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Research paper

Drug delivery to the brain using surfactant-coated poly(lactide-co-glycolide) nanoparticles: Influence of the formulation parameters

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ABSTRACT

Poly(lactide-co-glycolide) (PLGA) nanoparticles coated with poloxamer 188 (Pluronic® F-68) or polysorbate 80 (Tween® 80) enable an efficient brain delivery of the drugs after intravenous injection. This ability was evidenced by two different pharmacological test systems employing as model drugs the anti-tumour antibiotic doxorubicin and the agonist of opioid receptors loperamide, which being P-gp substrates can cross the blood–brain barrier (BBB) only in pharmacologically insignificant amounts: binding of doxorubicin to the surfactant-coated PLGA nanoparticles, however, enabled a high anti-tumour effect against an intracranial 101/8 glioblastoma in rats, and the penetration of nanoparticle-bound loperamide into the brain was demonstrated by the induction of central analgesic effects in mice. Both pharmacological tests could demonstrate that therapeutic amounts of the drugs were delivered to the sites of action in the brain and showed the high efficiency of the surfactant-coated PLGA nanoparticles for brain delivery. The results of the study also demonstrated that the efficacy of brain delivery by nanoparticles not only is influenced by the coating surfactants but also by other formulation parameters such as core polymer, drug, and stabilizer.

1. Introduction

In the past few decades, tremendous efforts have been focused on the chemotherapy of the diseases of the CNS. However, in many cases, the success of the treatment remained poor. One of the obstacles for successful chemotherapy is the ineffective drug delivery to the site of pathology within the CNS due to existence of the blood–brain barrier (BBB), which limits the entry of many substances into the brain. The approaches attempting to reach efficient concentrations of the drug in the brain include high-dose therapy or invasive methods (such as intraventricular drug infusion or temporary BBB disruption in the case of brain tumours). Clearly, in both cases, the therapeutic effect is achieved at the expense of deleterious side effects. So far, the non-invasive systemic delivery of the drugs to the brain remains a challenge that gives rise to the development of new drug-targeting technologies.

Among other strategies, the non-invasive systemic drug delivery to the brain by means of the nanoparticulate carriers appears to be a promising option. As evidenced by a number of studies, poly(butyl cyanoacrylate) (PBCA) nanoparticles coated with polysorbate 80 (Tween® 80) facilitate the brain delivery of a number of drugs that are unable to cross the BBB in free form [1]. Subsequently, polysorbate 80 was considered to be a 'gold standard' coating surfactant for brain delivery and proved to be effective for different types of the nanoparticles such as poly(alkyl cyanoacrylate) [2], human serum albumin [3], and solid lipid nanoparticles [4].

It was further shown that the surfactant coating is the key factor for the successful brain delivery [5]. The polysorbate 80-coated PBCA nanoparticles selectively adsorb certain plasma proteins (in particular, apolipoproteins E and B) from the blood, and these proteins promote receptor-mediated endocytosis of the particles by the endothelial cells forming the BBB, thus facilitating the delivery of the nanoparticle-bound drug into the brain. Indeed, binding of dalargin or loperamide to PBCA nanoparticles coated with polysorbate 80 and/or apolipoproteins E and B induced considerable CNS effects (analgesia), whereas the free drugs that are unable to cross the BBB were ineffective [6,7].

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The effectiveness of the polysorbate 80-coated PBCA nanoparticles for brain delivery was most clearly demonstrated by the high anti-tumour effect of the nanoparticle-bound doxorubicin against intracranial glioblastoma in rats [8]. Further studies, however, demonstrated that brain delivery by means of the nanoparticles is not restricted to coating with polysorbates. Poloxamer 188 (Pluronic® F-68) also considerably enhanced the anti-tumour action of doxorubicin bound to PBCA nanoparticles against intracranial glioblastoma [9,10]. In fact, poloxamer 188 and polysorbate 80 appeared to be similarly effective for brain delivery by the PBCA nanoparticles. This phenomenon was attributed to the enhanced adsorption of the plasma apolipoprotein A–I on the nanoparticle surface as a result of the coating of the particles with these surfactants. It was hypothesized that the correlation between the adsorption of apolipoprotein A–I on the nanoparticle surface and the efficient delivery of doxorubicin to the brain was due to the interaction of the particles with the scavenger receptor SR-BI at the BBB endothelium facilitated by this plasma protein, indicating that in addition of apolipoproteins E and B, apolipoprotein A–I also enables a drug delivery across the BBB with the surfactant-coated nanoparticles. Since apolipoproteins A–I and apolipoprotein E/B use different receptors and mechanisms for their interaction with the BBB [6,10], the involvement of these proteins in brain delivery by the PBCA nanoparticles suggests the versatility of the mechanisms by which plasma proteins influence the in vivo behavior and fate of the particles.

In any case, the earlier mentioned data indicate that the surfactant-coated PBCA nanoparticles appear to be an efficient and also relatively safe [11,12] carrier for brain delivery. However, this technology has certain limitations. First of all, although poly(alkyl cyanoacrylates) nanoparticles readily absorb amphiphilic substances (such as doxorubicin) that are stable under the preparation conditions (acidic aqueous media), the encapsulation of highly hydrophilic or, oppositely, poorly soluble substances is considerably less effective [13]. Moreover, the fast - within a few hours - biodegradation of poly(alkyl cyanoacrylates) nanoparticles [14], while being a welcome property in terms of safety, limits their application for the controlled release of many drugs.

Consequently, other types of nanocarriers, such as the vectorized nanoparticles made of human serum albumin, solid lipids, or poly(lactide-co-glycolide) (PLGA), have been developed, and their efficacy for brain delivery has been evidenced [15–18]. In comparison with the surfactant-coated nanoparticles, these carriers, derivatized with specific ligands (i.e. apolipoprotein E, transferrin or antisense oligonucleotides), represent much more complex - second generation - delivery systems. At the same time, the more simple approach using the surfactant-coated nanoparticles may yield similar results.

Accordingly, the objective of the present study was to investigate the feasibility of drug delivery to the brain using the surfactant-coated PLGA nanoparticles. In order to enable a comparison of the results, the experimental modalities that have proved to be successful for the PBCA nanoparticles also were employed here, and, for this reason, doxorubicin and loperamide served as the model drugs and polysorbate 80 and poloxamer 188 - as the coating surfactants.

2. Materials and methods

2.1. Materials

Doxorubicin HCl was purchased from Yick-Vick (Hong Kong, China). Poly(lactide-co-glycolide) (Resomer® RG 502H, lactide/glycolide = 50:50, i.v. 0.16–0.24 dl/g) was purchased from Boehringer Ingelheim (Ingelheim, Germany). Poly(lactide-co-glycolide) with acid end groups (Lactel®, lactide/glycolide = 50:50, i.v. 0,20 dl/g) was purchased from Lactel Absorbable Polymers (Durect Corp., Pelham, AL, USA). Polysorbate 80 (Tween® 80) was supplied by ICI Chemical (Essen, Germany). Loperamide, poloxamer 188 (Pluronic® F-68), poly(vinyl alcohol) (PVA, MW 30–70 kDa, 88% hydrolyzed), and human serum albumin (HSA, fraction V 96–99%) were purchased from Sigma (Steinheim, Germany). Other chemicals and solvents used in this study were of analytical grade.

2.2. Preparation of drug-loaded nanoparticles

2.2.1. Doxorubicin-loaded nanoparticles

Poly(lactide-co-glycolide) with acid end groups (Lactel®, 500 mg) was dissolved in 3 ml of dichloromethane. Doxorubicin (25 mg) was dissolved in 2 ml of 0.001 N HCl. These solutions were combined and emulsified using an UltraTurrax disperser (IKA, Germany). The obtained emulsion was added into a 1% solution of PVA in water (Dox-PLGA/PVA) or into a 1% solution of HSA in PBS (pH 7.2) (Dox-PLGA/HSA) and homogenized at 600 bar using a high pressure homogenizer (Emulsiflex C-5, Avestin Inc., Canada). The homogenization procedure was repeated three times, and then the organic solvent was removed using a rotary evaporator. The resulting nanosuspension was filtered through a G2 sintered glass filter and freeze-dried after addition of 5% of mannitol used as a cryoprotector.

2.2.2. Loperamide-loaded nanoparticles

Poly(lactide-co-glycolide) (Resomer® RG 502H, 250 мг) and loperamide (25 mg) were dissolved in 5 ml of dichloromethane, and this solution was added to a 1% aqueous solution of PVA (Lop-PLGA/PVA) or 1% HSA solution in PBS (Lop-PLGA/HSA). The mixture was first homogenized using an UltraTurrax disperser, and then the primary emulsion was homogenized at 600 bar using an Emulsiflex C-5 homogenizer and processed as described earlier.

2.3. Characterization of the nanoparticles

All measurements were done after freeze-drying of the samples.

2.3.1. Measurement of the particle size

Average particle size, polydispersity of the size distribution, and the zeta-potential were measured using a Malvern Zetasizer 3000HSA (Malvern, Worcs, UK). The cell temperature was 25 °C; the scattering angle was 90°. The samples were dispersed in purified deionized water (Milli-Q water) and diluted at least 50-fold for better measurement and suitable signal intensity.

2.3.2. Evaluation of the drug encapsulation efficiency and drug content

The drug encapsulation efficiency was evaluated by an indirect method, i.e. by assay of free drug after the separation of nanoparticles by ultrafiltration (Ultrafree MC centrifugal filter units, 30,000 NMWL, Millipore). The concentration of doxorubicin was measured by spectrophotometry at 480 nm. The assay of loperamide was carried out by HPLC as described by Chen et al. [19] using a Luna C18 column (250 mm × 4.6 mm, 5 μm, Phenomenex, Aschaffenburg, Germany). The flow rate was 1.0 ml/min, the mixture of acetonitrile–sodium phosphate buffer (40:60) (pH 2.3, 20 mM) was used as the mobile phase. The detection wavelength was 195 nm. The retention time of loperamide was 22 min.

For the determination of the doxorubicin content and encapsulation efficacy, the freeze-dried formulations were dissolved in DMSO containing 0.004% HCl to compensate for the basicity of this solvent. The insoluble material was separated by centrifugation (15 min at 16,000g), and the concentration of doxorubicin was measured spectrophotometrically at 480 nm. In the case of loperamide, the samples were dissolved in DMSO and then diluted with

acetonitrile; the drug concentrations were analyzed by HPLC, as described earlier.

The encapsulation efficiency (%) was calculated as the ratio of the drug content in the samples to the initial drug amount added.

2.3.3. Release of doxorubicin

The freeze-dried nanoparticles were reconstituted to the initial volume with water. The resulting suspension was diluted 25-fold with water, and this mixture was incubated at 37 °C under continuous shaking. At predetermined intervals, the 400 μl-samples were taken (three samples per time point), while the same amount of water was added back to the release medium. The doxorubicin concentration in the samples was determined after the nanoparticle separation, as described earlier.

2.4. In vivo studies

The animal experiments were performed in compliance with the German Tierschutzgesetz and the Allgemeine Verwaltungsvorschrift zur Durchführung des Tierschutzgesetzes and were authorized by the Regierungspräsidium Darmstadt (V56–19c 20/15 – F 116/11) and Russian Guidelines for Animal Experiments and Welfare authorized by the Russian Ministry of Health (267/19.06.2003 and 284/29.04.2002).

2.4.1. Chemotherapy of rat glioblastoma using doxorubicin formulations

2.4.1.1. Animals. Adult male Wistar rats weighing 180–220 g obtained from the animal breeding unit of the Russian Academy of Medical Sciences (Kryukovo, Moscow region) were caged in groups of five and acclimatized for 1 week. They were fed ad libitum with standard laboratory food and water throughout the experiment.

2.4.1.2. Intracranial inoculation of rat glioblastoma. The tumour implantation was performed as described by Steiniger et al. [8]. The animals were anesthetized by intraperitoneal injection of 100 mg/kg ketamine and 10 mg/kg xylazine. Through a midline sagittal incision, a burr hole of 1.5 mm in diameter was made with a dental drill 2 mm posterior to the right coronal suture and 2 mm lateral to the sagittal midline. Tumour cells ($\sim 10^6$) from the frozen stock were introduced into the cavity of the right lateral ventricle, using a tuberculin syringe (B. Braun, Melsungen, Germany) linked to a 21-gauge needle. The scalp incision was closed with surgical glue (Turbo 2000 Kleber Universal, Boldt Co, Wermelskirchen, Germany). After development of distinctive clinical signs of disease (usually day 14 to day 18), the animals were sacrificed by carbon dioxide asphyxiation and the brain was removed. The tumour tissue was excised and homogenized with a scalpel. This fresh tumour tissue was inoculated into the brain of new experimental animal as described earlier.

2.4.1.3. Drug treatment. Tumour-bearing animals were randomized into seven groups (n = 10–12) and received the following formulations: (1) doxorubicin bound to uncoated PLGA/PVA nanoparticles (Dox-PLGA/PVA); (2) doxorubicin bound to PLGA/PVA nanoparticles coated with polysorbate 80 (Dox-PLGA/PVA+Ps80); (3) doxorubicin bound to PLGA/PVA nanoparticles coated with poloxamer 188 (Dox-PLGA/PVA+P188); (4) doxorubicin bound to uncoated PLGA/HSA nanoparticles; (5) doxorubicin bound to PLGA/HSA nanoparticles coated with poloxamer 188 (Dox-PLGA/HSA+P188); and (6) doxorubicin in aqueous solution (Dox). These formulations were injected i.v. into the tail vein using the dose regimen of 3×1.5 mg/kg on days 2, 5, and 8 post tumour implantation. One group was used as untreated control.

Coating of the nanoparticles with the surfactant was performed immediately before administration in animals. For coating, the freeze-dried formulations were resuspended in 1% of either poloxamer 188 or polysorbate 80, and the suspensions were incubated for 30 min.

The animals were followed up for survival. The long-term survivors were sacrificed 100 days post tumour implantation and subjected to necropsy.

2.4.2. Evaluation of the analgesic effect produced by loperamide formulations

2.4.2.1. Animals. Female ICR (CD1) mice (23–28 g) obtained from Harlan Winkelmann (Borchen, Germany) or female Balb/c mice (20–25 g) obtained from the animal breeding unit of the Russian Academy of Medical Sciences (Kryukovo, Moscow region, Russia) were divided in groups (n = 10) and acclimatized for 1 week.

2.4.2.2. Tail-flick test. The tail-flick test measures nociceptive threshold of animals as they respond to the application of heat to a small area of their tails by withdrawing the tails, time for withdrawal being the measure of the nociception.

Loperamide formulations were administered in the dose of 7 mg/kg into the tail vein. The following formulations were used: (1) loperamide bound to non-coated PLGA/PVA nanoparticles (Lop-PLGA/PVA); (2) loperamide bound to PLGA/PVA nanoparticles coated with polysorbate 80 (Lop-PLGA/PVA+Ps80); (3) loperamide bound to PLGA/PVA nanoparticles coated with poloxamer 188 (Lop-PLGA/PVA+P188); (4) loperamide bound to PLGA/HSA nanoparticles coated with poloxamer 188 (Lop-PLGA/HSA+P188); (5) loperamide bound to PLGA/HSA nanoparticles coated with polysorbate 80 (Lop-PLGA/HSA+Ps80); (6) loperamide solution in 2.6% aqueous ethanol (Lop); and (7) loperamide solution in 1% poloxamer 188 (Lop+P188).

For surfactant coating, the freeze-dried nanoparticle formulations were redispersed in a 1% surfactant solution in water and incubated for 30 min at ambient temperature. The concentration of loperamide in the injection solutions was 0.7 mg/ml.

The analgesic effect of the loperamide formulations was measured using the tail-flick analgesia meter (Ugo Basile, Italy). To prevent injuries, the test was automatically truncated after 10 s (cut-off time). The response time for each animal was measured before (=pre-drug latency) and 15, 30, 60, 90, 120, and 180 min after dosing (=post-drug latency). The maximal possible effect (% MPE) was calculated using the following equation:

$$\%\ \mathrm{MPE} = \frac{\text{Post-drug latency} - \text{pre-druglatency}}{\text{Cut-off time} - \text{pre-drug latency}} \times 100\%$$

2.5. Statistical analysis

The statistical significance of the differences of the mean values was tested using the t-test in EXCEL. The data are presented as mean ± sd. A p value of less than 0.05 was considered to be statistically significant.

3. Results

3.1. Nanoparticulate formulations

The PLGA nanoparticles were prepared by a multistep emulsification–solvent evaporation technique. Two different stabilizers were used in this study, polyvinylalcohol (PVA) and human serum albumin (HSA). PVA by far has been the most commonly used surfactant in PLGA micro/nanoparticle formulations displaying a high affinity to these nanoparticles and yielding an excellent stability against aggregation [20]. However, due to safety issues, PVA may not be the optimal choice for parenteral formulations. For this

reason, HSA, an undoubtedly safe surfactant, also was used for the nanoparticle preparation.

Due to different physicochemical properties of doxorubicin hydrochloride and of loperamide, i.e. the first is fairly soluble in water [21], while the solubility of the latter is only 2 μg/ml [22], various modifications of the technique were employed for the loading of these drugs in the nanoparticles. The modified techniques were established in preliminary experiments and proved to enable an efficient encapsulation.

The loperamide-loaded nanoparticles were prepared by a standard procedure where both, the drug and the polymer, were present in the organic phase. For encapsulation of doxorubicin in the PVA-stabilized nanoparticles, the double emulsion (w/o/w) technique was used. As shown by preliminary experiments, at the initial drug-to-polymer ratio of 1:20, (wt./wt.) this technique allowed the increase in loading of doxorubicin to 70–75% versus 40–50% obtained by employment of a simple o/w emulsion (data not shown). In the case of the HSA-stabilized nanoparticles, PBS (pH 7.2) was used as an external phase instead of water. The lower solubility of doxorubicin in PBS facilitated its distribution into the organic phase, thus contributing to the efficacy of the encapsulation. This method indeed allowed an over twofold increase of doxorubicin encapsulation compared to using water as the external phase (>90% versus 30–40%, data not shown).

The physicochemical parameters of the drug-loaded nanoparticles are listed in Table 1. It can be seen that all procedures enabled an efficient drug loading, which in the case of Dox-PLGA/HSA almost reached 100%. The size of the particles stabilized with HSA was higher, when compared to PVA. Coating of the nanoparticles with the surfactants did not influence their size or zeta-potential. All formulations were stable upon freeze-drying.

The kinetics of doxorubicin release from PLGA nanoparticles in water is shown in Fig. 1. Both formulations displayed similar biphasic release profiles with a considerable burst release effect. Indeed, after 1 h, the percentage of free drug reached 60% (Dox-PLGA/PVA) or 40% (Dox-PLGA/HSA). The higher concentration in the case of Dox-PLGA/PVA at this time obviously is explainable by the fact that this formulation initially contained 25% of unbound drug. During the steady-state phase, the drug concentration was slightly increasing. After 24 h, approximately 25% of doxorubicin was still bound to the nanoparticles, which correlates with the results of Birnbaum et al. [20].

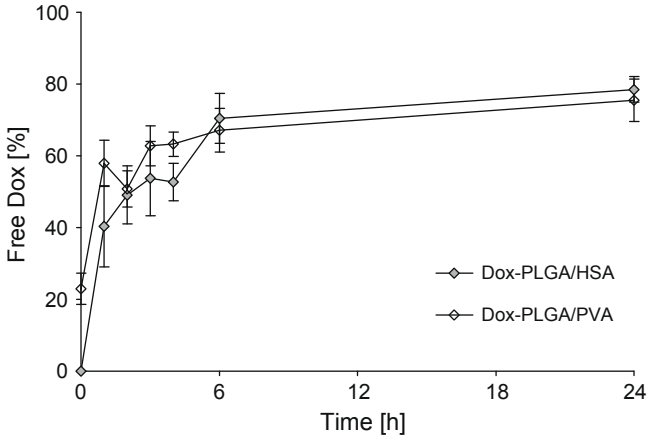

Fig. 1. In vitro release of doxorubicin from PLGA nanoparticles prepared using polyvinylalcohol (Dox-PLGA/PVA) or human serum albumin (Dox-PLGA/HSA) as stabilizers ($n = 3$).

3.2. Chemotherapy of brain tumour using doxorubicin formulations

The orthotopic 101/8 rat glioblastoma model and the treatment regimen used in this experiment have proved to be adequate experimental modalities for the evaluation of the anti-tumour efficacy of the nanoparticle-based formulations of doxorubicin [8,10,23]. As mentioned earlier, the previous studies demonstrated that the key factor of the efficient chemotherapy of 101/8 glioblastoma using doxorubicin bound to PBCA nanoparticles is the coating of their surface with the appropriate surfactants - polysorbate 80 or poloxamer 188. Accordingly, in the present study, the influence of these surfactants on the anti-tumour effect of the PLGA-based formulations was analyzed in the same brain tumour model. Additionally, the influence of another formulation parameter, such as the type of stabilizer, was investigated.

As shown in Fig. 2, all formulations extended the survival times of the tumour-bearing animals when compared to control. Dox-PLGA/PVA+P188 was most effective: a long-term remission (>100 days without tumour) was observed in 40% (4/10) of the animals treated with this formulation. Dox-PLGA/HSA+P188 also exhibited a high anti-tumour effect and produced long-term remission in 25% (3/12) of the animals, this effect being comparable to that of the doxorubicin formulations based on PBCA nanoparticles coated with poloxamer 188 or polysorbate 80 that also produced over 20% of long-term survivors in this model [8–10]. Coating of the PLGA/PVA nanoparticles with polysorbate 80 was not as effective: this formulation yielded only one single long-term survivor; all other animals died before day 40. Necropsy of the long-term survivors performed 100 days post tumour implantation revealed no signs of tumour growth. The efficacy of uncoated nanoparticles (Dox-PLGA/PVA and Dox-PLGA/HSA) was similar to that of doxorubicin in solution (Dox): only single animals in these groups survived after day 30, and all of these animals died between days 33 and 65.

3.3. Antinociceptive effect of loperamide formulations

The purpose of this study also was to investigate the influence of the stabilizer and the surfactant coating on the ability of the nanoparticle-bound loperamide to induce the antinociceptive

Table 1
Physicochemical parameters of the drug-loaded PLGA nanoparticles.

Formulation	Drug: polymer (w/w)	Size (nm)/polydispersity			Zeta-potential (mV)			Loading (%)
		Before coating	After coating with P188	After coating with Ps80	Before coating	After coating with P188	After coating with Ps80	
Dox-PLGA/PVA	1:20	238.6/0.125	243.4/0.211	239.9/0.187	+11.8	+6.0	+8.2	75
Dox-PLGA/HSA	1:20	401.7/0.302	408.6/0.289	412.0/0.311	+9.5	+8.1	+16.2	97
Lop-PLGA/PVA	1:10	177.7/0.286	168.5/0.346	166.9/0.266	−11.4	−17.9	−25.0	77
Lop-PLGA/HSA	1:10	288.9/0.047	287.7/0.077	292.4/0.092	−11.9	−17.5	−18.9	82

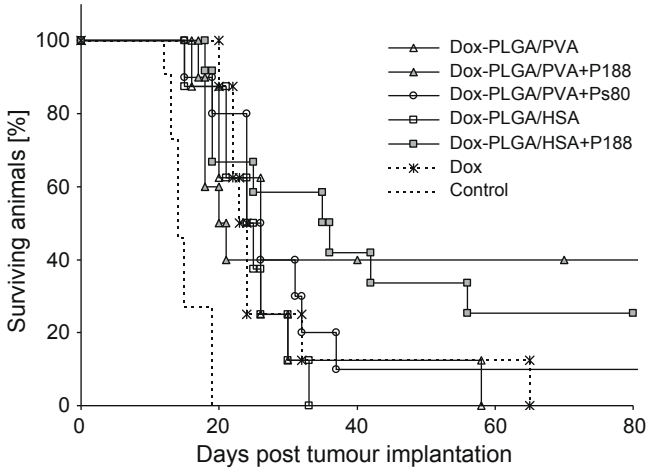

Fig. 2. Kaplan–Meier survival plot of rats with intracranially transplanted 101/8 glioblastoma after i.v. administration of doxorubicin formulations ($n = 10–12$): doxorubicin loaded in uncoated poly(lactide-co-glycolide) (PLGA) nanoparticles stabilized with PVA (Dox-PLGA/PVA); doxorubicin loaded in PLGA nanoparticles stabilized with PVA and coated with poloxamer 188 (Dox-PLGA/PVA+P188); doxorubicin loaded in PLGA nanoparticles stabilized with PVA and coated with polysorbate 80 (Dox-PLGA/PVA+Ps80); doxorubicin loaded in uncoated PLGA nanoparticles stabilized with HSA (Dox-PLGA/HSA); doxorubicin loaded in PLGA nanoparticles stabilized with HSA and coated with poloxamer 188 (Dox-PLGA/HSA+P188); and doxorubicin in aqueous solution (Dox), untreated animals (control).

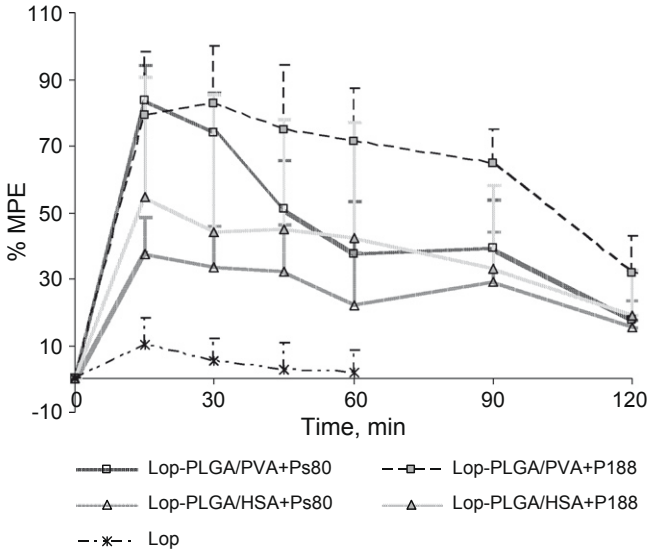

Fig. 4. Antinociceptive effects of loperamide formulations determined by the tail-flick test after i.v. injection (7.0 mg/kg) in female Balb/c mice ($n = 10$): loperamide bound to poloxamer 188-coated (Lop-PLGA/PVA+P188) or polysorbate 80-coated (Lop-PLGA/PVA+Ps80) poly(lactide-co-glycolide) (PLGA) nanoparticles stabilized by PVA; loperamide bound to poloxamer 188-coated (Lop-PLGA/HSA+P188) or polysorbate 80-coated (Lop-PLGA/HSA+Ps80) nanoparticles stabilized by HSA; and loperamide in ethanolic solution (Lop).

(analgesic) effect, which would be indicative of its ability to reach the CNS. Antinociception produced by the intravenous injection of the different formulations of loperamide was tested using the tail-flick test, as described by Hekmatara et al. [13]. The antinociceptive effects of the formulations measured as percentage of a maximal possible effect (% MPE) are presented in Figs. 3 and 4. The experiment was performed in two steps: the first experiment was focused on the evaluation of the influence that poloxamer 188

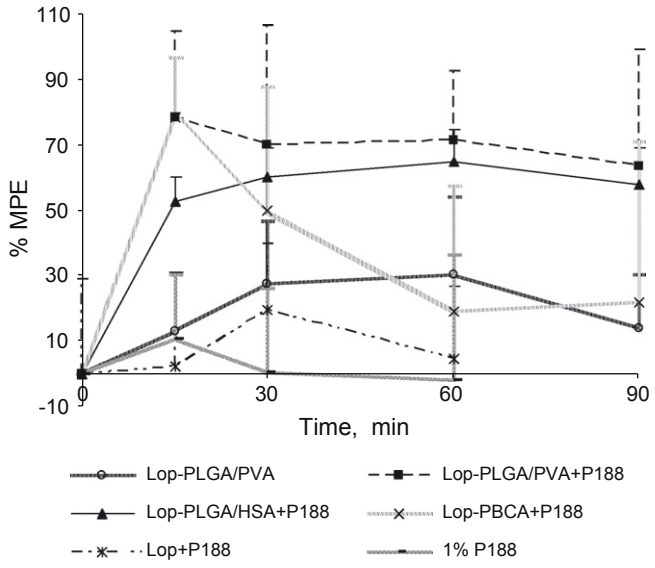

Fig. 3. Antinociceptive effects of loperamide formulations determined by the tail-flick test after i.v. injection (7.0 mg/kg) in female ICR (CD1) mice ($n = 10$): loperamide bound to non-coated (Lop-PLGA/PVA) or poloxamer 188-coated (Lop-PLGA/PVA+P188) poly(lactide-co-glycolide) (PLGA) nanoparticles stabilized by PVA; loperamide bound to poloxamer 188-coated PLGA nanoparticles stabilized by HSA (Lop-PLGA/HSA+P188); loperamide in 1% poloxamer 188 solution (Lop+P188); and blank 1% solution of poloxamer 188. Loperamide bound to PBCA nanoparticles coated with polysorbate 80 (Lop-PBCA+Ps80) is shown as a reference formulation (data from [13]).

might exert on the different formulations of loperamide (Fig. 3). In the second experiment, the influence of different surfactants and stabilizers was compared (Fig. 4).

It can be seen that the most pronounced nociceptive effect in both experiments was achieved by loperamide bound to PLGA/PVA nanoparticles coated with poloxamer 188. The effect produced by this formulation reached its maximum of 80% MPE 15 min after injection, then slightly decreased to 70% and was maintained at this level for at least 60 min; the effect was still measurable after 120 min (Figs. 3 and 4). The polysorbate 80-coated PLGA/PVA nanoparticles also produced a considerable but less prolonged analgesic effect that exceeded 80% after 15 min. However, after 60 min, it declined to 40% (Fig. 4). Thus Lop-PLGA/PVA+Ps80 displayed the pharmacodynamic profile that was similar to that of Lop-PBCA+Ps80 shown in Fig. 3 as a reference formulation. The effect of Lop-PLGA/HSA+P188 reached ~50% MPE after 30 min and remained at the level of 40–50% for 90 min (Figs. 3 and 4). Analgesia produced by PLGA/HSA nanoparticles coated with Ps 80 was visibly lower (30–40% MPE). The same effect was produced by the uncoated PLGA/PVA nanoparticles. In general, the influence of the surfactants on the performance of the HSA-stabilized particles was less pronounced, when compared to the particles stabilized by PVA. The antinociceptive effect in mice was associated with certain behavioral changes that are typical for opiates such as the Straub effect and periods of hyperactivity followed by akinesia. These observations are indicative of the central action produced by the nanoparticle-bound loperamide.

Loperamide in solution of 1% poloxamer 188 induced only marginal effects. The solution of loperamide in aqueous ethanol, as well as the blank 1% solution of poloxamer 188, was ineffective. After 60 min, the evaluation of these groups was truncated to avoid unnecessary stress to the animals.

4. Discussion

As mentioned earlier, the objective of the present study was to investigate the ability of surfactant-coated PLGA nanoparticles to

deliver drugs to the brain. The two model drugs chosen for this study, doxorubicin and loperamide, are P-gp substrates, and, therefore, their concentrations in the brain after intravenous injection normally do not reach levels that are sufficient for the induction of considerable pharmacological effects [24]. Hence, the observed pharmacological effects produced by the nanoparticulate formulations of these drugs in the brain, i.e. the high anti-tumour effect against the intracranial glioblastoma in the case of doxorubicin (Fig. 2) and the considerable analgesia in the case of loperamide (Figs. 3 and 4), provide evidence that with the help of the coated nanoparticles both drugs could successfully traverse the BBB. The pronounced manifestation of these pharmacological effects also implies that the concentrations of these drugs in the brain reached therapeutically significant levels.

The experimental results additionally reveal the influence of the formulation parameters on the efficacy of brain delivery and the conformity in these parameters between drugs with different physicochemical properties, i.e. doxorubicin and loperamide. In both cases, the most pronounced effects were achieved by binding of these drugs to PVA-stabilized PLGA nanoparticles coated with poloxamer 188. This carrier type enabled the highest anti-tumour effect of doxorubicin against intracranial glioblastoma manifested as long-term remission (tumour-free period) in 40% animals. Accordingly, loperamide bound to PLGA/PVA nanoparticles coated with poloxamer 188 also yielded the most pronounced and long-lasting antinociceptive effect. Considerable, although slightly lower effects also were produced by the HSA-stabilized nanoparticles coated with poloxamer 188. The polysorbate 80-coated PLGA/PVA nanoparticles were in general less effective. In the group treated with Dox-PLGA/PVA+Ps80, a long-term remission was achieved only in 1/10 animals. However, loperamide bound to PLGA/PVA+Ps80 still induced a considerable, though short-acting analgesia. In contrast, the uncoated nanoparticles induced almost no effects.

In order to investigate a possible correlation between PLGA and PBCA nanoparticles, the design of the present study was similar to previous experiments, employing the same drugs, the same animal models, and even the same surfactants. The correlation was indeed found: both surfactants, poloxamer 188 and polysorbate 80, enabled brain delivery for both types of the nanoparticles, whereas uncoated particles were ineffective. However, the efficacy of surfactants varied depending on the particle type. In the case of the PBCA nanoparticles, both surfactants enabled similarly pronounced pharmacological effects with loperamide [13,25] as well as with doxorubicin [8,10], i.e. similarly effective brain delivery. In particular, the concentration of doxorubicin delivered to the brain by the PBCA nanoparticles coated with polysorbate 80 reached the very high concentration of 6 μg/g tissue, whereas the concentration of free doxorubicin in the brain remained below the detection limit of 0.1 μg/ml [26]. At the same time, polysorbate 80, when used for the coating of the PLGA particles, appeared to be less effective when compared to poloxamer 188. It is also noteworthy that in the case of loperamide, the effect of the PLGA-based formulation was considerably prolonged compared to the PBCA nanoparticles [13], which is probably explained by a faster degradation of the latter.

As described by Tosi et al. [18], a nociceptive effect of loperamide also was achieved by binding the drug to the PLGA nanoparticles modified by an analogue of synthetic opioid peptides, which enabled penetration of these particles into the brain. Although the direct comparison of the results obtained by Tosi et al. with the results of the present study is not possible due to unlike experimental conditions, the difference between the pharmacodynamic profiles and, especially, the different onsets of the nociceptive effect suggest that the surfactant-coated and the vectorized nanoparticles may access the CNS by different mechanisms.

Finally, the conformity of the efficacy data obtained for two drugs with different types of pharmacological action and different physicochemical properties indicates that the surfactant-coated PLGA nanoparticles are an efficient carrier suitable for delivery of various agents across the BBB.

Furthermore, it is now well known that the body distribution of the intravenously administered nanoparticles and, in particular, their ability to reach the brain is governed by the protein adsorption pattern, where the latter depends on the surface properties of the particles. In concert with this statement and the observations made previously [9,10], the results of this study demonstrate that the efficacy of brain delivery by nanoparticles is influenced not only by the coating surfactants but also by other constituents of the particles such as the core polymer, drug, and stabilizer, which are exposed on the surface and can alter its properties.

5. Conclusion

This is the first study showing that coating of drug-loaded PLGA nanoparticles with pharmaceutical surfactants such as poloxamer 188 and polysorbate 80 enables the delivery of drugs into the brain. The pronounced pharmacological effects of the two model drugs in the brain enabled by the nanoparticulate formulations clearly indicated that therapeutic amounts of the drugs were delivered to the sites of action within the CNS, thus suggesting high efficiency of this carrier for brain delivery.

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References

[1] J. Kreuter, Nanoparticulate systems for brain delivery of drugs, Adv. Drug Deliv. Rev. 47 (2001) 65–81.
[2] P. Calvo, B. Gouritin, H. Chacun, D. Desmaele, J. D'Angelo, J.P. Noel, D. Georgin, E. Fattal, J.P. Andreux, P. Couvreur, Long-circulating PEGylated polycyanoacrylate nanoparticles as new drug carrier for brain delivery, Pharmacol. Res. 18 (2001) 1157–1166.
[3] K. Michaelis, M.M. Hoffmann, S. Dreis, E. Herbert, R.N. Alyautdin, M. Michaelis, J. Kreuter, K. Langer, Covalent linkage of apolipoprotein E to albumin nanoparticles strongly enhances drug transport into the brain, J. Pharmacol. Exp. Ther. 317 (2006) 1246–1253.
[4] T.M. Goppert, R.H. Müller, Polysorbate-stabilized solid lipid nanoparticles as colloidal carriers for intravenous targeting of drugs to the brain: comparison of plasma protein adsorption patterns, J. Drug Target. 13 (2005) 179–187.
[5] J. Kreuter, P. Ramge, V. Petrov, S. Hamm, S.E. Gelperina, B. Engelhardt, R. Alyautdin, H. von Briesen, D.J. Begley, Direct evidence that polysorbate-80-coated poly(butylcyanoacrylate) nanoparticles deliver drugs to the CNS via specific mechanisms requiring prior binding of drug to the nanoparticles, Pharmaceut. Res. 20 (2003) 409–416.
[6] J. Kreuter, D. Shamenkov, V. Petrov, P. Ramge, K. Cychutek, C. Koch-Brandt, R. Alyautdin, Apolipoprotein-mediated transport of nanoparticle-bound drugs across the blood–brain barrier, J. Drug Target. 10 (2002) 317–325.
[7] J. Kreuter, Influence of the surface properties on nanoparticle-mediated transport of drugs to the brain, J. Nanosci. Nanotechnol. 4 (2004) 484–488.
[8] S.C. Steiniger, J. Kreuter, A.S. Khalansky, I.N. Skidan, A.I. Bobruskin, Z.S. Smirnova, S.E. Severin, R. Uhl, M. Kock, K.D. Geiger, S.E. Gelperina, Chemotherapy of glioblastoma in rats using doxorubicin-loaded nanoparticles, Int. J. Cancer 109 (2004) 759–767.
[9] A. Ambruosi, S. Gelperina, A. Khalansky, S. Tanski, A. Theisen, J. Kreuter, Influence of surfactants, polymer and doxorubicin loading on the anti-tumour effect of poly(butyl cyanoacrylate) nanoparticles in a rat glioma model, J. Microencapsul. 23 (2006) 582–592.
[10] B. Petri, A. Bootz, A. Khalansky, T. Hekmatara, R. Müller, R. Uhl, J. Kreuter, S. Gelperina, Chemotherapy of brain tumour using doxorubicin bound to surfactant-coated poly(butyl cyanoacrylate) nanoparticles: revisiting the role of surfactants, J. Control. Release 117 (2007) 51–58.
[11] E. Pereverzeva, I. Treschalin, D. Bodyagin, O. Maksimenko, K. Langer, S. Dreis, B. Asmussen, J. Kreuter, S. Gelperina, Influence of the formulation on the tolerance profile of nanoparticle-bound doxorubicin in healthy rats: focus on cardio- and testicular toxicity, Int. J. Pharm. 337 (2007) 346–356.

[12] E. Pereverzeva, I. Treschalin, D. Bodyagin, O. Maksimenko, J. Kreuter, S. Gelperina, Intravenous tolerance of a nanoparticle-based formulation of doxorubicin in healthy rats, Toxicol. Lett. 178 (2008) 9–19.
[13] T. Hekmatara, S. Gelperina, V. Vogel, S.-R. Yang, J. Kreuter, Encapsulation of water-insoluble drugs in poly(butyl cyanoacrylate) nanoparticles, J. Nanosci. Nanotechnol. 9 (2009) 5091–5098.
[14] C. Vauthier, D. Labarre, G. Ponchel, Design aspects of poly(alkylcyanoacrylate) nanoparticles for drug delivery, J. Drug Target. 15 (2007) 641–663.
[15] A. Zensi, D. Begley, C. Pontikis, C. Legros, L. Mihoreanu, S. Wagner, C. Büchel, H. von Briesen, J. Kreuter, Albumin nanoparticles targeted with Apo E enter the CNS by transcytosis and are delivered to neurones, J. Control. Release 137 (2009) 78–86.
[16] A. Brioschi, S. Calderoni, L. Pradotto, M. Guido, A. Strada, F. Zenga, C. Benech, F. Benech, L. Serpe, G. Zara, C. Musicanti, A. Ducati, M. Gasco, A. Mauro, Solid lipid nanoparticles carrying oligonucleotides inhibit vascular endothelial growth factor expression in rat glioma models, J. Nanoneurosci. 1 (2008) 65–74.
[17] L. Constantino, F. Gandolfi, G. Tosi, F. Rivasi, M.A. Vandelli, F. Forni, Peptide-derivatized biodegradable nanoparticles able to cross the blood–brain barrier, J. Control. Release 108 (2005) 84–96.
[18] G. Tosi, L. Costantino, F. Rivasi, B. Ruozi, E. Leo, A.V. Vergoni, R. Tacchi, A. Bertolini, M.A. Vandelli, F. Forni, Targeting the central nervous system: in vivo experiments with peptide-derivatized nanoparticles loaded with loperamide and Rhodamine-123, J. Control. Release 122 (2007) 1–9.
[19] H. Chen, F. Gaul, D. Guo, A. Maycock, Determination of loperamide in rat plasma and bovine serum albumin by LC, J. Pharm. Biomed. Anal. 22 (2000) 555–561.
[20] D. Birnbaum, J. Kosmala, L. Brannon-Peppas, Optimization of preparation techniques for poly(lactic acid-co-glycolic acid), J. Nanoparticle Res. 2 (2000) 173–181.
[21] European Pharmacopoeia, vol. 6.0, 2008, Doxorubicin hydrochloride, 2392.
[22] Merck Index, vol. 13th ed., 2001, Loperamide, 5592.
[23] T. Hekmatara, C. Bernreuther, A. Khalansky, A. Theisen, J. Weissenberger, J. Matschke, S. Gelperina, J. Kreuter, M. Glatzel, Efficient systemic therapy of rat glioblastoma by nanoparticle-bound doxorubicin is due to antiangiogenic effects, Clin. Neuropathol. 28 (2009) 153–164.
[24] D.J. Begley, ABC transporters and the blood–brain barrier, Curr. Pharm. Des. 10 (2004) 1295–1312.
[25] R.N. Alyautdin, V.E. Petrov, K. Langer, A. Berthold, D.A. Kharkevich, J. Kreuter, Delivery of loperamide across the blood–brain barrier with polysorbate 80-coated polybutylcyanoacrylate nanoparticles, Pharm. Res. 14 (1997) 325–328.
[26] A.E. Gulyaev, S.E. Gelperina, I.N. Skidan, A.S. Antropov, G.Y. Kivman, J. Kreuter, Significant transport of doxorubicin into the brain with polysorbate 80-coated nanoparticles, Pharm. Res. 16 (1999) 1564–1569.