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Efficacy of Oral Dalargin-loaded Nanoparticle Delivery across the Blood–Brain Barrier

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SCHROEDER, U., P. SOMMERFELD AND B. A. SABEL. *Efficacy of oral dalargin-loaded nanoparticle delivery across the blood–brain barrier*. PEPTIDES **19**(4) 777–780, 1998.—The Leu-enkephalin dalargin normally does not penetrate the blood–brain barrier (BBB) when given intravenously. To transport dalargin across the blood–brain barrier, the peptide was adsorbed onto the surface of poly(butyl)cyanoacrylate nanoparticles and coated with polysorbate 80. After systemic administration the central analgesia was measured by hot plate test. Furthermore, nanoparticles were fabricated with different stabilizers. After the adsorption of the peptide on polysorbate 85 stabilized nanoparticles analgesia was observable after intravenously and oral application even when nanoparticles were not coated. Thus, our data support the usefulness of nanoparticles as a method to deliver drugs to the brain. © 1998 Elsevier Science Inc.

Analgesia Blood–brain barrier Dalargin Hot plate Nanoparticles Mice

THE pharmacological treatment of neurological and psychiatric disorders is often complicated by the inability of 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 unionised 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 (19,21). Furthermore, peptides and proteins can cross the BBB by saturable transport systems (4). Such saturable transport systems have been described for cytokines like MIP-1α and MIP-1β (3) or interleukin-1α (18).

Many attempts were made to overcome this diffusion limiting blood–brain barrier. Most are characterized by methods such as osmotic BBB opening (10,19) or the use of biologically active agents such as histamine, serotonin, substance P, free oxygen radicals, nitric oxide, calcium entry blocker, bradykinin, 5-hydroxytryptamine, cytokines, metalloproteinases, endothelin-1 or others (1,9). In this context, drug carriers such as liposomes (22) and nanoparticles (7,14) for targeted drug delivery have been examined. We and others have developed a nanoparticle system for drug loading, which can, after adsorption and coating with polysorbate 80, cross the BBB (14,20). The nanoparticles were adsorbed with dalargin, a Leu-enkephalin analogue (Tyr-D-Ala-Gly-Phe-Leu-Arg), containing D-Ala in second position in order to prevent enzymatic destruction. Dalargin shows good stability in the blood stream. Normally, the topical injection of this peptide induces analgesic action whereas the systemic administration of this peptide shows no effect on central analgesic mechanisms (11).

For nanoparticles it is clinically relevant, however, whether they are able to deliver therapeutic drugs via the oral route. Colloidal drug carriers are one approach to enhance the oral uptake of these substances. It is theoretically possible that such carriers can protect a drug from degradation in the gut (8).

To find a new technology for nanoparticle application with drugs which do not cross the BBB in the present study, we modified the nanoparticle fabrication method. Dalargin-

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loaded nanoparticles were applied intravenously (IV) or orally and central dalargin-induced analgesia was used as an indicator of BBB passage.

METHOD

Nanoparticle Preparation

Nanoparticles were prepared according to the method described (12) with some modifications. Briefly, an acidic polymerization medium containing different kinds of stabilizers (1% stabilizer in 0.01 N HCl) was used. As stabilizers Tween 85 (polysorbate 85), dextran 12,000 and Pluronic-F 68 (poloxamer 188) were selected. One percent of butylcyanoacrylate (Sichelwerke, Hannover, Germany) was added under constant magnetic stirring at 600 rpm. After a 4 h polymerization period the nanoparticle suspension was neutralized with 0.1 N NaOH to complete the polymerization and was then ultracentrifuged. The determination of particle size was achieved by means of photon correlation spectroscopy with an AutoSizer Lo-c (Malvern Instruments Ltd., UK). The nanoparticle suspension was lyophilized in the presence of 4% mannitol as cryoprotector (Alpha 1-4, Martin Christ Gefriertrocknungsanlagen, Germany).

Drug Loading

Thirty mg lyophilized nanoparticles were resuspended in 5 ml 10 mM phosphate-buffered saline (PBS). Dalargin (Tyr-D-Ala-Gly-Phe-Leu-Arg; Bachem, Germany) was added in a concentration of 1.0 mg/ml suspension. The peptide was allowed to adsorb onto the nanoparticle surface for 3 h and the amount of drug was determined as previously described (20). For coating 0.01% of polysorbate 80 (relative to the total suspension volume) was added and incubated for 30 min. The dalargin-loaded nanoparticles were given IV (0.1 ml/10 g). For the oral application 60 mg lyophilized nanoparticles were resuspended in 5.0 ml of 10 mM PBS and then 1.0 mg dalargin/ml solution was added.

Animals

A total of 180 male NMRI mice (Harlan/Winkelmann, Germany; 30–34 g bwt.) were used and housed in plastic cages, food and water ad lib. The animals were maintained in temperature- and humidity-controlled rooms with a 12: 12-h light/dark cycle.

Analgesic Studies

Analgesic effect was measured using the hot plate test, in which the animal was placed on a hot plate (Ugo Basile, Italy; 54°C) and the time for licking the hindlimb (maximal 30 s) was recorded. The hot plate latency was determined 5, 15, 30, 45, and 90 min after intravenous injection of dalargin-loaded nanoparticles. After oral application the latency was observed 30, 45, 60, 90, 120, and 150 min thereafter.

Statistics

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

RESULTS

Nanoparticles

We could demonstrate that nanoparticles (NP) can be fabricated with stabilizer other than dextran 70,000 as described previously (20). The average size (nm) and polydispersity for the different stabilizers is as follows: dextran 12,000-stabilized NP: 203 nm/0.197; poloxamer 188-stabilized NP: 305.5/0.046; and tween 85-stabilized NP: 288.9/0.340. The dextran 12,000 and poloxamer 188-stabilized NP showed a very small polydispersity with a monomodal distribution. Therefore, stabilizers other than dextran 70,000 may be particularly useful.

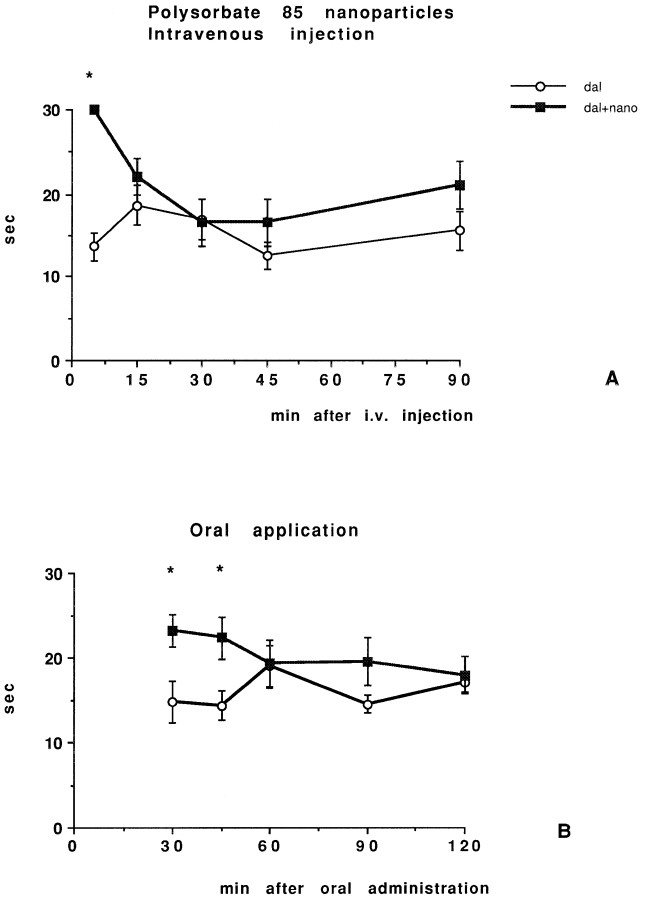

FIG. 1. Analgesic activity after intravenous (A) and oral (B) application of dalargin-loaded nanoparticles, which were stabilized with polysorbate 85 (means ± SEM; $*p < 0.05$ vs. dalargin alone); y-axis: seconds to withdraw paw from hot plate.

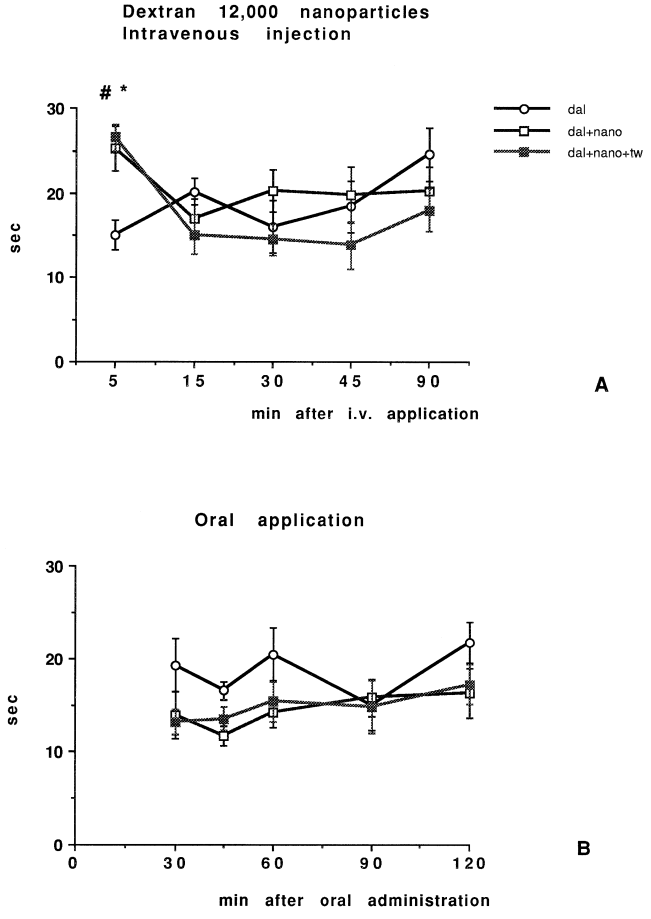

FIG. 2. Analgesic activity after intravenous (A) and oral (B) application of dalargin-loaded nanoparticles, which were stabilized with dextran 12,000 (means ± SEM; *#$p < 0.05$ vs. dalargin alone); y-axis: seconds to withdraw paw from hot plate.

Hot Plate Test

Generally, it could demonstrated in previous studies (2,14,20) and in this study that administration of NP themselves do not produce analgesia.

Tween 85-stabilized NP. After loading of these NPs with dalargin the suspension was injected intravenously. As seen in Figure 1A the NP application led to a drastic enhancement of analgesic activity immediately 5 min after the IV injection; $F(1, 18) = 7.82$, $p < 0.02$. In addition, all animals showed so-called ''Straub'' tail erection (15) phenomenon. The results of the oral NP application is depicted in Figure 1B. Because of the altered pharmacokinetics after oral application other time points following the drug application were used. We were able to demonstrate a centrally-induced analgesic effect at time points of 30 min [$F(1, 15) = 6.80$, $p < 0.02$] and 45 min [$F(1, 15) = 6.62$, $p < 0.02$] even when drug-loaded NP were given orally.

Dextran 12,000-stabilized NP. The IV application of the drug-loaded NPs led to an enhanced analgesic effect. This effect was also observable, when the drug-loaded NPs were coated with polysorbate 80 (Fig. 2A) but no ''Straub'' phenomenon was observed [$F(2, 24) = 9.63$, $p < 0.01$]. The oral treatment with NP stabilized in this way showed a light analgesic effect that failed to be statistically significant (Fig. 2B).

Poloxamer 188-stabilized NP. The IV application of these drug-loaded NP had only analgesic activity when it was coated with polysorbate 80 (Fig. 3A); [$F(2, 24) = 9.63$, $p < 0.01$] whereas the oral application was without any analgesic effect (Fig. 3B).

DISCUSSION

The main finding is that polysorbate 85 (Tween 85) stabilized and dalargin-loaded nanoparticles are able to induce a central analgesic effect after IV application as well as after oral treatment. According to the literature data (2,14) demonstrating that the analgesic effect of dalargin-loaded nanoparticles can be blocked by the central μ-opiate antagonist naloxone, it can be

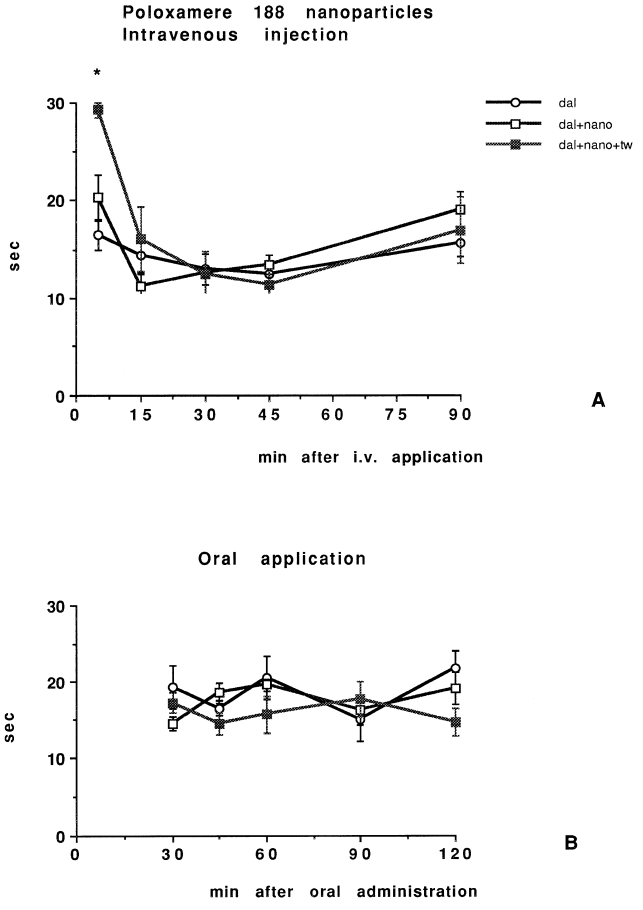

FIG. 3. Analgesic activity after intravenous (A) and oral (B) application of dalargin-loaded nanoparticles, which were stabilized with poloxamer 188 (means ± SEM; *$p < 0.05$ vs. dalargin alone); y-axis: seconds to withdraw paw from hot plate.

concluded that analgesia of dalargin is mediated by central mechanisms. The mechanisms whereby dalargin is released from the nanoparticle surface is not yet known and we can only speculate at this point. In general, peptides produce their central effects by (i) crossing the cerebral capillary endothelium forming the blood–brain barrier by either a passive diffusion or by a specific receptor-mediated mechanism; (ii) penetrating the fenestrated capillaries of the circumventricular organs (6), or (iii) the brain may have taken up dalargin via endothelial uptake by phagocytosis (2). It is also possible that degradation products of the nanoparticles may act as absorption enhancers (16).

The analgesic effect measured after oral application offers new possibilities for drug-targeting of potent CNS active drugs when they are bound to such nanoparticles. Increases in oral bioavailability by PBCA nanoparticles or nanocapsule preparations were already described (5,13). Avarol, a cytostatic and antiviral drug was bound to the nanoparticles and given orally. The resulting avarol blood levels showed an 8 to 9-fold increase in bioavailability compared to the avarol solution alone. In this context it was postulated, that the enhancement in absorption seems to be mainly caused by an adhesion of the PBCA particles to the intestinal mucosa, similarly to poly(hydroxypropyl methacrylate) nanoparticles (17).

Whatever the mechanism may be, the main finding of our studies is that oral applied nanoparticle-bound dalargin can cross the BBB and induce analgesia. Therefore, nanoparticles represent a novel tool to deliver drugs across the BBB. Furthermore, the results suggest that the nanoparticles can be use as an oral drug delivery method.

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