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Research paper

Baclofen-loaded solid lipid nanoparticles: Preparation, electrophysiological assessment of efficacy, pharmacokinetic and tissue distribution in rats after intraperitoneal administration

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ABSTRACT

Intrathecal baclofen administration is the reference treatment for spasticity of spinal or cerebral origin, but the risk of infection or catheter dysfunctions are important limits. To explore the possibility of alternative administration routes, we studied a new preparation comprising solid lipid nanoparticles (SLN) incorporating baclofen (baclofen-SLN). We used SLN because they are able to give a sustained release and to target the CNS. Wistar rats were injected intraperitoneally with baclofen-SLN or baclofen solution (baclofen-sol group) at increasing dosages. At different times up to 4 h, efficacy was tested by the H-reflex and two scales evaluating sedation and motor symptoms due to spinal lesions. Rats were killed and baclofen concentration determined in blood and tissues. Physiological solution or unloaded SLN was used as controls. After baclofen-SLN injection, the effect, consisting in a greater and earlier reduction of the H/M ratio than baclofen-sol group and controls, was statistically significant from a dose of 5 mg/kg and was inversely correlated with dose. Clinical effect of baclofen-SLN on both the behavioral scales was greater than that of baclofen-sol and lasted until 4th hour. Compared with baclofen-sol, baclofen-SLN produced significantly higher drug concentrations in plasma from 2nd hour until 4th hour with a linear decrement and in the brain at all times. In conclusion, our study demonstrated the efficacy of a novel formulation of baclofen, which exploits the advantages of SLN preparations. However, for clinical purposes, high baclofen concentrations in brain tissue and sedation may be unwanted effects, requiring further studies and optimization of dosages.

1. Introduction

Baclofen is the reference treatment for spasticity of spinal or cerebral origin [1]. The oral administration route is mostly used for less severe forms of spasticity. In some cases, oral treatment may become insufficient or the doses required may be too high, with increased risk of adverse effects. To treat severely disabling spasticity, a small amount of baclofen must be injected continually into the intrathecal space, as lifetime treatment [2]. Until now, the only solution available to solve this challenging drug delivery problem is to use electronic pumps surgically implanted in the patient's body and connected to indwelling catheters. However, the need for surgery, the risk of infection (estimated range from 0.5% to about 2%) or catheter dysfunctions (about 4%), and several side effects (nausea, drowsiness, dizziness, sedation, constipation, fatigue and muscular hypotonia) still limit the number of potentially treatable patients for prolonged periods [3]. In recent years, long-lasting sustained-release baclofen formulations have been developed to overcome some of these limitations. Among these formulations, baclofen-loaded microspheres constituted of polylactide-co-glycolide (PLGA) have been injected into the intrathecal space in rabbits, providing long-lasting sustained drug release, and have been found to be well tolerated [4].

Baclofen is a very hydrophilic drug. In fact, its octanol–water distribution coefficient (D), a parameter indicating lipophilicity, is very low ($\mathrm{Log}\, D = -0.96$). After oral administration, more than

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80–90% of it is absorbed by the stomach and bowel and then eliminated by urinary excretion. Failure of oral medication to produce sufficient relief of spasticity is due to the poor passage of the drug across the blood–brain barrier (BBB) [5].

In order to explore the possibility of alternative and efficacious administration routes, we studied a new pharmaceutical preparation consisting of solid lipid nanoparticles (SLN) incorporating baclofen (baclofen-SLN).

SLN are prepared from warm microemulsions and can incorporate hydrophilic and/or lipophilic drugs [6,7]. "Stealth"-SLN, loaded or not with drugs, can be prepared so as to avoid their recognition by the reticuloendothelial system; this strategy notably increases the mean residence time of the drug [8]. Both "stealth"- and "non-stealth"-SLN can pass the blood–brain barrier [9,10]. We have successfully administered SLN to laboratory animals via the duodenal [11–14], parenteral [9,10], and ocular [15] routes, and to humans via the oral and transdermal routes [16]. SLN operate as a nanoparticulate drug delivery system (NDDS). They are targeted to the lymph after duodenal administration. In previous studies, we have shown that doxorubicin and tobramycin SLN formulations increase the drug concentration in the brain in an animal experimental model [9,12]. They are also internalized into all the cell lines tested within a few minutes.

The purpose of this research was to evaluate the efficacy of baclofen-SLN and to test the possibility of achieving a more favorable pharmacological profile in comparison with baclofen in solution. In this context, we evaluated the efficacy of baclofen-SLN by means of H-reflex modulation study and animal behavioral characterization and correlated these data with plasma, liver, and brain tissue concentrations. H-reflex electrophysiological measurement and behavioral alteration scales are considered a reliable method to evaluate baclofen efficacy and adverse effects in animal models [4], indispensable premises for further developments and studies in human physiopathology. In particular, H-reflex in rats is a non-invasive, reproducible, and reliable technique to study the physiological and pharmacological modulation of α-motoneurons and is recordable without the influence of anesthesia [17–20], while scales focusing on spontaneous or evoked movements, alertness, and responsiveness of rats are affordable and rapid methods to evaluate clinical effects on motor activity and sedation [21–23]. A dispersion of baclofen-SLN, administered by the intraperitoneal route, was compared with baclofen in solution in order to discriminate the pharmacokinetics and neurophysiological characteristics of the baclofen-SLN preparation.

2. Materials and methods

2.1. Baclofen included in solid lipid nanoparticles (SLN)

2.1.1. Materials

Baclofen was from Sigma Aldrich (Milan, Italy), stearic acid was from Merck (Hohenbrunn, Germany), Epikuron 200 (92% phosphatidylcholine) was from Cargill (Hamburg, Germany), and sodium taurocholate was from PCA (Basaluzzo, Italy). The other chemicals were of analytical grade.

2.1.2. Preparation of baclofen-loaded SLN and unloaded SLN

Baclofen-solid lipid nanoparticles were prepared at 70° from a multiple (w/o/w) warm microemulsion: the components were baclofen, water, stearic acid as oil phase, epikuron 200 as surfactant, propionic acid, butyric acid, and sodium taurocholate as cosurfactants. Baclofen has been dissolved in the internal aqueous phase. The warm w/o/w clear microemulsion was dispersed in cold water at 2–3° under mechanical stirring obtaining baclofen-SLN dispersion that was then washed three times with a diluted lysine aqueous solution by tangential flow filtration using Vivaflow 50 Sartorius system (RC membrane – cutoff 100 kDa, 1 volume added and same volume removed). The washed baclofen-SLN dispersion was then added of threalose as cryoprotecting agent and went for freeze-drying.

Concentration of baclofen in third washing water has been determined to evaluate free baclofen present in baclofen-SLN dispersion. The freeze-dried SLN were reconstituted with half of original water. Total baclofen concentration was determined on reconstituted dispersion before administration. Drug-unloaded SLN were prepared as above.

2.1.3. Characterization of SLN

The average diameter and polydispersity index of baclofen-SLN and unloaded SLN were determined by photocorrelation spectroscopy using a Zetasizer 3000 HS_A (Malvern Instruments, UK) at a fixed angle of 90° and at a temperature of 25 °C. Zeta potential was determined by laser Doppler electrophoresis using the Zetasizer 3000 HS_A. To determine average diameter and zeta potential, SLN dispersion samples were diluted with water.

2.1.4. Determination of baclofen concentration in baclofen-SLN and in washing waters

The SLN dispersion was dissolved in methanol and diluted with mobile phase. Three samples were filtered through a 0.45-μm nylon syringe filter before analysis. The amount of baclofen present in the dispersion was determined by HPLC-UV, according to the methods described. The analytical column was a Tracer Extrasil ODS1 15 × 0.46, 5 μm particle size (Teknokroma, Spain). The mobile phase was 0.01 M monobasic potassium phosphate (pH 3.5)/acetonitrile (80:20 v/v). The analysis was performed at room temperature with a 1.0 ml/min flow rate, detection wavelength 220 nm. The same method was used to determine baclofen concentration in washing waters: value obtained from third washing water was taken as concentration of free baclofen in baclofen-SLN dispersion.

2.2. Efficacy testing

2.2.1. Animals

The experiment was performed on healthy male Wistar rats (age 9 weeks; weight 285 ± 4 g), according to the current European Directive (*EC Directive 86/609/EEC*) for animal experiments and under the approval of local Ethics Committee, as follows: at time 0, corresponding to 9 a.m., 4 groups of four rats each were injected intraperitoneally either with physiological solution (control group) or with unloaded SLN at 10 ml/kg, or else with baclofen-SLN or baclofen in solution (baclofen-sol) at increasing doses (2.5, 5, 7.5, 8.5, 10 mg/kg).

2.2.2. H-reflex examination

The H-reflex was determined in conscious rats held manually in a supine position on a specific device to obtain a 90° angle of both knee and ankle joints. Surface silver electrodes (diameter 2 mm) were firmly positioned with adhesive patches upon the skin of the left leg, previously shaved, in the area overlying the soleus muscle. A needle reference electrode was inserted into the tail. Stimulation electrodes were positioned on the skin overlying the femur, following the techniques described [20]. Stimulus duration was 0.2 ms, while intensity was adjusted to obtain the highest basal H/M amplitude ratio (ratio between maximal amplitudes of the H-reflex and M-wave). Stimulation was applied on muscles devoid of any recognizable electromyographic activity, in order to prevent reflex suppression due to tonic muscle contraction. In order to exclude post-activation depression, corresponding to a reduced H wave amplitude after sequential trials, the inter-stimulus interval

Table 1
Experimental allergic encephalitis (EAE) scale and Wilson sedation scale scores.

Score	EAE scale	Wilson sedation scale
0	No clinical sign	–
1	Flat tail	Fully awake and oriented
2	Hindlimb paresis	Drowsy
3	Hindlimb paralysis	Eyes closed but rousable to command
4	Hindlimb paralysis + forelimb paresis	Eyes closed but rousable to mild physical stimulation
5	Death	Eyes closed but unrousable to mild physical stimulation

was 30 s. Ten H-reflex and M-wave peak-to-peak amplitude measurements were made, and the maximum values were used to calculate the H/M ratio. The H/M amplitude ratio was determined for each group at time 0 and 1 h, 2 h, and 4 h after injection.

2.2.3. Characterization of animal behavior

Two validated scales were used to characterize and quantify behavioral changes after baclofen injection: (a) the scale used in experimental allergic encephalitis (EAE) animal models, specifically validated for motor symptoms due to spinal cord lesions; higher scores indicate severe spinal motoneuron inhibition [21]; (b) the Wilson sedation scale, an analogous of sedation rating scale used in a rat model of induced sedation [22,23]; higher scores indicate deeper sedation. The scoring charts for both scales are summarized in Table 1. The EAE scale and the Wilson sedation scale were administered to each group at time 0 and at 1 h, 2 h, and 4 h after injection.

2.3. Pharmacological assay

2.3.1. Animals and sample preparation

Two rat groups of 3 rats each were killed with CO_2 for blood and tissue collection, 1 h, 2 h, and 4 h after baclofen-sol and baclofen-SLN injection at 7.5 mg/kg. Blood was stored in the tubes containing disodium EDTA (2 mg/ml). Plasma samples were stored at −80 °C. Brain, spinal cord, and liver tissues were collected and frozen in nitrogen liquid for subsequent detection of baclofen concentration. Brain, liver, and spinal cord were homogenized (500 mg of tissue in 2000 μl of MeOH and 500 μl of phosphate buffer ($Na_2H_2PO_4$, 50 mM, pH 7.4) and then stored at −80 °C.

2.3.2. Standards and solutions

A stock standard solution of Baclofen (100 μg/ml) was prepared in water, and all serial dilutions were prepared with water. In *n*-hexanol was dissolved 0.8% tetrapentylammonium bromide and 0.4% tetrapropylammonium bromide.

2.3.3. High-performance liquid chromatography (HPLC) apparatus

An Agilent 1100 series HPLC system equipped with a variable wavelength detector was used for baclofen detection, according to the method described in the literature [24]. Baclofen was monitored at 220 nm. Separation was performed on a 250 mm × 4.0 mm (5 μm) Nucleosil 100-C18 column (Agilent). The mobile phase consisted of acetonitrile/0.1 M phosphate buffer (20:80) adjusted to pH 3 with phosphoric acid. And 20 mg/l sodium octanesulphonate and 10 mg/l Na_2EDTA were dissolved in the buffer (0.1 M $Na_2H_2PO_4$). The flow rate of the mobile phase was 1 ml/min. Chromatography was performed at 25 °C. Detection limit was 20 ng/ml.

2.3.4. Extraction procedure

To 0.5 ml of plasma (or to 1 ml of homogenized tissue) were added 3 ml of *n*-hexanol and 0.5 ml of phosphate buffer ($Na_2H_2PO_4$ 50 mM) pH 8.55. After 2 min shaking and 10 min centrifugation at 10 °C, the organic phase was separated and 0.5 ml of 0.001 M HCl was added. After 2 min shaking and 10 min centrifugation at 10 °C, the aqueous phase was separated and filtered through a 2-μm nylon filter, and then an aliquot of 30 μl was injected into the HPLC system.

2.4. Statistical analysis

Comparison among groups was performed by ANOVA and Post-Hoc (Duncan) tests, as appropriate. Correlations between neurophysiological or clinical effects and doses were performed by the Spearman correlation test. NCSS-PASS™ statistical software package was used for the analysis.

3. Results

3.1. SLN characterization

The average diameter of baclofen-SLN dispersion obtained after reconstitution of freeze-dried powder with half of original water volume was 161.4 nm, while the polydispersity index was 0.27 and the zeta potential was −35.2 mV; baclofen concentration in reconstituted baclofen-SLN dispersion was 1.7 mg/ml, while in third washing waters free baclofen was 0.11 mg/ml.

The average diameter of unloaded SLN dispersion was 156.8 nm, polydispersity index was 0.22 and zeta potential was −37 mV.

3.2. Efficacy testing

A decrease in the H/M amplitude ratio during the first hours of daytime after awakening is a normal physiological finding in rats and was confirmed in our control group. H/M amplitude ratio after unloaded SLN injection showed no statistically significant differences from the control group at each time point (Fig. 1). This comparison was performed in order to confirm the absence of any effect of drug-free SLN upon H-reflex. Only control group was then used for successive comparisons.

Conversely, significant and dose-dependent variations of H/M amplitude after baclofen-SLN administration were found, as shown in Fig. 2. One hour and 2 h after injection, the effect, consisting in a greater reduction of the H/M ratio than in the control group (spinal reflex hypoexcitability), was statistically significant starting from a dose of 5 mg/kg and was inversely correlated with dose ($r = -0.56$; $p = 0.03$). There was no H/M ratio reduction in rats treated with

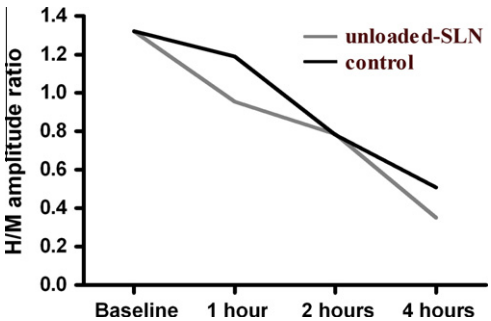

Fig. 1. Physiologic reduction of H/M amplitude ratio during the morning hours in rats, compared with SLN-unloaded injections. No statistically significant difference is present at each time point.

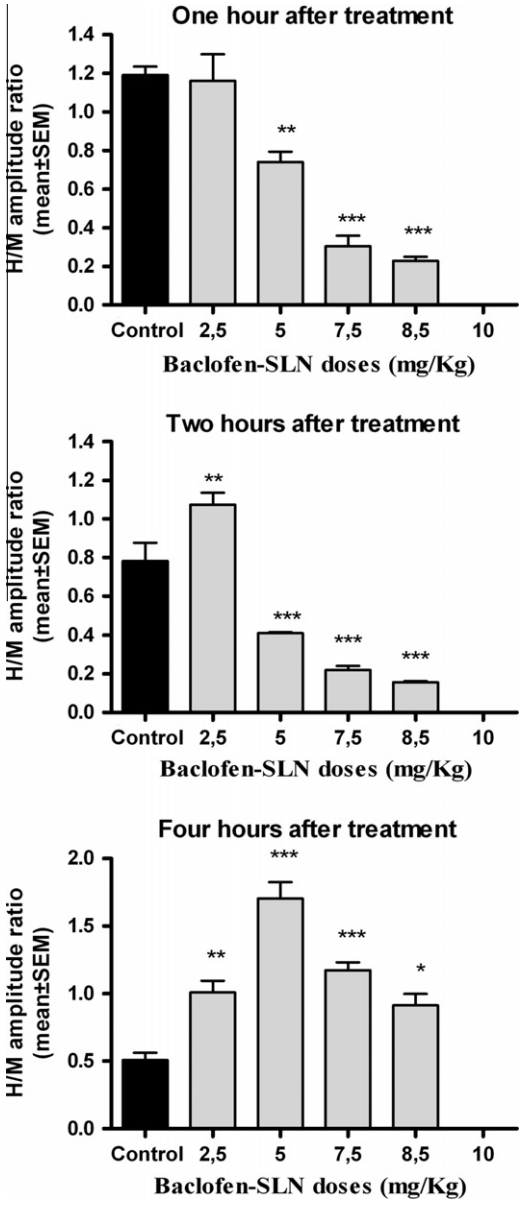

Fig. 2. H/M amplitude ratios after baclofen-SLN administration, at increasing doses, compared with control group. * Indicates $0.01 \leqslant p < 0.05$; ** indicates $0.001 \leqslant p < 0.01$; *** indicates $p < 0.001$; no sign indicates not statistically significant test.

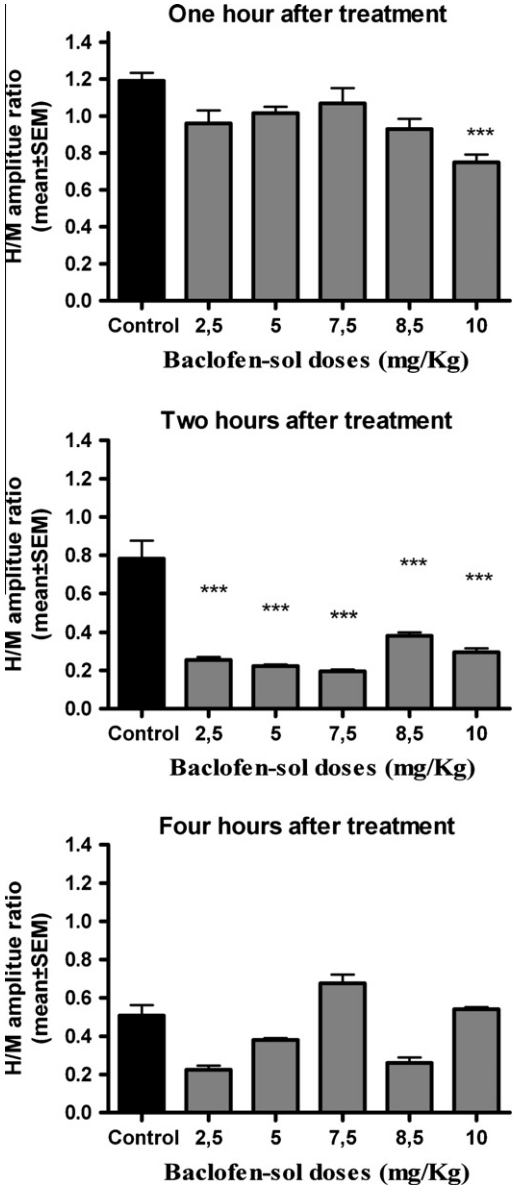

Fig. 3. H/M amplitude ratios after baclofen-sol administration, at increasing doses, compared with control group. *** Indicates $p < 0.001$; no sign indicates not statistically significant test.

2.5 mg/kg baclofen-SLN, while the H-reflex was persistently absent at 10 mg/kg. Interestingly, 4 h after injection, an increased H/M ratio compared with the control group (spinal reflex hyperexcitability) occurred at all doses except 10 mg/kg, at which dose the H-reflex remained unelicitable. Only one rat of the three injected with baclofen-SLN 10 mg/kg survived. In this case, the H-reflex became elicitable 24 h after treatment, when the rat had recovered.

As shown in Fig. 3, after baclofen-sol injection, reduction of H/M ratio was greater than in the control group starting from a dose of 2.5 mg/kg. However, unlike baclofen-SLN, the effect appeared only after 2 h and did not show a clear dose-dependent relationship. Moreover, the H-reflex was not completely abolished at 10 mg/kg, and after 4 h, the H/M amplitude ratio did not show statistically significant differences from control group, at all doses (termination of any recognizable effect on spinal reflex excitability).

Fig. 4 shows the mean H/M amplitude ratio, including all doses of baclofen-SLN and baclofen-sol except 10 mg/kg, compared with values for the control group. Mean profile of H-reflex confirmed an overall effect of baclofen-SLN at 1st and 2nd hour followed by a rebound effect at 4th hours (increased H/M ratio, indicating spinal reflex hyperexcitability), these difference being statistically significant compared with the control group. Baclofen-sol, on the contrary, produced a significant decrease in the mean profile of H-reflex later, at 2th hour, while, at 4th hour, H-reflex was comparable with that of the control group, indicating a recovery of normal spinal reflex excitability.

Behavioral characterization of rats after baclofen-SLN or baclofen-sol injections is summarized in Table 2. Mean scores on the two clinical scales including all doses are shown in Fig. 5. After baclofen-SLN injection, mean scores on both scales were higher than those for the baclofen-sol group. Interestingly, clinical effects (EAE scale score) were detectable after the lowest dose of baclofen-SLN (2.5 mg/kg) but only after a higher dose of baclofen-sol (7.5 mg/kg). Four hours after the injection, only rats treated with the higher doses of baclofen-SLN still presented significant clinical signs, consisting in sedation (8.5 mg/kg) or complete paralysis and

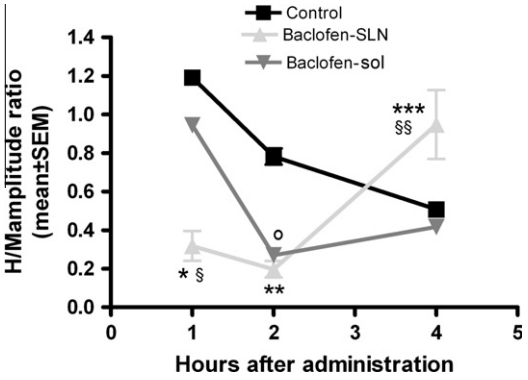

Fig. 4. Mean H/M amplitude ratio considering all dosages of baclofen-SLN, all dosages of baclofen-sol, and control group. * Indicates baclofen-SLN vs. control at 1st hour, $p < 0.001$; ** indicates baclofen-SLN vs. control at 2nd hour, $p < 0.001$; *** indicates baclofen-SLN vs. control at 3rd hour, $p < 0.01$; § indicates baclofen-SLN vs. baclofen-sol at 1st hour, $p < 0.001$; §§ indicates baclofen-SLN vs. baclofen-sol at 3rd hour, $p < 0.01$; ° indicates baclofen-sol vs. control at 2nd hour, $p < 0.001$.

Table 2
Behavior characterization of rat after baclofen-SLN and baclofen-sol injections, at increasing dosages.

	1 h after injection		2 h after injection		4 h after injection	
	EAE scale score	Wilson sedation scale score	EAE scale score	Wilson sedation scale score	EAE scale score	Wilson sedation scale score
Baclofen-SLN doses (mg/kg)						
2.5	1	1	0	1	0	1
5	3	1	0	2	0	1
7.5	3	2	2	3	0	1
8.5	4	2	2	3	1	2
10	4	5	4	5	4	5
Baclofen-sol doses (mg/kg)						
2.5	0	1	0	1	0	1
5	0	1	0	1	0	1
7.5	1	2	0	1	0	1
8.5	1	2	0	2	0	1
10	4	3	2	2	0	1

piloerection (10 mg/kg). Conversely, no clinical effect was measured 4 h after baclofen-sol injection, when all the rats had completely recovered, even after the highest dose (10 mg/kg).

3.3. Pharmacological assay

Liver, plasma, and brain concentrations of baclofen after baclofen-SLN and baclofen-sol injection at 7.5 mg/kg are shown in Fig. 6. Administration of Baclofen-SLN yielded a lower plasma concentration of baclofen after 1 hour compared with baclofen-sol. Notably, after 2 and 4 h, only baclofen-SLN administration produced

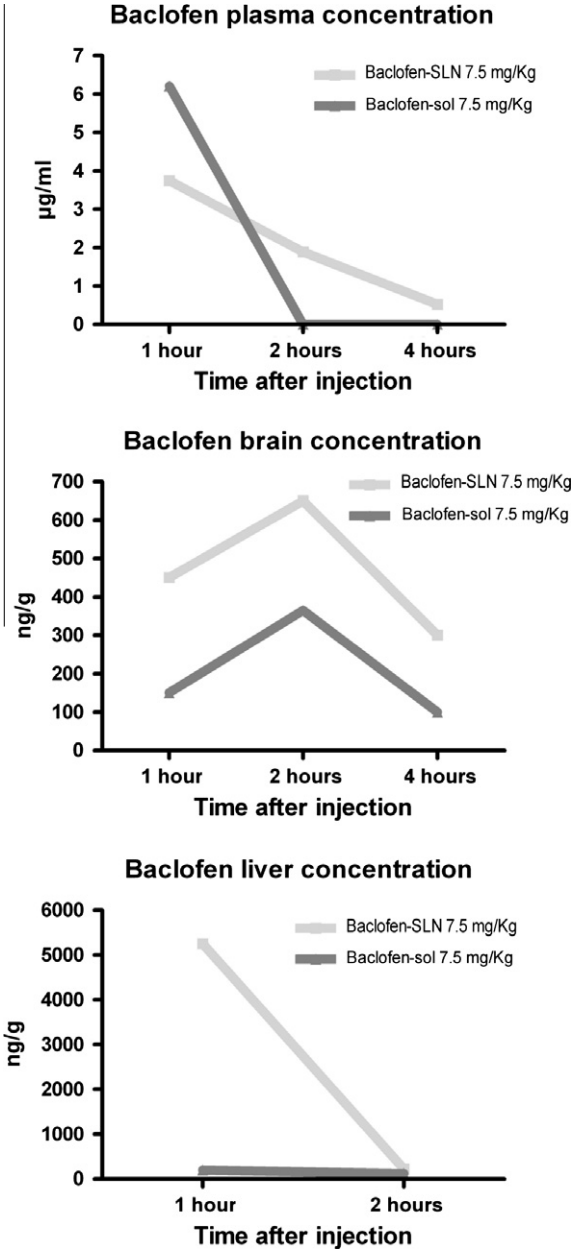

Fig. 6. Plasma, brain, and liver concentrations of baclofen after baclofen-SLN and baclofen-sol injection at 7.5 mg/kg.

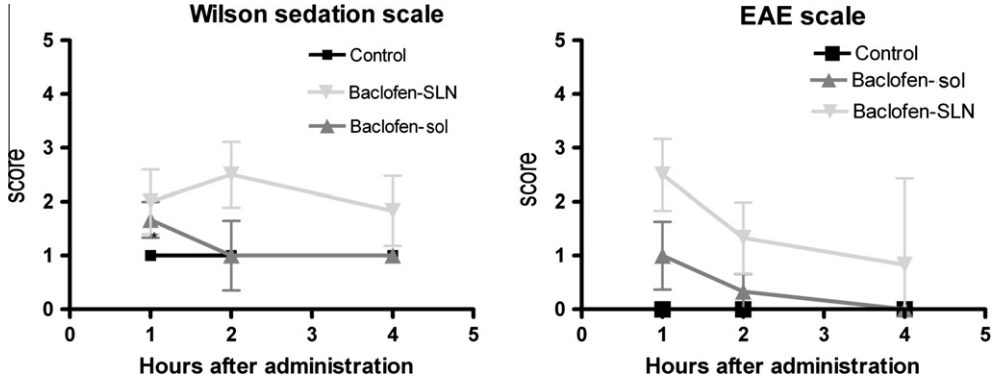

Fig. 5. Mean behavioral scale scores considering all dosages of baclofen-SLN, all dosages of baclofen-sol, and control group.

measurable baclofen plasma concentrations, with an almost linear decrease in baclofen that continued steadily for 4 h. In the liver, elimination of baclofen-SLN was slower than that of solution, probably due to the longer-lasting presence of the nanoparticles in the blood. In the brain, both formulations gave a maximum value after two hours, but concentrations after SLN administration were almost twice those after solution administration. The baclofen concentration was undetectable in spinal tissue for all doses tested and at all time points.

4. Discussion

Continuous intrathecal baclofen is an important treatment for severe spasticity in neurological disorders, but it requires implanted devices to deliver the drug into the cerebrospinal fluid, so that the risks related to surgical procedures may limit its use. Moreover, the oral route is still not a valid alternative to intrathecal administration for severe spasticity management [2,3,25].

Our study demonstrated the efficacy and promising pharmacological profile of a novel formulation of baclofen, which exploits the advantages of SLN preparations. Baclofen, as is well known, is a highly hydrophilic drug, but, despite this characteristic, a sufficient amount of the drug was incorporated into the SLN. A significant loading of baclofen in SLN dispersion was achieved by dispersion in cold water of w/o/w microemulsion where drug was dissolved in water of internal warm water in oil (w_1/o) microemulsion. Baclofen concentration determined in washing waters showed a low amount of free baclofen in SLN dispersion comparing to total baclofen, assessing an evident incorporation of drug in solid lipid nanoparticles.

SLN can incorporate either hydrophilic or lipophilic drugs and present the peculiarity of acting as a reservoir for their sustained release [6,7]. SLN have been successfully administered in animals and healthy humans via different routes [9,14–16], achieving prolonged therapeutic blood levels of the included drug.

Intraperitoneal injection of baclofen-SLN in rats showed both electrophysiological and clinical effects, which were dose dependent. To assess baclofen activity, it is necessary to employ either an animal model of spasticity or a non-injured animal model. Spasticity, defined as "a motor disorder characterized by a velocity-dependent increase in tonic stretch reflexes" (hyperexcitability of the stretch reflex), is one of the clinical signs of the upper motor neuron syndrome. In animal models, it is difficult to obtain all the components of spasticity after a surgical lesion; thus, as described in other studies, we decided to examine baclofen activity in non-injured, healthy rats. We used the H-reflex (H/M ratio) as a reproducible measure of spinal reflex excitability and a reliable marker of the antispastic effects of baclofen [26,27]. Baclofen has been shown to reduce resistive torque, joint stiffness, stretch reflex responses, and H/M ratio in a dose- and time-dependent manner consistent with clinical findings [28,29]. In particular, after intrathecal bolus administration, the H/M ratio progressively decreases within minutes [30,31] and remains significantly reduced or absent for several hours [32–34]. It is important to consider that in freely moving rats, the H-reflex amplitude displays a circadian variation, without any change in background motoneuron tone: it is highest around noon and lowest around midnight [35]. This circadian rhythm of the H-reflex appears to depend in part on the descending influence from the brain that is conveyed by the main corticospinal tract, while the rubrospinal, vestubulospinal, and reticulospinal or dorsal column ascending transection has been shown not to affect the rhythm amplitude or phase [19].

Taking into account this physiological H/M reduction, baclofen-SLN showed an antispastic effect similar to baclofen-sol, but with a few peculiarities. First, the effect was reached earlier (at 1 h) so that the curve of the H-reflex modulation against time was shifted down and leftward (Fig. 4). Second, the effect was more strongly dose dependent, and the highest dose (10 mg/kg) produced a prolonged suppression of the H-reflex. On the whole, these data suggest an early effect of baclofen-SLN on spinal reflex excitability and, more interestingly, a dose-dependent modulation of this effect, which is less marked after the administration of standard baclofen formulations. Clinical scores for spinal cord involvement confirmed this data and, in addition, indicated a more marked and more prolonged effect of baclofen-SLN compared with baclofen-sol: unlike baclofen-sol, after 4 h the basal clinical score for spinal cord involvement had not been completely recovered. This is in agreement with findings that baclofen-SLN gave a measurable plasma concentration of baclofen until 4 h. SLN have some peculiarities, such as providing sustained release of the incorporated drug and passing the BBB [9,10]. The sustained release is clearly apparent in this study in the plasma (Fig. 6) where a decreasing release, practically linear, was observed.

Surprisingly, at all doses of baclofen-SLN except 2.5 mg/kg and 10 mg/kg, a rebound increase of the H/M ratio occurred at 4 h, indicating spinal reflex hyperexcitability. As clinical signs of spinal cord involvement were still present at that time point, a possible explanation could lie in the concomitant cortical effects of baclofen-SLN: this is supported by the finding that baclofen-SLN produced higher scores for sedation and higher brain tissue baclofen concentrations than standard formulation. In the brain, both formulations (baclofen in solution and in SLN) gave maximum values after two hours, but the baclofen concentration after SLN administration was almost double that after administration of the solution.

On the whole, these data suggest that there is improved passage through the BBB, as has also been reported in studies on doxorubicin and tobramycin [9,12]. It is known that baclofen acts on GABA(B) receptors, which are widely expressed in the brain and spinal cord [36]. GABA(B) receptor activation could result in a presynaptic inhibition of transmitter release as well as a postsynaptic increase in potassium conductance [37–39]. Numerous studies have shown that baclofen depresses the release of transmitters in the CNS and has a hyperpolarizing action on pyramidal cells, due to an increase in calcium conductance [40–42]. The cortical effects after baclofen-SLN administration indicate that this formulation passes the blood–brain barrier in higher amounts than the same dose of baclofen-sol, thus inducing prolonged inhibition of cortical modulation on spinal reflex. The balance of spinal and cortical effects of baclofen could explain our findings. Soon after administration, the effect the on spinal cord is prevalent and the H-reflex amplitude decreases; subsequently, the cortical effect predominates, cortical inhibition over spinal motoneuron vanishes, and facilitation of the H-reflex occurs. For clinical purposes, this effect of baclofen-SLN is obviously unwanted. However, it should be noted that baclofen-sol also produced sedation in our study, although to a lesser extent and corresponding to lower plasma concentrations compared with baclofen-SLN.

In conclusion, more precocious and more prolonged spinal and cortical effects of baclofen-SLN compared with baclofen-sol can be attributed to higher and more prolonged drug concentrations in the plasma and, primarily, in CNS, also depending on dosages of the drug included in SLN. Taking in account these data, further research will be directed toward obtaining a detailed definition of the pharmacokinetic profile, after optimizing dosages and concentrations of baclofen included in SLN, in order to preserve the prolonged antispastic effect, peculiar of this new formulation, but eliminating any clinically significant cortical effects. As was previously stated, SLN can also be prepared in a "stealth" version to increase their residence time in the blood. Further efforts will also be directed to evaluating the duodenal administration route as a new way to deliver this drug to spastic patients, as it is known that

unloaded SLN administered by that route are targeted to the lymph and some of the incorporated drug can reach the brain.

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