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Note

Haloperidol-loaded polysorbate-coated polymeric nanocapsules increase its efficacy in the antipsychotic treatment in rats

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ABSTRACT

Haloperidol is an antipsychotic drug associated with the development of movement disorders. We evaluated the effect of its nanoencapsulation on its pharmacological activity and motor side effects. Haloperidol-loaded polysorbate-coated nanocapsules (H-NC) showed nanometric size, negative zeta potential and low polydispersity indices and high encapsulation efficiency (>95%). Rats received a single dose of H-NC (0.2 mg/kg ip) and four doses of D,L-amphetamine, AMPH (8.0 mg/kg ip), injected every 3 h (0, 3, 6 and 9 h). The AMPH-induced stereotyped movements were quantified in the intervals of 15 min after each of four doses of AMPH, demonstrating greater pharmacological efficacy of the H-NC over free haloperidol (FH). The acute motor side effects were evaluated 1 h after a single dose of H-NC or its free solution (0.2 mg/kg ip). The group treated with H-NC presented lower extrapyramidal effects (catalepsy and oral dyskinesia) than those treated with FH. In the last experimental set, rats sub-chronically treated with a daily dose of H-NC (0.2 mg/kg ip) for 28 days showed a lower incidence of extrapyramidal effects than those treated with the free drug (0.2 mg/kg ip). Our findings showed the potential of using H-NC in the development of a nanomedicine aimed at increasing the efficacy of this antipsychotic drug and reducing its side effects.

1. Introduction

Haloperidol is an antipsychotic drug that causes movement disorders such as parkinsonism and tardive dyskinesia, which is characterized by involuntary movements frequently irreversible and disabling.

Body weight gain and diabetes development are side effects of the more recent atypical neuroleptics, so that typical neuroleptics such as haloperidol are still the most widely used drugs to treat psychiatric disorders.

Polymeric nanoparticles have attracted attention as drug delivery systems and can be employed to carry and release drugs at controlled rate in specific body sites [1], especially in the central nervous system (CNS). We hypothesized that by loading haloperidol in polysorbate-coated nanocapsules, we could improve its pharmacological efficacy and/or reduce its side effects, proposing its use as a nanomedicine. To the best of our findings, no study has been previously designed to evaluate the improved efficacy of haloperidol by its nanoencapsulation in polymeric nanoparticles.

2. Materials and methods

2.1. Preparation and characterization of nanocapsules suspension

2.1.1. Preparation of nanocapsules suspension

Haloperidol-loaded nanocapsules (H-NC) were prepared by interfacial deposition of preformed polymer [2]. Blank nanocapsules (B-NC) were prepared as controls using the same protocol of H-NC, but omitting the presence of the drug. A free suspension of haloperidol (0.25 mg/mL) was prepared in water using 5% (w/v) of polysorbate 80.

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2.1.2. Physicochemical characterization

Particle sizes, polydispersity indices and zeta potential were measured by photon correlation spectroscopy using Zetasizer® Nano Series equipment (Zetasizer Nanoseries ZEN 3600, Malvern Instruments, UK).

2.1.3. Determination of drug content and encapsulation efficiency

Drug content (mg/mL) was assayed by HPLC (Gemini RP-18 column (150 × 4.60 mm, 5 μm) and a Shimadzu instrument (UV–VIS SPD-10AVP Module). The mobile phase consisted of methanol/potassium phosphate monobasic pH 4.0 (60:40% v/v) and was pumped through the system at a flow rate of 1.0 mL/min. The volume injected was 20 μL, and haloperidol was detected at 254 nm. HPLC assay demonstrated that this method was linear ($r = 0.9979$) in the range of 5–40 μg/mL and precise (RSD: 2.58% for repeatability and 0.63% for intermediate precision). Free drug was determined in the clear supernatant following separation of nanocapsules by a combined ultrafiltration–centrifugation technique. Encapsulation efficiency (%) was calculated by the difference between the total and free drug concentrations determined in the nanocapsule suspension and in the ultrafiltrate, respectively, using the HPLC method described above.

2.2. In vivo experiments

This study was approved by the Animal Ethical Committee (Universidade Federal de Santa Maria-22/2010), which is affiliated to the Brazilian college of animal experimentation (COBEA) in accordance with international rules of ethics in research.

2.2.1. Experiment 1: pseudo-psychosis induced by D,L-amphetamine (AMPH)

AMPH treatment promotes a set of positive symptoms similar to schizophrenia. Five groups of male Wistar rats ($n = 7$) were allocated in mirrored individual cages, allowing the assessment when the animal was facing away from the observer. Considering the abbreviations: AMPH (amphetamine), FH (free haloperidol), H-NC (haloperidol-loaded nanocapsules), B-NC (blank nanocapsules) and C (control), the following groups were designated and treated with the suspensions: AMPH + FH; AMPH + H-NC; AMPH + B-NC; AMPH; and C. All groups received their first D,L-amphetamine administration (8 mg/kg ip) at time 0 (hour 0), except the control group (C), which received saline. After 30 min of AMPH or saline administration, the FH, H-NC and B-NC received a single injection of each nanocapsule suspension (0.2 mg/Kg body weight ip). The AMPH and C groups received a single injection of Tween 80® suspension (5% ip). Three, 6 and 9 h after the first AMPH administration, the rats received the second, third and fourth AMPH or saline administration. The duration of the experiment was 12 h, and every 3 h, a new dose of AMPH was administered. Two observers quantified the stereotyped head behavior every 15 min according to Ujike et al. [3] scale scores: 0, no head movement; 1, normal head movement and normal exploration; 2, increased rate of head movement with hyperactivity; 3, discontinuous repetitive and stereotyped up-down head movement; 4, continuous stereotyped head movement with occasional break; 5, continuous and intense stereotyped head movement at one location. Results are expressed as stereotyped behavior scores during the 3 h following each AMPH administration.

2.2.2. Experiment 2: motor side effects induced by acute haloperidol administration

Twenty-eight rats were divided in four groups ($n = 7$), as described below: (C group): received an ip injection of polysorbate 80 suspension 5% (v/v); (B-NC group): received an injection of blank nanocapsule suspension; (FH group): received an injection of free haloperidol suspension; (H-NC group): received an injection of haloperidol-loaded nanocapsules suspension. All drugs were administered at the dose of 0.2 mg/kg ip, and the behavioral tests were performed 1 h after the administration as follows:

(A) *Catalepsy*: Only the haloperidol-treated rats (FH and H-NC groups) were individually placed on a wire inclined grid (45° relative to the bench top) and observed for 60 s. The amount of time spent in this atypical position was recorded for three times, with an interval of 5 min between them. If the animal did not move, it was removed from the grid and returned to it. If it did not move within 60 s, it was removed again and returned to the grid. At the end, the mean time spent by the rat without moving was calculated for each test.

(B) *Oral dyskinesia (OD)*: Immediately after the catalepsy test, the rats were allocated in mirrored individual cages, allowing the assessment when the animal was facing away from the observer. OD was quantified by the frequency of vacuous chewing movement (VCM), which was recorded for three sets of 5 min with 5-min intervals. Observers were blind to the treatment.

2.2.3. Experiment 3: motor side effects induced by sub-chronic haloperidol administration

Twenty-eight rats were divided in the same four groups ($n = 7$) described above (C, B-NC, FH and H-NC). All drugs were administered in the dose of 0.2 mg/kg ip once a day for 28 days. The same behavioral tests (measurement of catalepsy and OD) were performed on the 7th, 14th, 21st and 28th days of treatment. On these days, the drugs were administrated after the behavioral tests, differently from Experiment 2.

2.3. Statistical analysis

AMPH-induced stereotyped behavior data (experiment 1) were analyzed by Kruskal–Wallis followed by Mann–Whitney *U*-test. Differences among the three observed times after each AMPH administration were analyzed by Wilcoxon matched pairs test. Acute and sub-chronic haloperidol-induced OD and catalepsy measurement data (experiments 2 and 3, respectively) were analyzed by one-way ANOVA followed by Duncan's multiple range test, if necessary. Differences among the groups at the same time were analyzed by paired samples *t*-test. A value of $p < 0.05$ was considered as statistically significant.

3. Results and discussion

The physicochemical characteristics of polymeric nanocapsules are shown in Table 1. All formulations appeared macroscopically homogeneous similar to a milky bluish opalescent fluid (Tyndall effect). Haloperidol was efficiently encapsulated (95 ± 1%) using the method of interfacial deposition of a preformed polymer (poly-ε-caprolactone), which was chosen due to its biodegradability and biocompatible properties. Both nanocapsule suspensions (B-NC and H-NC) presented particles in the sub-micrometric range (between 200 and 300 nm), low polydispersity (⩽0.25), negative zeta potentials, acid pH values and drug content near 100% of the theoretical (0.25 mg/mL). These values are in agreement with the diameters observed for nanocapsules prepared using the preformed polymers by the interfacial deposition method [4]. The negative zeta potential values (∼−8 mV) are a consequence of the particle coating with polysorbate 80, presenting a negative surface density of charge due to the presence of oxygen atoms in their molecules.

Efforts have been made by our group to reduce the motor disorders induced by haloperidol [5]. Studies have demonstrated that

Table 1
Physicochemical characteristics of blank and haloperidol-loaded nanocapsules (B-NC and H-NC).

Formulation	Particle size (nm)	PDI[a]	Zeta potential (mV)	pH	Drug content (mg/mL)
B-NC	280 ± 15	0.25 ± 0.1	−8.3 ± 0.5	6.3 ± 0.2	–
H-NC	230 ± 22	0.14 ± 0.1	−8.1 ± 0.2	6.5 ± 0.2	0.25 ± 0.2

Mean ± SD: represents the variation between the different batches ($n = 3$).
[a] PDI: polydispersity index.

drug-loaded nanocarriers are an efficient tool in drug delivery [6], promoting its permeation across the blood–brain barrier [7] and suggesting their use to deliver haloperidol to the brain. The results of experiment 1 show the head movement scores of AMPH-treated rats (Table 2). AMPH treatment increased the head movements at the three observed times after each AMPH administration, when compared to the control. Wilcoxon pairs test showed that the stereotyped behavior was modified by time after each AMPH administration. In addition, AMPH reached its maximum effect 1 h after each administration and this effect began to decrease 3 h later, indicating a new administration for psychosis maintenance. This procedure was thus performed every 3 h for 12 h for a full view of the responses to the haloperidol treatments (H-NC and FH) and proved to be adequate to evaluate the antipsychotic efficacy of haloperidol.

The effects of H-NC versus FH solution on the percentage of AMPH-induced stereotyped behavior are shown in Fig. 1. Kruskal–Wallis analysis revealed significant differences at the three observed times after the 1st ($p < 0.001$), 2nd ($p < 0.001$), 3rd ($p < 0.001$) and 4th ($p < 0.001$) AMPH administration, respectively. Rats treated with B-NC showed no reduction in head movements at any observation following each AMPH administration. On the other hand, both H-NC and FH showed reduced behavioral scores at all observed times after the 1st and 2nd AMPH administration. Following the 3rd dose, only the H-NC group showed decreased movement scores. From 2 h after this AMPH administration, only H-NC reduced the movements in relation to the B-NC group. Regarding the differences between the two haloperidol-treated groups, the H-NC group showed a greater decrease in stereotyped movements than the FH group in almost all observations, except 3 h after the 1st and 1 h after the 4th AMPH administration. The Wilcoxon test indicated that the effects of B-NC, FH and H-NC on the AMPH-induced stereotyped behavior were modified by time after each AMPH administration (Fig. 1A–C). After the last dose of AMPH, the stereotyped behavior was reduced at 3 h only, for all groups (B-NC, FH and H-NC) (Fig. 1D).

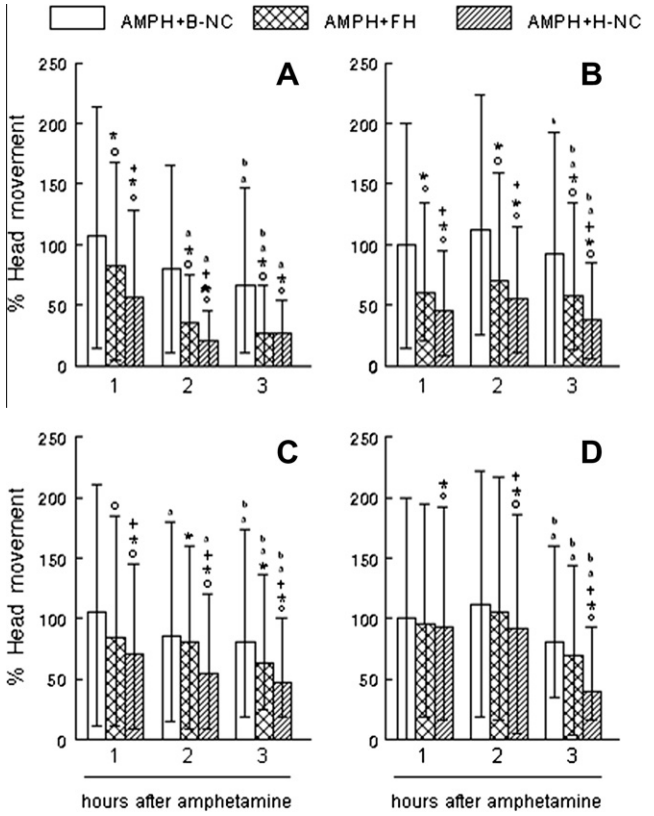

Fig. 1. Effect of blank nanocapsules (B-NC), free haloperidol (FH) and haloperidol-loaded nanocapsules (H-NC) on amphetamine (AMPH)-induced stereotyped behavior in rats ($n = 7$). The behavioral evaluation was expressed as stereotyped behavior scores for 3 h following the first (A), second (B), third (C) and fourth (D) AMPH administration. Data are expressed as median (lower/upper quartile) of % amphetamine-induced stereotyped behavior. The lowercase letters show significant differences among the times within the same AMPH administration; symbols show significant difference among treatments within the same AMPH administration. *Different from AMPH; different from AMPH+B-NC group; +different from AMPH+FH group; [a]different from 1 h; [b]different from 2 h.

Table 2
Stereotyped behavior amphetamine (AMPH) induced in rats ($n = 7$). Data are represented in scores scale.

AMPH administrations	Hours	Head movement	
		C group	A group
	1	0.2 (0/0.5)	3.5 (3/3.5)
1	2	0.2 (0/0.2)	5 (4/5)[a]
	3	NS	3.7 (3.7/4)
	1	0 (0/0.2)	5 (5/5)
2	2	NS	4.2 (4/5)
	3	NS	3.2 (3/3.5)[a,b]
	1	NS	4.7 (4.5/4.7)
3	2	NS	5 (4.7/5)
	3	NS	3.7 (3.5/4)[a,b]
	1	NS	5 (5/5)
4	2	NS	4.5 (4.5/4.5)
	3	NS	3.7 (3.5/4)

NS: No stereotypy. Data are expressed as median (lower/upper quartile). The lowercase letters show significant differences among the times within the same treatment.
[a] Different from 1 h.
[b] Different from 2 h.

With a single sub-therapeutic dose (0.2 mg/kg), haloperidol-loaded nanocapsules showed higher antipsychotic effects evidenced by the decrease in AMPH-induced stereotyped movements when compared to the FH group. As haloperidol is a potent antipsychotic, a low dose was chosen to allow the observation of the efficacy of H-NC compared to FH at the same dose. In our study, both groups treated with haloperidol (H-NC and FH) decreased the AMPH-induced stereotyped behavior, but the H-NC group showed a more prolonged antipsychotic action at the equivalent dose. These findings are in agreement with a recent study [8], which also showed stronger biological effects in brain using drug delivery systems composed of polymeric nanocapsules. In order to evaluate if this prolonged antipsychotic effect was accompanied

by adverse effects, motor effects were monitored after acute and sub-chronic haloperidol courses (experiments 2 and 3, respectively). The motor side effects induced by acute haloperidol treatment (H-NC or FH) are shown in Fig. 2. Duncan's test of the OD data showed an increase of 95% in the VCM frequency ($p < 0.05$) after the administration of FH when compared to the C group (C). H-NC or B-NC showed unchanged behavior in relation to the C group regarding this orofacial parameter (Fig. 2A). Duncan's test showed that the H-NC treatment led to a decrease in the immobility time (31%) in relation to the FH group ($p < 0.05$) (Fig. 2B). Taken together, these results showed that FH treatment caused extrapyramidal effects, such as OD and catalepsy. On the other hand, the H-NC group showed less motor side effects than the group treated with the free drug. Subsequently, we evaluated the motor side effects induced by sub-chronic haloperidol treatment (H-NC and FH) (Fig. 3). Post hoc tests of OD showed a significant increase in the VCM frequency after FH administration when compared to the C group ($p < 0.05$). The groups treated with H-NC and B-NC showed unchanged behavior in relation to the C group regarding this orofacial parameter at all analyzed times (Fig. 3A). Paired *t*-test indicated that there were no significant modifications in the C, B-NC and H-NC groups along the time. However, there was a significant increase in the orofacial parameter in the FH group at day 21 when compared to days 7 and 28.

Duncan's test of catalepsy showed a lower immobility time (23%) in the H-NC-treated group than in the FH group at day 28 ($p < 0.05$) (Fig. 3B). The paired test indicated that both FH and H-NC groups showed an increase in catalepsy time at days 21 and 28 when compared to day 7 ($p < 0.05$) (Fig. 3B). These results were similar to those observed after H-NC acute administration and confirm that H-NC causes less extrapyramidal effects than FH does. The mechanism behind drug delivery into the brain by colloidal carriers remains uncertain. Studies have given support to the role of polysorbates on the endocytosis processes. Polysorbate acts mainly as an anchor for the apolipoprotein-overcoated nanoparticles and thus would mimic lipoprotein particles and could interact with and then be taken up by the brain capillary endothelial cells via receptor-mediated endocytosis and/or transcytosis [9]. In fact, nanoparticles coated with polysorbate adsorb apoliprotein E. Apart from this, the surfactant polysorbate 80 is an inhibitor of P-glycoprotein, representing an important constituent of the BBB. The molecular basis for the barrier function of the BBB is a group of drug efflux transporters such as P-glycoprotein, which hinder the access of some drugs into the CNS. The mechanism involved in this barrier function is by extruding drugs from the brain and is a major obstacle for many pharmacological agents. Furthermore, the coating of polymeric nanoparticles by polysorbate 80 can change their particle surface (hydrophobic to hydrophilic), avoiding their opsonization by plasma proteins. Thus, H-NC could maintain haloperidol blood levels for a longer time, explaining its effect for at least 12 h, i.e., about three additional hours when compared to the free suspension.

So, the most important result was the significant decrease in the motor side effects after H-NC administrations, which are commonly related to dopamine receptors blockade at the nigro-striatal system. Thereby, our results suggest that H-NC targeted the drug to the mesocorticolimbic region (related to psychotic symptoms), reaching the nigro-striatal system in lower concentration and minimizing the extrapyramidal disorders. Haloperidol levels in brain

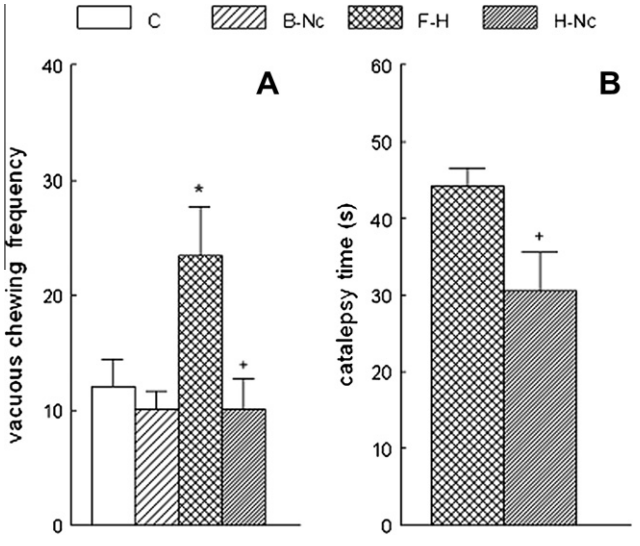

Fig. 2. Acute effect of free haloperidol or haloperidol-loaded nanocapsules on the development of vacuous chewing movements (A) and catalepsy (B) in rats. Both behavioral parameters were evaluated 1 h after haloperidol administration. Data are expressed as mean + SEM ($n = 7$). C – control group; B-NC – blank nanocapsules group; FH – free haloperidol; H-NC – haloperidol-loaded nanocapsules. *Different from C group; +different from FH group.

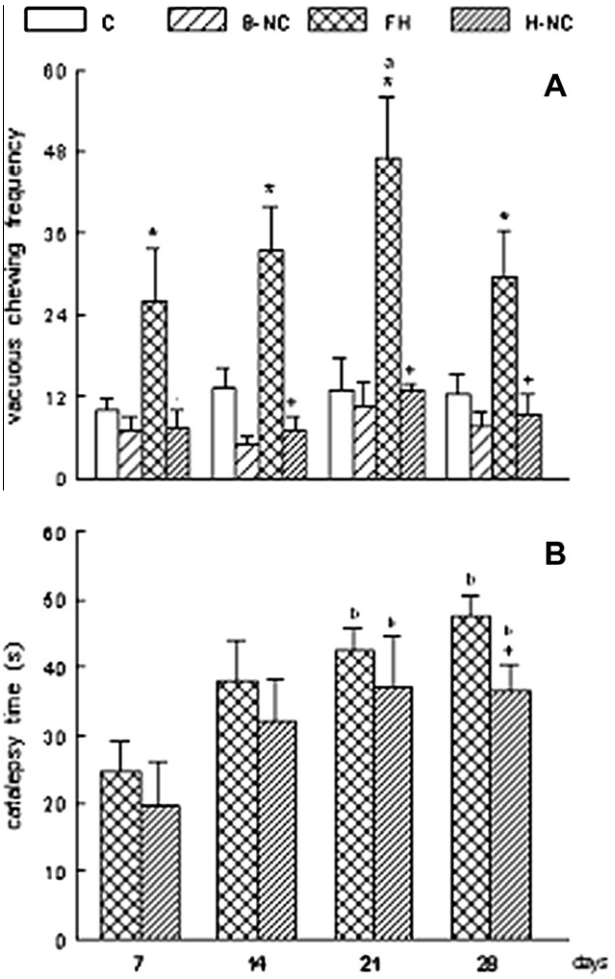

Fig. 3. Sub-chronic effect of free haloperidol or haloperidol-loaded nanocapsules on the development of vacuous chewing movements (A) and catalepsy (B) in rats. Both behavioral parameters were evaluated on days 7, 14, 21 and 28 after daily haloperidol administration. Data are expressed as mean + SEM ($n = 7$). C – control group; B-NC – blank nanocapsules group; FH – free haloperidol; H-NC – haloperidol-loaded nanocapsules. The lowercase letters show significant differences among the times within the same treatment; symbols show significant difference among treatments within the same time. *Different from C group; +different from FH group; [a]different from days 7 and 28; [b]different from day 7.

dopaminergic regions need to be studied in order to confirm this hypothesis.

The feasibility of delivering drugs into the brain using polymeric nanoparticles may open new perspectives for the treatment of diseases such as schizophrenia, mainly by the possibility of achieving their biological activity at low doses. Recently, the prolonged antipsychotic effects and reduced extrapyramidal effects of risperidone-loaded nanoparticles, an atypical antipsychotic, were demonstrated [10]. Here, we are demonstrating for the first time the design of a nanomedicine as an alternative to the administration of haloperidol, which is the most widely used drug to treat mental disorders, and its nanoencapsulation in polymeric systems is a promising therapeutic tool.

In summary, our data clearly demonstrated the feasibility of preparing H-NC to improve its therapeutic efficacy. The antipsychotic effect of H-NC was maintained for a longer time, and the acute and sub-chronic extrapyramidal motor disorders were reduced when compared to the administration of FH suspension. The exact mechanisms related to these findings should be investigated in further studies.

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