Nanoparticle Technology for Delivery of Drugs Across the Blood–Brain Barrier

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Abstract □ The Leu-enkephalin dalargin and the Met-enkephalin kyotorphin normally do not cross the blood–brain barrier (BBB) when given systemically. To transport these neuropeptides across the BBB they were adsorbed onto the surface of poly(butylcyanoacrylate) nanoparticles (NPs) and the NPs were coated with polysorbate 80. Central analgesia was measured by the hot plate test in mice. The antidepressant amitriptyline, which normally penetrates the BBB, was used to examine the versatility of the NP method. The concentration of amitriptyline in serum and brain of mice was determined by a gas chromatographic method. Furthermore, NPs were fabricated with different stabilizers. After the adsorption of the peptides on polysorbate 85-stabilized NPs, analgesia was noted after intravenous application when NPs were not coated. The amitriptyline level was significantly enhanced in brain when the substance was adsorbed onto the NP and coated or when the particles were stabilized with polysorbate 85.

Introduction

The pharmacological treatment of neurological and psychiatric disorders is often complicated by the inability of potent drugs to pass the blood–brain barrier (BBB), which is formed by the endothelium of the brain vessels, the basal membrane, and neuroglial cells. Physicochemical properties of drugs, such as lipophilicity and molecular weight, determine to what extent drugs can cross the BBB. Drugs or compounds that are not ionized at physiological pH, lipophilic, and of low molecular weight can cross the BBB by diffusion mechanisms. Other essential compounds, such as amino acids, neuropeptides, and hexoses, normally need specific carriers to permeate into the brain.[1,2] Furthermore, peptides and proteins can cross the BBB by saturable transport systems[3] that have been described for cytokines such as MIP-1α and MIP-1β[4] or interleukin-1α.[5]

To overcome the limited access of drugs to the brain different methods have been developed that achieve BBB penetration. Most of these methods are characterized, for instance, by osmotic BBB opening[1,6] or by the use of biologically active agents (e.g., histamine, serotonin, substance P, free oxygen radicals, nitric oxide, calcium entry blocker, bradykinin, 5-hydroxytryptamine, cytokines, metalloproteinases, endothelin-1, etc.).[7,8] The use of so-called drug carriers, such as liposomes[9] and nanoparticles,[10,11] for targeted drug delivery has been examined. One of the main problems in the targeted drug delivery is the rapid opsonization and uptake of the injected carrier systems by the reticuloendothelial system, by macrophages in liver and spleen. In the case of nanoparticles (NPs), it could be demonstrated in vivo that the body distribution of intravenously (iv) applied NPs can be altered by coating these particles with nonionic surfactants, such as polysorbate, poloxamers, and poloxamines.[12]

In the present study, the possibility of using dextran 70 000-stabilized and polysorbate 80-coated NPs for the delivery of two central analgesic peptides to the brain was compared with an alternative method using polysorbate 85-stabilized NPs. The following central analgesic-acting peptides were tested: (i) dalargin (Tyr-D-Ala-Gly-Phe-Leu-Arg), a Leu-enkephalin analogue, and (ii) kyotorphin (L-Tyr-L-Arg). Both substances are known not to cross the BBB because of their hydrophilicity.[13,14] A further advantage of the NP technology may be a reduction of therapeutic dose, which reduces the side effects of therapeutic drugs. Therefore, the aim of our study was to demonstrate whether NPs are suitable to enhance the concentration of drugs in brain and serum. Amitriptyline, a tricyclic antidepressant, that normally does penetrate the BBB, was chosen as a model drug.

Experimental Section

Nanoparticle Preparation—Nanoparticles were prepared according to the method described by Kreuter[15] with some modifications. Briefly, an acidic polymerization medium containing different stabilizers (1% stabilizer in 0.01 N HCl) was used. Dextran 70 000 (Sigma, Germany) and Tween 85 (polysorbate 85; Erbslöh KG, Germany) were used as stabilizers. Stabilization means that the compound (dextran 70 000 or polysorbate 85) was incorporated into the NP. Butylcyanoacrylate 1%; (Sichelwerke, Hannover, Germany) was added under constant magnetic stirring at 600 rpm. After a 4-h polymerization period, the NP suspension was neutralized with 0.1 N NaOH to complete the polymerization and was then purified by centrifugation. The determination of particle size was achieved by photon correlation spectroscopy with an AutoSizer Lo-c (Malvern Instruments Ltd., U.K.). The NP suspension was lyophilized in the presence of 4% mannitol as cryoprotector (Alpha 1–4, Martin Christ Gefriertrocknungsanlagen, Germany).

Drug Loading—An amount of 30 mg of lyophilized NPs was resuspended in 5 mL of 10 mM phosphate-buffered saline (PBS). Dalargin (Tyr-D-Ala-Gly-Phe-Leu-Arg; Bachem, Germany) and kyotorphin (Tyr-D-Arg; Bachem, Germany) were each added in a concentration of 1.0 mg/mL suspension. Amitriptyline (Sigma, Germany) was used in a concentration of 2.0 mg/mL solution. The substances were allowed to adsorb onto the NP surface for 3 h, and the amount of adsorbed drug was determined as previously described.[16] Therefore, the NP–drug suspension was ultracentrifuged and the amount of the free drug in supernatant was measured by ultraviolet (UV) spectrophotometry (DU 7 spectrophotometer; Beckman Instruments, USA). For coating, 0.01% polysorbate 80 (relative to the total suspension volume) was added and incubated for 30 min. The term "coated" means the compound (polysorbate 80; Erbslöh KG, Germany) was added to the particle surface at its formation. The drug-loaded NPs were given iv (0.1 mL/10 g).

Animals—All procedures were approved by the animal experimentation committee, according to the requirements of the National Act on the use of experimental animals (Germany). Male

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Table 1—Analgesic Activity (Latency of Hindlimb Licking) following Intravenous Application of Dalargin-Loaded Nanoparticles[a]

stabilizer	group	latency of hindlimb licking (s) 5 min	15 min	30 min	45 min	90 min
dextran 70 000	empty nanoparticles	12.4 ± 1.94	9.2 ± 1.45	8.0 ± 0.91	9.1 ± 0.63	16.7 ± 2.82
	10 mg/kg dalargin	14.7 ± 3.29	13.0 ± 1.82	13.0 ± 2.48	12.2 ± 1.74	16.2 ± 3.02
	10 mg/kg dalargin + nanoparticles	17.9 ± 2.14	13.2 ± 0.82	14.5 ± 2.50	16.1 ± 1.95	17.6 ± 1.72
	10 mg/kg dalargin + nanoparticles + polysorbate 80	27.5 ± 1.57[b]	26.7 ± 1.69[b]	17.8 ± 3.28	19.2 ± 3.16	15.9 ± 2.40
polysorbate 85	empty nanoparticles	15.0 ± 2.09	13.7 ± 2.43	11.4 ± 1.47	11.7 ± 1.98	16.5 ± 1.64
	10 mg/kg dalargin	13.6 ± 1.81	18.7 ± 2.40	17.0 ± 2.46	12.5 ± 1.67	15.6 ± 2.44
	10 mg/kg dalargin + nanoparticles	30.0 ± 0.00[b]	22.1 ± 2.12	16.6 ± 2.85	16.6 ± 2.85	21.1 ± 2.88

[a] Dalargin concentration was 10.0 mg/kg; values are expressed as means ± SEM. [b] Significantly different ($p < 0.05$) compared with dalargin alone and empty nanoparticles.

Table 2—Analgesic Activity (Latency of Hindlimb Licking) following Intravenous Application Kyotorphin-Loaded Nanoparticles[a]

stablizer	group	latency of hindlimb licking(s) 5 min	15 min	30 min	45 min	90 min
dextran 70 000	empty nanoparticles	11.8 ± 1.26	18.0 ± 2.86	12.0 ± 2.10	10.7 ± 1.41	14.5 ± 2.85
	10 mg/kg kyotorphin	10.9 ± 0.69	12.6 ± 1.63	14.6 ± 2.05	12.5 ± 1.76	17.9 ± 3.08
	10 mg/kg kyotorphin + nanoparticles	22.8 ± 2.84[b]	19.6 ± 3.08	18.3 ± 2.77	17.3 ± 2.31	16.4 ± 1.82
	10 mg/kg kyotorphin +nanoparticles + polysorbate 80	21.9 ± 2.09[b]	13.7 ± 1.55[b]	18.6 ± 2.00	17.6 ± 1.50	18.2 ± 2.97
polysorbate 85	empty nanoparticles	10.0 ± 2.52	9.28 ± 2.01	12.7 ± 2.83	12.7 ± 3.45	16.2 ± 3.29
	10 mg/kg kyotorphin	10.0 ± 0.99	16.0 ± 1.74	14.2 ± 2.55	16.6 ± 2.89	21.4 ± 2.65
	10 mg/kg kyotorphin + nanoparticles	14.8 ± 3.22	19.0 ± 2.97	16.3 ± 3.25	14.3 ± 1.99	13.0 ± 0.19
	empty nanoparticles	10.0 ± 2.52	9.28 ± 2.01	12.7 ± 2.83	12.7 ± 3.45	16.2 ± 3.29

[a] Kyotorphin concentration was 10.0 mg/kg; values are expressed as means ± SEM. [b] Statistically different ($p < 0.05$) compared with kyotorphin alone and empty nanoparticles.

Table 3—Area Under the Curve (AUC) Concentration versus Time after Intravenous Application of Amitriptyline-Loaded Nanoparticles

stabilizer	group	AUC (brain) μg/g·min	AUC (serum) μg/mL·min
dextran 70 000	amitriptyline	998.5	85.43
	amitriptyline + nanoparticles	684.5	73.33
	amitriptyline + nanoparticles + polysorbate 80	1154.0	69.7
polysorbate 85	amitriptyline	455.6	38.65
	amitriptyline + nanoparticles	994.3	86.33

NMRI mice (Harlan/Winkelmann, Germany; 30−34 g body weight) were used. Mice were housed in plastic cages, given food and water ad libitum, and were maintained in temperature- and humidity-controlled rooms with a 12:12 h light/dark cycle.

Analgesic Studies—Analgesic effect was measured by the hot plate test, in which the animal was placed on a hot plate (Ugo Basile, Italy; 54 °C) and the time until onset of licking the hindlimb (hot plate latency, maximum 30 s) was recorded. The hot plate latency was determined 5, 15, 30, 45, and 90 min after iv injection of peptide (dalargin and kyotorphin)-loaded NPs.

Kinetic Studies—Amitriptyline concentrations in serum and brain were estimated 5, 20, and 60 min after iv injection. The serum levels of amitriptyline and its metabolite in animals were measured by gas−liquid chromatography (GLC) with nitrogen−phosphorus selective detection.[17] The method was adapted for the assay of amitriptyline and metabolite in brain. Linear calibration curves passing through the origin and with correlative coefficients $r > 0.99$ were found for serum. The precision of the assay in both compartments as determined by the coefficient of variation of 12 subsequent measurements at concentrations of 0.5 and 2.0 μg/mL (amitriptyline in serum), 0.05 and 0.2 μg/mL (nortriptyline in serum), 5 and 20 μg/g (amitriptyline in brain) and 0.1 and 0.5 μg/g (nortriptyline in brain), was <7% (amitriptyline) and <12% (nortriptyline), respectively. Testing the accuracy revealed values between 94 and 114%. For the data analysis, the area under the curve (AUC) of concentration versus time was calculated.

Statistics—Statistical significance was determined by a one-way ANOVA and subsequent post hoc Tukey comparison.

Results

Nanoparticles—The NPs fabricated in our laboratory had the following size (nm) and polydispersities: dextran 70 000-stabilized NP: 195.2/0.282- and polysorbate-85-stabilized NP: 288.9/0.340, respectively.

Hot Plate Test—As in previous studies,[11,16,18,19] we found that administration of NPs themselves do not produce analgesia.

The iv application of dalargin bound to dextran 70 000-stabilized NPs with a polysorbate 80 coat was followed by a hot plate latency enhancement of ~85% at 5 and 15 min after iv application (Table 1) compared with dalargin alone. After loading of polysorbate 85-stabilized NPs with dalargin, the suspension was injected iv. As seen in Table 1, the NP application led to a drastic enhancement of analgesic activity immediately (5 min) after the iv injection. In addition, all animals showed the so-called "Straub" (tail erection[20]) phenomenon.

Kyotorphin bound to dextran 70 000-stabilized NPs and coated with polysorbate 80 showed the following effects (Table 2): When kyotorphin was adsorbed onto NPs with subsequent coating, a significant enhancement of analgesia could be demonstrated in both groups compared with the kyotorphin-treated animals alone. The adsorption onto the surface of polysorbate 85-stabilized NPs did not vary the central analgesic effect (Table 2).

Kinetic Studies—The area under the curve (AUC) of concentration versus time after adsorption of 20.0 mg/kg amitriptyline onto the surface of dextran 70 000-stabilizied NPs was enhanced in brain when the drug−NP mixture was coated with polysorbate 80, whereas the serum AUC was decreased (Table 3). Furthermore, the AUC in brain and serum were enhanced after adsorption of amitriptyline

onto the surface of polysorbate 85-stabilized NPs that did not have a coating (Table 3).

Discussion

In the present study, the analgesic latency on the hot plate was monitored after administration of drug-loaded NPs. In the case of dalargin, the peptide-adsorbed particles (dextran 70 000-stabilized) that were coated with polysorbate 80 were capable of inducing analgesia. Polysorbate 85-stabilized and dalargin-loaded NPs without coating were also able to induce a significant central analgesic effect after iv application. Because previous reports[11,18] show that the analgesic effect of dalargin-loaded NPs can be blocked by the central μ-opiate antagonist naloxone, it can be concluded that dalargin-induced analgesia is mediated by central mechanisms.

Kyotorphin-loaded NPs induced central analgesic effects only when its adsorption was realized with dextran 70 000-stabilized NPs. In contrast, both kinds of amitriptyline-loaded NPs led to an enhanced level of the antidepressant concentration in brain. Furthermore, when amitriptyline was adsorbed onto the surface of polysorbate 85-stabilized NPs, the serum level was also increased. A point of discussion may be that the NPs application led to another body distribution; namely, a reduction of the uptake by either the reticuloendothelial system or by macrophages in liver and spleen.

The search for tools to overcome the limited penetration of drugs through the BBB is an important problem in the central nervous system pharmacology. In previous studies[16,19] we have demonstrated that drug-loaded NPs can cross the BBB as intact molecules and act on central nervous system.

The presumed enhancement of the transport across the BBB of dalargin as well as kyotorphin could be a result of different mechanisms. The binding of NPs to the inner endothelial cells of brain capillaries and the subsequent transport by passive diffusion may be caused by a larger concentration gradient. On the other hand, the neuropeptides can enter the brain via phagocytosis processes of endothelial uptake.[18] The enhanced concentration gradient may be an additional reason for the increased amount of amitriptyline determined in the brain. Furthermore, it is possible that degradation products of the NPs may act as absorption enhancers.[21]

The mechanisms whereby substances are released from the NP surface into the brain are still unknown and one can only speculate at this point. Nevertheless, we postulate that the NPs represent a very interesting alternative to deliver drugs to the brain and to other organs (i.e., lung) because of their great potential versatility for different drugs. The NPs seem to be a good tool for drug delivery through physiological barriers, especially the BBB. Furthermore, the NPs can be used for reducing the dose of a drug while maintaining its therapeutic effects.

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