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Research paper

In vitro/in vivo characterization of melt-molded gabapentin-loaded poly(epsilon-caprolactone) implants for sustained release in animal studies

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ABSTRACT

Gabapentin (GBP) is a water soluble low molecular weight drug with anticonvulsivant and antinociceptive activity. In animal models, systemic administration regimes resembling chronic exposure to this drug (50 mg/kg, twice a day during one week), induce memory impairment. Aiming to gain further insight on the mechanisms involved in this process, a monolithic implant that releases constant plasma levels during one-week was designed. GBP-loaded poly(epsilon-caprolactone) matrices were produced by means of a simple and reproducible melt-molding/compression procedure. In vitro release studies firstly comprised uncoated implants that displayed release profiles according to a pseudo-first order model. In order to further regulate the release, two-sided coated implants where drug-free layers would perform as membranes controlling the delivery rate were prepared. A more moderated burst effect and a relatively linear (zero-order) release between days 1 and 7 were apparent. Implants were investigated in vivo and the plasma levels monitored during 10 days. Findings indicated that after a more pronounced release during day 1 and the achievement of the levels in blood comparable to a twice-a-day intraperitoneal management, relatively constant levels were attained until day 7. Overall results support the usefulness of this manufacturing method for the production of implants to attain more prolonged GBP release profiles in memory animal studies.

1. Introduction

Long-term pharmacotherapy employed in the treatment of different pathologies may affect the memory and learning processes [1]. In this context, aiming to characterize the cognitive effects associated with the chronic exposure to newly developed or already commercialized drugs, a number of preclinical studies are usually conducted [2,3]. Most of this work comprises the administration of a single or limited number of doses (administered at different time points) and the latter evaluation of the effect they have on certain behavior. However, in the case of drugs involved in the treatment of chronic diseases, these studies are of partial relevance. Having expressed this, only a few preclinical studies that evaluate the effect of chronic treatments on the cognitive aspects are available in the literature [4,5]. This is probably due to time-consuming administration schedules and the large number of animals required in this kind of experiment.

Epilepsy is a very common and complex pathology characterized by anomalous neuronal discharges [6]. Chronic treatment with antiepileptic drugs aims to minimize the recurrence of convulsions by reducing the neuronal activity and excitability. At the same time, their inherent mechanism of action can eventually affect different neurobiological systems related to the cognitive process. From this perspective, both the disease and its treatment trigger cognitive impairment [7,8] that could be especially critical in the earlier stages of the learning process [4]. Some clinical studies evidenced the negative influence of gabapentin (GBP), a well-known add-on antiepileptic drug used under chronic management, on memory performance after standard administration regimes [9–12]. In contrast, other scientists proclaimed that this agent had minimal effects on cognitive functions and improved memory and attention in different tests [13]. GBP was approved for use in the US in the early nineties [14,15]. Due to a relatively fast renal clearance it is administered several times a day [16] in epilepsy, neuropathic pain and also in off-label treatments [17,18], being all these chronic regimes.

In the recent years, the potential contribution of GBP to memory deficit or enhancement has been a matter of comprehensive investigations by research groups worldwide. We previously evaluated

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the effect of a single GBP intraperitoneal injection (i.p.) on the step-through inhibitory avoidance task in mice [19–21]. Findings indicated improved performance retention as compared to controls. More recently, the effect of a single administration of 50 mg/kg of GBP was compared to the response resulting from repeated similar doses of the drug [22]. Interestingly, whereas a single injection immediately after training not only preserved but also improved the avoiding performance on day 8, the administration of 2 doses daily during one week after training led to amnesia. Aiming to discern whether these opposed effects stem from constant low drug plasma levels or, by the contrary, from plasma concentration peaks induced by repeated administrations, we present the design of a drug delivery system (DDS) that assures for at least 1-week sustained plasma levels of GBP, similar to those attained after the redistribution phase of an i.p. administration [22].

Poly(ε-caprolactone) (PCL) is a substantially hydrophobic and semi-crystalline polyester that, due to its proven biocompatibility, is extensively used in several biomedical applications [23]. PCL-based implants display high permeability to many drugs and remarkable biocompatibility, becoming a very attractive biomaterial in the design of drug delivery systems [24–26]. Due to its low water affinity, PCL displays longer degradation times than other polyesters [27,28]. Independently of the molecular weight, PCL presents a relatively low glass transition temperature around −60 °C and a melting point ~60 °C [29]. These thermal properties were capitalized in order to fabricate DDS by means of processes such as melt molding, injection or extrusion without risking neither the polymer nor the drug molecular integrity due to an aggressive heating treatment [30,31].

Taking advantage of the unique properties of PCL, we describe herein a straightforward methodology (melt molding/compression) to produce GBP-loaded implants for the sustained release of the drug and suitable for pharmacological studies in animal models. Drug-free and drug-containing implants were produced and the release primarily evaluated in vitro. Finally, implants were inserted subcutaneously in mice and plasma levels assayed along 10 days. The relatively slow degradation kinetics of the implants permitted its surgical removal after the release assays in order to perform additional characterizations of the matrix and the analysis of unreleased drug.

2. Materials and methods

Poly(ε-caprolactone) (PCL, MW 14 kDa; Sigma, St. Louis, MO), gabapentin (GBP, Triquim SA, Argentina) and solvents (analytical grade) were used as received. *O*-phthaldialdehyde and mercaptoethanol were purchased from Sigma.

2.1. Implant preparation

In order produce GBP-loaded PCL implants (350 mg total weight, 10, 15 and 20 wt% drug) polymer and drug powders were mixed in different weight ratios, poured into a stainless steel mold (11 mm in diameter), tightly compressed with a plunger, heated in a preheated oven (70 °C, 1 h) and compressed (0.713 Kg/cm^2) during the thermal treatment. Then, molds were cooled (4 °C, 30 min) and finally solid samples removed to obtain disc-shaped implants (11 mm diameter, 3.3 mm thickness, Fig. 1). In order to fine tune the release kinetics, two-sided coated implants (450 mg total weight, 11 mm diameter, 4.3 mm thickness) were produced. In this case, drug-free PCL powder (50 mg) was introduced into the mold prior to the addition of the polymer/GBP mixture. Finally, an identical amount of a drug-free PCL was added and the melt-molding/compression procedure performed. Thus, the system was heated only once the three layers of powder were placed into the mold. GBP-free matrices were manufactured similarly and were used as controls. In order to investigate the thermal stability of GBP under these conditions, drug powder was exposed to heating (70 and 80 °C, 1 h) and titrated (see below). A similar stability analysis was performed on GBP after the production of the implants. Samples containing 70 mg drug were dissolved in dichloromethane (2 mL, Sintorgan, Argentina), extracted with distilled water (3 × 2 mL) and the aqueous fractions analyzed for GBP content. Assays were performed by triplicate and results expressed as Mean ± SD. In order to quantify GBP, 10 min prior to the analysis, the drug was derivatized with o-phthaldialdehyde as previously described (see below) [22,32].

2.2. Thermal analysis

Samples (4–7 mg) of native PCL, GBP, drug-free and drug-containing implants were sealed in 40 μl Al-crucible pans and analyzed by Differential Scanning Calorimetry (DSC, Mettler TA-400 differential scanning calorimeter) in a single heating ramp (25–210 °C, 10 °C/min). Values of enthalpy were normalized to the content of the correspondent component in the sample. ΔH_m of 100% crystalline PCL was 139.5 J/g.

2.3. In vitro release

Release profiles were assayed in vitro using a static method. Specimens ($n = 3$) were immersed in phosphate-buffered saline (10 mL, PBS, pH 7.4) and incubated at 37 °C. At different time points, supernatant solution was removed, replaced by fresh medium and the GBP content determined by HPLC after a convenient dilution. Results are expressed as Mean ± SD.

2.4. In vivo release

The in vivo release profiles were assessed in CF-1 male mice ($n = 19$, age 60–70 days; weight 25–30 g, FUCAL, Buenos Aires, Argentina). Animals were individually identified, housed in stainless steel cages and kept in a conditioned room (21–23 °C, 12 h light/dark cycle). Access to fresh dry food and tap water, both conveniently renewed every 24 h, was unrestricted. Experiments were performed in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals (NIH Publication No. 80-23/96), the guidelines for the use of animals in neuroscience and behavioral research (Institute for Laboratory Animal Research Division on Earth and Life Studies, National Research Council) and local regulations. All efforts were made in order to minimize animal suffering and to reduce the number of animals used. Implants were inserted in a subcutaneous pocket in the side of the mice. After implantation, incisions were sutured. During the experiments, mice were monitored for physical condition (weight and behavior). At each time point, blood samples were extracted from the ophthalmic sinus using heparinized capillaries and GBP plasma levels were determined. Each animal was bled 2–4 times at different time points along the study. In order to characterize the in vivo GBP release, the implants were recovered from mice at different time points and dried under mild conditions (48 h at 37 °C). The implant weight loss was assessed and the drug still entrapped in the implants extracted and analyzed (see below).

2.5. Chromatographic method for gabapentin analysis

Citrate buffer, sodium borate, 2-mercaptoethanol and *O*-phthaldialdehyde solutions were successively added as previously published [22] and the samples (20 μL) injected into a liquid chromatography separation unit consisting of a Waters 515 HPLC pump (Waters, Milford, MA), a C18 column (Phenomenex, 5-μm, C18, 250 × 4.6 mm;

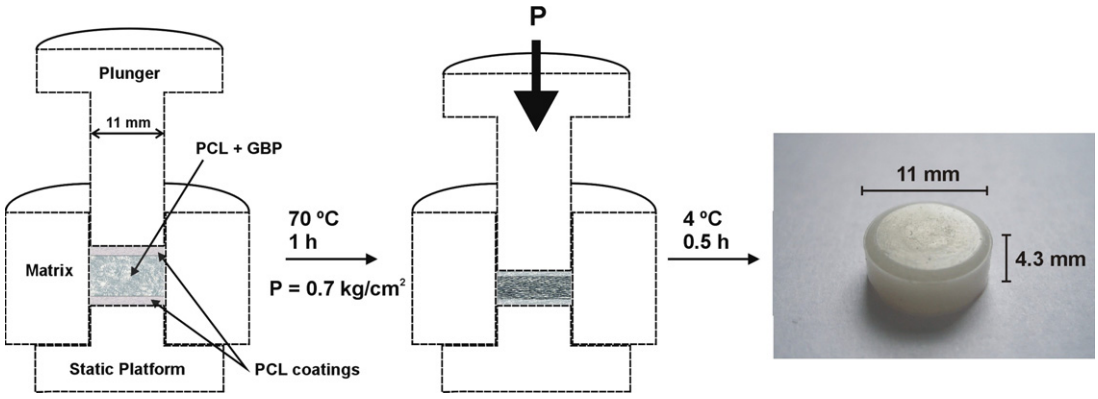

Fig. 1. Fabrication process of a coated GBP-loaded PCL implant by means of a melt-molding/compression technique. In order to produce an uncoated matrix, drug/polymer mixtures were directly poured into the mold. Cross sections of the stainless steel molds are represented.

Phenomenex Co., Torrance, CA), and a fluorescence detector ($\lambda_{excitation}$ = 230 nm, $\lambda_{emission}$ = 420 nm, Model FL-45A, Bioanalytical Systems, West Lafayette, IN). The mobile phase was an acetonitrile:0.02 mM sodium phosphate buffer mixture (1:1, pH 4) at a flow rate of 1.4 mL/min. In order to analyze GBP plasma levels, blood samples (100 μL) were centrifuged (3000 rpm, 5 min, 4 °C) and the plasma isolated. Proteins removal was carried out by the successive addition of acetonitrile (30 μL) and zinc sulfate solution (10 μL, 10%). Then, samples were centrifuged again (4000 rpm, 5 min., 4 °C) and the supernatant assayed for GBP as detailed above. The chromatographic method was linear in the range of 0.1–100 μg/mL and the coefficient of variation was less than 20% at the lowest concentration. The limit of quantification of GBP was 100 ng/mL.

3. Results

In order to assess the pharmacological effect of GBP on cognitive performance in mice, this study aimed to design a GBP-containing polymeric delivery system that would (A) assure low and constant plasma levels in the 1–5 μg/mL range during 1 week and (B) prevent the repetitive exposure to the extremely high concentrations (>100 μg/mL) observed immediately after the intraperitoneal administration [22].

Disc-shaped GBP-loaded PCL implants containing 10–20 wt% drug were produced by a simple and straightforward melt-molding/compression method (Fig. 1). The dimensions of the implants (11 mm diameter, 3.3–4.3 mm thickness) were planned considering the implantation site.

3.1. Thermal analysis

Crystallinity strongly affects both the drug release kinetics and the degradation. In order to characterize the matrix, the thermal behavior of drug-loaded implants after the melt-molding process was analyzed by DSC and compared to the properties of raw PCL (powder), GBP and a drug-free implant (Fig. 2). T_m and ΔH_m values are summarized in Table 1. Native PCL showed a T_m = 64 °C and a ΔH_m value of 94.7 J/g that represented a 67.9% crystallinity. As observed in drug-free implants, the fabrication procedure negligibly affected the T_m. In contrast, the enthalpy involved in the transition decreased to 80.2 J/g (57.5% crystallinity). In the drug-containing matrix, ΔH_m values were similar to the observed for drug-free PCL implants and pure GBP, respectively. Complementary information on the stability of the drug to the thermal treatment was obtained by evaluating GBP recovery extents in the implants, immediately after their production. Results demonstrated a 100.3 ± 2.0 % drug recovery and supported the compatibility of the heating process. Similar findings were apparent for pure GBP.

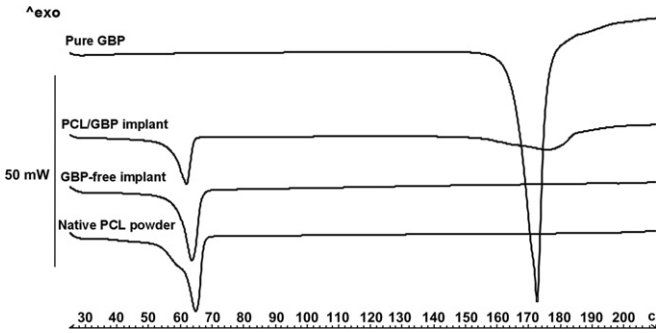

Fig. 2. Representative DSC thermograms of native GBP, PCL implants containing 20% GBP, GBP-free PCL implants and native PCL powder.

Table 1
T_m and ΔH_m values of pure PCL and GBP and the drug-free and drug-loaded implants

Sample	Native PCL powder	GBP-free PCL implant	Native GBP	GBP-loaded PCL implant	
				PCL	GBP
T_m (°C)	64	63	172	61	176
ΔH_m (J/g)	94.7	80.2	456.4[a]	83.0	456.3
% Crystallinity	67.9	57.5	100	59.5	100

T_m: melting temperature.
ΔH_m: melting enthalpy.
[a] This enthalpy value was considered 100% crystallinity.

3.2. In vitro GBP release

The study of the in vitro release kinetics was a central task in order to fine tune the properties of the implants towards the in vivo experiments. Fig. 3A shows the cumulative release of GBP from uncoated drug-loaded implants over a period of 10 days. During the first day of the assay, a faster release was observed. Then, rates gradually slowed down to follow a constant regime. This was apparent especially in 15 and 20% systems. In contrast, after day 4, 10% matrices released almost negligible amounts of drug. This fact was even more evident when the release was represented on a daily basis (Fig. 3B). In Fig. 3C, the cumulative release was plotted against the time square root ($t^{1/2}$) according to the Higuchi model. The linear curve for 15 and 20% GBP-loaded implants indicated that the release followed a pseudo first-order profile (note R^2 = 0.997 in both cases, R2 in graph). In contrast, 10% samples slightly deviated from linearity. Application of the Korsmeyer–Peppas model (SigmaPlot® Software 2001, SPSS Inc.) was done

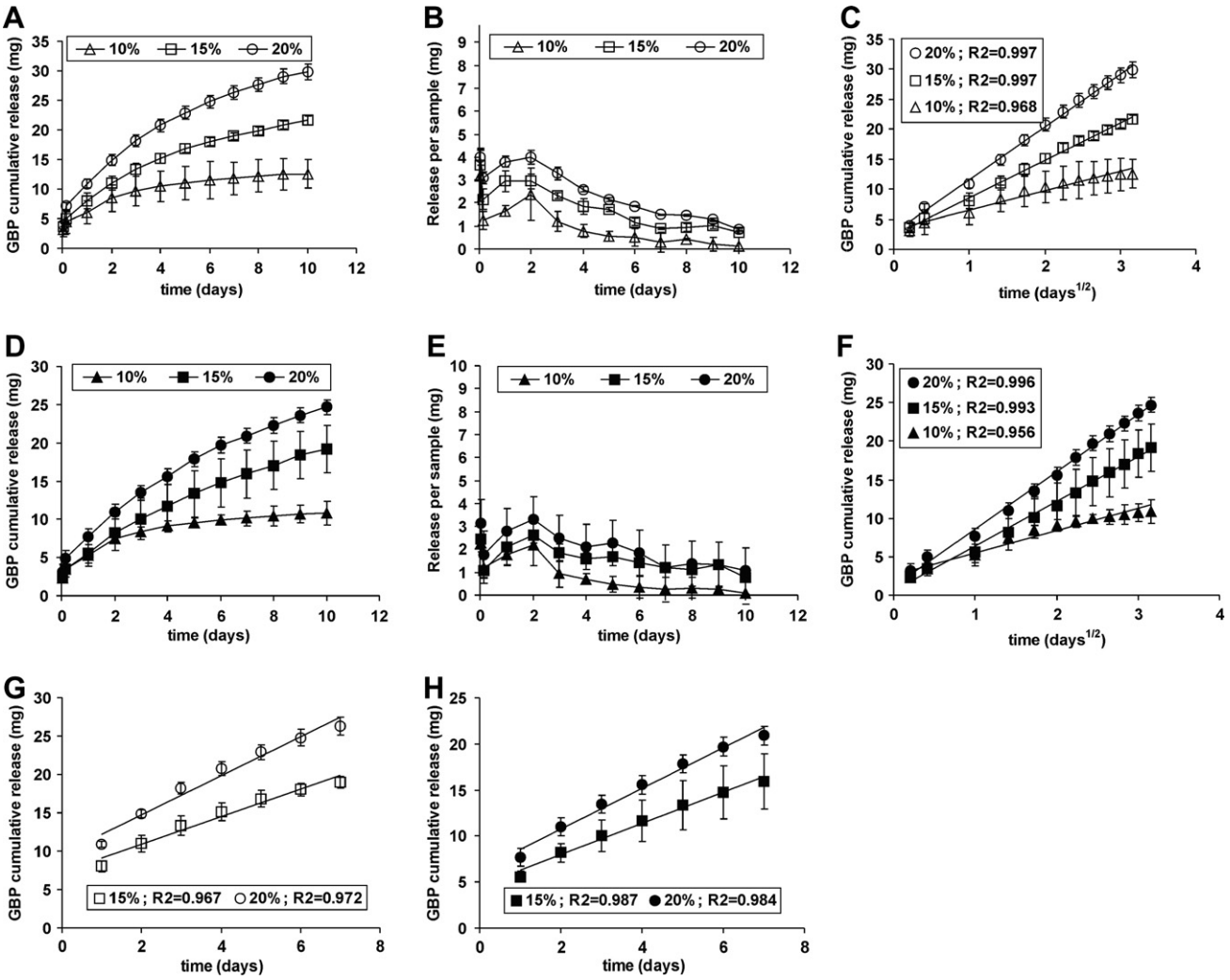

Fig. 3. In vitro release profiles of GBP from the manufactured implants. (A) Cumulative release of the uncoated systems. (B) Release per sample of the uncoated systems. (C) Cumulative release of the uncoated systems graphed against the time square root ($t^{1/2}$) according to the Higuchi model. (D) Cumulative release of the coated systems. (E) Release per sample of the coated systems. (F) Cumulative release of the coated systems graphed as in (C). (G) Cumulative release of uncoated systems between days 1 and 7. (H) Cumulative release of coated systems between days 1 and 7. Means ± SD of triplicate assays are shown. When available, R^2 values (R^2) are shown in graph legends.

considering samples as cylinders. Results showed values of n (release exponents) <0.45 for all the uncoated samples, supporting a combined release model comprising Fickian diffusion and other mechanisms (Table 2).

In order to achieve a more controllable drug delivery immediately after implantation, two-sided (top and bottom) coated implants were prepared by modifying the preparation protocol; a small amount of drug-free polymer powder was poured into the mold before and after the addition of the drug/polymer mixture. Afterwards, the cumulative release of coated specimens was investigated (Fig. 3D). Coated samples rendered a more moderated burst effect followed by a slight decline in the slope of the curve that resulted in an almost steady delivery. It is worth remarking that the smaller drug-loaded free surface area in a direct contact with the release medium resulted in a more controlled delivery than the one attained with the uncoated implants, releasing lower drug amounts during the initial sampling times as compared to uncoated systems with similar drug load (Fig. 3E). For example, on day 1, uncoated and coated 20% GBP-loaded implants released about 12 and 7 mg drug, respectively. Also here, 15 and 20% specimens displayed an overall pseudo-first order release (Fig. 3F, $R^2 > 0.99$). It is noteworthy that between days 1 and 7 both uncoated and coated 15 and 20% samples approximated to a zero-order release profile, with $R^2 > 0.96$ (Fig. 3G and H). Then, release data were processed by Korsmeyer–Peppas. In the case of 10% coated specimens, n values were similar to those calculated for

Table 2
Curve fitting analysis of GBP-loaded PCL implants

Formulation	GBP content (wt%)	Zero-order model $M = k_0 \cdot t$		Higuchi model $M = D \cdot t^{0.5}$		Korsmeyer–Peppas model, $M_t/M_\infty = k \cdot t^n < 0.6$		
		k_0	r^a	D	r^a	k	n	r^a
Uncoated	10	2.494	0.844	9.293	0.967	0.200	0.270	0.993
	15	3.289	0.934	11.821	0.997	0.169	0.388	0.996
	20	3.498	0.932	12.591	0.997	0.171	0.402	0.997
Coated	10	2.220	0.818	8.363	0.958	0.167	0.286	0.995
	15	3.130	0.971	11.015	0.993	0.115	0.499	0.996
	20	2.837	0.940	10.14	0.992	0.119	0.472	0.996

Zero order model: M, amount released; k_o, zero order release constant.
Higuchi model: M, amount released; D, diffusion rate constant.
Korsmeyer–Peppas model: M_t, amount of drug released at time t; M_∞, amount of drug at infinity; M_t/M_∞, fraction of released drug; k, kinetic constant; n, release exponent.
[a] Regression.

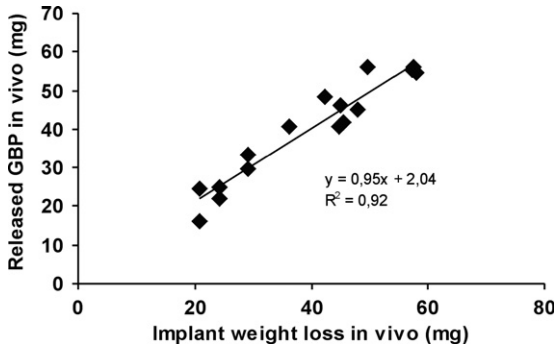

Fig. 4. Correlation between the implant weight loss in vivo and the in vivo GBP release from coated 20% GBP-loaded implants. Previous to determine the final weight of the implants, they were dried 48 h at 37 °C. In vivo GBP release was calculated from the remaining amount of GBP in the dried recovered implants. Individual data from matrices explanted at different time points along the 10 day in vivo assay are represented.

uncoated samples. Contrary to that, for more concentrated coated systems (15% and 20%), n approximated to 0.45 (Table 2). Fig. 4.

3.3. In vivo GBP release

In vivo release experiments involve a more complex system and extrapolation from in vitro assays is not always possible. In order to demonstrate the feasibility to achieve the desired GBP levels over a 1-week time period, implants were inserted into a subcutaneous pocket generated on the side of mice and the drug in plasma analyzed at different time points. The in vivo experiments were limited to a coated 20 wt% GBP-loaded systems due to a more fine-tunable burst release in comparison to uncoated ones and the relatively linear delivery found between days 1 and 7 (in vitro studies). Mice did not show any weight or behavioral change during the whole study. As opposed to the in vitro experiments where the amount of released drug was calculated from the supernatant, the amount of GBP released in vivo could not be calculated from the plasma drug concentrations. A priori, degradation of PCL was not expected and any weight loss in the implant should necessarily stem from the drug delivered to the medium. Drug-free implants assayed for weight loss in vitro showed only a 2% weight loss during more than 2 months (not shown), confirming the high stability of the polymeric system. In vivo, drug-free controls lost 0.9% of the initial weight during the first week of the assay (not shown). Matrices were explanted on Days 3, 7 and 10 and evaluated for appearance and weight loss. The shape and the dimensions of explanted implants remained unchanged. In addition, either in the in vitro and the in vivo specimens, the release of the water-soluble GBP left behind a similar pattern of clefts homogenously distributed along the piece (not shown). Then, the drug that remained entrapped in the implant at the end of the assay was extracted, analyzed and used to indirectly calculate the amount of drug released. From the correlation curve it was apparent that the weight loss exclusively depended on the GBP release (see slope ~1, Fig. 5). Blood samples were processed and serum levels determined (Fig. 5A). After slightly higher plasma levels during the first 3 h of the study (probably due to the direct exposure of drug-loaded areas at the edges of the implant; see detail in Fig. 5A), GBP plasma concentrations reached values of about 5 μg/mL. Thereafter, concentrations remained in the 2–3 μg/mL range between days 1 and 7 indicating a constant release. These findings agreed with the expected plasma levels (between 1 and 5 μg/mL). An additional goal of the implants was to prevent the exposure to very high GBP levels (>100 μg/mL) observed upon injection in the twice-a-day i.p. management. It is worth remarking that our results indicated that

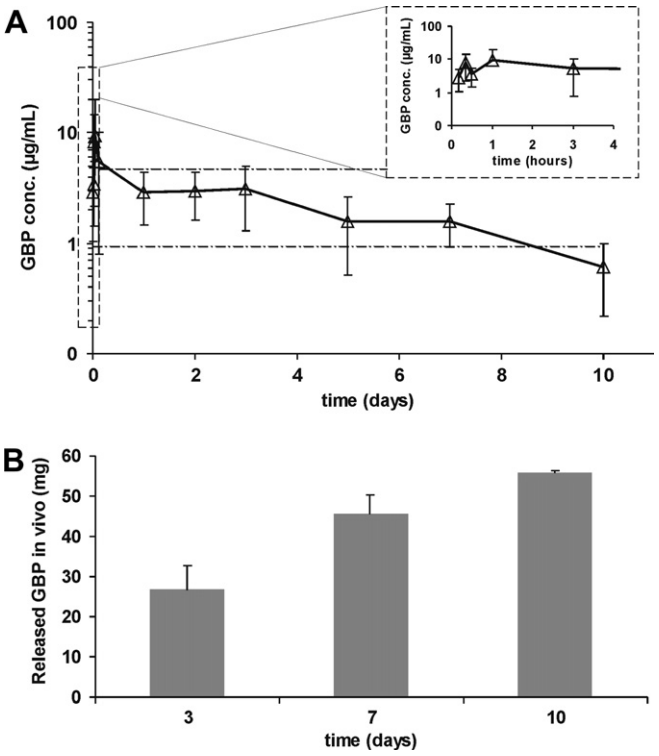

Fig. 5. In vivo GBP release from coated 20% GBP-loaded implants. (A) Means ± SD plasma concentration values are represented. Horizontal dotted lines delimit the concentration range between 1 and 5 μg/mL. (B) Cumulative in vivo release profile as determined from the remaining amount of gabapentin into the recovered implants. Means ± SD of 3–7 values are represented.

only 3 out of 27 individual plasma samples analyzed during the first 3 h after the subcutaneous implantation displayed concentrations in the 10–30 μg/mL range, being all the others between 1 and 10 μg/mL. After Day 7, a more pronounced decay to values below 1 μg/mL was apparent. Fig. 5B represents the GBP cumulative release versus time in vivo. Even though data prior to day 3 are not available, findings suggested a faster release during the first three days of the assay (25 mg) with a later decline to follow a linear release (zero-order profile). Remarkably, released amounts of drug were higher than those observed in the in vitro static assays.

4. Discussion

Due to discrepancies about the effect of GBP under chronic regimes on cognitive performance, our research group has dedicated efforts to investigate the consequences of such therapy in the long-term range. Paradoxically, in order to perform these studies, drugs are often administered in one single dose or utmost in a limited number. The fact that GBP is clinically used under a long-term course makes these studies of limited value. In this context, Blake and co-workers compared the behavior shown by mice exposed to (A) a single injection of GBP or (B) a 1-week routine (twice-a-day, every 12 h) [22]. In the former, short-lasting (1 h) high GBP plasma concentrations (up to 140 μg/mL 10 min after 50 mg/kg i.p. injections [22]) followed by low levels (~1 to 5 μg/mL, detectable 1–4 h post-injection), resulted in memory enhancement. Contrary to this, the 1-week regimen that results in a repetitive peak-trough profile resulted in cognitive impairment. Aiming to obtain additional information about the mechanisms involved in memory enhancement/impairment phenomena, GBP-loaded PCL monolithic implants were prepared by a melt-molding/compression technique and the release kinetics in vitro and in vivo thoroughly

investigated. The main features of the delivery systems were the attainment of GBP plasma levels comparable to those observed after the redistribution phase of an i.p. administration (1–5 μg/mL) though without the initial exacerbated burst effect.

Several devices were considered in order to achieve this goal. Preclinical studies frequently employ osmotic minipumps subcutaneously implanted [33]. However, these devices are usually expensive, their drug release rate is limited by the solubility of the loaded drug and, in specific cases where a localized delivery is desired, their performance is less efficient than polymer matrices inserted in the area of release. In the case of GBP, the design of pro-drugs [34] and gastroretentive tablets [35] enabled to reduce the administration frequency in humans. In animals, these approaches are less practical than parenteral devices. We are interested in exploring the possibility of using polymer-based systems in order to conduct preclinical studies. In this framework, this work reports on the sustained delivery of a water-soluble and low molecular weight drug, GBP, currently under investigation in our group.

The unique thermal properties of PCL (low T_g and T_m) enabled a simple, fast and reproducible fabrication process that rendered disc-shaped implants. In addition, the relatively high stability of the polymer to hydrolysis enabled explantation for further structural and drug content analysis (see below). It is worth stressing that different thicknesses may eventually affect the long-term degradation profile of the implant. In general, for implants displaying identical diameters, the thicker the implant is, the longer the degradation process. Nevertheless, the goal of this study was to design a system that would enable the correlation between the implant weight loss in vivo (determined after the implant removal) and the released amount of GBP. In this context, degradation studies were out of the scope of this work.

In order to obtain mechanically stable matrices, while constraining crystallization that usually hinders diffusion and drug release, PCL with an intermediate molecular weight (14 kDa) was used. The produced implants presented only 57.5% crystallinity. In general, in systems where a good drug/polymer interaction takes place, polymer crystallization extents raise as drug crystals play the role of nuclei in a heterogeneous nucleation process [36]. In the PCL/GBP system, a negligible drug-polymer interaction was observed as expressed by similar ΔH_m values apparent for both drug-free and drug-loaded implants (Table 1). This phenomenon was expected due to the contrasting properties of the components: high and low hydrophilicity of the drug and the polymer, respectively. GBP is a low molecular weight drug that displays a melting point above 170 °C. Degradation of GBP to its lactam derivative could occur in an aqueous medium under certain conditions of pH and temperature [37]. This degradation leads to a clear change in the retention time by HPLC [33,38]. Findings of drug recovery assays upon exposure to the thermal treatment showed 100% drug recovery. In addition, analyses did not show the presence of any secondary elution peak belonging to the degradation product. Accordingly, we assumed that since the drug was heated under water-free conditions, it maintained the initial potency.

As previously detailed, the goal of the work was to attain a relatively constant GBP release during a period of 7 days. In order to primarily evaluate the release kinetics, drug-loaded specimens were incubated in PBS and the drug released under static conditions was assayed over time. This stage also enabled to adjust the properties of the implant in view of the in vivo studies. Findings obtained with uncoated systems depended on the drug initial load. In general, due to (A) a fall in the implant drug content, especially in the outer layers, and (B) an increase in the path length that drug molecules residing in the inner parts of the matrix need to go through, a decrease in GBP release rate was observed over time. Drug delivery from monolithic matrices depends on the hydrophilic/hydrophobic balance of the surface [39]. A more hydrophilic surface that favors wetting, leads to better solubilization at the superficial level and to a faster release due to the formation of pores that enhance drug diffusion. Systems containing 10% GBP showed a fast release until day 2. Then, the amounts of delivered drug rapidly declined and stayed at a minimal level during the rest of the study (probably due to a sharp decrease in the concentration gradient) (Fig. 3A). In contrast, more concentrated samples (15% and 20%) extended the release times until Day 10 and kinetics agreed with the model described by Higuchi (see the linear relationship between the cumulative GBP released versus the square root of the time, Fig. 3C) [40]. In addition, data were processed applying the Korsmeyer-Peppas model (Table 2). Findings indicated that the release approached to a Fickian diffusion profile, though deviation from $n = 0.45$ supported the presence of a partial diffusion mechanism through water-filled pores [41]. In 10% samples, due to the low amount of the water soluble GBP a relatively low porosity was originated, resulting in a plateau on Day 4. Implants with higher GBP contents (a readily water soluble drug) increased the water affinity of the surface and also rendered a more porous structure that enabled more prolonged release times as opposed to the less concentrated implants [39]. In order to perform in vivo studies, we were interested in systems combining a more moderated initial release (that enables the attainment of targeted plasma levels) with a zero-order profile after the burst effect. This behavior would assure concentration levels within a narrow range during at least 1 week. Thus, two-sided coated systems were prepared and evaluated [39]. According to our design, matrices comprised (A) a drug-free polymer layer on the top and the bottom of the implant that would expectedly act as a rate controlling membrane and (B) a relatively small drug-containing free surface area in direct contact with the medium (at the edges of the disc-shaped system) that would result in a relatively attenuated burst effect. This structure would enable to reach the desired concentration range promptly after implantation. In contrast, completely coated implants may have displayed a very limited initial burst release and thus prevented the attainment of active plasma concentrations, immediately after implantation. In the case of samples containing lower drug loads (10%), in vitro release profiles were similar to the uncoated ones. However, cumulative concentrations at each time point were lower than in the case of uncoated specimens. These findings stemmed from the restricted porosity generated in the uncoated systems (similar in coated ones) and a fast gradient decrease. Contrary to that, more concentrated specimens (15% and 20%) approached the designed delivery profiles and after an initial faster release (Day 2) an almost linear curve was apparent until Day 7 (Fig. 3G and H). Cumulative GBP release curves of uncoated and coated specimens between Days 1 and 7 versus time were also fitted to zero-order kinetics. While 10% concentration deviated substantially from linearity, all the samples containing 15 and 20% GBP showed $R^2 > 0.96$, supporting the constant drug-content independent release.

In vitro investigations constitute a useful first approximation to the in vivo model, though prediction of in vivo behaviors based on them is sometimes difficult. Accordingly, drug-containing implants were inserted in subcutaneous pockets in the side of mice and GBP serum concentrations followed up during 10 days. At selected time points, animals were euthanized and implants removed for further weight and GBP content analysis. As a preliminary indication of the biocompatibility of the implant, a visual inspection of the implantation site was performed. No evidence of inflammatory response or infection was found. This finding was in full agreement with the profuse bibliography supporting the biocompatibility of PCL in contact with different tissues and organs [23,42]. Removal of the specimens was simple as formation of a fibrotic capsule usually occurs about 3–4 weeks after implantation of biocompatible matrices [43]. Studies by Baratti and collaborators using a twice-a-day

management administered about 3 mg/mouse/day attained plasma concentrations in the 1–5 μg/mL range 1–4 h after the injection [22]. The task of this work was to obtain similar steady levels during 1 week, though preventing the extremely high post-injection concentrations. 20% coated samples released about 2.2–2.5 mg/day in vitro that approximated to the total dose administered daily in the mentioned study [22]. In order to associate the released amount of GBP at each time point to the levels measured in plasma, a correlation between the implant weight loss (as determined after removal from mice) and the drug that remained entrapped in the matrix after the in vivo experiments was carried out (Fig. 4). The accurate correlation established was proven to be a useful tool to follow up the delivery without the need of extracting and quantifying the remaining drug in the implants.

Due to a higher molecular hydrophobicity, PCL is more stable to hydrolysis than other polyesters such as polylactic and polyglycolic acid [44] and, in general, degrades in vivo after several months to several years, depending on the structural properties of the polymer (e.g., molecular weight, crystallinity) [23] and the presence of modifications leading to a more hydrophilic matrix. In vitro studies in our laboratory indicated that drug-free implants remained unchanged for at least 2 months in full agreement with very recent reports using PCL with identical MW that indicated stability for at least 5 months [45]. Findings showed that 20% GBP-loaded coated implants displayed a pronounced increase in the released levels in vivo (almost 4.5 mg/mouse/day) as compared to the same system in vitro (~2.5 mg/day). As described above, due to the low erosion of the PCL-made implant, release was mainly dictated by gradient-driven passive diffusion. Thus, the presence of sink conditions in vivo, the relatively fast clearance of the drug by kidney filtration and the accumulation in CNS [22] led to an enhanced delivery due to the constant restitution of the gradient as opposed to the in vitro experiments. More importantly, plasma levels measured along the time indicated not only controlled and stable concentrations in the expected range (1–5 μg/mL), but also the absence of the concentration peaks found after the i.p. administration (Fig. 5A) [22]. Other studies using similar matrices for the localized release of the hydrophilic antitumoral agent topotecan to the posterior segment of the eye are underway [46].

5. Summary

GBP-loaded implants were prepared by means of a melt-molding/compression technique. In vitro preliminary investigations were performed using uncoated implants, showing their release profile in agreement with the Higuchi diffusion model (pseudo-first order). Aiming to fine tune the release profile, particularly during the first hours upon implantation, coated implants were also developed. While a more moderated burst effect was observed during day 1, a zero-order delivery profile between days 1 and 7 was apparent. Finally, the most concentrated coated implants were inserted subcutaneously in mice and the drug levels followed up during 10 days. Even though the behavior coincided with the in vitro assays, higher release levels were attained.

Overall results support the use of PCL-based implants produced by means of this simple, relatively low cost, reproducible and fast methodology for the sustained administration of hydrophilic drugs in preclinical pharmacological studies in vivo. Provided that the stability of the candidate drugs is not affected by the manufacturing process, selected dose regimes for several hydrophilic molecules could be fulfilled by manipulating simple parameters in the production method like the size of the molds, the drug-to-matrix ratio and the coating of the implants. Additionally, taking advantage of the monolithic nature of the PCL implants, a straightforward method to evaluate released drug amounts in vivo is proposed by assessing the implant weight loss. Memory performance studies are being conducted in our laboratory and results will be published separately.

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