Effects of Apolipoproteins on Dalargin Transport Across the Blood-Brain Barrier

D. A. Shamenkov, V. E. Petrov, and R. N. Alyautdin

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Antinociceptive activity of dalargin (7.5 mg/kg) adsorbed on poly(butyl)cyanoacrylate nanoparticles with different coating was studied on outbred albino mice by the tail-flick test. Poly(butyl)cyanoacrylate nanoparticles without coating did not increase the antinociceptive activity of dalargin and hence, did not increase its transport across the blood-brain barrier. Poly(butyl)cyanoacrylate nanoparticles coated with apolipoprotein B, apolipoprotein E, and polysorbate 80 increased the transport of dalargin across the blood-brain barrier. Delivery of dalargin to the brain was most effective in case of using poly(butyl)cyanoacrylate nanoparticles with polysorbate 80 coating and subsequent supercoating with apolipoprotein E.

Key Words: *apolipoprotein E; apolipoprotein B; nanoparticles; blood-brain barrier; dalargin*

The blood-brain barrier is low permeable for the majority of drugs [3,4]. This is explained by the presence of tight junctions between endotheliocytes in the cerebral capillaries and their poor fenestration, the factors impeding filtration, and the presence of glycoprotein P extruding the substance from the endotheliocyte cytoplasm [11]. An approach for facilitating drug transport through the blood-brain barrier is adsorption of drugs on poly(butyl)cyanoacrylate nanoparticles (PBCA-NP) coated with polysorbate-80 (PS-80) [1,2,9,10,13-15].

We studied the effects of apolipoproteins on dalargin transport across the blood-brain barrier.

MATERIALS AND METHODS

Experiments were carried out on outbred albino mice (18-22 g). The animals were kept in cages 10 per cage at 12:12 h day:night regimen at 20-22°C with free access to water and food. Considering the specific circadian rhythms of mice and in order to minimize their effects on the results, the analgesimetric studies were carried out at the same hours of the day (at 14.00).

PBCA-NP were prepared and lyophilized as described previously [1].

In order to prepare a suspension of PBCA-NP with adsorbed dalargin, nanoparticles were resuspended in phosphate buffer at constant stirring and 5-min ultrasonic treatment (by an UZDN ultrasonic disperser), so that the concentration of PBCA-NP were 20 mg/ml. Dalargin (0.75-1.00 mg/ml) was then added to the suspension and incubated for 4 h with regular stirring.

In order to prepare suspension of PBCA-NP with adsorbed dalargin coated with PS-80, 1% PS-80 solution was added to the suspension of PBCA-NP with adsorbed dalargin and incubated at constant stirring for 30 min.

In order to prepare suspension of PBCA-NP with adsorbed dalargin coated with apolipoprotein (apo), solutions of apoA-II (12.5 μg/ml), apoB (12.5 μg/ml), apoC-II (12.5 μg/ml), apoE (12.5 μg/ml), and apoJ (12.5 μg/ml) were added to the suspension of

Department of Pharmacology, Pharmaceutical Faculty, I. M. Sechenov Moscow Medical Academy. ***Address for correspondence:*** dshamenkov@yandex.ru. D. A. Shamenkov

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PBCA-NP with adsorbed dalargin and incubated for 60 min at constant stirring.

Suspension of PBCA-NP with adsorbed dalargin coated with PS-80 with apolipoprotein supercoating was prepared by adding solutions of apoA-II, apoB, apoC-II, apoE, or apoJ (in concentrations of 12.5 μg/ml) to the suspension with subsequent 60-min incubation.

Antinociceptive effect of dalargin (in different dosage forms) was studied in the tail-flick test using an Iitic-Inc., mod. 33 Tail flick analgesimeter as described previously [1]. Painful thermal stimulation of the skin with a light beam from an incandescent lamp focused with a lens was inflicted to the distal third of animal's tail; this contact-free method reduced the probability of animal reaction to tactile stimulation. Animal reaction to nociceptive stimulation manifested by sharp flick of the tail from the light beam. In animals with unchanged pain sensitivity the tail flick latency (TFL, the period from the start of stimulation to the tail flick response) was 1.5-3.2 sec. The drug possessing antinociceptive activity increased TFL.

The animals were placed into individual transparent plastic boxes; after 30-min adaptation TFL was measured before drug injection. After 15 min control measurement of TFL, dalargin (0.2 ml/animal) in different dosage forms was injected into the lateral caudal vein. All animals received the same drug in the same dose.

Fifteen minutes after drug injection TFL was measured; the measurements were carried out at 15-30-min intervals (on minutes 15, 30, 45, 60, 90) and the development of the antinociceptive effect was traced over time. Analgesimetry was stopped when the TFL returned to the level before the drug injection.

Antinociceptive effect was evaluated by comparing the mean TFL in the group before the drug injection (TFL 1) with the mean TFL values after injection of the dosage form (TFL 2). The significance of differences was evaluated using Student's *t* test. For each TFL 2 the percent of maximum effect (PME) was calculated by the formula:

$$\text{PME}=\frac{\text{TFL 2-TFL 1}}{\text{TTTPS-TFL 1}}\times 100\%,$$

where TTTPS is the threshold time of thermal painful stimulation.

The animals (n=75) were divided at random into 15 groups, 5 per group. All mice received injections into the lateral caudal vein: group 1) 0.2 ml PBCA-NP suspension (placebo); 2) 7.5 mg/kg dalargin; 3) 7.5 mg/kg dalargin with PS-80; 4) 7.5 mg/kg dalargin adsorbed on PBCA-NP; 5) 7.5 mg/kg dalargin adsorbed on PBCA-NP coated with apoA-II (12.5 μg/kg); 6) 7.5 mg/kg dalargin adsorbed on PBCA-NP coated with apoB (12.5 μg/kg); 7) 7.5 mg/kg dalargin adsorbed on PBCA-NP coated with apoC-II (12.5 μg/kg); 8) 7.5 mg/kg dalargin adsorbed on PBCA-NP coated with apoE (12.5 μg/kg); 9) 7.5 mg/kg dalargin adsorbed on PBCA-NP coated with apoJ (12.5 μg/kg); 10) 7.5 mg/kg dalargin adsorbed on PBCA-NP coated with PS-80; 11) 7.5 mg/kg dalargin adsorbed on PBCA-NP coated with PS-80 and apoA-II (12.5 μg/kg); 12) 7.5 mg/kg dalargin adsorbed on PBCA-NP coated with PS-80 and apoB (12.5 μg/kg); 13) 7.5 mg/kg dalargin adsorbed on PBCA-NP coated with PS-80 and apoC-II (12.5 μg/kg); 14) 7.5 mg/kg dalargin adsorbed on PBCA-NP coated with PS-80 and apoE (12.5 μg/kg); 15) 7.5 mg/kg dalargin adsorbed on PBCA-NP coated with PS-80 and apoJ (12.5 μg/kg). Analgesimetry with calculation of PME was carried out after injection of dosage forms.

RESULTS

A significant increase in PME was observed on minute 30 in groups 6 and 8 (apoB and apoE coating; Table 1) in comparison with control groups (1-4). However, this effect was less pronounced than in group 10 (PS-80 coating). On the other hand, the maximum increase of PME was detected in groups 12, 14, and 15 (PS-80 coating with apoB, apoE, and apoJ supercoating, respectively). In group 14, the analgesic effect was long-lasting (observed during the entire period of the study). In group 11 (PS-80 coating with apoA-II supercoating), analgesia developed by the 90th min of observation (Table 1).

The results indicate that apoA-II, apoC-II, and apoJ had a negligible effect on the transport of dalargin in PBCA-NP through the blood-brain barrier, while apoB and apoE increased it, though their effect was lower than that of PS-80. The most pronounced increase of dalargin transport into the brain was attained in case of PBCA-NP coated with PS-80 with apoB and apoE supercoating. This coating of PBCA-NP resulted in not only increase, but also prolongation of the antinociceptive effect of dalargin.

Uncoated PBCA-NP exhibited poor capacity for dalargin transportation across the blood-brain barrier [8], which was confirmed by zero antinociceptive effect of dalargin in group 4. Low transporting capacity of PBCA-NP without special coating can be explained by the fact that nanoparticles can be captured by liver cells, which results in reduction of their blood concentration and hence,

TABLE 1. Antinociceptive Activity of Dalargin (7.5 mg/kg) Adsorbed on PBCA-NP with Different Types of Coating (*M*±*m*)

Group	PME				
	after 15 min	after 30 min	after 45 min	after 60 min	after 90 min
1	3.8±3.3	1.5±9.0	0.75±3.20	3.9±4.3	-2.0±9.8
2	2.3±4.6	10.0±9.8	9.3±2.8	4.7±5.1	2.0±6.1
3	4.8±1.7	8.3±2.3	7.8±2.3	6.1±4.2	6.6±2.6
4	5.7±5.1	5.0±9.4	4.7±9.1	6.9±11.1	3.8±9.1
5	5.29±2.00	2.97±6.28	5.98±7.64	5.94±7.26	9.44±12.50
6	6.76±5.26	25.17±4.31*	37.74±6.61*	27.10±8.82	17.54±8.17*
7	8.39±2.19	7.19±3.64	3.65±5.67	7.26±5.18	1.14±7.67
8	38.8±13.7*	36.08±11.63*	29.70±5.57*	19.59±7.47	2.03±6.49
9	3.32±2.58	5.84±15.86	10.89±8.64	13.73±13.56	5.0±5.0
10	35.2±5.8	50.4±4.1	49.5±4.5	36.5±13.7	7.1±6.3
11	1.98±9.56	0.50±10.58	12.81±16.80	18.29±21.81	48.80±13.24**
12	30.87±19.43	74.68±15.81**	58.71±8.03**	45.09±18.55	25.51±16.44
13	7.76±2.56	22.24±9.36	49.48±10.88	16.19±16.55	3.72±8.58
14	61.39±8.59**	62.09±6.91**	64.52±13.98	62.33±11.82**	51.73±12.90**
15	18.49±27.2	53.37±28.69	51.51±16.68	36.33±19.73	19.39±19.1

Note. $p<0.05$ compared to *group 1, +group 10.

a lower concentration of the transported substances near the cerebral capillary endotheliocytes [12]. It should be noted that receptor-dependent endocytosis is one of the main mechanisms of PBCA-NP penetration through the blood-brain barrier, and therefore, the particles should carry endocytosis activators (for example, apolipoproteins) on their surface. Nanoparticles with adsorbed substances without activators are not recognized by specific receptors and their endocytosis is minor or absent, which make impossible creation of sufficient concentrations of the transported drug in the brain [7].

Coating of PBCA-NP with PS-80 significantly increases transporting function of nanoparticles, which is seen from the pronounced antinociceptive effect in group 10. This is confirmed by the data indicating that coating of PBCA-NP with PS-80 and supercoating with apoE (group 14) was more effective than PS-80 coating (group 10) or apoE coating (group 8). The most possible explanation is that due to this coating nanoparticles become similar to LDL particles. The nanoparticle via apoE on its surface binds to apoE receptors on endotheliocyte membrane and then, reacting with LDL receptors, undergoes receptor-dependent endocytosis [5]. After this the drug can be released inside the cells and diffuse into the brain or the particles can be translocated by transcytosis [6].

Relatively low capacity of apoA-II, apoC-II, and apoJ to increase the transport of dalargin adsorbed on PBCA-NP into the brain can be explained by partial desorption of these apolipoproteins from the surface of the particles.

Hence, PBCA-NP without special treatment virtually do not improve the transport of dalargin into the brain. The delivery of dalargin adsorbed on PBCA-NP can be improved by coating the particles with apoB, apoE, and PS-80. The maximum increase in the delivery of dalargin adsorbed on PBCA-NP is attained by coating the particles with PS-80 and subsequent supercoating with apoE.

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