thus $C = 257$ μg/l for K_{0_1} and 128 μg/l for K_{0_2}. These two theoretical concentrations after steady state which were calculated from in vitro data have been compared to the concentrations obtained in vivo with our model after implantation of 100 mg of the same microparticles (Fig. 4). As the in vitro release profile of baclofen from the microspheres is a sigmoidal curve, considering all the data from day 3 (after the burst effect) to day 174 tends to overestimate the mean release rate, this could be a reason why the calculated In vivo concentration (257 μg/l) is slightly over the mean observed in vivo concentration (200 μg/l). Previous studies have shed light on the discrepancies between in vivo and in vitro release behavior of PLGA microparticles, in particularly in the central nervous system (Chen et al., 1997). Here we found a pretty good agreement between our animal model and in vitro continuous flow apparatus. The in vitro/in vivo correlation has now to be fully studied and validated, particularly over different types of microparticles, i.e. different release behavior and over the full range of the release period (174 days).

4. Conclusion

This study allowed us to set-up a goat model designed for intrathecal baclofen biopharmaceutic sustained release studies. Silicone implanted catheters were well tolerated, did not interfere with behavioral testings and allowed us to perform a scaling dose pharmacokinetic analysis and regular sampling during 28 days. The pharmacokinetic parameters determined in the goat for baclofen in blood and CSF were similar to the human ones. Within the 100–560 μg dose range, the AUC was proportional to the dose injected as it is described in humans. However clearance and volume of distribution were found smaller in the goat. The ability of the microparticles to maintain therapeutic levels of baclofen in the intrathecal space for 28 days has been demonstrated. Unfortunately, the catheters were not functional for a longer duration and these sampling systems have to be improved to obtain data over a longer period. The determination of the pharmacokinetic parameters allowed us to determine theoretical in vivo baclofen level derived from in vitro release data. The good agreement between theoretical and observed values in vivo proved the predictive value of the in vitro continuous flow system for this application. This study is a first step for the development of in vivo/in vitro correlation of baclofen microparticles designed for intrathecal injection, thus being a milestone in the development of long term sustained release dosage form of baclofen for the treatment of severe spasticity.

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