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Dedicated to the memory of
Professor Ioan Silaghi-Dumitrescu (1950 – 2009)

ANTINOCICEPTIVE EFFECT OF MORPHICEPTIN LOADED POLY(BUTYL CYANOACRYLATE) NANOPARTICLES

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Morphiceptin, a μ-peptide opioid agonist, as well as other neuropeptides, normally does not penetrate the blood–brain barrier (BBB) by itself. However, for more than a decade, surfactant coated nanoparticles have been reported as specific carriers able to transport drugs across the BBB. To investigate the antinociceptive effect following the systemic administration of morphiceptin using poly(butyl cyanoacrylate) nanoparticulate delivery systems (PBCA-NPs) overcoated with Polysorbate 80 (PS 80) surfactant, different formulations of loaded nanoparticles were prepared, depending on the moment of drug addition – during or after butyl cyanoacrylate polymerization. Dispersions of empty nanoparticles, uncoated nanoparticles and a mixture of morphiceptin (MF) and surfactant solution were also injected intraperitoneal in mice at the same dosage as controls. The *in vivo* measurements of central antinociceptive effect of morphiceptin using a thermoalgesic test (hot plate), following the administration, demonstrated a significant analgesia for the formulations implying nanoparticles. Differences were observed depending on the type of loading and surface properties of the particles. It was also found that the coating of the nanoparticles with PS 80 surfactant increases the BBB penetration and, as a consequence, the antinociceptive effect, confirming the effectiveness of colloidal carriers in protein delivery across the blood-brain barrier.

INTRODUCTION

Morphiceptin (MF) is an opioid tetrapeptide and a potent and highly selective μ-opioid receptor agonist. Several studies pointed out that opioid peptides within the same family inhibit pain without inducing some of the side effects specific to classical opioid analgesics as morphine. Atypical opioid peptides such as endomorphins and morphiceptin are less likely to induce respiratory depression and cardiovascular effects at effective antinociceptive doses, two of the most menacing side effects of the opioid analgesics. However, morphiceptin, similar with other opioid peptides, cannot be delivered into the central nervous system (CNS) in sufficient amount to elicit analgesia when administered systemically, because its targeted delivery is severely restricted

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by the diffusion limiting blood–brain barrier (BBB). Under normal conditions the BBB, formed by tight endothelial cell junctions of brain capillary, acts as a barrier to toxic agents and safeguards the integrity of the brain, but the same mechanisms that protect the brain from foreign substances also restrict the entry of many potentially therapeutic agents. It is widely accepted that only the compounds which are unionized at physiological pH, lipophilic, and of low molecular mass can cross the BBB by diffusion mechanisms, and other compounds such as amino acids, neuropeptides, and hexoses need specific carrier proteins to pass into the brain. Thus, the BBB prevents the administration of a large number of therapeutic compounds, including antibiotics, antineoplastic agents and CNS active drugs, to the central nervous system, even if in certain pathological situations the BBB is partly disrupted. In this context, the difficulty encountered by morphiceptin in readily penetrating the BBB (to gain access to the brain and spinal cord in order to exercise its analgesic activity), as well as its biological instability, make difficult its development for clinical use.[1, 2]

Several invasive (disruption of the BBB, direct drug delivery, intracerebral implantation of controlled release systems) and non-invasive (of chemical or biological nature) strategies were developed to circumvent the BBB and deliver drugs into the brain. Unfortunately, invasive techniques are associated with increased risk of infection and high neurosurgical cost, and most of chemical and biological techniques are too expensive and the efficacy of such technologies is not remarkable.[3, 4] However, with the advances in nanotechnology, colloidal carriers (micelles, emulsions, liposomes and nanoparticles – nanospheres and nanocapsules) became a research subject of increasing interest. Their potential as non-invasive routes for the brain delivery of drugs consists mainly in the possibility to target specific brain tissues thanks to carrier surface linked ligands.[3, 5] Other clinical advantages offered by colloidal carriers include decreased drug dose, reduced drug side effects, increased drug viability and thus improved patient quality of life. In this context, one of the most attractive and innovative alternatives to transport and deliver entrapped or adsorbed drugs into CNS consists in the use of nanoparticulate carriers. They can easily enter brain capillaries before reaching the surface of brain microvascular endothelial cells, when the surface of these colloids is modified in a proper way. The prolonged blood circulation of the surface-modified colloidal particles enhances exposure of the BBB, which favors the interaction and penetration into brain endothelial cells. Consequently, for more than a decade, surfactant coated nanoparticles have been reported to successfully transport drugs across the BBB and recently the method was applied for some opioid peptides, too.[6, 7, 8, 9] As an example, polysorbate 80 (PS 80) coated poly(alkyl cyanoacrylate) nanoparticles have been proved to successfully transport several compounds – including dalargin[10] hexapeptide, kytorphin dipeptide, loperamide[11], tubocurarine[12], doxorubicin[13], and the NMDA receptor[14] – across the BBB. The surface coating of nanoparticles is considered to be an indispensable property for brain targeting effect, the protein adsorption pattern depending on the surface properties of the particles and on the chemistry of the surface-modifying agent.[15] Few surfactants have been tested and the efficiency of PS 80 as surface coating reagent in facilitating brain delivery of nanoparticles against similar systems involving different surfactants – such as polysorbate 20, 40, 60, poloxamer, poloxamine, Cremopor EZ and RH-40 – was proved.[16]

The objective of the present study was to determine if the known colloidal carrier, i.e., the poly(butyl cyanoacrylate) nanoparticulate system PBCA-NPs/PS-80, enables the brain delivery of morphiceptin, following systemic administration, and to gain further insight into the factors governing the phenomenon of drug delivery to the brain by this route.

EXPERIMENTAL

Materials

n-Butyl cyanoacrylate (BCA) monomer was kindly supplied by Sichelwerke, Hannover, Germany. Dextran 70 (D, molecular mass 70,000 g/mol) was obtained from Fluka, Switzerland. Tween 80 (Polysorbate 80 - PS 80) and morphiceptin (MF) were purchased from Sigma-Aldrich Chemie GmbH, Germany. All other chemicals used in the study were of analytical grade. Bi-distilled water was used in all experiments.

Nanoparticles preparation and drug loading

The preparation of nanoparticles was performed under sterile conditions, to avoid peptide degradation by bacterial proteases.[6] Two different formulations of MF loaded nanoparticles (NPs) were considered, depending on the

moment of drug addition – during (MF-NP, entrapped drug) or after (MF/NP, adsorbed drug) polymerization (Table 1).

A. MF-NP formulation

1 mL BCA was added in droplet with a syringe to 100 mL hydrochloric solution (pH = 2.2) containing 1.5 w/v % D, under stirring (1100 rpm), at 20 °C. 30 min after the onset of the polymerization an appropriate amount of MF was added (15 mg MF in 1.2 mL bi-distilled water, yielding 0.15 mg MF/mL). The reaction mixture was maintained under stirring for 5 h, and then a 0.1 N sodium hydroxide aqueous solution was added for neutralization. The mixture was vigorously stirred for one more hour to complete polymerization. The dispersion was filtered (G3 glass filter, Schott AG, Mainz, Germany), subjected to repeated centrifugation and washing (three cycles at 10,000 x g, for 20 min each, 70 mL washing water), and submitted to size and structure analysis. Solid content was evaluated by centrifugation followed by drying for 3 aliquots of 10 mL from the filtered dispersion. The polymerization yield was approximated relative to total final reaction mixture volume.

B. MF/NP formulation

1% (v/v) BCA was added in droplet with a syringe to a HCl medium (pH = 2.2) containing 1.5 w/v % D and the reaction mixture was stirred for 6 h with a magnetic stirrer (1100 rpm) to promote polymerization. The reaction was completed by neutralization of the mixture with 0.1 N sodium hydroxide solution and kept under stirring overnight. After filtration through a G3 glass filter, the fine dispersion was subjected to lyophilization with a CHRIST freeze dryer, Alpha 1-4 LSC type. To evaluate the polymerization yield, 100 mg lyophilized NPs were redispersed in bi-distilled water and then were three times submitted to centrifugation – washing cycles (at 17,000 x g, for 10 min with a Hettich® EBA 21 centrifuge, Germany). The total volume of washing water was of 6 mL. The content in free, soluble dextran was found to be of 56 wt%. Two different sets of samples, submitted to different purification procedures, according to earlier[16] and recent/current protocols, were prepared for administration (Table 1).

For the first set (samples B1-B3), 100 mg lyophilized NPs (containing 44% PBCA NPs) were resuspended by ultrasonication in 4.5 mL isotonic saline. An appropriate amount of morphiceptin solved in 0.5 mL saline was then added, to achieve a final content of 1.2 mg/mL, and the mixture was stirred (700 rpm) for 6 h at 20 ºC (sample B2). PS-80 was then added to yield a final 1% surfactant solution (relative to the total suspension volume) and the mixture was incubated for 30 min (sample B3).

For the second set of samples (B4-B6), 100 mg lyophilized NP were re-dispersed in 2 mL saline serum and thoroughly purified, in order to remove residual monomer by threefold centrifugation at 17,000 x g, for 10 min, and washing with isotonic saline after resuspension by ultrasonication. The thoroughly purified PBCA-NPs were redispersed in an appropriate amount of saline serum to reach a final volume of dispersion of 5 mL (sample B4). The before mentioned procedure was then applied in order to obtain sample B5 (MF loaded NPs) and sample B6 (MF loaded NPs covered with PS-80) with a final dispersion volume of 5 mL and 1.2 mg MF/mL.

Characterization

Aliquots of aqueous dispersions of unloaded nanoparticles (before and after lyophilization), MF loaded nanoparticles, coated or uncoated with PS 80, were subjected to size particle analysis and spectroscopic investigation (^{1}H-NMR, FT-IR) after centrifugation and drying, respectively. ^{1}H-NMR and Fourier transform infrared (FT-IR) spectra were registered with an Avance DRX400 (Bruker) spectrometer working at 400 MHz and with a Vertex 70 (Bruker) spectrophotometer, respectively. DMSO-d_6 or $CDCl_3$ were used as solvents and TMS as internal standard.

For the drug loaded nanoparticles, aliquots from the prepared dispersions were centrifuged and the supernatant was analyzed by UV-Vis spectroscopy (λ=275 nm), SPECORD 200 Analytic Jena instrument, in order to evaluate the amount of free drug (M_1) as compared to the loaded drug percent obtained from ^{1}H-NMR data. The loading efficiency was estimated as the ratio of the amount of bound drug to that of the originally added drug (M_0) by the equation $E = [(M_0-M_1)/M_0]$ x 100%. For MF loaded NPs covered with PS-80 this characterization alternative couldn't be applied due to the superposition of MF and surfactant absorptions.

The particle size analysis (Dn – mean numerical diameter, SD – standard deviation) was performed with a Laser Diffraction Particle Size Analyzer Shimadzu-Sald 700. The aqueous nanoparticulate dispersion was added to the sample dispersion unit containing a stirrer, and was stirred in order to minimize the inter-particle interactions. The analysis was performed trice and the average value was considered.

For samples B4-B6, the recovered supernatant and washing water resulted after the centrifugation cycles performed for further purification were subjected to the determination of the unreacted monomer amount by analytical methods (bromometry). An amount as high as 9.5 wt% monomer was found, relative to processed lyophilized NPs.

***In vivo* testing of MF/NP particles**

All animal experimental procedures employed in the present study were strictly in accordance with the European Community guidelines regarding ethics. The animal breeding facility of the Central Drug Testing Laboratory, "Gr. T. Popa" University of Medicine and Pharmacy, Iasi, supplied adult male Swiss mice with an average weight of 20 g ± 2 g. The animals were housed in a temperature-controlled room (21 ± 2ºC) with a 12 hours/12 hours light/dark cycle, 4 mice per cage, and allowed to acclimate for at least 24 hours before use, with free access to food and water.

Drugs administration

Different groups of 8 mice each were intraperitoneally injected with one of the following formulations:

a) saline suspension of empty PBCA-NPs (Table 1, formulations B1, B4, groups 1 and 4)

b) uncoated MF-loaded NPs (Table 1, formulations B2, B5, groups 2 and 5)

c) PS-80 coated MF-loaded NPs (Table 1, formulations B3 and B6, groups 3 and 6)

d) a mixture of MF and PS 80 (group 7)

e) commercially available MF lyophilized powder solution in saline (group 8) and

f) equal volume of 0.9% saline for a control/reference group (group 9).

The nanoparticulate systems were administered in an amount of 200 mg lyophilized NPs/kg, with the before mentioned composition. For the further purified systems, as mentioned before the free dextran was priory removed by repeated centrifugation/saline washing cycles, the remained nanoparticles (88 mg/kg) being carefully recovered and

subjected to specific procedures according to the envisaged final formulation. In all cases, the morphiceptin dose was of 12 mg/kg.

Analgesic testing

The antinociceptive effect of the tested substances was evaluated by hot plate test, a behavioral test that quantifies the thermal nociception. The test was performed on mice using an Ugo Basile hot plate device. The rectangular metal surface was heated to a temperature of 55 ± 0.5 °C. A chronometer measured the latency observed from the time when the mouse was placed on the heated surface until the first overt behavioral sign of nociception such as (i) the mouse licking a hind paw, (ii) vocalization, or (iii) an escape response. The timer is stopped by a foot-operated pedal and the mouse is immediately removed from the hot plate. Analgesic measurements were performed before drug administration (base line) and at 15, 30, 60, 90 and 120 minutes after the drug or 0.9 % saline (control group) was administered. Treatments that produced a significant increase in the nociceptive thresholds were considered to be antinociceptive.

Statistical methods

A *cut-off time* (maximum time allowed so no injuries could afflict the animal) of 60 seconds was considered for the hot plate test. Data were expressed as the mean ± standard deviation for each measurement time. Differences between treatment groups were analyzed using ANOVA one-way method for comparison at each time point, followed by Bonferroni post-hoc tests. The *p* values less than 0.05 were used to indicate a significant difference for all tests.

RESULTS AND DISCUSSION

Generally, BCA can be polymerized by two techniques, the dispersion polymerization (DP) and emulsion polymerization (EP), both involving the basic mechanism of anionic polymerization.[1, 6, 9, 17] Nanoparticles with an average particle size of about 300 nm and a broad particle size distribution are generally yielded by dispersion polymerization, while EP nanoparticles show an average particle size of up to 200 nm with a narrow particle size distribution. The characteristics of the resulting particles can be controlled adjusting the polymerization mixture recipe, the pH and the stirring rate. Our choice was dispersion polymerization, using dextran as a stabilizer and also cryoprotector, the dextran shell favoring drug adsorption.[7, 9, 18, 19]

The dependence of BBB permeation efficacy on PBCA-NPs size was recently clearly demonstrated.[20] The data agree with the hypothesis considering that the mechanism for surface-coating of the nanoparticles to overcome the BBB supposes that the coated NPs mimic LDL (low density lipoprotein) following the intravenous administration, PS 80 playing a special role as an anchor between NPs and the apolipoprotein, especially ApoE. NPs combined with the apolipoprotein are considered as LDL and LDL receptor-mediated transcytosis transports drug-loaded nanoparticles across the BBB. It seems that this is why nanoparticles of sizes similar to LDL are more efficient than any other types. The size range of LDL was reported to be from 20 to 25 nm.[21] As a consequence, after preliminary polymerization experiments, the preparation of PBCA-NPs was performed by adding 1% (v/v) butyl cyanoacrylate monomer to a 1.5% w/v solution of dextran 70 in 0.01 N hydrochloric acid (stirring rate: 1100 rpm, 20°C) – a recipe yielding nanoparticles with a mean diameter as low as 19 nm and a narrow size distribution with a SD = 5.5 nm (Figure 1).

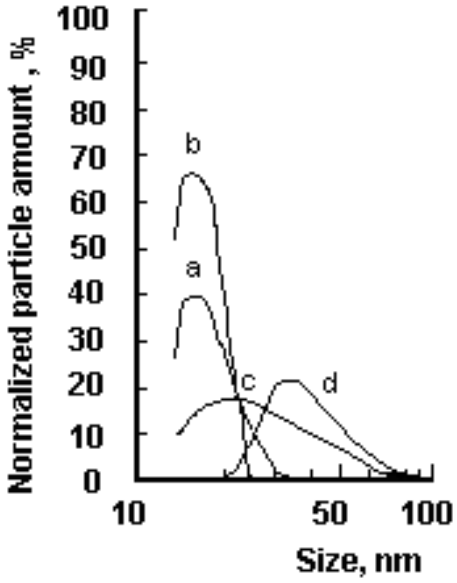

Fig. 1 – PBCA-NPs size distribution. Empty nanoparticles (a) before lyophilization and (b) 6 months after lyophilization and PS-80 coated MF/PBCA-NPs (c) sample B3 and (d) sample B6 (Table 1).

According to ^{1}H-NMR data, the inclusion of MF in PBCA-NPs during the polymerization process gave rise to drug structure modification, most probably by covalent bonding of the peptide on the polymeric support, the resulted MF-NP product being not recommended for injectible administration. Low values were obtained for the polymerization yield, as well as for the amount of the loaded drug into the NPs. The nanoparticles size distribution was large in this case (Table 1).

The adsorption of MF on presynthesized PBCA-NPs gave better results (Table 1).

The nanoparticles stored at -20°C maintained their characteristics as long as six months after lyophilization (Figure 1, plots a, b). However, during the preparation of drug loaded nanoparticles at the concentration imposed by the adopted formulation, an agglomeration tendency was observed before or after drug loading. This instability problem was solved by surfactant addition, the colloidal system generally regaining the initial characteristics (Table 1, Figure 1). One should note that even after further purification, the PBCA-NPs retained about 6 wt% dextran, this suggesting a core-shell structure.

The amount of loaded drug was evaluated by comparing the areas of specific NMR signals appearing in the 6 to 9 ppm range, attributed to MF, with those of the signals situated in the 1 to 4 ppm domain ranging, mainly ascertained to PBCA (Figures 2, 3). The calculations indicate a MF loading on dextran-PBCA-NPs of about 2.8 wt/wt % before surfactant addition (20.4 % loading efficiency), and of only 1.2 % after surfactant coating. Apparently, the loading process is not influenced by the presence or absence of free dextran or residual monomer in the final system.

Table 1

Characteristics of loaded and unloaded NP-PBCA nanoparticles and *in vivo* test arrangement

Sample		Polymer yield %	Dn[a] nm	SD[a] nm	Group	MF[b] %	Coating[c]	Dn[d] nm	SD[d] nm
A		35	59	53	-	0.6	-	-	-
B	B1	73	19	5	1	-	uc	1318	19
	B2				2	2.8	uc	1000	20
	B3				3	1.2	c	23	6
	B4				4	-	uc	912	38
	B5				5	2.7	uc	345	37
	B6				6	1.1	c	35	5

[a] according to measurements before and after lyophilization and redispersion in deionized water
[b] loaded morphiceptin according to the evaluation results from ^{1}H-NMR data
[c] coating with PS 80: c-coated, uc-uncoated
[d] according to size analysis data after sample preparation for administration

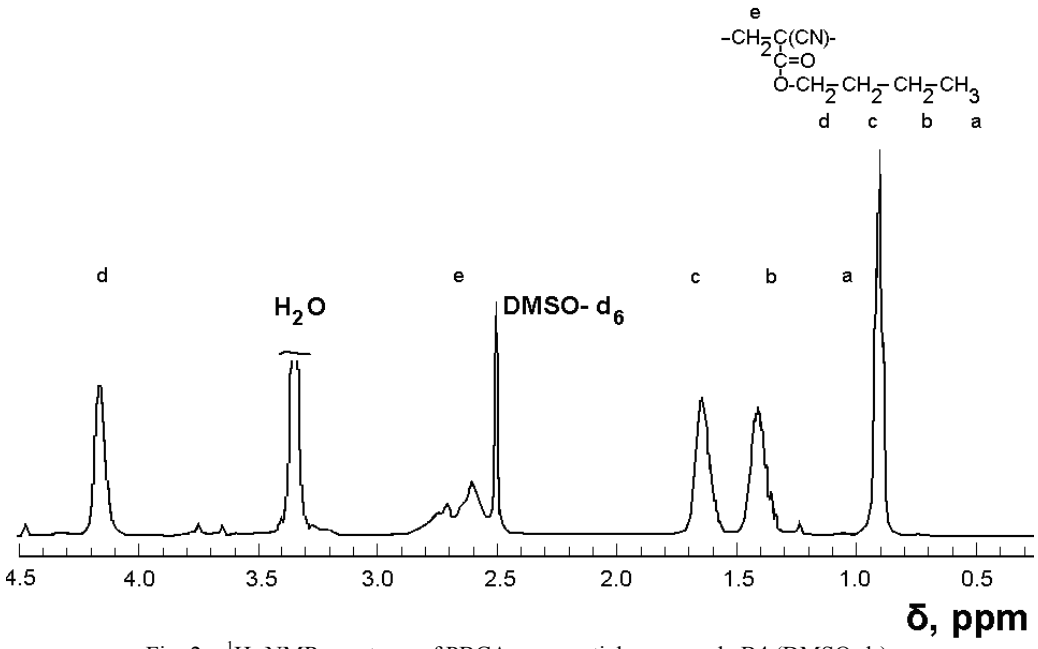

Fig. 2 – ^{1}H- NMR spectrum of PBCA nanoparticles – sample B4 (DMSO-d_6).

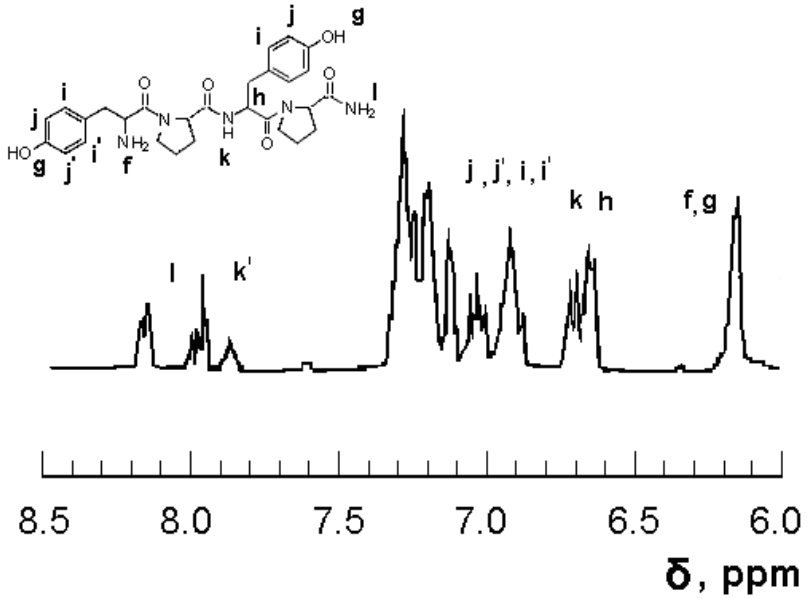

Fig. 3 – ^{1}H- NMR spectrum and signals attribution
for morphiceptin loaded PBCA nanoparticles (detail) – sample B3 (Table 1). Solvent: DMSO-d_6.

The results of antinociceptive testing following intraperitoneal injection show different effects, depending on the applied formulation and on the purity of the polymeric carrier (Table 2).

The nociceptive response latencies to the applied thermal nociceptive stimulus clearly demonstrate that free MF does not penetrate into the brain in amounts able to induce an antinociceptive effect. The analgesic effect of this treatment (group 8) is not different from that induced by the control sample (group 9), when systemically administered. The simple mixing of MF with pure PS 80 is not sufficient to ensure the transport of drug through the BBB barrier, as shown by the lack of antinociceptive effect (Figure 4).

Empty nanoparticles (groups 1 and 3) were also not able to evoke a prominent analgesic activity. There is also no significant difference in the analgesic effect between the empty nanoparticles (groups 1 and 3) submitted to different purification procedures, this suggesting that monomer traces are not involved in a decisive manner in system's behavior. The amount of nanoparticles injected in the present study is without apparent toxic effect in the intact animal and is below the suggested LD50 of 230 mg/kg.[22] However, MF-PS 80 solutions and empty PBCA-NPs suspensions cause a slight BBB disturbance as compared to control saline solution, which may be considered as a minimum response to a polymeric chemical agent administration/BBB penetration.

Table 2

Antinociceptive effect in mice (mean and standard error of latency) following intraperitoneal injection (*n=8*)

Group	0 min	15 min	30 min	60 min	90 min	120 min
1	9.516±0.649	10.066±0.750	10.75±0.954	10.9±1.021	12.316±1.034*	11.9±0.909*
2	9.25±0.463	10.15±0.887	11.2±1.261	11.316±1.182**	12.866±0.781*	12.333±0.527*
3	9.233± 0.708	11.133±1.040**	12.1±0.456*	11.95±0.361**	12.883±0.923*	12.55±0.671*
4	9.783±0.462	10.85±0.450	11.716±0.796**	11.933±0.539**	11.266±1.102	10.266±0.524
5	10.066±0.546	13.683±0.343*	14.45±0.472*	14.166±0.344*	11.233±0.877	10.15±0.983
6	9.316±0.453	13.633±0.445*	14.433±0.496*	14.183±0.699*	12.15±2.448*	10.816±1.396**
7	9.85±0.450	10.85±1.052	11.133±1.121	11.5±1.031	11±0.712	10.616±0.556
8	9.233± 0.417	8.083±0.685	8.383±0.617	9.016±0.402	8.516±0.567	8.683±0.397
9	8.733±0.697	8.45±0.700	9.166±2.154	8.716±1.108	9.0167±0.793	8.933±0.768

* The mean difference is significant at the 0.01 level when compared to control
** The mean difference is significant at the 0.05 level when compared to control

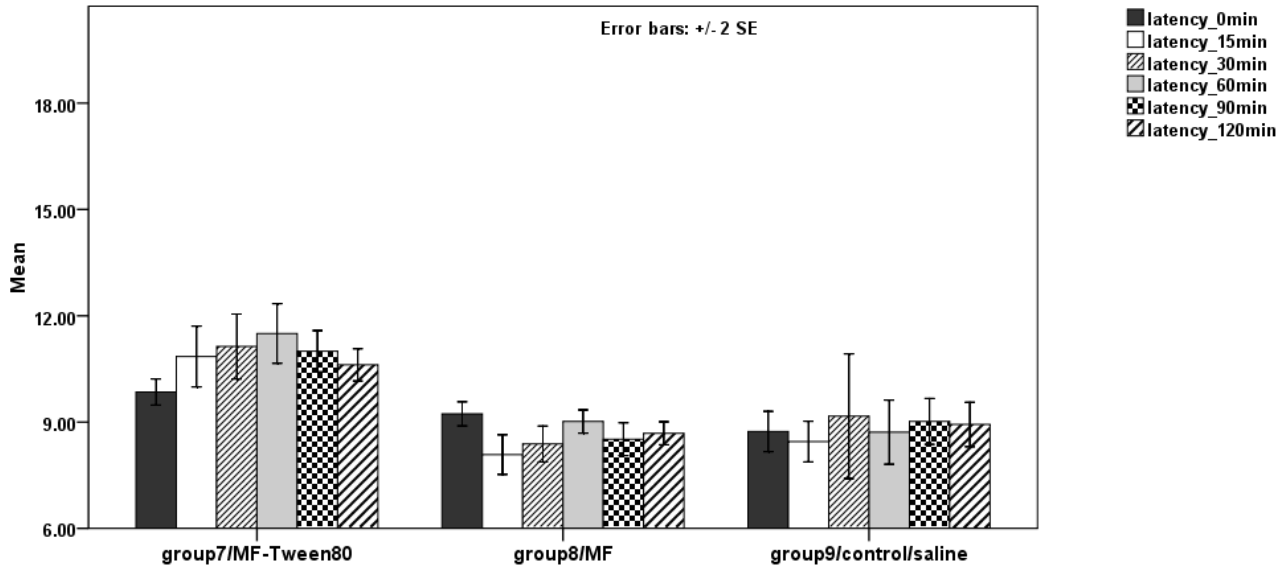

Fig. 4 – Hot plate test. Antinociceptive effect in mice (latency in seconds) after intraperitoneal injection of MF-PS 80 mixture (group 7), MF solution (group 8) and isotonic saline (group 9).
(*p < 0.01 when compared to saline control)

Both formulations with MF adsorbed on the surfactant-coated or uncoated PBCA-NPs were able to induce analgesia after ~30 min from the administration moment (Figure 5 and 6). A further purification of the particles increases the analgesic effect (see the specific behavior of groups 2 and 3 as compared to 5 and 6).

For groups 2 and 3, the latency increased slower as compared to groups 5 and 6, but showed a prolonged duration of the analgesic effect. Considering the high D content of the administered formulations for groups 1-3 (free D included), one possible reason for the specific behavior may be considered the free diffusion of most injected formulations, allowed as effect of the disruption of the BBB by infusion of hyperosmotic solutions, resulting in tight junctions opening. The slow onset of the analgesic effect after MF binding to nanoparticles and the administration of this formulation intravenously is consistent with a direct interaction of nanoparticles with and subsequent uptake by the brain blood capillary endothelial cells.

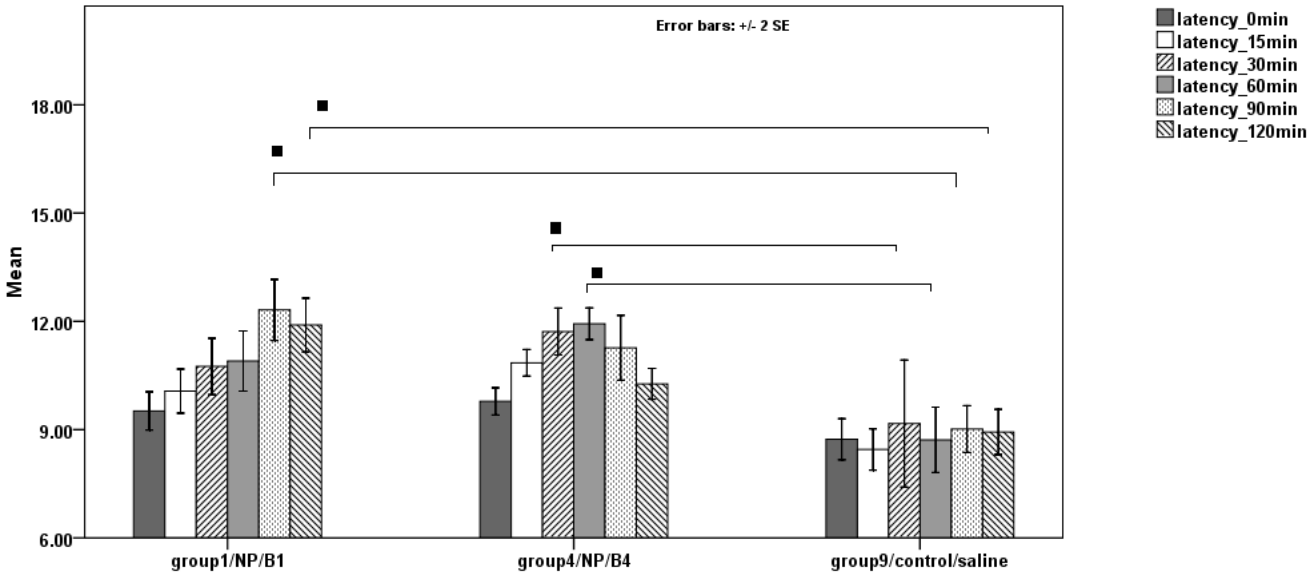

Fig. 5 – Hot plate test. Antinociceptive effect in mice (latency in seconds) after intraperitoneal injection of empty NPs prepared according the two procedures: samples B1 and B4 as compared to isotonic saline as reference (*p < 0.01).

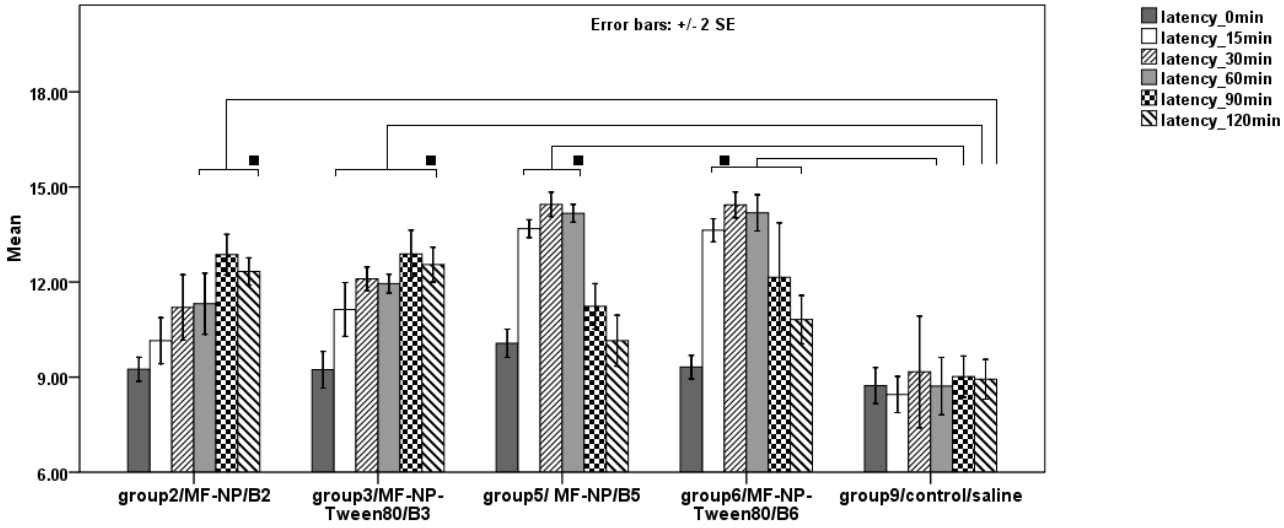

Fig. 6 – Hot plate test. Antinociceptive effect in mice (latency in seconds) after intraperitoneal injection of morphiceptin loaded PBCA nanoparticles uncoated (groups 2 and 4) or coated with PS 80 (groups 3 and 6) as compared to isotonic saline (group 9) (*p < 0.01).

As for the targeting efficiency, surface chemistry is of highest importance.[3] Even if apparently the difference between the PS 80 coated and uncoated particles is a minimum one, it is to note that the amount of the adsorbed μ-peptide opioid is nearly half on the PBCA-NPs carrier in the surfactant-containing system, most probably its micellar trapping reducing the free drug concentration available for transcellular flux. Micellar trapping effects were observed for high surfactant concentrations in formulations based on polymeric micelles as drug delivery systems.[3] Similar drug loading lowering effect by surfactant coating of NPs carriers was reported by J. C. Olivier.[23]

The efficiency was not so remarkable mainly due to the low drug loading capacity of PBCA-NPs (~20 %). Thus, although plenty of nanoparticles might have entered the central nervous system, according to the mentioned effect of empty particles administration, only a limited amount of peptide is transported across the BBB, and the administered drug dose is known to be important.

These results support previous studies that have demonstrated the delivery of a number of drugs to the CNS using Tween 80 coated PBCA nanoparticles as a delivery system. The delivery mechanism remains unclear.

Due to the diameter of the nanoparticles, approximately 20 nm, the movement of the MF/NP/PS 80 complex is expected to be the result of a process different from the simple tight junction disruption or modification followed by diffusion. Earlier studies have shown internalization of PS 80-coated PBCA-NPs by cerebral endothelial cells *in vitro,* which may be a critical step in the delivery of adsorbed drug to the brain.[24-26] Some investigations indicated that the PS 80-coated PBCA-NPs are inducing changes at cell membranes level, not detected to the same degree for uncoated PBCA nanoparticles.[24, 27-30] According to other authors, PBCA-NPs are able to increase brain uptake of drugs simply by nonspecifically opening the tight junctions between the brain endothelial cells through a generalized toxic effect as a result of breakdown products of the polymer nanoparticle.[23] However, most authors consider that PBCA nanoparticles exert no generalized toxic effect on BBB cells.[24, 27-31]

Here, the improved action of MF/NP/PS 80 system as compared to uncoated nanoparticles points on the importance of surface properties modification by surfactant presence and its effect on drug delivery mechanism, and supports the hypothesis that if there are indeed modifications induced at the level of the BBB cells, these changes are mainly due to the used emulsifier (PS 80) and less likely to NPs chemical nature. However, knowing that different delivery pathways may occur depending on drug, carrier and surfactant nature and ratio,[11] further studies are required to elucidate the BBB permeation mechanism for the studied system. Work is under way in order to increase the loading efficiency.

CONCLUSIONS

In vivo experiments with mice have demonstrated that the analgesic effect of morphiceptin delivered *via* systemic administration can be obtained only when the morphiceptin drug

is preadsorbed onto PBCA-NPs, the effect being enhanced by their coating with polysorbate 80. When formulated according to the described procedure, this μ-peptide opioid is delivered to the brain following intravenous administration and exerts a biologic effect which is comparable to direct CNS injection. Low size and high cleanness nanoparticles are strongly recommended to reach a high targeted delivery.

It was also evidenced that the presence of free osmotic agents – like dextran – in the administered formulation may influence the delivery efficiency by affecting, most probably, the BBB crossing mechanism.

The mechanism of this delivery seems to be, accordingly to most authors, an endocytic uptake by the endothelial cells in the brain blood capillaries. Even if the present results partially support the above mechanism, more direct evidences are required to draw conclusions.

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