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European Journal of Pharmaceutics and Biopharmaceutics 61 (2005) 171–180
European Journal of Pharmaceutics and Biopharmaceutics
www.elsevier.com/locate/ejpb

Research paper

Baclofen-loaded microspheres in gel suspensions for intrathecal drug delivery: In vitro and in vivo evaluation

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Received 17 February 2005; accepted in revised form 18 April 2005
Available online 20 June 2005

Abstract

Severe spasticity is a very disabling disorder treated by continuous baclofen intrathecal infusion which unfortunately remains an expensive and uncomfortable treatment. In order to address these issues, new sustained release formulations designed for intrathecal baclofen delivery were sought with the aim of minimising the burst effect of baclofen which can lead to toxicity.

Baclofen was encapsulated in poly(lactide-co-glycolide) (PLGA) microspheres which were then dispersed in chitosan thermosensitive gels, Pluronic® PF-127 gels, carboxymethylcellulose solutions or Ringer lactate solution. The release rate was assessed in vitro using continuous flow cells and in vivo after intrathecal injection in goats: baclofen was quantified in cerebrospinal fluid (CSF) and plasma, and the associated pharmacological effect was evaluated. The results showed that the burst effect was reduced by at least a factor of 2 in vitro, after microsphere dispersion in viscous media. In vivo, PF-127 gel was found to be the best vehicle to reduce the burst effect by a factor of 10 in CSF, and by a factor of 2 in plasma. The toxic effect of baclofen due to the burst effect was reduced by the dispersion in PF127 gels. Therapeutic levels of baclofen in CSF were maintained during at least 1 month.

Keywords: PLGA microsphere; Baclofen; Burst effect; Polymer gel; Intrathecal administration

1. Introduction

Intrathecal baclofen is the reference treatment for severe spasticity, a chronic affection of spinal or cerebral origin. This drug, as well as morphine, is the most chronically injected drug in the intrathecal space. Nowadays, the only way to continuously administer small drug amounts to the spinal cord over months is to use surgically implanted electronic pumps connected to spinal catheters. The costs of implantation and follow up of these pumps average $US 28,000 per patient [1]. Moreover, the risks of spinal infection or catheter malfunctioning are not insignificant and may lead to an interruption of treatment and toxic effects [2–8]. To solve these issues and to extend the number of patients treated, sustained release dosage forms have been investigated for controlled spinal drug delivery [9]. Among these devices, microspheres and implants are the only dosage forms able to sustain drug delivery over weeks or months, thus greatly limiting the number of injections. For intrathecal drug delivery, microspheres, having a size which allows their injection via a small needle, seem very promising to maximise the safety and comfort for the patient. Microsphere formulations designed for spinal drug delivery have been investigated during the last decade [10]. In most cases, the encapsulated drugs were local anaesthetics [11–13] and their evaluation in vivo was performed after epidural injection

Abbreviations: CMC, carboxymethylcellulose; CSF, cerebrospinal fluid; LC/MSMS, liquid chromatography tandem mass spectrometry; LOD, limit of detection; LOQ, limit of quantification; MS, microspheres; PLGA, poly (lactide-co-glycolide).

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0939-6411/$ - see front matter © 2005 Published by Elsevier B.V.
doi:10.1016/j.ejpb.2005.04.004

over a period no longer than a few days [14]. Baclofen microsphere formulations was studied by our group [15]. The feasibility of injecting these particles in the intrathecal space of rabbits has already been assessed [16]. Recently [17], baclofen microsphere formulation has been improved in order to fulfill industrial and toxicological requirements. The repeatability of the manufacturing process was optimised and methylene chloride was replaced by ethyl acetate as the solvent of the PLGA 85:15 which is a low-degrading, biocompatible material. Moreover, the tolerance and sustained pharmacological effect of these new preparations were confirmed in a rabbit model of baclofen activity [17].

The principal remaining issue is the limiting of the burst effect (16% within the first 24 h at best) that can be a source of toxicity as baclofen unfortunately has a narrow therapeutic index. Many ideas have been proposed to prevent the burst effect of drug delivery systems (for a review see [18]): surface extraction [19], surface coating [20], non-uniform drug loading [21], surface modification [22]. Unfortunately the solutions proposed to limit burst effect involve costly additional steps or are not simple to implement. Instead of modifying the formulation, the idea proposed in the present study was to use thermosensitive gels to disperse the microspheres prior to injection. Indeed, these viscous media are known to sustain drug release during a few days [23]. They have been used to control drug release from liposomes [24]. The question of their ability to normalise the release from microparticulate systems has just very recently been demonstrated for lidocaine encapsulated in microparticles and dispersed in poloxamer gel [25]. In the current investigations, the suspension media were chosen for their biocompatibility and their ability to become highly viscous at 37 °C, but not too viscous at 20 °C, to allow a reliable suspension of the microspheres and injection via a 15G needle into the intrathecal space. Furthermore, suspension media that led to serious swelling after tissue injection were discarded in order to avoid possible tissue damage. Thus, two types of thermosensitive gels were evaluated: (i) thermosensitive neutralised chitosan gel previously studied by A. Chenite [26,27] which have shown sustained release properties for various drugs [28] and (ii) extensively investigated Pluronic® gels, which have been previously used via epidural route to safely sustain the release of ibuprofen or lidocaine [29] or to prevent chronic adhesive arachnoiditis [30].

The goal of the current investigations was first to study the effect of various suspension media on the burst effect in vitro. Then, the most promising gel formulation was selected and pharmacokinetic studies were performed in vivo in a large animal model (goat) and compared to diluted carboxymethyl cellulose solution previously used to suspend microspheres prior to intracerebral implantation [31].

2. Materials and methods

2.1. Materials

Microspheres were prepared using poly (DL lactide-co-glycolide), Medisorb® 85/15, obtained from Alkermes (Cincinnati, Ohio, USA), 88% hydrolysed poly(vinyl alcohol), Rhodoviol® 4/125 from Merck Eurolab (Paris, France), and ethyl acetate reagent grade from Sigma Aldrich (Saint Quentin-Fallavier, France). Baclofen powder was purchased from Heumann (Feucht, Germany). Pluronic® PF-127 (poloxamer 407) was a kind gift from BASF (Ludwigshafen, Germany). Medium weight chitosan (cat Nr C-3646) having a deacetylation degree higher than 85%, came from Sigma Aldrich (Saint Quentin-Fallavier, France).

For animal anaesthesia, subcutaneous morphine chlorhydrate (morphine Aguettant, Lyon, France), intravenous ketamine 1% (Clorketam®, Vetoquinol, Lure, France) and xylazine 2% (Rompun®, Bayer Pharma, Puteaux, France), intratracheal Halothane (Halothane, Belamont, Paris, France), and intrathecal lidocaine 1% (Xylocaine®, Astra, Reuil Malmaison, France) were used. During the first 4 days after surgery, 20 μl/kg marbofloxacine 10% (Marboxyl®, Vetoquinol, Lure, France) were injected intramuscularly to prevent infection.

Silicone catheters were kindly given by Medtronic, Inc. within intrathecal sets (Indura™ 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 inch of the filed needle were cleaned and sterilised with alcohol.

2.2. Microsphere preparation

Microspheres (MS) were prepared using a solvent extraction process previously described [17]. Briefly, Baclofen (100 mg ± 5 mg) was suspended in 4 ml ethyl acetate and homogenised using an Ultra-Turrax® at 22,000 rpm for 2 min. PLGA (400 mg ± 5 mg) was gently dissolved in the baclofen suspension under magnetic stirring at room temperature for 30 min. The resulting organic suspension was then emulsified in a 5% poly (vinyl alcohol) aqueous solution maintained at 4 °C in a 250 ml reactor under paddle stirring at 1000 rpm. Two additional millilitres of ethyl acetate were used to rinse the vial in which the organic suspension was made, and were added to the emulsion. After 2 min, the extraction of ethyl acetate from the dispersed phase of the emulsion was carried on by pouring 100 ml of distilled water in one minute into the emulsion. Finally the extraction was completed by transferring the emulsion into 2 l of water under paddle stirring at 500 rpm. Microspheres were then isolated by filtration under nitrogen pressure using ethyl cellulose membranes (Millipore, Guyancourt, France) with 3 μm pores. Separation was carried out within less than one minute. Finally the microspheres were freeze-dried overnight.

The volume size of the microparticles assessed by Coulter® size analyser (Multisizer, Coultronics, France) was 30.05 ± 1.76 μm [17].

The drug content was determined by LC/MSMS after dissolution of 10 mg MS in 1 ml of chloroform followed by liquid/liquid extraction with 3 ml of water [17].

2.3. Suspension medium preparation

Microspheres were suspended in four types of suspension medium prior to in vitro and in vivo evaluation. In all cases, the concentration of microspheres in the vehicle was fixed to 50 mg/ml. This choice is a compromise between the maximal injectable volume in the intrathecal space (2–5 ml) [10] and the mean intrathecal baclofen dose to treat spasticity (160 μg/day) [32] considering a drug loading of 11% (w/w) in the microparticles and a drug delivery over 3–6 months. If high baclofen dose is desired, concentration of microparticles in the vehicle will have to be enhanced.

The first medium used was Phosphate Buffer Saline pH 7.35 (replaced by sterile Ringer lactate solution from Aguettant, Lyon, France for in vivo studies). The second type of suspension medium was previously used and evaluated for microsphere suspension in the central nervous system [31] and consisted of two aqueous solutions of either 1% or 0.5% (w/v) carboxymethylcellulose (CMC) of low viscosity (Coopérative Pharmaceutique, France), 1% (w/v) polysorbate 80 (Prolabo, Paris, France) and 4% (w/v) mannitol (Coopérative Pharmaceutique, France). This media was sterilised by autoclave at 133 °C during 20 min. The third type of suspension medium was a 1.8% (w/w) thermosensitive chitosan gel, prepared in aseptic conditions, as described previously by Chenite and co-workers [27,28] and consisted of 2.2% chitosan acid solution neutralised by 0.5 ml of a 45% (w/w) α-, β-glycerophosphate solution. The fourth and last type of suspension medium prepared were solutions of increasing quantities (17–25% w/w) of poloxamer 407 (Pluronic® PF-127). These solutions were prepared in aseptic conditions, as previously described [33] by dissolution in sterile water at 2 °C with overnight maturation at 4 °C.

2.4. Viscosimetry of the suspension media

The viscosimetry of the suspension media was studied using a RV1 Couette viscosimeter (RotoVisco® 1, Thermo Haake, Champlan, France), with a Din Ti 41 mobile cylinder allowing viscosimetric measurements from 0.1 mPa s to 100 Pa s. The temperature and the rotation speed of the RV1 viscosimeter were controlled by the software Rheowin Pro 2.96 (Haake). For thermosensitive gels, the experiment was divided in five consecutive steps:

1. temperature equilibration at 11 °C ± 0.1 °C,
2. ramp-up from 1 to 300 s^{-1} rotation speed for 120 s followed by a ramp-down back to 1 s^{-1},
3. at the rotation speed of 50 s^{-1} the temperature was increased in 20 steps from 11 °C to 38.5 °C with an equilibration time of 90 s and five measurements of viscosimetry for each step,
4. temperature equilibration was taken at 37 °C ± 0.1 °C,
5. ramp-up and ramp-down was carried out at 37 °C with the same conditions as the experiment at 11 °C.

This procedure allowed the determination of gelation temperature and the viscosimetric behaviour study at 11 °C and 37 °C.

2.5. In vitro release experiments

In vitro release experiments were performed in triplicate with Sotax® (Basel, Switzerland) continuous-flow cells connected to a IPCN Ismatec® peristaltic pump. To investigate the effect of suspension media on burst effect, a suspension of 100 mg MS was made in a 2 ml suspension medium and injected in a dialysis bag with a 10,000 Da cut-off point. Baclofen powder (11 mg) dispersed in same volume of CMC or PF 127 was used as control. The bag was rapidly inserted in the continuous flow cell and the peristaltic pump was started. All release experiments were carried out in a PBS buffer at pH 7.35 ± 0.05. The pH of the buffer was controlled twice a week during the release procedure. The flow rate of the PBS buffer was set at 85 μl/min as previously performed on free flowing baclofen microparticles [17]. The collected fractions were stored at 4 °C prior to analysis.

2.6. Baclofen determination

Baclofen was determined using liquid chromatography tandem mass spectrometry (LC/MSMS) in PBS buffer following in vitro release experiments and in cerebrospinal fluid (CSF) or plasma after in vivo release experiments. Determination of baclofen was carried out using electrospray tandem mass spectrometry. Analysis was performed on a triple quadrupole Quatro-Micro mass spectrometer (Waters™, St-Quentin-en-Yvelines, France) equipped with an atmospheric ionisation source via an ion spray interface. Baclofen was separated on a Waters™ X-Terra MS C8 5 μm 100 × 2.1 mm column. The mobile phase was a mixture of water (60%) and acetonitrile (40%). Solvent flow was set at 250 μl/min and a 20 μl sample was injected. The quantification was based on the transition 214.1–151.2. 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 using a solid phase extraction procedure previously described [34] after a 4-fold 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). The purified plasma samples were then treated like the CSF samples, but using a specific dosage curve. For CSF

and plasma, the analytical method was validated following the guidelines from the Société Francaise des Sciences et Techniques Pharmaceutiques [35]. In particular, limits of quantification (LOQ), limits of detection (LOD) and linearity were determined for every type of matrix. Linearity was at least demonstrated between 1 μg/l and 1 mg/l for the three matrices (CSF, plasma and PBS buffer). LOQ was found to be 4 μg/l and 1 μg/l in CSF or PBS buffer and corresponded to the smallest amount of baclofen having a signal to noise ratio over 10 and a relative error below 15%.

2.7. Animals

In vivo experiments were performed on adult Saanen Goats weighting 54–80 kg. Animals were handled and cared for in accordance with the European Directive No. 86/609 and the principles of laboratory animals care published by the NIH. The experimental protocol was carried out in compliance with French regulations and with local ethical committee guidelines for animal research. Before surgery, animal health was checked by a veterinary doctor. Animals, given free access to water and hay, were housed separately but in such a way so that they could keep in visual and auditory contact with each other.

2.8. Catheter and microsphere implantation procedures

The animal was weighed and a 20×30 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 using a laryngoscope. Following inflation of the cuff, the tube was connected to a halothane/oxygen source, and a gastric tube was introduced to avoid entry of rumen juices into the trachea. The goat was laid on its left side; the shaved area was decontaminated with alcohol and 10% povidone iodine. The legs of the animal were tied together to ensure that the rachis was in a convex position. A skin incision was made between L4 and L5. A Tuohy needle containing the lumen guard was slowly inserted perpendicularly into the spinal column. One ml lidocaine was then injected directly through the Tuohy needle, into the intrathecal space. After 5 min, a 2 ml microsphere suspension was injected via the needle followed by a 1 ml suspension medium alone to flush the dead volume of the needle. The catheter was then slowly inserted through the lumen of the needle and thus guided in the intrathecal space along 10 cm. A 15 cm long part of the implanted catheter was left hanging out of the animal. The CSF flows directly from the outer tip of the catheter indicating its correct position. Finally, the Tuohy needle was withdrawn leaving the catheter in place. The Luer customised connection was adapted at the outer tip and a stopper was screwed into place. The catheter was then stitched to the skin. A compress was placed on the catheter and a rectangular sheet of Elastoplast® was applied and stitched to the skin with non-biodegradable stitches. A prophylactic antibiotic treatment was then injected intramuscularly each day for 4 days after surgery at the dose of 0.02 ml/kg 10% marbofloxacin. The entire procedure varied from 30 to 90 min.

2.9. In vivo release, behaviour and tolerance assessment

Two series of experiments were performed for in vivo release evaluation: burst effect determination over 2 days and a 4 week follow-up with indwelling catheters. During these experiments, the behaviour and health status of the animals were carefully noted. Gait ability and posture were noted. If hind limb paralysis occurred, its duration was noted. Goat's behaviour after microspheres implantation was compared to a scale defined after increasing baclofen bolus doses injection in order to evaluate the importance of the burst effect by its pharmacological activity.

The goats were divided into four groups: one goat received MS in 0.5% CMC, four goats received MS in 1% CMC, three goats received MS in 21% PF-127 and one goat received only 20% of the reference dose in Ringer lactate suspension. The reference dose of MS injected was 1.5 mg/kg, i.e. 165 μg encapsulated baclofen per kilo. Following the previously described surgical procedure, baclofen-loaded microspheres were suspended in 2 ml of various described carriers as soon as the Tuohy needle was in place and were injected between the fifth and sixth lumbar vertebrae under deep anaesthesia.

CSF was sampled after microsphere implantation using the implanted catheter at 19 and 43 h for burst effect studies and then at days 3, 6, 9, 13, 17, 22 and 28. For each time point, the first 150 μl sample was discarded since it corresponded to twice the dead volume of the catheter. 150 μl was then sampled through a 22 μm filter avoiding the possibility to collect microparticles and kept at −20 °C before LC/MSMS analysis. Thus, a total volume of 300 μl, corresponding to 1% of the CSF goat volume [36], was sampled for each time point.

3. Results and discussion

3.1. In vitro characterisation of microspheres

Five 450 mg batches of baclofen microspheres (MS) were produced using the production method described. The drug loading determined by LC/MSMS analysis after microsphere dissolution and liquid/liquid extraction was $10.8 \pm 0.5\%$ (coefficient of variation 4.6%). The corresponding encapsulation efficiency was then $52.6 \pm 2.5\%$ (coefficient of variation 4.7%). Then the five batches were pooled to obtain a sufficient amount for all the remaining

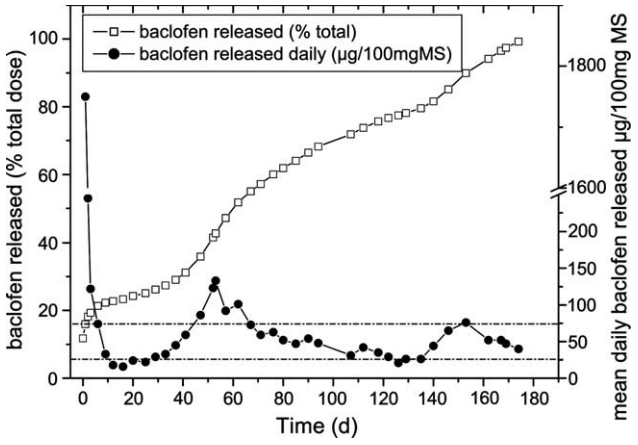

Fig. 1. Time course of baclofen released from microparticles in continuous flow cells performed in triplicate. The results are presented as baclofen released as a percentage of the total dose encapsulated (open squares) and as the mean daily dose delivered by 100 mg of microspheres (closed circles). The dot–dash lines represent 25 and 75 µg baclofen dose delivered daily. Scale does not allow the representation of standard deviations which were inferior to 3% of the measurements for each time point.

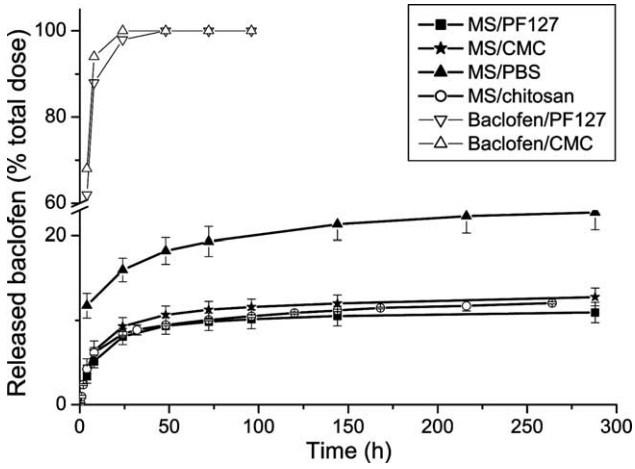

Fig. 2. Effect of MS suspension medium on the in vitro release rate of baclofen from PLGA microspheres under continuous flow (85 µl/min).

experiments. The drug loading of the pooled batch was measured and found to be 10.9%.

The in vitro release from microspheres (Fig. 1) under a very slow continuous flow of PBS buffer (85 µl/min) was assessed for 6 months (data from [17]). The baclofen doses mainly ranged from 25 and 75 µg per day (for 100 mg MS), except during the first three days (16% burst effect equivalent to a dose of 1766 µg of baclofen), and from D45 to D66 (75–150 µg/day). These amounts of delivered baclofen are consistent with the range of the dose injected for human treatment; a mean of 160 µg/day [32], if extrapolated to 300 mg of implanted microspheres. However, the safety of an intrathecal injection of 300 mg microspheres and its feasibility remain to be investigated. In our study only a quantity around 100 mg (depending on animal weight) was used because the animals were not spastic and no therapeutic effect was thus expected. To evaluate the burst effect of our preparation without impairment to animal comfort, it was preferred to work with a sub-therapeutic dose.

3.2. In vitro drug release from MS suspensions

In order to modify the release profile of baclofen to obtain a rate as constant as possible and especially to reduce the burst effect, the MS were suspended in different media and the release rates of these preparations were recorded in a continuous flow apparatus, designed to mimic the intrathecal space (Fig. 2). The effect of different viscous suspension media on the burst effect was similar: the amount of baclofen released from microspheres was divided by at least two when suspended in CMC or thermosensitive gels in comparison to PBS buffer. In both cases ‘sink conditions’ were not wanted since they are hardly reached in vivo due to both the poor water solubility of baclofen in water (4 g/l at pH 7) and the reduced volume of the intrathecal space.

For a more acute comparison between suspension media, the mean release rates were calculated for days 1, 3, 6, 9, 12 and 14 and are presented in Table 1. These rates calculated for viscous media are also expressed as percentage ratios of the rates calculated for microsphere suspensions in PBS buffer. These results show that the maximum reduction effect on release rates was obtained for PF-127 gels. The suspension media formed a barrier between the microparticles and the environment where baclofen was released. This barrier could limit the release of baclofen by various mechanisms: (1) the flow of water entering into the polymer matrix could be reduced and therefore, could limit baclofen crystal dissolution and matrix solvatation; (2) the diffusion of drug molecules from the microparticles into the surrounding environment could be limited by physical or chemical interaction. The first hypothesis should here be preferred if one considers the results obtained with control formulation where free flowing powder of baclofen was dispersed in CMC or PF-127 gel. In these cases, baclofen release was not really limited by the suspension media, a near 100% release was indeed observed after the first day if baclofen powder was suspended in CMC or PF127 (Fig. 2). This suggests that the effect of suspension media on the release rate of baclofen from microparticles may not be due to a limitation of baclofen diffusion but on a reduced availability of the external media to the microparticles. Furthermore, this could explain why similar results were found with suspension media of different viscosity.

To check this parameter, the behaviour of the suspension media under various shear rates was investigated (Table 2). At 37 °C, the highest viscosity was observed with PF-127 gel, which was the only suspension medium to display plastic flow properties thus having a practical yield point. The practical yield point, corresponding to the threshold shear stress for flowing properties of the gel (τo), was

Table 1
In vitro mean baclofen release rates measured from microsphere (MS) suspensions in various media: PBS buffer, CMC 1%, 21% PF-127 gel, chitosan gel (Chit.)

Day	Mean baclofen release rate (μg/day/100 mg MS)				% released/reference (MS/PBS)		
	MS/PBS	MS/PF-127	MS/CMC	MS/Chit.	MS/PF-127	MS/CMC	MS/Chit.
1	1750.0	886.0	1026.0	1106.0	50.6	58.6	63.2
3	122.0	55.8	67.0	77.4	45.7	54.9	63.4
6	74.1	21.1	23.6	38.7	28.5	31.8	52.2
9	37.9	Nd	Nd	17.6	Nd	Nd	46.4
12	18.0	7.6	7.3	21.6	42.2	40.6	120.0
14	16.1	8.4	13.1	Nd	52.2	81.4	Nd

The release rate is expressed in μg of baclofen for one day and 100 mg microspheres.

extrapolated using the Casson model which fitted well with the experimental flow curve ($r > 0.98$). These properties of the PF-127 gel could be very useful to avoid the gel flowing under reduced shear stress, especially when injected in the intrathecal space. The PF-127 gel displayed both the highest viscosity at 37 °C and the greatest impact on in vitro baclofen release from microspheres. These results confirm that viscosity should play a role in the reduction of release rates from microsphere preparations, but it is suspected that this physical parameter is only secondary for the reduction of release rates in vitro.

It was not possible to formulate a chitosan gel having a gelation temperature under 44 °C. This may be due to the origin and the particular properties of the chitosan used. It has been shown that a variation in the degree of deacetylation or in the pH of the chitosan solution can have a major impact on the gelation temperature [27]. The chitosan used in this study had a deacetylation degree (dda) of at least 85% (manufacturer's specification), but the actual deacetylation degree was not assessed and was probably less than the 91% dda used in the Chenite study [27], the limit value for dda identified to obtain a gelation temperature of 37 °C. However, the possibility to obtain different rheological behaviour depending on the batch used, since chitosan is derived from natural products, does not make this material easy to use. Furthermore, the incidence on the burst effect was lower if chitosan gels were used. Therefore, 21% PF-127 gels displaying interesting rheological properties, associated with a useful effect on the release rate of baclofen, and known for its compatibility with neural tissue [29,37-39], was preferred to chitosan gels for the in vivo evaluation against CMC suspension medium as a reference.

3.3. Burst effect after implantation of MS suspension

After the evaluation of baclofen release in continuous flow cells, a pharmacokinetic study was conducted in large laboratory animals to evaluate the concentration reached in vivo and the pharmacological tolerance of these preparations. It is necessary to use animals having a volume of intrathecal space similar to that of humans in order to obtain predictive pharmacokinetic data after intrathecal injection [40]. The diffusion, distribution, toxicity and elimination of the drug are related to the volume of the intrathecal space and to the volume of vehicle injected [40–42]. Hence, using a large animal model allowed us to investigate the dosage form under similar conditions as it would be for humans.

The goats were divided into four groups, each of them receiving MS suspended in a different suspension medium (0.5% CMC, 1% CMC, 21% PF-127 gel and Ringer lactate solution). All the goats received the same dose of encapsulated baclofen (165 μ/kg), except in the group of Ringer lactate solution where a high release rate was expected (as obtained in vitro with PBS buffer). For this reason, the dose was only a fifth of that administered to the other groups.

Table 2
Viscosimetric properties of microsphere suspension media

	Flow properties	Viscosity (mPa s)	Gel. temp.	Yield point (Pa)
Ringer lactate	Newtonian	1.0	No gelation	No yield point
CMC 0.5%	Newtonian	2.3	No gelation	No yield point
CMC 1%		4.0	No gelation	No yield point
PF-127 17%	Newtonian (11 °C) Plastic (37 °C)	690.0	30.6 °C	28.95
PF-127 19%		789.0	29.9 °C	36.52
PF-127 21%		1,219.0	28.3 °C	48.53
PF-127 23%		1,277.0	25.7 °C	48.77
Chitosan gel	Pseudoplastic	240.0	44.0 °C	No yield point

Flow properties were investigated at 11 and 37 °C. Apparent viscosity has been measured at 37 °C under shear rate of 50 s^{-1}. For gelation temperature (Gel. temp.) determination, the first temperature value leading to at least a 10-fold increase in viscosity was considered.

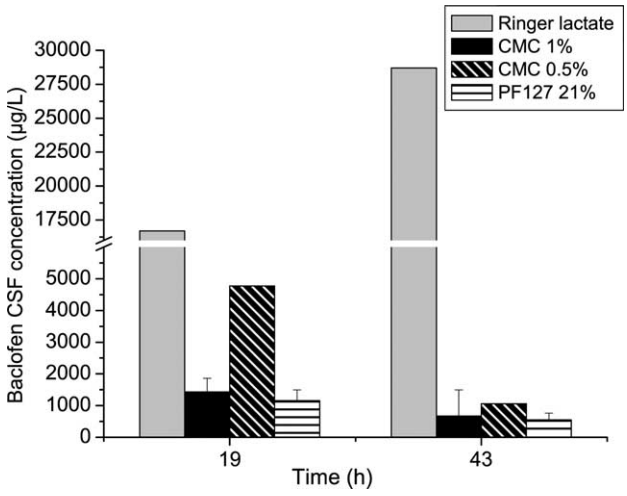

Fig. 3. Baclofen CSF concentration after intrathecal implantation of 11% (w/w) baclofen-loaded microsphere suspensions in various vehicles. Data have been normalised for an injected dose of 10 mg encapsulated baclofen and are presented as mean baclofen concentration in CSF ± standard deviation of data if relevant, determined using LC/MSMS. ($n=3$ for each time point except for CMC 0.5% and Ringer lactate).

Following the previously-described surgical procedure, baclofen-loaded microspheres were suspended in 2 ml of the vehicles described above, as soon as the Tuohy needle was in place, and were injected between the fifth and sixth lumbar vertebrae under deep anaesthesia. After MS implantation, cerebrospinal fluid was sampled at 19 and 43 h, and the baclofen concentration was assessed by LC/MSMS. The sampling procedure was set-up in order to avoid microparticles sampling: the sampling site was 15 cm from the injection point and a 22 μm filter was used. The burst effect observed in vivo during the first 2 days was highly dependent on the suspension medium (Fig. 3). The concentration in CSF after MS implantation in Ringer lactate suspension was 10 times higher than the concentration found after implantation of MS in CMC 1% or 21% PF-127 gel. On the second day, this ratio was around 50. These discrepancies between in vivo and in vitro data (where the ratio was only about 2) demonstrated that the suspension medium had an effect not only on the reduction of the release rate of the drug, but also on the distribution of the preparation in the intrathecal space. It is possible that the viscosity of the suspension medium might contribute to a limitation of the spreading in the subarachnoid space, thus decreasing the observed baclofen concentration in cerebrospinal fluid sampled at a 10 cm distance (at the L3/L2 level). The fact that the concentration observed after the second day was higher when the MS were suspended in Ringer lactate, even though this was not observed in vitro in PBS buffer, was consistent with the hypothesis of a role of the MS distribution in the baclofen concentration measured in CSF. In other words, the observed concentration in the sample was not only related to baclofen release from the dosage forms but was also dependent on its spreading in the spinal canal as previously described by Kroin et al. [43]. However, the viscosities of the 1% CMC preparation and the 21% PF-127 gel at 37 °C were very different even though the CSF baclofen concentration decreased by the same order for both vehicles in comparison to 0.5% CMC or Ringer lactate solution. There might be a viscosity threshold above which the distribution of the microspheres would be significantly affected. This threshold should range between the viscosity of CMC 1% and CMC 0.5%, i.e. between 4.3 and 2.3 mPa s (Table 2).

In order to obtain an evaluation of the released amount of baclofen while being less dependent on its distribution in the spinal canal, pharmacological observations of the animals were performed. After intrathecal lumbar injection in bolus, non encapsulated baclofen (commercial solution: Lioresal®) produces a relaxation of hind limb muscles, until hind limb paralysis occurs for bolus doses over 400 μg in goats, and unconsciousness for doses over 1600 μg. The goat having received MS in Ringer lactate suspension displayed pharmacological signs of baclofen over dosage: drowsiness and hind limb paralysis for an encapsulated baclofen dose of 2.2 mg (20 mg of 10.9% baclofen-loaded MS implanted intrathecally). These signs were observed for a bolus dose over 400 μg, i.e. 20% of the dose injected; the burst effect was also at least 20% in vivo. The goats having received 100 mg MS in 1% CMC or PF-127 thermosensitive gel displayed the same signs, but for a dose five times higher (11 mg encapsulated baclofen implanted). In the latter case, a 20% burst would have corresponded to a 2200 μg bolus dose related to unconsciousness but this side effect was not observed. Instead, the goats displayed hind limb paralysis and drowsiness for 3–5 days. These pharmacological signs corresponded to a bolus dose of between 400 and 1600 μg. The burst effect in vivo could thus be evaluated between 4 and 15%. The evaluation of the in vivo burst effect using pharmacological signs of baclofen activity (or toxicity) was more consistent with what was observed from in vitro experiments. Indeed, the pharmacological signs of baclofen activity/toxicity were consistent with a rapid release of baclofen from microspheres lasting around 96 h, not only the first 24 h.

Pharmacological observation has the advantage of being less dependent on the distribution of the vehicle, but the data obtained are semi-quantitative. In order to obtain indirect quantitative data on the burst effect in vivo, without the bias of vehicle distribution, the variation of baclofen plasma concentrations after intrathecal injection of the dosage form was monitored. During the first 2 days, baclofen was detectable and quantifiable in plasma and the drug concentration was related to the suspension medium used (Fig. 4). On the third day, baclofen concentration was below the LOQ (i.e. <4 μg/l) for all suspension media, and from day 6 to day 28, baclofen concentration was below the LOD (i.e. <1 μg/l).

The results of the plasma concentration follow-up confirmed in part the data obtained with cerebrospinal

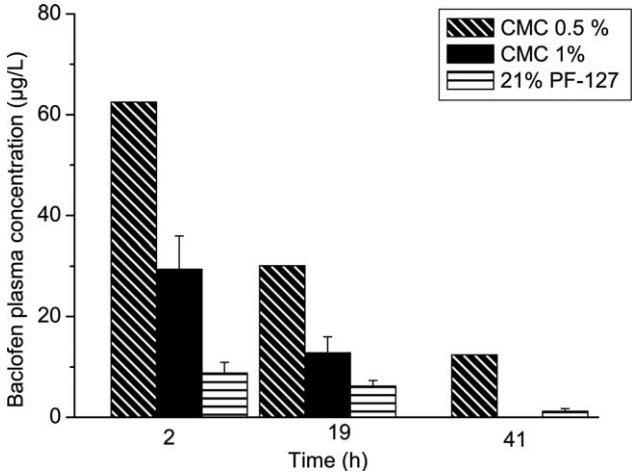

Fig. 4. Baclofen plasma concentration after implantation of 100 mg 11% loaded microspheres in the intrathecal space for 3 types of suspension medium: CMC 0.5%, CMC 1% and PF-127 21% ($n=3$ for each time point except for CMC 0.5%).

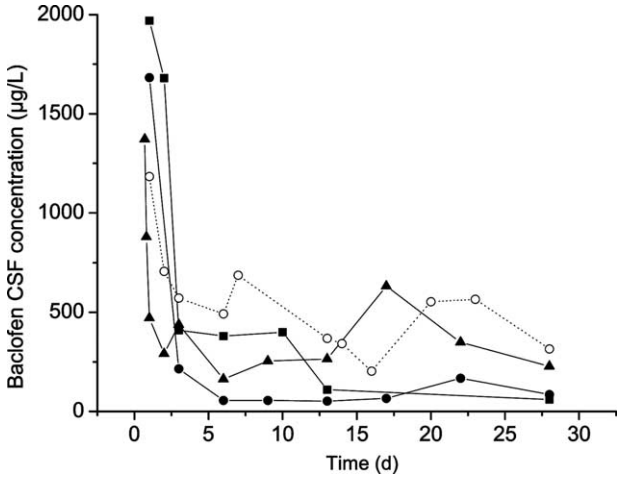

Fig. 5. Baclofen concentration in CSF during 28 days after implantation of 100 mg 11% loaded microspheres in the intrathecal space of four goats. Microspheres were resuspended in CMC 1% (closed symbols) and in 21% PF-127 gel (open symbols).

fluid. The burst effect was reduced as the CMC concentration rose from 0.5 to 1%, and even more when MS were suspended in 21% PF-127. The combination between the observed concentration and modification of the MS distribution along the intrathecal space was minimised, since the drug was detected in plasma after its removal from the spinal compartment. The authors are well aware that baclofen concentrations observed in plasma are related to the baclofen concentrations in CSF, but that a strict relationship remains difficult to establish. The elimination rates from CSF and from plasma are however comparable, since baclofen mean residence time (MRT) in goat plasma is 4.6 ± 0.9 h for an intravenous 10 mg dose, and 2.3 ± 1.0 h in CSF for a baclofen intrathecal dose of 200–560 µg [44]. The follow-up of baclofen concentrations in plasma is then useful to compare burst effect of preparations injected in the intrathecal space.

To summarise, it is worth noticing that the pharmacological effect was related to the baclofen concentrations measured in plasma and in CSF. However, concentrations observed in CSF samples were probably mostly impaired by the distribution of the dosage forms in this unmixed compartment. Concentration in the CSF thus depended on the release rate and the spreading of the drug and its vehicle along the spinal canal. On the contrary, pharmacological effects and plasmatic concentrations were related to the release rate of baclofen only. Our results make it possible to conclude that the PF-127 gel could reduce the burst effect by a factor of 2 in vitro and in vivo, and could limit the distribution of the dosage form along the spinal cord in comparison to less viscous media having no yield point.

3.4. One month follow-up with implanted catheters

Two groups of three goats receiving MS dispersed either in PF-127 or CMC 1% had a follow-up of the baclofen concentration in CSF using implanted catheters. Unfortunately 2 goats of the PF-127 group suffered catheter dysfunction on D3 and D16. The data obtained for the other four goats are presented in Fig. 5. The shape of the baclofen concentration versus the time curve in the CSF was similar for all the animals: the burst effect was responsible for high baclofen concentrations in the CSF during 3–5 days and then the concentration came to a plateau value. Inter-individual differences were observed in the CMC group. Unfortunately, the comparison was not possible with the other group since only one goat could have a 28 day follow-up. The ratio between high baclofen concentrations due to the burst effect and equilibrated CSF concentrations was higher for the MS/CMC group in comparison to the goat having received the MS in suspension in PF-127. It is worth noticing that these concentrations were determined in aliquots sampled 10 cm away from the implantation area. The baclofen concentration near the implantation site of the microspheres was likely to be higher. It has been previously shown using radionuclides that the decrease of the concentration of a water soluble substance such as baclofen was 43% over 20 cm in the spinal cord [43]. It has to be emphasised that the measured baclofen concentrations greatly depend on the sampling site and the volume of CSF removed; it is therefore difficult to compare concentration data from one study to another and especially to the CSF baclofen concentrations measured in clinical studies. The interpretation of Fig. 5 should thus only be limited to the relative curve patterns and the ratio between burst effect and plateau value which show, however, a sustained release of baclofen over at least 28 days was observed. This result could explain the prolonged pharmaceutical effect of baclofen observed in the rabbit in a previous study by our group [17]. Unfortunately, implanted catheters were not fully functional for a time longer than 28 days. The implanted catheter technique still has to be improved to

obtain reliable data to characterise the long-term release from intrathecally implanted microspheres and thus to confirm or infirm these preliminary results.

4. Conclusion

This study was aimed at evaluating the release rate of baclofen from microspheres suspended in solutions showing various viscosity values. The in vitro and in vivo release studies have shown that viscous reconstituting media, and in particular thermosensitive PF-127 gels, can be used efficiently to reduce the burst effect. Following three different approaches, it was demonstrated that the burst effect in vivo was reduced by at least a factor of 2 which still remain insufficient to avoid the side effect of the drug during the first days after implantation. The concentration of baclofen in the CSF was maintained during at least one month. This study also contributed to verify that viscous media had an impact on distribution along the spinal canal, which has been already described [9]. Poloxamer gels, and especially PF-127 gels, have shown very interesting properties in controlling drug release and being safe, when injected via epidural or intrathecal route [29,37,38]. The ability of these compounds to reduce the burst effect in vivo is promising for future applications of long-term, sustained spinal delivery of drugs. In the foreseen clinical applications, these being spasticity treatment and chronic pain relief, the burst effect has to be completely eliminated to allow repeated injections of the dosage forms. Therefore, an optimisation study is now needed to improve these properties and to find a solution to the still unsolved issue of the parenteral dosage forms, the problem being the accelerated release of the drug in the first few hours following injection.

Acknowledgements

The authors are grateful to Francis Prual, Stephane Madec and Delphine Holopherne for their technical assistance on animal handling and surgical procedures.

This work was granted by French ministry of Research and technology and by Ethypharm SA.

The authors wish to thank the 'Comité du Maine et Loire de la Ligue contre le Cancer' for their financial support.

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