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ACUTE TOXICITY INVESTIGATION AND THE EFFECTS OF ORIGINAL MAGNESIUM NANOVESICLES ON THE MEMORY PROCESSES PERFORMANCE IN RATS

Tarțău Liliana[1], Lupușoru Cătălina Elena[1], Ciubotariu Diana[1], Melnig V.[2]

[1.] Department of Pharmacology, "Gr. T. Popa" University of Medicine and Pharmacy, Iasi, Romania
[2.] Department of Physics – COMB Laboratory, Faculty of Physics, "Al. I. Cuza" University, Iasi, Romania

Abstract. The purposes of this study were to evaluate in vivo acute toxicity of original magnesium chloride nanoparticulate formulations and to investigate their effects in cognitive processes in rats. The experiments were carried out on white Wistar rats (200-250g), divided into 3 groups of 7 animals each, treated orally (using an eso-gastric device), 7 consecutive daily administration as follows: Group I (Control): distilled water 0,1ml/10g weight; Group II (Mg): magnesium chloride 200mg/kbw; Group III (Mg ves): magnesium chloride 200mg/kbw entrapped in soft vesicles. At the end of the experiment, blood samples were taken from retro-orbital plexus to assess blood count, phagocytic capacity of peripheral neutrophils and serum complement activity, to investigate acute toxicity of the new nanoparticulate formulations. Spatial memory performance was assessed by recording spontaneous alternation behaviour in a single session in Y-maze. Experimental protocols were implemented according to recommendations of the University Committee for Research and Ethical Issues. Data were statistically analyzed with SPSS software for Windows version 17.0 and ANOVA one-way method. Results: Laboratory analysis showed no significant differences of blood count, phagocytic capacity of peripheral neutrophils and serum complement activity values, between new carrier formulations that entrapped magnesium chloride in lipid vesicles and non entrapped substance. Research results provided evidence that magnesium chloride entrapped vesicles, significantly increased spontaneous alternation percent in Y-maze test, which suggest the improvement of short-term memory. Conclusions: Biocompatibility evaluation proved that magnesium chloride lipid vesicles may be very suitable for in vivo use in the contexts of drug delivery systems. Oral administration during 7 days of soft vesicles entrapping magnesium chloride, 7 consecutive days administration of magnesium entrapped in lipid vesicles resulted in an enhancement of cognitive functions in rats.
Keywords: magnesium chloride nanovesicles, acute toxicity, Y-maze test

Introduction

Progresses in nanotechnology have opened up multiple possibilities for the creation of new or improved drug systems. Vesicles are versatile carriers formed by molecules that self-assemble capable of drug effect prolongation. Vesicles are hollow spheres, commonly used to encapsulate labile hydrophilic molecules within their interior, and are important to be studied for developing new drug delivery vehicles to facilitate the absorption through the intestinal wall and transport substances into the blood.[1,2] They are of great interest for applications ranging from drug delivery and controlled release to separations and sensing.

Magnesium is one of the most wide-spread elements in nature. It is the fourth major cation in the human body (after Ca^{2+}, Na^{+} and K^{+}), and the second most abundant within the cells (after K^{+}). Magnesium ion is equally important to the central nervous system (spinal cord) and to the brain itself. It is well known that magnesium ions (Mg^{2+}) have a well-established depressant effect on the central nervous system and on neuromuscular transmission. [3,4]. Rodent studies suggest that magnesium has a complex relationship with aggressive behaviours[5].

Given the close conceptual associations between stress, anxiety and depression which are intrinsic to most animal models and testing paradigms, a

Liliana Tarțău
16 Universitatii Str., Iasi, Romania
e-mail: lylytartau@yahoo.com

reasonable hypothesis emerges whereby magnesium may be exerting its antidepressant effect through anxiolytic mechanisms[6].

Progresses in nanotechnology have opened up innumerable possibilities for the creation of new or improved drug systems. Determining acute systemic toxicity is usually an initial step in the assessment and evaluation of the new formulation biocompatibility. Undesirable possible effects of new nanoparticulate systems on the blood may include hemolysis, thrombus formation, alterations in coagulation parameters, biochemical disturbances and immunological changes. Acute systemic toxicity investigation is similar to the nonspecific toxicity test that aims to look for possible undesirable changes in the blood caused directly by a new nanoparticulate formulation administration.

The **goals** of our study are the acute toxicity evaluation of lipid vesicles coated in chitosan which entrap magnesium chloride inside and the investigation of their effects on cognitive processes in rats.

The influence of liposome structure on blood count, phagocytic capacity of peripheral neutrophils, glutamic oxaloacetic transaminase, glutamic pyruvic transaminase and lactic dehydrogenase enzyme activities in vivo was assessed. Changes within different blood cells and the biochemical parameter levels were estimated after 7 consecutive days of magnesium chloride vesicles oral administration. In addition to this, the effects of magnesium chloride loaded vesicles on rats cognitive processes were investigated.

Material and method

Substances: The lipid used, Egg Yolk L-α-phosphatidylcholine (L-α-lecithin), approximately 99% (TLC) pure, chitosan (biocompatible and biodegradable polymer) and magnesium chloride, were obtained from Sigma-Aldrich Company.

Using an original methodology we achieved the entrapment with a large efficiency, of magnesium chloride within the lipid vesicles stabilized with polysaccharide chitosan.[7] The polymer binds to vesicles, causing the bilayer to rigidify and in turn driving a decrease in the size of unilamellar vesicles.[8] The soft matter vesicles made by lipid-tramadol-chitosan were prepared and thereafter, were dialyzed for 10 hours, to remove its acidity and gain a neutral pH.[9] The magnesium vesicles were physicochemical and structurally analyzed using Malvern Zetasizer Nano ZS ZEN-3500 model and visualized with a Nikon Ti Eclipse optical microscope.

Animals: Male white Wistar rats (200-250g) were used. Standard laboratory food and tap water were freely available, except during the time of the experiments.

Procedure: The experiments were carried out on white Wistar rats (200-250g), distributed into 3 groups of 7 animals each, treated orally (using an eso-gastric device), 7 consecutive daily administration as follows:

Group I (Control): distilled water 0,1ml/10g weight;

Group II (Mg): magnesium chloride 200mg/kbw;

Group III (Mg ves): magnesium chloride 200mg/kbw entrapped in soft vesicles.

At the end of the experiment, blood samples were taken from retro-orbitary plexus to assess blood count, phagocytic capacity of peripheral neutrophils and serum complement activity, to investigate acute toxicity of the new magnesium chloride nanoparticulate formulations.

Spatial memory performance was assessed by recording spontaneous alternation behaviour in a single session in Y-maze. Each rat was placed at the end of one arm and allowed to move freely through the maze during an 8 min session. Alternation was defined as "a consecutive entry in three different arms". The alternation percentage was computed with the following formula: "number of alternations" divided by "total number of arm visits" minus 2.[10]

Data were statistically analyzed with SPSS software for Windows version 17.0 and ANOVA one-way method. Experimental protocols were implemented according to recommendations of the "GR.T. Popa" University Committee for Research and Ethical Issues. Each animal was used once only and the duration of the experiments was kept as short as possible. For ethical reasons, all the animals were sacrificed at the end of the experiment. [11]

Results and discussions

Magnesium entrapped vesicles were found to have a mean Zeta potential of +36.1mV (Figure 1) and a mean size of 129.56nm (Table I). We can conclude that the systems of magnesium lipid vesicles correspond to the criteria of suspension solutions.

Laboratory analysis did not show significant differences of blood parameters (Table II), serum complement values nor phagocytic capacity of peripheral neutrophils (Table III), between magnesium chloride non-entrapped and entrapped in vesicles and control group, elements relevant for a good biocompatibility.

Statistical analysis of the results obtained in Y-maze test shows that: oral administration of magnesium chloride entrapped vesicles, during 7 days, significantly increased spontaneous alternation percent in Y-maze test, which suggest the improve-

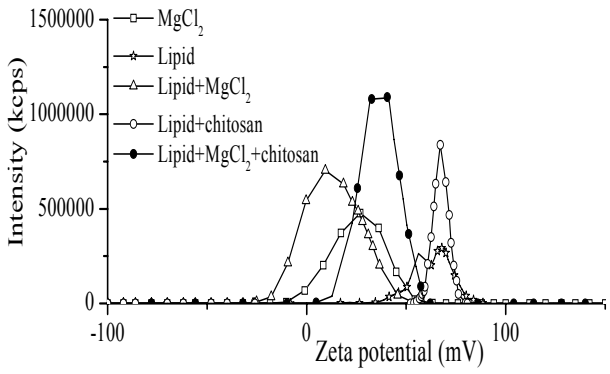

Figure 1. Zeta potential distribution of the magnesium chloride vesicles

System	Average dimension (nm)
Magnesium chloride	55.93
Lipid vesicles	81.16
Lipid + magnesium chloride vesicles	36.89
Lipid + magnesium chloride + chitosan vesicles	129.56

Table I. The average values of the magnesium chloride vesicles dimension

	Leucocyte formula (% values)				
	PMN	Ly	E	M	B
Control	29.4±0.2	58.7±0.3	0.4±0.05	3.7±0.1	0.2±0.1
Mg	29.1±0.3	58.6±0.4	0.5±0.05	3.8±0.1	0.2±0.05
Mg ves	29.3±0.4	58.5±0.2	0.4±0.1	3.7±0.1	0.2±0.05

Table II. Differential white cell count (leukocyte formula) determination in control rats group and in groups treated with magnesium chloride and magnesium chloride soft mater vesicles.
Values were expressed as mean ± S.E.M

	NBT (%)	Serum complement
Control	16.33±0.84	39±1.1
Mg	16.17±0.52	43±1.4
Mg ves	17.49±0.72	41±3.7

Table III. Phagocytic capacity of peripheral neutrophils (NBT%) and serum complement values in control rats group and in groups treated with magnesium chloride and magnesium chloride soft mater vesicles.
Values were expressed as mean ± S.E.M

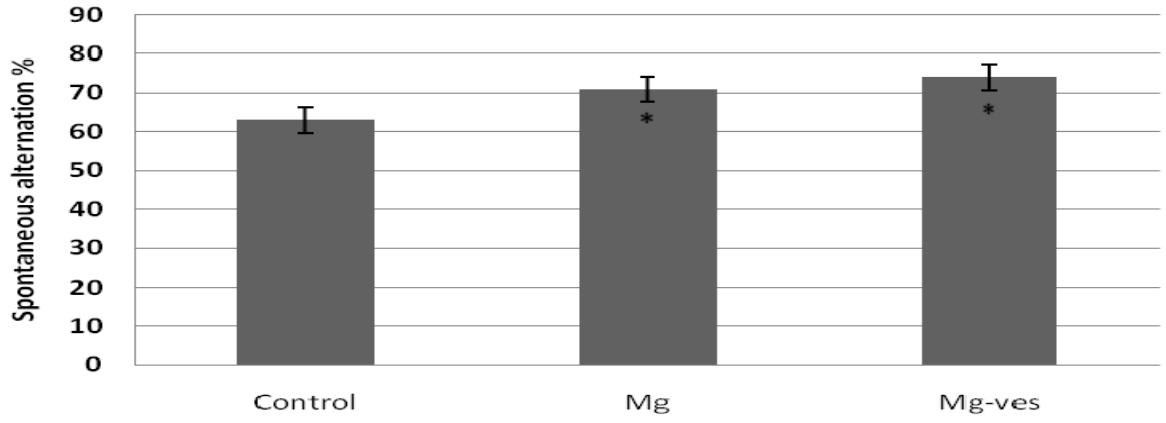

Figure 2. Spontaneous alternation % in Y-maze test. Values are means ± S.E.M. (n=7 animals per group)
*p<0.001 vs. control group

ment of short-term memory (Figure 2).

Regarding this new nanoparticulate formulation we previously demonstrated that in Activity Cage test, oral administration of 1 mmol/kbw magnesium chloride entrapped in soft vesicles resulted in a prolonged reduction of vertical movements' number and those in horizontal plane, both variations being statistically significant, compared to the

control group and to the same dose of magnesium chloride non entrapped. These results reflect the decrease of escape attempts number, within the context, significant diminution of exploratory and self-maintenance spontaneous behaviour. [12] This diminishing of both global motor behaviour and the number of escape attempts, could be correlated somehow to general behavioural inhibition or to sedation.

Conclusions

We achieved a method of magnesium chloride incorporation into vesicles which will transport and release them, in a controlled manner, in the animal body.

The present paper describes the acute toxicity study on the magnesium chloride soft vesicles and the effects of this new carrier system, on cognitive processes in rats.

It was demonstrated that magnesium chloride vesicles determined similar immune responses with both control group and magnesium chloride, after oral subacute administration in rats, indicative of good in vivo biocompatibility.

In order to evaluate the effects on spatial memory, using a behavioural model for measuring the willingness of rodents to explore new environments, we determined that in our experimental conditions, orally administration for 7 consecutive days, of magnesium chloride entrapped in lipid vesicles have been associated with the enhancement of cognitive functions in rats.

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References

1. Barauskas J, Johnsson M, Tiberg F. Self-Assembled Lipid Superstructures: Beyond Vesicles and Liposomes, *Nano Lett.*, **2005, 5 (8), 1615–1619.**

2. Dean AS. Liposomal Drug Delivery. *Journal of Infusion Nursing*, **March/April, 2007, Vol. 30, Issue 2, pp. 89-95.**

3. Durlach J, Bac P, Bara M et al. Physiopathology of symptomatic and latent forms of central nervous system hyperexcitability due to magnesium deficiency: a current general scheme. *Magnes. Res.*, **2000, 13, 293-302.**

4. Nechifor M Porr PJ. Magnesium involvements in biology and pharmacotherapy, **2003, Casa Cartii de Stiinta, Cluj-Napoca, 160-77.**

5. Saris NL, Mervaala E, Karppanen H et al. Magnesium: an update on physiological, clinical and analytical concepts. *Clin. Chim. Acta*, **2000, 294, 1-26.**

6. Fromm L, Pharm B, Heath DL et al. Magnesium Attenuates Post-Traumatic Depression/Anxiety Following Diffuse Traumatic Brain Injury in Rats. *J. Am. Coll. Nutr.*, **2004, 23, 529S–533S.**

7. Foda NH, Ellaithy HM, Tadros MI. Optimization of Biodegradable sponges as controlled release drug matrices. I. Effect of moisture level on chitosan sponge mechanical properties. *Drug Development and Industrial Pharmacy*, **2004, 30, (4), 369-379.**

8. Discher DE, Eisenberg A. Polymer vesicles. *Science*, **2002, 297 (5583): 967–973.**

9. Gârlea A, Popa MI, Pohoaţă V et al. Ibuprofen/ketoprofen entrapment in chitosan based vesicle carrier. *Rom. J. Biophys*, **2007, 17, 157-168.**

10. Detrait E, Brohez C, Hanon E et al. Automation of Continuous Spontaneous Alternation to Increase the Throughput for In Vivo Screening of Cognitive Enhancers. Optimization of the Ethovision System for the Y-maze Test in Mice. *Proceedings of Measuring Behavior,* **Eds. A.J. Spink, F. Grieco, O.E. Krips, L.W.S. Loijens, L.P.J.J. Noldus, and P.H. Zimmerman, 2010.**

11. AVMA Guidelines on Euthanasia (Formerly Report of the AVMA Panel on Euthanasia), **2007 June, p. 6-7.**

12. Bîndar D, Tarţău L, Gârlea A et al. Effects of magnesium soft matter vesicles carrier on behavioral manifestations in mice. *Romanian Journal of Biophysics*, **2010, Vol. 20., No. 1, pp. 23-35.**