ORIGINAL ARTICLE

THE EFFECTS OF SOFT MATTER VESICLES ENTRAPPING MAGNESIUM CHLORIDE IN NOCICEPTIVE REACTIVITY IN MICE

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Abstract

The study investigates the effects of magnesium nanovesicles on cutaneous nociception assessed using tail immersion test in mice. Nanoparticles were prepared by magnesium chloride immobilization inside lipid vesicles followed by stabilization with chitosan. The experiment was carried out on white Swiss mice, divided into 3 groups of 7 animals each, treated orally 7 consecutive days. In tail immersion test, the latency time response was recorded 15, 30, 60, 90 minutes, 2, 4, 6, 8, 10 and 12 hours after substances administration. We developed new carrier formulations that entrapped magnesium chloride in lipid vesicles with high efficacy and we proved that high stability magnesium nanovesicles can be designed. The administration of magnesium nanovesicles displayed a prolonged antinociceptive effect compared with the non-entrapped substance in tail immersion test.

Rezumat

Studiul investighează efectele nanoveziculelor care încorporează magneziu asupra nocicepției cutanate la șoarece, folosind testul imersiei cozii. Nanoparticulele au fost realizate prin încorporarea clorurii de magneziu în interiorul veziculelor lipidice, urmată de stabilizarea cu chitosan. Experimentul a fost efectuat pe șoareci albi Swiss, care au fost împărțiți în 3 loturi de câte 7 animale pe lot, care au fost tratate pe cale orală timp de 7 zile consecutiv. La testul imersiei cozii latența timpului de răspuns a fost înregistrată la 15, 30, 60, 90 minute, 2, 4, 6, 8, 10 și 12 ore de la administrarea substanțelor. Astfel, am creat noi formule de transport de o înaltă eficiență care înglobează clorura de magneziu în vezicule lipidice, dovedind că pot fi realizate nanoveziculele cu magneziu cu o înaltă stabilitate. La testul imersiei cozii administrarea nanoveziculelor cu magneziu asigură un efect antinociceptiv prelungit comparativ cu substanța neîncorporată.

Keywords: magnesium, soft matter vesicles, tail immersion test, nociception

Introduction

Magnesium is one of the most wide-spread elements in the environment, being the fourth major cation in the organism and the second most abundant within the cells. Electrophysiology studies show that the motor nerves that transmit messages by electrical impulse from the brain to the muscles are dependent on magnesium for the ability to conduct electrical messages [9]. Magnesium also acts as a non-competitive NMDA receptor antagonist by blocking the NMDA receptor channels implicated in the mediation of nociceptive pathways [3].

Nanoparticles designed for drug delivery are defined as submicrometer-sized colloidal particles. The advantages of using vesicles as drug carriers are associated with their physicochemical properties, such as: ultra-small size, large surface area to mass ratio and high reactivity [5]. Literature reports limited studies about the use of magnesium chloride in the preparation of vesicles: one of them refers to surface stabilization properties of phospholipid vesicles demonstrated by various concentration of magnesium cation, and another one to highly monodisperse colloidal magnesium nanoparticles prepared by dispersion method in the presence of hexadecylamine [4].

The goals of this study were the preparation and stabilization of chitosan lipid vesicles with entrapped magnesium chloride, followed by their application in testing cutaneous nociceptive reactivity, after oral administration in mice.

Materials and Methods

The substances were obtained from Sigma-Aldrich Company: the lipid used - egg yolk L-α-phosphatidylcholine (L-α-lecithin), approximately 99 % pure; chitosan (biocompatible and biodegradable polymer, N-deacetylation grade = 79.7%, mean molecular weight M_w = 310.00 g/mol, polydispersity index = 3.26); magnesium chloride. The 0.5 (*w/w*) chitosan solutions were prepared in a 0.5 % (*v/v)* acetic acid. The other solutions were prepared in distilled water. The soft matter vesicles were designed by immobilization of magnesium chloride inside lipid vesicles; the preparation method implied dissolution of the lipid in chloroform, followed by solvent removal through evaporation, which lead to dry lipid film formation. The film was then hydrated, by adding aqueous solution of magnesium chloride (12 mg/mL concentration). In the final phase of the experiment, the vesicles were stabilized with a 0.5 % chitosan solution [2]. Surface morphology and size distribution of nanoparticles were studied using a Malvern Zetasizer Nano ZS, ZEN-3500 apparatus. Images were recorded with a Nikon Coolpix 950 camera, maximum resolution of 1600×1200 (1.92 Mpx), 3 × optical zoom.

The *in vivo* experiment was carried out on white Swiss mice (20 – 25 g), divided into 3 groups of 7 animals each, treated orally (using an eso-gastric device), during 7 consecutive days, as follows: Group I (control): 0,1 ml/10 g body weight distilled water; Group II (Mg): 1 mmol/kgbw magnesium chloride; Group III (Mg-vesicles): 1 mmol/kgbw magnesium chloride entrapped in soft vesicles.

The investigation of cutaneous nociception was performed using the tail immersion test [8], an experimental model based on noxious thermal stimulation [11] of mouse tail with hot water (56°C), followed by measurement of the response latency period (counting the time that the animal takes to move its tail away from the heat source) - 15, 30, 60, 90 minutes, 2, 4, 6, 8, 10 and 12 hours after substances administration. The analgesic effect was measured after 7 days because from the data obtained in previous experiments the equilibrium concentration is achieved after 7 days of substances administration. The baseline latency for control group (before drug administration) in the tail immersion test was 4.2 ± 0.15 seconds (mean ± standard error of mean, SEM). The recommended cut-off time of 12 seconds was used to prevent tail damage. Differences between the experimental and baseline latencies are interpreted as an index of analgesia. An increase of the latency period for the animal to flick its tail is indicative of analgesia, while a decrease in the latency response is indicative of hyperalgesia. Response latency data from tail reactivity measurements were converted to per cent of maximum possible effect (% MPE) according to the formula [7]:

$$\%\ MPE = \frac{observed\ latency - baseline\ latency}{cut\ off\ time - baseline\ latency} \times 100$$

The data were interpreted as mean with its corresponding confidence limits (95%) and were presented in graphs as means ± SEM of latency time (seconds) for seven mice, and the statistical significance of results was interpreted using the ANOVA method implemented in SPSS Statistics 13.0 software. p-values less than 0.05 were considered statistically significant compared to the control group.

The experimental protocol was approved and implemented according to recommendations of the University "Grigore T. Popa" Iasi Committee for Research and Ethical Issues, and to the guidelines of IASP Committee for Research and Ethical Issues [12].

Results and Discussion

The obtained nanoparticulate formulations (Mg-vesicles) were visualized and monitored for their morphological attributes (Figure 1).

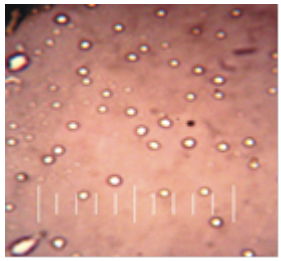

Figure 1.
Optical microscopy visualization of Mg-vesicles (dimensioning scale = 10 μm, maximum resolution of 1600 × 1200 (1.92 Mpx), 3 × optical zoom)

Using an original methodology we achieved the entrapment with high efficacy of magnesium chloride within the lipid vesicles stabilized with polysaccharide chitosan [1]. The soft matter vesicles prepared by us proved to possess a high stability in aqueous solution. After addition of chitosan lipid solution, the vesicles became spherical, very stable, and the solution was clear. Magnesium lipid vesicles were found to have a mean Zeta potential of + 36.1 mV and a mean size of 129.56 nm. We estimate that the systems correspond to the criteria of nanosuspensions and the presence of chitosan resulted in positively charged, more confined vesicles, generating repulsion forces, thus increasing stability.

Substantial numbers of studies on the biomedical use of chitosan as a drug carrier have been reported.

Chitosan can reside longer in the stomach, being a good candidate for formulation into gastroretentive specific drug delivery systems [6]. It also can be cross-linked to various degrees to modulate drug diffusion through matrix models and hence to achieve sustained delivery of drugs. In our experimental conditions chitosan determined all vesicles to become positively charged and more confined, which lead to repulsion forces, therefore increasing the stability of vesicles. Chitosan acts like an additional transport barrier which enables a slower, extended release rate of the encapsulated substances from the vesicles.

In the tail immersion test magnesium chloride 1 mmol/kgbw determined a rapid and statistically significant increase of the latency time period of the response compared to the control group ($p < 0.05$). This effect was maximal after 60 minutes in the experiment, lasted for 120 minutes after substance oral administration, and gradually decreased afterwards. Using soft vesicles as carriers for magnesium chloride we noticed an increase of the latency reaction, statistically significant ($p < 0.05$) compared to the control group, that began after 90 minutes, with a peak effect between 2 and 4 hours interval ($p < 0.05$) and maintained for more than 8 hours after this new formulation administration in this cutaneous pain model in mice (Figure 2).

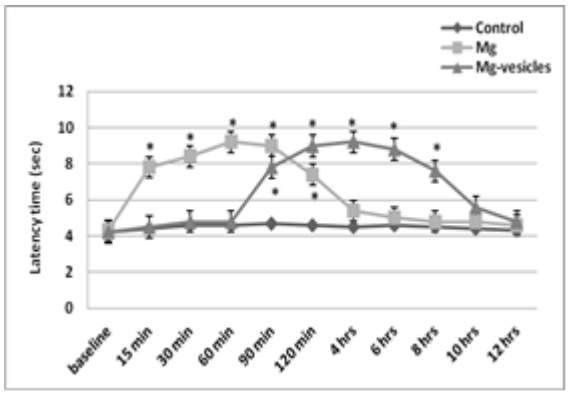

Figure 2.
The latency time period of Mg and Mg-vesicles response to thermal noxious stimulus in the tail immersion test
Each point is the mean ± SEM of latency time (seconds) for seven mice. *$p < 0.05$, **$p < 0.01$ *vs.* control group

Additionally, we used the percentage of maximum possible effect (% MPE) to quantify the intensity of antinociception, which helps to corroborate and confirm the obtained results. The administration of Mg 1 mmol/kgbw resulted in an increase in % MPE statistically significant in the first 2 hours after drug administration. Its maximum antinociception was observed after 60-minutes mark (% MPE_{60} = 63.6 ± 1.2 %), statistically significant compared to the % MPE_{60} (5.1 ± 1.3 %) of the control group in the tail immersion test. Mg-vesicles produced an increase in % MPE statistically significant after 90 minutes in the tail immersion assay. These effects were prolonged for approximately 6 hours, with a significant increase to a sub-maximal level (more than 60 % of MPE) in the interval between 2 hours (% MPE_{60} = 61.5 ± 0.7 %) and 4 hours (% MPE_{60} = 64.1 ± 1.1 %) after substance administration, compared to the control group (% MPE_{60} = 5.1 ± 1.3 %, respectively 3.8 ± 0.3 %) and the Mg group (% MPE_{60} = 40.3 ± 1.1 %, respectively 14.3 ± 0.9 %) (Figure 3).

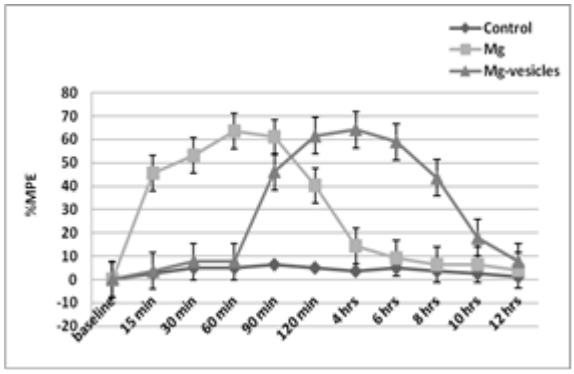

Figure 3.
Time course of the maximum possible effect (% MPE) of Mg, Mg-vesicles and control group on the tail-withdrawal latency
Each point is the mean ± SEM of percentage of maximum possible effect (% MPE) for seven mice

In a previous study, we demonstrated that magnesium entrapped in soft matter vesicles proved analgesic effect in tail flick test, a classical cutaneous pain model in mice [10].

Conclusions

Using a standardized somatic pain model (tail immersion test) we have established that oral administration of Mg-vesicles resulted in significant antinociceptive effects that started after 90 minute, with a maximum intensity between 2 and 4 hours, and prolonged for more than 6 hours after substance administration. Our experimental study demonstrated that the use of soft matter vesicles as carriers for magnesium chloride presents the advantage of a sustained release of drug, compared to the non-entrapped substance, in the tail immersion test.

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