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The neuroleptic activity of haloperidol increases after its solubilization in surfactant micelles

Micelles as microcontainers for drug targeting

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It has been suggested to use surfactant micelles as microcontainers for increasing the efficiency of neuroleptic targeting from blood flow into the brain The neuroleptic action of haloperidol, intraperitoneally injected into mice in micellar solution of non-ionic block copolymer surfactant (pluronic P-85) in water, increased several-fold if compared with that observed for haloperidol aqueous solution Incorporation of brain-specific antibodies into haloperidol-containing micelles resulted in additional drastic increase (more than by 2 orders of magnitude) in the drug effect

Neuroleptics, Haloperidol, Hematoencephalic barrier, Directed transport, Micelle, Pluronic P-85

1. INTRODUCTION

Direct transport of neuroleptics from blood flow across hematoencephalic barrier (HEB) into the brain is one of the key problems of experimental and practical psychiatry. It is known that after their administration in the blood flow, neuroleptics may affect biological targets of the brain. However, the efficiency of their penetration through HEB is very low as a rule. Therefore, higher doses of neuroleptics are required to achieve a therapeutic effect, which usually cause undesirable side effects [1,2].

To enhance the efficiency of transport of neuroleptics across HEB, we have suggested to solubilize them in surfactant micelles. Such micelles, spontaneously forming upon solution of surfactants in water, are capable to incorporate large amounts of non-polar and amphiphilic substances [3], including the majority of neuroleptics used in the present day psychiatry. In other words, surfactant micelles can act as universal 'microcontainers' for storage and transport of these physiologically active substances.

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Abbreviations HEB, hematoencephalitic barrier, pluronic P-85, poly(55)(oxypropylene)dipoly(8)(oxyethylene), BPEA, butylpoly(25)(oxypropylene)dipoly(25)(oxyethylene); Ins, insulin, anti-ADG Ab, murine monoclonal antibodies to alcohol dehydrogenase; anti-GFA Ab, murine polyclonal antibodies to glia fibrillar acid antigen

It is well known that the permeability of biological membranes is increased by surfactants. Therefore, it seems reasonable to expect that interaction of the micellar microcontainer, carrying the solubilized neuroleptic molecules, with HEB membranes might be accompanied by effective penetration of this neuroleptic through HEB. For ensuring selective interaction of the micelles with HEB constituents, the micelle-forming surfactant molecules can be covalently bound to target-recognizing molecules (hormones, antibodies, etc.), specific with respect to HEB membranes.

The present work illustrates an applicability of the above-suggested principle in experiments on enhancement of the haloperidol action.

2. MATERIALS AND METHODS

For preparation of micelles and solubilization of haloperidol, we used a non-ionic polymeric surfactant, pluronic P-85 (poly(55)(oxypropylene)dipoly(8)(oxyethylene)) (Serva, FRG) Under experimental conditions (37°C, pH 6 0), solubilization of haloperidol, registered by changes in its absorption and fluorescence spectra, is observed at pluronic concentrations higher than 4 5% This value corresponds to the pluronic critical micellization concentration, determined independently

A pluronic analog, butylpoly(25)(oxypropylene)poly(20)(oxyethylene) ether of 2-hydroxyacetaldehyde (BPEA), kindly prepared by Dr I N Topchieva (Polymer Science Department, Moscow State University), was used as an 'anchor' moiety for incorporation of target-recognizing molecules into the micelles Using the reductive amination reaction [4] carried out in the presence of cyanborhydride (Sigma, USA) we synthesized BPEA conjugates with insulin (Ins), murine monoclonal antibodies to alcohol dehydrogenase (anti-ADG Ab) and murine polyclonal antibodies to glia fibrillar acid antigen of

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Table 1

Biological activity of haloperidol in the physiologic solution and in micellar solutions of various compositions

Tested solutions	LD_{95}[a] (mg/kg body weight)
Physiologic solution	75 ±5
10% pluronic P-85	15 ±2
10% pluronic P-85 + 1 mg/ml BPEA	15 ±2
10% pluronic P-85 + 2 4 mg/ml Ins	15 ±2
10% pluronic P-85 + 2 4 mg/ml Ins-BPEA conjugate	3 0±0 5

[a] Here and below the LD_{95} values were calculated using the probit method on the basis of 9–10 concentration points (7–10 mice for each point)

Table 2

Effect of antibodies and their conjugates with BPEA on the biological activity of haloperidol, solubilized in 10% pluronic P-85

Micellar solution contains	LD_{95} (mg/kg body weight)
0 6 mg/ml anti-ADG Ab	13 ±2
0 6 mg/ml anti-ADG Ab-BPEA conjugate	13 ±2
0 04 mg/ml anti-GFA Ab	5 2 ±1 1
0 4 mg/ml anti-GFA Ab	0 8 ±0 1
0 04 mg/ml anti-GFA Ab-BPEA conjugate	4 0 ±0 9
0 4 mg/ml anti-GFA Ab-BPEA conjugate	0 15±0 03

brain glial cells (anti-GFA Ab) Ins was purchased from Sigma (USA), anti-ADG Ab were a gift from Dr P G Sveshnikov (Research Center of Molecular Diagnostics, USSR Ministry of Health), anti-GFA Ab were separated from monospecific antiserum by immunoaffinity chromatography [5]

The conjugates obtained were purified by gel filtration on Ultragel A-202 (Ins-BPEA) and Ultragel A-44 (anti-ADG Ab-BPEA, anti-GFA Ab-BPEA) By the data of indirect enzyme immunoassay using peroxidase-labeled antispecies antibodies, the titres of BPEA-modified anti-ADG Ab and anti-GFA Ab after their modification with BPEA were 40–50% of the initial values

Haloperidol was dissolved in the micellar solutions (10% pluronic) of various compositions or in the physiologic solution, pH 6 0 (tables 1, 2) Concentration of haloperidol in all preparations obtained was 6–7 mg/ml To study the neuroleptic action in vivo, the preparations were incubated at 37°C for 1 h, and then introduced intraperitoneally into wild grey mice (18–19 g body weight) The control group of mice were treated with the physiologic solution or with corresponding micellar systems without haloperidol

For quantitative evaluation of the biological action of haloperidol, its lethal dose (LD_{95}) was determined using the common technique [6]

3. RESULTS AND DISCUSSION

Administration of heloperidol-containing preparations was accompanied by development of a specific neurolepsy of various degrees described in detail in [6]. In control mice no deviations from the norm were observed.

As is seen in table 1, solubilization of haloperidol in the pluronic micelles results in a 5-fold increase in its toxicity (a decrease in LD_{95}). Incorporation of the Ins-BPEA conjugate into the micelles is followed by a 25-fold increase in the toxicity. We suppose that the Ins moiety exposed on the surface of the micelles can be bound with insulin receptors on the surface of the cells, thus enhancing interaction of the drug-containing micelles with corresponding membranes, in particular with those of HEB. Non-modified Ins, that in contrast to the Ins-BPEA conjugate is not incorporated into the micelles, does not affect the toxicity of haloperidol.

It is known that insulin receptors are presented on the surface of practically all types of cells [7]. Therefore, the insulin vectors of the haloperidol-containing micelles cannot selectively address them to the brain. We assumed that brain-specific antibodies might serve for selective targeting of micellar neuroleptic carriers.

Table 2 illustrates the effect of antibodies on the biological activity of haloperidol solubilized in the micelles. It can be seen that addition of anti-ADG Ab and their conjugate with BPEA, incapable of specific interaction with brain antigens, to the system does not increase haloperidol toxicity. Its LD_{95} is equal to that observed for haloperidol solubilized in pluronic solution in the absence of the antibodies. In contrast, addition of the anti-GFA Ab-BPEA conjugate drastically enhances the neuroleptic action of haloperidol and increases its toxicity. In this case, specific neurolepsy syndromes are observed at haloperidol concentrations which are dozens of times lower than upon injection of its aqueous solutions. The toxicity of the neuroleptic becomes nearly 500-fold higher than that determined for aqueous solution of haloperidol.

It should be mentioned that considerable elevation of the biological activity of solubilized haloperidol was also observed when non-BPEA-modified anti-GFA Ab were added to the micellar solution. This phenomenon apparently correlates with the discovered ability of non-modified antibodies to bind with the pluronic micelles (data not presented here). However, this ability to bind with the micelles might vary for the antibodies from different sources. In this respect we presume that the proposed method of antibodies coupling with micelles through BPEA anchor could become universal for targeting of micellar microcontainers.

The results obtained suggest that the above-described approach can be used for the directed transport of various physiologically active substances.

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