Therapeutics, Pharmacology and Clinical Toxicology
Vol XIV, Number 2, June 2010
Pages 113-116

ORIGINAL PAPERS

EXPERIMENTAL RESEARCH ON THE EFFECTS OF NANO-VESICLES ENCAPSULATING KETOPROFEN IN A VISCERAL PAIN MODEL IN MICE

Liliana Tarțău[1], R.V. Lupușoru[2], D. Bindar[3], V. Melnig[3]

[1.] *Pharmacology - Algesiology Department, Gr. T. Popa University of Medicine and Pharmacy, Iaşi, Romania*
[2.] *Pathophysiology Department, Gr. T. Popa University of Medicine and Pharmacy, Iaşi, Romania*
[3.] *Department of Physics - COMB Laboratory, Faculty of Physics, Al. I. Cuza University, Iaşi, Romania*

Abstract. Nanoparticles designed for drug delivery are defined as submicrometer-sized colloidal particles in which the active principle is dissolved, entrapped or encapsulated, and to which the active principle is absorbed or attached. Generally, nanoparticles can be used to provide targeted delivery of active substances, to sustain drug effect in target tissue, to decrease its adverse effects. Ketoprofen is one of the most potent non-steroidal anti-inflammatory drug frequently prescribed worldwide. Literature data describe various possibilities for the design of ketoprofen nanoparticulate formulations, which are characterized and studied regarding active substances' delivery, but only a few were investigated for the in vivo effects. Aim: This study investigated the effects of ketoprofen lipid vesicles in a visceral pain model in mice. Method: The soft matter vesicles made of lipid-ketoprofen-chitosan were prepared using an original method, and were physicochemically analyzed using a Malvern Zetasizer Nano ZS, ZEN-3500 model. The experiments were carried out on white Swiss mice (20-25 g), divided into 3 groups of 7 animals each, treated orally: Group I (Control): distilled water 0.3 ml, Group II (KET): 15 mg/kbw ketoprofen, Group III (KET-ves): 15 mg/kbw ketoprofen entrapped in vesicles. The nociceptive visceral testing was performed using the mouse model of acute pain writhing test. The experimental protocol was implemented according to the recommendations of the University Committee for Research and Ethical Issues, and to the guidelines of IASP Committee for Research and Ethical Issues. The data were statistically analyzed with SPSS software for Windows version 17.0 and ANOVA method. Results: It was demonstrated that the soft matter vesicles are capable to entrap ketoprofen water solution, with a large efficiency. The vesicles' dimensions vary from tens of nanometers to hundreds, and the solution has a moderate stability. Oral administration of ketoprofen resulted in a decrease of behavioral manifestations induced by chemical noxious stimuli, statistically significant immediately in the experiment, effect present for 120 minutes.

Using soft vesicles entrapping ketoprofen, we obtained the reduction of writhes number with an onset at 3 hours and prolonged for 5 hours after substance administration. Conclusions: The use of soft matter vesicles as carriers for ketoprofen presented the advantage of drugs' sustained release, compared to non-entrapped substances in the writhing test.

Keywords: ketoprofen loaded vesicles, nociception, in vivo, writhing test

General data

Nanoparticle drug carriers consist of solid biodegradable particles ranging in size from 10 to 1000 nm in which the active principle is dissolved, entrapped or encapsulated, and to which the active principle is absorbed or attached. Nanoparticles have been studied extensively as particulate carriers in different pathophysiologic conditions. Generally, nanoparticles can be used to provide targeted delivery of active substances, to sustain drug effect in target tissue, to decrease its adverse effects. The important technological

Liliana Tarțău
Gr.T. Popa University of Medicine and Pharmacy
Universitatii No. 16 Street, Iasi, Romania
email: lylytartau@yahoo.com

advantages of vesicles used as drug carriers are the high stability and carrier capacity, feasibility of incorporation of both hydrophilic and hydrophobic substances, and feasibility of variable routes of administration[1,2].

Literature data describe various possibilities for the design of analgesic loaded nanoparticles, especially for non-steroidal anti-inflammatory drugs (NSAIDs). Many methods for preparing microcapsules have been developed, some are based exclusively on physical phenomena and some utilize polymerization reactions to produce a capsule shell while others combine physical and chemical phenomena. Most of the analgesic formulations were characterized and studied regarding active substances' delivery, but only a few were investigated for the *in vivo* effects, and some data were controversial[3]. In the literature there are limited studies reported about the design of ketoprofen carrier systems, some of them referring to: ketoprofen-loaded albumin microspheres[4], ketoprofen-loaded sodium alginate beads manufactured by prilling[5], Eudragit RSPO microspheres containing ketoprofen prepared by solvent evaporation technique using acetone-liquid paraffin[6], ketoprofen spray-dried microspheres using the polymer Eudragit[7], ketoprofen-loaded polystyrene microparticles with polyvinylpyrrolidone prepared by emulsion solvent evaporation method [8], ketoprofen chitosan/alginate microcapsules prepared by an air extrusion method[9].

This paper presents the obtaining and the stabilization of lipid vesicles coated in chitosan which entrapped ketoprofen inside, aiming towards their use for *in vivo* testing of the effects of ketoprofen soft matter vesicles carrier in a visceral pain model in mice.

Methods

Substances

The lipid used, Egg Yolk L-α-phosphatidylcholine (L-α-lecithin), approximately 99% (TLC) pure, chitosan (biocompatible and biodegradable polymer) and ketoprofen, were obtained from Sigma-Aldrich Company. The 0.5 (*w*/*w*) chitosan solutions were prepared in a 0.5% (*v*/*v*) acetic acid. All solutions were made using distilled water, purchased from Sicomed, Romania.

Vesicles' preparation and morphologic analysis

The soft matter vesicles designed after ketoprofen immobilization inside lipid vesicles, were obtained by dissolving the lipid in chloroform and removing the solvent by evaporation, which lead to a dry lipid film. The film was then hydrated, by adding distilled water with ketoprofen. In the end, the vesicles were stabilized with a 0.5% biodegradable polymer chitosan solution[10]. The N-deacetylation degree of utilized chitosan was 79.7%, the average molecular weight was M_w = 310.000g/mol and the polydispersity index was 3.26. The ketoprofen vesicles were characterized for size and Zeta potential by Malvern Zetasizer Nano ZS ZEN-3500. Values of viscosity and refractive index were set at 0.6864cP and 1.330, respectively.

Animals

Male white Swiss mice (20-25g) were used. Lighting was on a 12 hours light/dark cycle (lights on at 6:00 a.m.), with standard laboratory food and tap water freely available, except during the time of the experiments. Before the experiment, the mice were placed on a raised wire mesh, under a clear plastic box and allowed 2 hours to acclimate to the testing room.

Procedure

The experiment was carried out on 8 identical groups of 3 lots of 7 white Swiss mice (20-25g) that received the substances orally, at the same moment:

Group I (Control) served as normal control and received distilled water 0.1 ml/10g body weight,

Group II (KET) served as reference group and received ketoprofen 15 mg/kbw,

Groups III (KET ves) served as treatment group and received ketoprofen vesicles 15 mg/kbw.

The model of visceral pain consists of writhing test with acetic acid 0.6%. Intraperitoneal injection of the irritant agent (acetic acid) causes typical stretching responses named writhes (abdominal constrictions and full extension of hind limbs) as a pain reaction[11].

Acetic acid (0.6%) was administered at the dose of 1 ml/kbw to the lots of each group, according to the following schedule: Group I – half an hour, Group II – 1 hour, Group III – 2 hours, Group IV – 3 hours, Group V – 4 hours, Group VI – 5 hours, Group VII – 6 hours, respectively Group VIII – 8 hours after substances' administration. After a five minutes interval for proper absorption of acetic acid, the mice were observed for specific contractions of the body referred to as 'writhing', which is an indication of pain sensation in test animals. A comparison of writhing was made

between control, simple substance and entrapped substance group. For each investigation timeline, the number of writhes of each mice induced by chemical noxious stimulus were counted within 10 minutes[12].

Compared to the control animals, decreases in the number of writhes per mice are indicative of analgesia, while increases in the number of behavioral manifestations are indicative of hyperalgesia. The antinociceptive effects of ketoprofen lipid vesicles were measured by calculating the mean reduction in the number of abdominal contractions, compared to the control group[13].

The pain inhibition percentage was calculated according to the following formula[11]:

Pain inhibition percentage = (Writhes No. control-Writhes No. tested)/Writhes No. tested)x100

The data were expressed as mean ± SEM of 7 animals. Results were analyzed statistically by SPSS for Windows version 17.0 and ANOVA method. P-values of under 0.05 were considered statistically significant compared to those of the controls.

The experimental protocol was implemented according to the recommendations of the University Committee for Research and Ethical Issues, and to the guidelines of the IASP Committee for Research and Ethical Issues[14].

Results and discussions

We achieved a method of incorporating ketoprofen into vesicles which will transport and release the analgesic drug in the animal body in a controlled fashion.

The vesicles' dimensions vary from tens of nanometers to hundreds (mean size 491 nm), and the solution has a moderate stability (mean Zeta potential +20mV).

Statistical analysis of the results obtained in the *writhing test* showed that:

- oral administration of ketoprofen 15 mg/kbw resulted in the gradual decrease of the number of writhes, immediately after acetic acid injection, statistically significant compared with the control group, effect prolonged around 3 hours after noxious peritoneal stimulation.
- ketoprofen entrapped in lipid vesicles decreased the number of behavioral manifestations statistically significant (*p<0.05) compared to the control group, but its effect was less intense than that of the non-entrapped ketoprofen, in the interval between 3 and 5 hours after substance administration (**figure 1**).

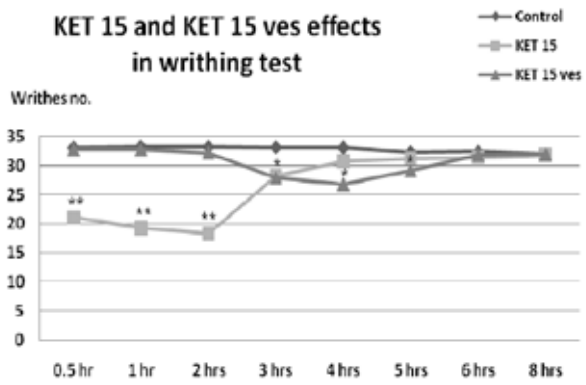

Figure 1. Effects of ketoprofen and ketoprofen lipid vesicles on acetic acid induced writhing in mice. The data are represented as mean ± SEM of 7 animals. *p< 0.05, **p< 0.01 compared to control

Both ketoprofen entrapped vesicles and the non-entrapped drug suppressed the frequency of acetic acid induced writhing in mice. Ketoprofen exhibited a maximum writhing inhibition percentage of 44.72%, whereas ketoprofen lipid vesicles showed a maximum writhing inhibition percentage of 23.98% (4 hours after substance administration), respectively. Oral administration of a non-entrapped ketoprofen resulted in a greater activity (36.07%-44.72%) in the interval between half an hour and 2 hours after substance administration, while the inhibition rate of behavioral manifestations was only 20.91% at 3 hours and 23.98% at 4 hours for ketoprofen entrapped in lipid vesicles (**figure 2**).

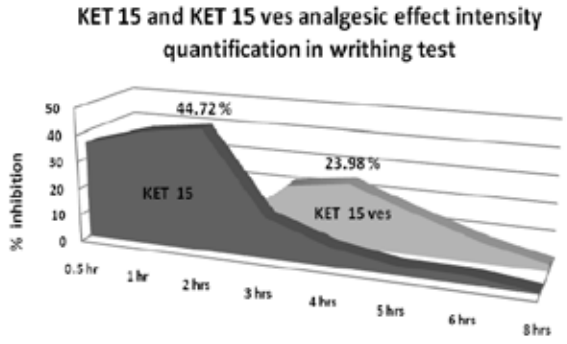

Figure 2. Pain inhibition percentage of ketoprofen and ketoprofen lipid vesicles on acetic acid induced writhing in mice. The data are represented as mean ± SEM of 7 animals.

Corroborating both *in vivo* tests, we observed a relative correlation between blood levels of ketoprofen entrapped in lipid vesicles and non-entrapped substance and their respective effects in a visceral pain model in mice.

Conclusions

We developed new carrier formulations that entrapped ketoprofen in lipid vesicles with a moderate stability of a solution.

The results of the present study demonstrated that both non-entrapped and vesicle-entrapped ketoprofen possessed antinociceptive activity in this nociceptive model in mice.

We can estimate than the use of soft matter vesicles as carriers for ketoprofen presented the advantage of sustained drug release, compared to the non-entrapped substance in the writhing test.

Acknowledgements: This work was financially supported by the Gr.T. Popa University of Medicine and Pharmacy, Iaşi, Romania, Internal Research Grant No. 16407/2009.

References

1. Zhang L, Gu FX, Chan JM et al. Nanoparticles in Medicine: Therapeutic Applications and Developments, *Clin Pharmacol Ther*, **2008, 83, 5:761–769.**

2. Morrow KJ, Bawa R, Wei C. Recent advances in basic and clinical nanomedicine, *Med Clin North Am*, **2007, 91, 5:805–843.**

3. Tarţău L, Wei C, Lupusoru RV, Lupusoru CE. Melnig V., Design of analgesic nanoparticulate systems and the advantages of their use in experimental nociception, in Hariton N.C. Edition, *Advancements of Medical Bioengineering and Informatics*, Gr.T. Popa University of Medicine and Pharmacy Publishing House, Iaşi, **2009, pp. 118-123.**

4. Mathew ST, Gayathri Devi S, Prasanth VV, Vinod B. Formulation and in vitro–in vivo evaluation of ketoprofen-loaded albumin microspheres for intramuscular administration, *J Microencapsul*, **2009, 26 (5):456-469.**

5. Del Gaudio P, Russo P et al. Encapsulation of Ketoprofen and Ketoprofen Lysinate by Prilling for Controlled Drug Release, *AAPS PharmSciTech*, **2009, 10 (4):1178-1185.**

6. Pandit SS, Hase DP, Bankar MM et al. Ketoprofen-loaded Eudragit RSPO microspheres: An influence of sodium carbonate on in vitro drug release and surface topology, *J Microencapsul*, **2009, 26 (3):195-201.**

7. Rassu G, Gavini E, Spada G et al. Ketoprofen Spray-dried Microspheres Based on Eudragit® RS and RL: Study of the Manufacturing Parameters, *Drug Develop Ind Pharm*, **2008, 34 (11):1178-1187.**

8. Rajesh KS, Bhowmik BB, Sa B. Effect of polyvinylpyrrolidone on physical characteristics of ketoprofen-loaded polystryrene microparticles, *Indian J Pharm Sci*, **2003, 65 (5):526-29.**

9. Tan TW, Hu B, Jin XH et al. Release Behavior of Ketoprofen from Chitosan/Alginate Microcapsules, *Journal of Bioactive and Compatible Polymers*, **2003, 18 (3):207-218.**

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

11. Shin JW, Hwang KS, Kim YK et al. Nonsteroidal Antiinflammatory Drugs Suppress Pain-Related Behaviors, but Not Referred Hyperalgesia of Visceral Pain in Mice, *Anaesth. Analg.,* **2006, 102, 1: 195-200.**

12. Le Bars D, Gozariu M, Cadden SW. Animal models of nociception, *Pharmacological Reviews*, **2001, 53: 597-652.**

13. Ness TJ. Models of visceral nociception, *Inst. Lab. Anim. Res. J.,* **1999, 40: 119-128.**

14. Zimmerman M. Ethical guidelines for investigations of experimental pain in conscious animals, *Pain*, **1983, 16:109-110.**