Indirect Evidence that Drug Brain Targeting Using Polysorbate 80-Coated Polybutylcyanoacrylate Nanoparticles Is Related to Toxicity

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Received August 6, 1999; accepted September 7, 1999

Purpose. To investigate the mechanism underlying the entry of the analgesic peptide dalargin into brain using biodegradable polybutylcyanoacrylate (PBCA) nanoparticles (NP) overcoated with polysorbate 80.
Methods. The investigations were carried out with PBCA NP and with non biodegradable polystyrene (PS) NP (200 nm diameter). Dalargin adsorption was assessed by HPLC. Its entry into the CNS in mice was evaluated using the tail-flick procedure. Locomotor activity measurements were performed to compare NP toxicities. BBB permeabilization by PBCA NP was studied *in vitro* using a coculture of bovine brain capillary endothelial cells and rat astrocytes.
Results. Dalargin loading was 11.7 μg/mg on PBCA NP and 16.5 μg/mg on PS NP. Adding polysorbate 80 to NP led to a complete desorption. Nevertheless, dalargin associated with PBCA NP and polysorbate 80 induced a potent and prolonged analgesia, which could not be obtained using PS NP in place of PBCA NP. Locomotor activity dramatically decreased in mice dosed with PBCA NP, but not with PS NP. PBCA NP also caused occasional mortality. *In vitro*, PBCA NP (10 μg/ml) induced a permeabilization of the BBB model.
Conclusions. A non specific permeabilization of the BBB, probably related to the toxicity of the carrier, may account for the CNS penetration of dalargin associated with PBCA NP and polysorbate 80.

KEY WORDS: polybutylcyanoacrylate; polystyrene; nanoparticles; blood brain barrier; dalargin; brain targeting.

INTRODUCTION

Brain targeting is one of the most challenging issues for the pharmaceutical research, as numbers of hydrophilic therapeutic agents, such as antibiotics, anticancer drugs or newly developed neuropeptides are unable to cross the blood brain barrier (BBB) (1). The BBB is formed by the brain vessel endothelial cells linked together by tight junctions and strictly controls drug transfer from blood to brain (2). Drug modification, osmotic opening of cerebral capillary endothelium and alternative routes for administration (e.g., intracerebral administration) have been successful approaches for drug delivery to the central nervous system (CNS) (3). Supramolecular carriers, such as liposomes or nanoparticles, could be useful tools for brain targeting, because they should be able to transport any entrapped drug to the brain, insuring in the mean time its protection from the administration site to the CNS. However, due to their size, these carriers are unable to extravasate through brain vessel endothelia via tight junctions (3) and attempts to target the CNS remain unsuccessful. Specific targeting of the transferrin transcytosis system using immunoliposomes has been proposed as a means to cross the BBB (4).

Easier to prepare, polybutylcyanoacrylate (PBCA) nanoparticles coated with a non ionic surfactant (polysorbate 80), were successfully used by Alyautdin *et al.* (5–7) and Kreuter *et al.* (8,9) to transport various drugs to the CNS. Dalargin, a Leu-enkephalin analogue peptide, which exhibits a potent analgesic activity after intracisternal administration, but not after IV administration, induced a prolonged central analgesia by the IV route when loaded onto this carrier (7–9). It was hypothesised that coated nanoparticles were transported across the BBB after endocytosis by the brain capillary endothelial cells (9). However, the mechanism underlying such an endocytosis remains obscure. Furthermore, some observations do not confirm preferential distribution of polysorbate 80-coated nanoparticles into the brain or the transcytosis hypothesis. Less than 1% of poly(methylmethacrylate) nanoparticles coated with polysorbate 80 were found in the brain (including brain vessels and the parenchyma) after IV administration, most of them ending up in the lung, the liver and the spleen, due to their uptake by the mononuclear phagocyte system (10). The early onset of pharmacological activity, 15 min with polysorbate 80-coated dalargin-loaded nanoparticles (7,8) and 5 min with polysorbate 80-coated nanoparticles loaded with loperamide (6) or with tubocurarine (5) is hardly compatible with the transcytosis hypothesis (11). In addition, drug adsorption onto nanoparticles may not be stable in the presence of the surfactant polysorbate 80 (12). Finally, the high dose of PBCA nanoparticles used (166 mg/kg body weight), close to the lethal dose 50% (230 mg/kg (13)) might induce toxicity that may limit the therapeutic applications of the carrier.

To clarify these points, PBCA nanoparticles loaded with dalargin and overcoated with polysorbate 80 were evaluated in comparison with non biodegradable and non toxic polystyrene (PS) nanoparticles. The stability of dalargin adsorption onto the nanoparticles was investigated in the presence of polysorbate 80. Antinociception experiments were carried out to check for CNS penetration of dalargin, and locomotor activity measurements to evaluate the nanoparticle toxicity. The mechanism underlying drug penetration through the BBB using PBCA nanoparticles was investigated *in vitro* on a BBB cell culture model.

MATERIALS AND METHODS

Animals

This work was done in accordance with the Principles of Laboratory Animal Care (NIH Publication #86-23, revised 1985). Male Swiss mice (20–25 g) obtained from Déprés Breeding Laboratories (St Doulchard, France) were housed in the animal breeding facilities of the laboratory (Authorization No.

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ABBREVIATIONS: DAL, dalargin; NP, nanoparticle; PBCA, polybutylcyanoacrylate; PBS, phosphate-buffered saline; PS, polystyrene; P80, polysorbate 80.

0724-8741/99/1200-1836$16.00/0

0028) for 4–6 days before experiments (10 per cage), maintained in a light-(12-hr light-dark cycle) and temperature-controlled environment, with food and water available *ad libitum*.

Materials

N-butyl-2-cyanoacrylate (Sicomet 6000) was a gift from Sichel-Werke (Hannover, Germany). Dalargin and polyoxyethylenesorbitan monooleate (polysorbate 80) were obtained from Sigma and dextran 70 (Mw ≈ 70,000) from Fluka (Saint-Quentin-Fallavier, France). Polystyrene nanoparticles (0.202 ± 0.01 μm diameter, 2.54% solid content) were from Polysciences Inc. (Warrington, USA). For HPLC, acetonitrile (chromatography grade) was from Fisher Scientific (Elancourt, France) and trifluoroacetic acid from Sigma. For cell culture, [^{3}H]-inulin (1.25 Ci/mmol) and [^{14}C]-sucrose (677 mCi/mmol) were obtained from Amersham (Les Ulis, France).

Preparation of PBCA Nanoparticles

PBCA nanoparticles were prepared by emulsion-polymerization (14). One ml butylcyanoacrylate monomer was added to 100 ml hydrochloric solution of dextran 70 0.5% (w/v), pH 2.5, filtered on 0.2 μm Sartorius filters (Bioblock Scientific, Illkirch, France). After a 3 hour polymerization time under constant magnetic stirring, the nanoparticle suspension was neutralized with sodium hydroxide solutions 1 N and 0.1 N and filtered through a glass microfiber 0.7 μm Whatman® GF/F filter (Polylabo, Strasbourg, France). Nanoparticles were then centrifuged at 57,000 g for 20 min at 4°C (Optima™ L70 Preparative Ultracentrifuge equipped with a rotor Ti. 70.1, Beckman) to remove the polymerization medium. Pellets were resuspended with 11 ml water (final volume: 12.77 ml). To insure a proper redispersion of the nanoparticle pellets, the preparations were vortexed, left at room temperature overnight and sonicated for 10 min (waterbath sonicator Bransonic 220). Nanoparticle mean diameter was determined to be 210 nm (polydispersity 0.075) with a Zetasizer 5000 (Malvern, Orsay, France). Solid content was evaluated by drying 3 aliquots of 200 μl suspension for 3 days at 40°C. Concentration was found to be 61.8 mg/ml, which is a yield of 79% from the initial monomer amount.

Preparation of Dalargin-Loaded Nanoparticles

PBCA or PS nanoparticles (13.5 mg/ml) and dalargin (0.75 mg/ml) were incubated together in a phosphate-buffered saline (PBS) pH 7.4 at room temperature for 3 hours under gentle magnetic stirring. For overcoating with polysorbate 80, one-percent (m/v) polysorbate 80 was added to the nanoparticle suspensions under gentle stirring, 30 min before injection to animals. PS nanoparticles precipitated in the presence of dalargin and PBS. This instability problem was solved by adding polysorbate 80 (1% m/v) after the incubation time and by waterbath sonication for 5 min, without modifying PS nanoparticle size. Injections of PS nanoparticles without polysorbate 80 as a control was therefore not carried out.

Determination of Drug Loading on PBCA and PS Nanoparticles

Dalargin loading was determined before and after the polysorbate 80-overcoating step. Nanoparticles were diluted to 1/5 with water and immediately centrifuged (112000 g for 30 min at 4°C). Unbound dalargin was determined in the collected supernatants by HPLC. A control dalargin solution in PBS (0.75 mg/ml) was used to ascertain that the peptide was not altered during the whole procedure. The peptide integrity was assessed by HPLC analysis by checking for concentrations and retention times.

Dalargin HPLC Determination

Dalargin was determined by isocratic reversed-phase HPLC using a Nucleosil C18 Column 5 μm Interchrom, 150 × 4 mm (Interchim, Montluçon, France), a mobile phase of 22.46:77.64 (m/m) acetonitrile-water containing 0.1% (v/v) trifluoroacetic acid at a flow rate of 1 ml/min, and a 215 nm detection wavelength. Injection volume was 20 μl and dalargin concentrations for calibration ranged from 10 to 150 μg/ml. Mean dalargin retention time was 3.4 min.

Animal Testing

Animal testing was conducted between 1.00 pm and 7.00 pm. The preparations used for each test are listed in Table I. Mice (10 per group) were treated with 10 ml preparation per kg body weight, so that doses of nanoparticles, dalargin and polysorbate 80 were respectively 135 mg/kg, 7.5 mg/kg and 100 mg/kg. PBS was used as control solution.

Antinociception Studies

Antinociception was assessed using the tail-flick test procedure of D' Amour and Smith (15), performed with an Analgesy Meter LE7106 (Letica Scientific Instruments, Harvard Biosciences, Les Ulis, France). A noxious radiant heat source was focused on the dorsal part of the tails of mice maintained in Perspex® boxes. Flick of the tail due to the heat stimulus exposed the light beam to a photodetector which in turn stopped a timer displaying the tail-flick latency. When mice did not react with an effective flick of the tail, the timer was stopped manually. The intensity of the heat beam was set to induce baseline latencies between 3.0 and 5.0 s. A cut-off latency of 10 s was employed to prevent any tissue damage.

Tail-flick baseline latencies were determined 30 min before injections. Injections were performed in one of the tail veins dilated by clamping and by plunging in water at 42°C. Care was taken to inject away from the impact point of the radiant heat. Tail-flick tests were performed from 10 min until 100 min post-injection. As repeating tail-flick test might lead to a sensitization to or to a learning of the nociceptive test (16), statistical analysis was performed by comparing at each time point tail-flick latencies of treated mice with the control latencies.

Locomotor Activity Measurements

Locomotor activities were evaluated using a modular automatic actimeter Panlab (Apelex, Bagneux, France) which detects the animal movements in a high frequency electromagnetic field and converts them into scores. Mice were placed on the actimeter 2.5 min after injections and, 5 min later, activities were recorded for 5 min. Statistical analysis was made by comparison with PBS control scores. For easier comparison,

Table I. Preparations Tested and *In Vivo* Tests Performed

	Abbreviation names of the preparations	Nanoparticles (mg/ml)	Dalargin (mg/ml)	Polysorbate 80 (mg/ml)	Tests*
PBCA nanoparticles	NP[PBCA]-DAL-P80	13.5	0.75	10	TF/LA
	NP[PBCA]-DAL	13.5	0.75	0	TF
	NP[PBCA]-P80	13.5	0	10	TF/LA
	NP[PBCA]	13.5	0	0	LA
PS nanoparticles	NP[PS]-DAL-P80	13.5	0.75	10	TF/LA
	NP[PS]-P80	13.5	0	10	TF/LA
dalargin solutions	DAL-P80	0	0.75	10	TF/LA
	DAL	0	0.75	0	TF/LA
control	PBS	0	0	0	TF/LA

* TF, tail-flick test; LA, locomotor activity measurements.

the locomotor activity data were expressed on Fig. 3 as the percentage of the mean control locomotor activities scaled to 100%.

In Vitro Studies on a BBB Model

The *in vitro* blood-brain barrier model consisted of a coculture of bovine brain capillary endothelial cells (BBCEC) and rat astrocytes as described by Dehouck *et al.* (17). The BBB model permeability was evaluated by measuring the flux of two paracellular markers, [^{3}H]-inulin (0.31 μCi/ml) and [^{14}C]-sucrose (0.05 μCi/ml) added to the luminal side of the BBCEC monolayer. Uncoated and polysorbate 80-coated PBCA nanoparticles (0.01 to 500 μg/ml) in serum-free culture medium (to avoid any nanoparticle degradation by serum esterases before experiments started), were added to the luminal side at time 0. The radioactivity penetrating through the BBCEC monolayer into the abluminal side was measured by liquid scintillation (Scintillation Counter Wallac 14110 Pharmacia, Guyancourt, France).

Diffusion of sucrose and inulin across the endothelial cell monolayers was expressed in clearance terms according to the method of Siflinger-Birnboim *et al.* (18) by dividing the cumulated amount of radiolabelled compounds recovered in the abluminal side by the initial concentration of the radiolabelled compounds in the luminal side. The mean cumulated cleared volumes were plotted versus time, which gives a linear relationship for an endothelial cell barrier established with tight junctions. The slope of the curve calculated by linear regression analysis corresponded to the clearance (μl/min) of the radiotracer.

Statistical Analysis

Results are given as the mean ± SEM. Statistical analysis was performed using Mann-Whitney test. $p < 0.05$ was considered as significant.

RESULTS

Dalargin Loading of Nanoparticles and Effect of Polysorbate 80

HPLC analyses of control dalargin solutions (0.75 mg/ml) did not show any alteration of the peptide after the incubation and the centrifugation steps (Table II). After the incubation with nanoparticles for 3 h, 21% of dalargin was adsorbed onto PBCA nanoparticles (11.7 μg dalargin per mg nanoparticles) and 30% onto PS nanoparticles (16.5 μg/mg) (Table II). Thirty minutes after the addition of polysorbate 80 (1% m/v) 100% dalargin was desorbed from both PS and PBCA nanoparticles.

Antinociception Studies

Baseline latencies (determined 30 min before injections) were never significantly different between groups (Figs. 1–2). One mouse (out of 10) died by 20 min after injection of dalargin-loaded polysorbate 80-coated PBCA nanoparticles and two died within 10 min after injection of polysorbate 80-coated PBCA nanoparticles.

After the administration of the dalargin solutions (Fig.1A & 1B), of the PS nanoparticle preparations (Fig. 1C & 1D), of the dalargin-loaded PBCA nanoparticles (Fig. 2B) or of the polysorbate 80-coated PBCA nanoparticles (Fig. 2C), latencies were not significantly different from PBS control latencies, indicating that these preparations did not induce any analgesia. With polysorbate 80, dalargin-loaded PBCA nanoparticles (Fig. 2A) induced a significant increase in latencies throughout the duration of the evaluation (100 min). At 10 min post-injection, all the mice (9/9) reached the cut-off latency. Thereafter, latencies gradually decreased over 100 min.

Locomotor Activity

One mouse (in 10) injected with polysorbate 80-coated nanoparticles (NP[PBCA]-P80) died after its activity was recorded. As shown on Fig. 3, no significant alteration of activity was observed with the dalargin solution (DAL), with dalargin mixed with polysorbate 80 (DAL-P80) or with the PS nanoparticle preparations (NP[PS]-P80 and NP[PS]-DAL-P80). However, all the PBCA nanoparticle preparations significantly reduced activity scores. Activities obtained with NP[PBCA], NP[PBCA]-P80 and NP[PBCA]-DAL-P80 were 30 ± 13%, 5 ± 3% and 12 ± 5% of the controls respectively. The decrease in locomotor activity was always associated with behavioral alterations: a short period (around 5 min) of hyperactivity and/or of aggressiveness against other mice was followed by a prolonged period (around one hour) of prostrate attitude with

Table II. Concentration and Percentage of Unbound Dalargin After 3 Hour Incubation at Room Temperature with or Without Nanoparticles (A) and After the Same Procedure, but Followed by a Further 30 min Incubation with 1% (m/v) Polysorbate 80 (B)

	A		B	
	Concentration (mg/ml ±SE)	Percentage (%)	Concentration (mg/ml ±SE)	Percentage (%)
Incubation with PBCA nanoparticles	0.592 ± 0.010	79 ± 1.3	0.755 ± 0.008	101 ± 1.1
Incubation with PS nanoparticles	0.527 ± 0.021	70 ± 2.8	0.760 ± 0.005	101 ± 0.7
Control (incubation without nanoparticles)	0.730 ± 0.033	97 ± 4.4	0.742 ± 0.016	99 ± 2.1

Note. Dalargin concentration was initially 0.75 mg/ml (for details see text).

eye watering. These signs could not be observed during antinociception experiments, as mice were enclosed within individual Perspex® boxes.

BBB Permeability Studies *In Vitro*

For uncoated PBCA nanoparticles (Fig. 4) and for polysorbate 80-coated nanoparticles (Fig. 5), no alteration of the BBB model permeability of [^{14}C]-sucrose was observed for concentrations up to 1.0 μg/ml: the relationship was linear between cleared volumes and incubation times, and clearances were not significantly different from controls (1.11 μl/min). For concentrations of 10 μg/ml and above, a dramatic increase in [^{14}C]-sucrose penetration was observed after 20 min incubation, indicating a progressive disruption of the tight junctions. Similar results were obtained with [^{3}H]-inulin (not shown). Thus, threshold concentrations for BBB opening were between 1 and 10 μg/ml for uncoated and polysorbate 80-coated PBCA nanoparticles.

DISCUSSION

In initial antinociception experiments (data not shown), mice were dosed with 166 mg/kg PBCA nanoparticles as in experiments by Kreuter *et al.* (8,9) and Alyautdin *et al.* (7). This dose caused a mortality of 3 to 4 mice out of 10 occurring within 20 min post-injection. Although this mortality was consistent with the 230 mg/kg LD_{50} (13), it has never been reported in the earlier works (7–9). The dry-weight content of our PBCA nanoparticle suspensions corresponded to a 79% yield. In the previous works (7–9) nanoparticle concentrations were not determined after filtration and a 100% yield was assumed. Therefore, the nanoparticle dose administered to the mice in our experiments was probably higher than in the earlier works, which would explain the higher toxicity. Consequently, PBCA nanoparticle dose was lowered to 135 mg/kg (≈79% of 166 mg/kg). Occasional deaths were nevertheless recorded. PBCA nanoparticle toxicity is a well-documented issue: it is mediated by toxic compounds released due to the rapid biodegradation of the PBCA polymer by the esterases present in biological

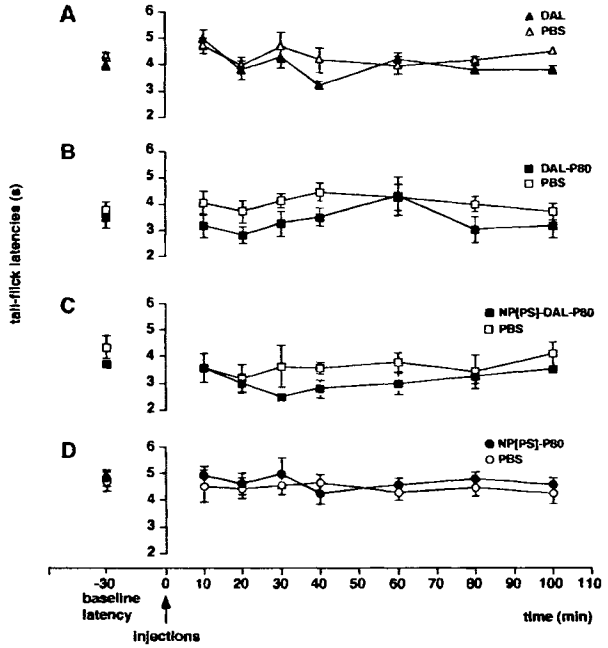

Fig. 1. Tail-flick latencies obtained after administration of dalargin preparations and PS nanoparticle preparations, and their respective PBS controls. Error bars indicate the standard error. For details about the preparations injected, see Table I.

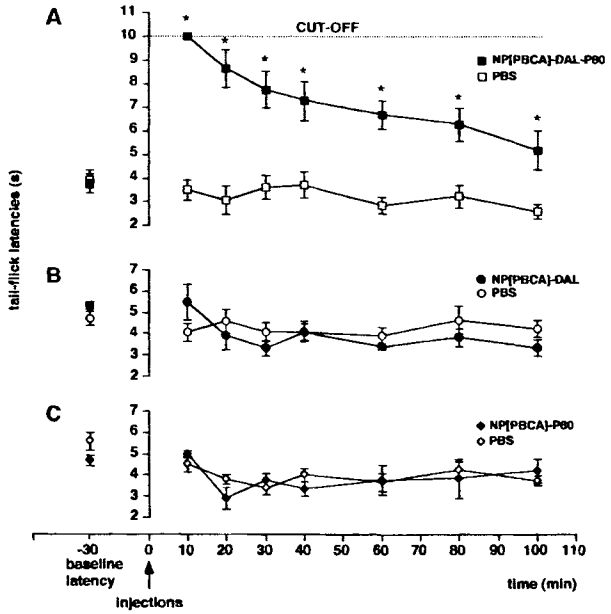

Fig. 2. Tail-flick latencies obtained after administration of PBCA nanoparticle preparations, and their respective PBS controls. Error bars indicate the standard error. * Significantly different from control ($p < 0.05$). For details about the preparations injected, see Table I.

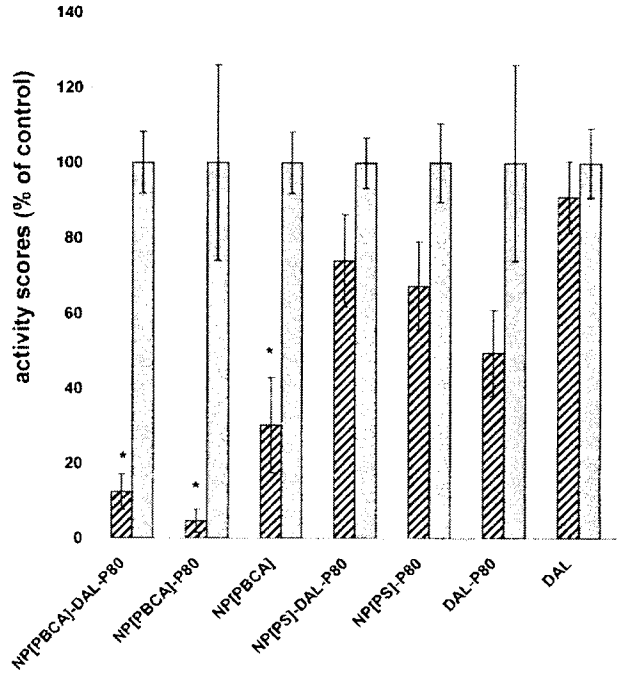

Fig. 3. Activity scores expressed as the percentage of control mean (hatched columns) with the respective controls scaled to 100% (grey columns). Error bars indicate the standard error. *Significantly different from control ($p < 0.05$). For details about the preparations injected, see Table I.

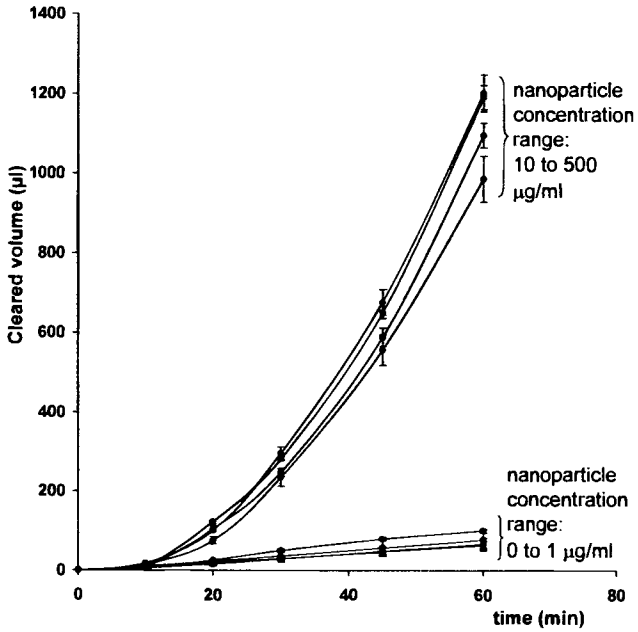

Fig. 5. Clearance of sucrose across the BBB model in the presence of various concentrations of polysorbate 80-coated PBCA nanoparticles.

fluids (19–22) and was observed for doses as low as 20 mg/kg (23).

To check whether the nanoparticle polymer was involved in dalargin penetration to the CNS, antinociception experiments were carried out with PBCA nanoparticles and with PS nanoparticles. The latter were selected for their non-biodegradability

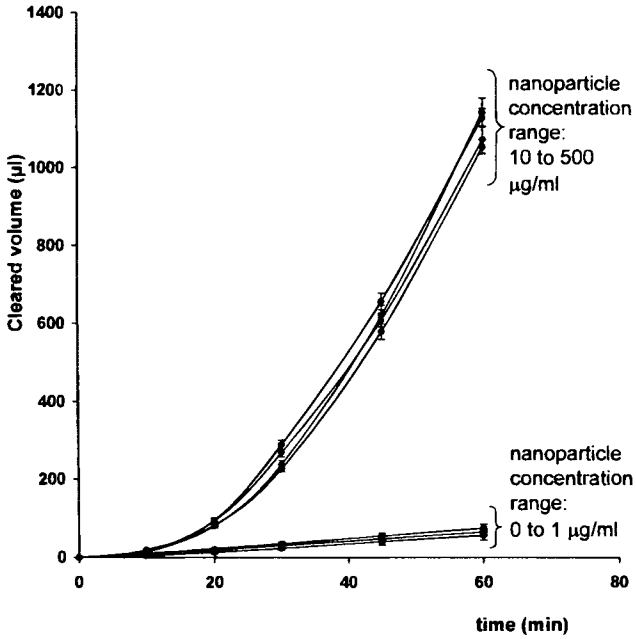

Fig. 4. Clearance of sucrose across the BBB model in the presence of various concentrations of PBCA nanoparticles.

(24), which prevented any release of potentially toxic compounds. The amount of dalargin adsorbed onto PBCA nanoparticles, 11.7 µg dalargin per mg, was in agreement with earlier studies (13.5 µg/mg, (7,9)). For PS nanoparticles, dalargin adsorbed was 16.5 µg/mg. Overcoating the nanoparticles with polysorbate 80 led to a complete desorption of the peptide from both nanoparticle types (Table II), probably by a competitive adsorption of the surfactant onto the nanoparticle surface, as already reported for a glycoprotein (orosomucoid) adsorbed onto polyisobutylcyanocrylate nanoparticles (12). Thus, the polysorbate 80-coated dalargin-loaded nanoparticle preparation should actually be considered as a simple mixture of nanoparticles, dalargin and polysorbate 80. This preparation nevertheless induced an early and significant analgesia (Fig. 2A), confirming the earlier results (7–9). Dalargin alone or mixed with polysorbate 80 (Fig. 1A & 1B) had no analgesic effect. Consequently, polysorbate 80-coated nanoparticles permitted dalargin entry to the CNS without needing the peptide to be adsorbed onto the nanoparticles. These data contradicted the transcytosis hypothesis and suggested that PBCA nanoparticles induced an alteration of the BBB permeability to polysorbate 80- associated dalargin. As shown previously (7–9), polysorbate 80 was necessary for dalargin entry to the CNS: dalargin-loaded PBCA nanoparticles without polysorbate 80 were ineffective (Fig. 2B). The antinociception effect was related to the polymer itself and not to the particulate nature of the carrier: replacing PBCA nanoparticles with PS nanoparticles abolished completely the CNS penetration of dalargin (Fig. 1C).

The experiments performed on the *in vitro* BBB model showed that PBCA nanoparticles (overcoated with polysorbate 80 or not) were able to open the tight junctions at concentrations of 10 µg/ml and above (Fig. 4 and 5). Considering that blood volume was 1.7 ml/0.02 kg in mice (25), PBCA nanoparticle concentrations reached in blood immediately after injections to mice should be around 1.5 mg/ml, therefore far above the

permeabilization threshold concentrations determined *in vitro*. With an estimated blood half-life of 6 min (22), nanoparticle blood concentration was likely to be maintained above the threshold concentrations for at least 60 min (10 blood half-lives), which would be consistent with the sustained antinociception observed *in vivo* (Fig. 2A). However, a discrepancy appeared between *in vivo* and *in vitro* results: PBCA nanoparticles without polysorbate 80 induced a BBB permeabilization *in vitro*, but did not induce analgesia *in vivo* when associated with dalargin. Polysorbate 80 has been shown to enhance the uptake of methotrexate into the brain of healthy animals, presumably by a direct effect on the BBB (26). Even though this effect was not observed for dalargin mixed with polysorbate 80 (Fig. 1B), polysorbate 80 may promote dalargin entry once the BBB has been disrupted by the PBCA nanoparticles. The *in vitro* model has proved useful in highlighting possible mechanisms by which the PBCA nanoparticles/polysorbate 80 may act on brain endothelium, but the simple assessment of *in vitro* permeability with sucrose and inulin may not fully reflect the situation *in vivo*, either for penetration of the hexapeptide dalargin, or for the particular effect of polysorbate 80 on penetration of the peptide across the BBB. Further studies both *in vivo* and *in vitro* are required for more complete understanding.

The toxicity of the PBCA nanoparticles may account for the *in vivo* and *in vitro* results. Toxicity was constantly observed after the administration of PBCA nanoparticles to mice: occasional death was recorded in accordance with the LD_{50} of the carrier (13). The mice surviving PBCA nanoparticle administrations presented a dramatic decrease in their activity scores (Fig. 3) and clear signs of discomfort. PS nanoparticles with dalargin and polysorbate 80 which never caused mortality or behavioral alterations were ineffective in inducing antinociception. Furthermore, the delay for inducing the mice's deaths, the behavioral alterations, the antinociception and the *in vitro* BBB permeabilization (which occurred at concentration cytotoxic for L929 fibroblasts (19)) were consistent with the kinetics of PBCA nanoparticle degradation (27) and of the release of toxic compounds (21). Therefore the antinociception effect of dalargin mixed with PBCA nanoparticles and polysorbate 80 may result, at least in part, from the toxicity of the carrier on the BBB and opening of the tight junctions.

As a conclusion, this work showed the following points: (1) polysorbate 80 desorbed dalargin from the PBCA nanoparticle surface, (2) analgesia was nevertheless observed with the preparation of PBCA nanoparticles-polysorbate 80-dalargin, (3) locomotor activity was dramatically reduced by PBCA nanoparticles and (4) PBCA nanoparticles opened the tight junctions of an *in vitro* BBB model. Both *in vitro and in vivo* results suggested that PBCA nanoparticles induced a non specific opening of the BBB which allowed, in the presence of polysorbate 80, the penetration of dalargin into the CNS. Although polysorbate 80-coated PBCA nanoparticles may be a useful tool to increase experimentally the penetration of drugs into the CNS, potential therapeutic applications may be limited as the high systemic nanoparticle concentration necessary to deliver drugs to the CNS is responsible for an important toxicity.

ACKNOWLEDGMENTS

The authors wish to thank Mr B. Floure from Malvern Instruments S.A. (Orsay, France) for providing nanoparticle size measurement facilities.

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