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ORIGINAL

Evaluation of the biocompatibility of solid lipid nanoparticles of Myristyl Myristate in human blood cells

Evaluación de la biocompatibilidad de nanopartículas lipídicas sólidas de Miristil Miristato en células sanguíneas humanas

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ABSTRACT

Introduction: solid lipid nanoparticles (SLNs) have emerged as promising drug delivery systems, standing out for their biocompatibility and stability.
Objective: to evaluate the biocompatibility of SLNs formulated with myristyl myristate (MM) in human lymphocytes through cytotoxicity, genotoxicity, and oxidative stress assays.
Method: SLNs were synthesized using sonication and characterized in terms of size, polydispersity index, and zeta potential. Concentrations ranging from 18,75 to 300,00 µg/ml were selected for in vitro assays. Cell viability was assessed using the MTT assay, while genotoxicity was analyzed through the Comet assay. Lipid peroxidation was measured by quantifying thiobarbituric acid reactive substances (TBARs).
Results: after 24 hours of exposure, cell viability remained above 90 % at all concentrations. However, after 48 hours, viability decreased at concentrations of 150 and 300 µg/ml. No significant DNA damage or changes in lipid peroxidation levels were observed under any tested condition.
Conclusion: these findings suggest that MM-based SLNs exhibit high in vitro biocompatibility, with no relevant short-term cytotoxic or genotoxic effects. However, further studies in in vivo models and under prolonged exposure conditions are necessary to assess their safety for biomedical applications.

Keywords: Solid Lipid Nanoparticles; Biocompatibility; Myristyl Myristate; Cytotoxicity; Genotoxicity; Oxidative Stress.

RESUMEN

Introducción: las nanopartículas lipídicas sólidas (SLNs) han surgido como sistemas prometedores para la administración de fármacos, destacándose por su biocompatibilidad y estabilidad.
Objetivo: evaluar la biocompatibilidad de SLNs formuladas con miristil miristato (MM) en linfocitos humanos mediante ensayos de citotoxicidad, genotoxicidad y estrés oxidativo.

Método: las SLNs se sintetizaron utilizando sonicación y se caracterizaron en términos de tamaño, índice de polidispersidad y potencial zeta. Se seleccionaron concentraciones entre 18,75 y 300,00 µg/ml para los ensayos in vitro. La viabilidad celular se evaluó mediante el ensayo MTT, mientras que la genotoxicidad se analizó con el ensayo Cometa. Además, se midió la peroxidación lipídica mediante la cuantificación de sustancias reactivas al ácido tiobarbitúrico (TBARs).
Resultados: los resultados indicaron que, tras 24 horas de exposición, la viabilidad celular se mantuvo superior al 90 % en todas las concentraciones. Sin embargo, a 48 horas, se observó una disminución en la viabilidad en concentraciones de 150 y 300 µg/ml. No se detectó daño significativo al ADN en ninguna de las condiciones evaluadas, ni cambios en los niveles de peroxidación lipídica.
Conclusión: estos hallazgos sugieren que las SLNs de MM presentan una alta biocompatibilidad en condiciones in vitro, sin efectos citotóxicos ni genotóxicos relevantes a corto plazo. No obstante, estudios adicionales en modelos in vivo y en exposiciones prolongadas serán necesarios para evaluar su seguridad en aplicaciones biomédicas.

Palabras clave: Nanopartículas Lipídicas Sólidas; Biocompatibilidad; Miristil Miristato; Citotoxicidad; Genotoxicidad; Estrés Oxidativo.

INTRODUCTION

Nanomedicine is an emerging field that combines nanotechnology with medicine to transform healthcare. Its development has enabled significant advances in biomedical research, optimizing targeted drug delivery and contributing to progress in regenerative medicine. It also offers new strategies for the diagnosis and treatment of various diseases.[1]

Nanoparticles (NPs) are innovative systems, ranging in size from 1-100 nm, designed to deliver drugs and diagnostic agents. Their unique properties can significantly alter the fate of drugs in the body by acting as specialized vehicles that prolong their circulation and promote their accumulation in specific organs.[2] several types of NPs exist, including lipid nanoparticles, polymeric micelles, polymerosomes, dendrimers, and liposomes. In addition, their ability to transport multiple drugs makes them optimal candidates for combination therapy, a key strategy for tackling drug resistance.[3]

Lipid nanoparticles (LNPs) represent a type of lipid-based nanocarrier used to deliver drugs or nucleic acids with hydrophobic or hydrophilic properties. Their versatility makes them applicable in various fields, including cancer and bacterial infection therapy, bioimaging, diagnostics, cosmetics, and agriculture.[4]

Among the variants of LNPs, solid lipid nanoparticles (SLNs) stand out as colloidal systems composed of solid lipids at room and body temperature. These nanoparticles consist of biodegradable lipids as the dispersed phase and a surfactant that acts as an emulsifier, which gives them stability and versatility for various biomedical applications.[4,5]

Solid lipid nanoparticles (SLNs) have several advantages, including controlled drug release, increased stability of the active ingredient, high loading efficiency, ability to incorporate lipophilic and hydrophilic compounds, biocompatibility, and feasibility for large-scale production.[6,7] Due to these properties, SLNs have been investigated in multiple therapeutic applications, such as cancer treatment, antimicrobial use, and targeting diseases of the central nervous system.[8]

To move towards their clinical use, it is essential to assess the toxicity and biocompatibility of SLNs through in vitro studies. However, the information available on their toxicological profile is still limited.[9] The biocompatibility of these nanoparticles can be analyzed through cytotoxicity, genotoxicity, and oxidative stress assays in cellular models.[5] Their possible toxic effects could be related to membrane disruption, alterations in membrane potential, protein oxidation, interference in energy transmission, formation of reactive oxygen species, and releasing toxic compounds.[10] In the present study, it has been proposed that their possible toxic effects could be related to the disruption of membranes, alterations in membrane potential, oxidation of proteins, interference in energy transmission, formation of reactive oxygen species, and the release of toxic compounds.[10] In the present study, the biocompatibility of these nanoparticles can be analyzed using cytotoxicity and genotoxicity assays.

Therefore, the present study will evaluate the biocompatibility of different concentrations of solid lipid nanoparticles of myristyl myristate (SLN of MM) in human cells using cytotoxicity, genotoxicity, and oxidative stress assays.

METHOD

Preparation of lipid nanoparticles

Solid lipid nanoparticles (SLNs) were prepared using myristyl myristate (Crodamol™ MM), donated by Croda

(Argentina). 400 mg of lipid (2,00 % w/v) was melted in a water bath at 60-70 °C. After 10 minutes, a hot aqueous solution (20 ml) containing 3,00 % (w/v) Pluronic® F68 was added to the lipid phase. Immediately, the mixture was sonicated for 30 minutes (50 % amplitude) using an ultrasonic processor (130 W, Cole-Parmer, USA) equipped with a 6 mm titanium tip. Finally, the dispersion was cooled to room temperature and stored at 4 °C.

Characterization of lipid nanoparticles

The nanoparticles' mean diameter and size distribution were determined by photon correlation spectroscopy (PCS) using a Nano ZS Zetasizer (Malvern Instruments Corp, UK) at 25 °C. The zeta potential was measured by laser Doppler anemometry with the same equipment, using 10 nm path lengths. The polydispersity index (PDI) was also calculated. The physical stability of the system was assessed by monitoring changes in mean size and zeta potential during storage at 4 °C in the dark for up to three months.

Selection of MM SLN concentrations

The concentrations used were selected based on the available literature. Five serial concentrations (18,75, 37,50, 75,00, 150,00, and 300,00 μg/ml) were established and used for in vitro assays. The selection was based on previous studies[11,12,13] and on the review by Doktorovova.[14]

Collection of human blood samples

After informed consent, blood samples (10 ml) were collected from three clinically healthy individuals (25-35 years) by venipuncture with a sterile heparinized syringe. Participants declared not to be suffering from infectious diseases or under medical and/or pharmacological treatment three months before sample collection. One ml of blood was separated to quantify thiobarbituric acid reactive substances (TBARs). At the same time, the remaining volume was used to isolate lymphocytes, on which cytotoxicity and genotoxicity assays were performed.

Lymphocyte collection and exposure to MM SLNs

Lymphocytes were isolated by density gradient separation using Ficoll type F-P (Sigma Co.). Cells were then cultured in 96-well plates with RPMI-1 640 medium supplemented with 10 % fetal bovine serum (FBS) and antibiotics. The cells were exposed to the different concentrations of SLNs (0,00, 18,75, 37,50, 75,00, 150,00, 300,00 μg/ml) for 24 and 48 hours in a 37°C incubator with 5 % CO_2 and controlled humidity.

Evaluation of cytotoxicity by MTT assay

The cytotoxicity of MM SLNs was assessed using an MTT assay. After exposure of lymphocytes to the different concentrations for 24 and 48 hours, they were centrifuged at 2 500 rpm; the supernatant was discarded and resuspended in 100 μl of fresh RPMI-1 640. 10 μl of MTT solution (1 mg/ml) was added. The cells were incubated and protected from light at 37°C with 5 % CO_2 for 4 hours. Then, 50 μl of dimethyl sulfoxide (DMSO) was added to dissolve the formazan crystals, and the absorbance was measured at 560 nm spectrophotometrically.

Comet assay (alkaline electrophoresis on single cells)

The Comet assay was performed on lymphocytes exposed to MM SLNs with more than 85 % viability. The method described by Singh et al.[15] was followed. Lymphocytes from each treatment were mixed with 75 μl of low melting point agarose (0,75 % in distilled water at 37 °C) and deposited on slides previously coated with a layer of standard melting point agarose (0,75 %). Subsequently, another layer of low melting point agarose was added and covered with coverslips.

After solidification, the slides were immersed in cold lysis solution (2,5 M NaCl, 100 mM EDTA, 10 mM Tris pH 10, 1 % Triton X-100, and 10 % DMSO) at 4 °C for at least 1 hour. They were then transferred to an electrophoresis cuvette with an alkaline solution (NaOH 300 mM, EDTA 1 mM, pH 13) and incubated in the dark at 4°C for 20 minutes for DNA unwinding. Electrophoresis was performed at 30 V and 250 mA for 25 minutes. Finally, samples were stained with 50 μl of ethidium bromide (20 μg/ml), and images of at least 100 cells per treatment were captured and analyzed with CometScore™ software.

Determination of thiobarbituric acid reactive substances (TBARs)

Quantification of TBARs was performed according to the method described by Buege & Aust[16] and modified by [17]. Samples of 1 ml of blood from the three donors, exposed to different concentrations of MM SLNs for 4 hours at 37°C in an incubator, were analyzed. Malondialdehyde (MDA) was used as a reference, obtained by hydrolysis of 1,1,3,3-tetramethoxypropane (TMP). The reading was made at 535 nm in a spectrophotometer (Metrolab 1600 DR).

Statistical analysis

The data obtained were analyzed using Generalised Linear Models. To compare treatments, Fisher's LSD multiple comparisons test was applied. Statistical analyses were performed using RStudio, version 1.1.463.

RESULTS

Characterization and stability of MM SLNs

The myristyl myristate solid lipid nanoparticles (MM SLNs) had a mean diameter of 118 nm, a polydispersity index (PDI) of less than 0,2, and a zeta potential of -4,0 mV, indicating a homogeneous size distribution and adequate colloidal stability. During storage at 4°C for three months, no significant changes in their physical characteristics were detected, suggesting good stability of the system.

Assessment of cell viability

Human lymphocytes exposed to different concentrations of MM SLNs (0,00; 18,75; 37,50; 75,00; 150,00; 300,00 μg/ml) for 24 hours showed cell survival above 90 % in all conditions evaluated. However, after 48 hours of exposure, a decrease in cell viability was observed at the highest concentrations (150,00 and 300,00 μg/ml), with values below 85 % (figure 1).

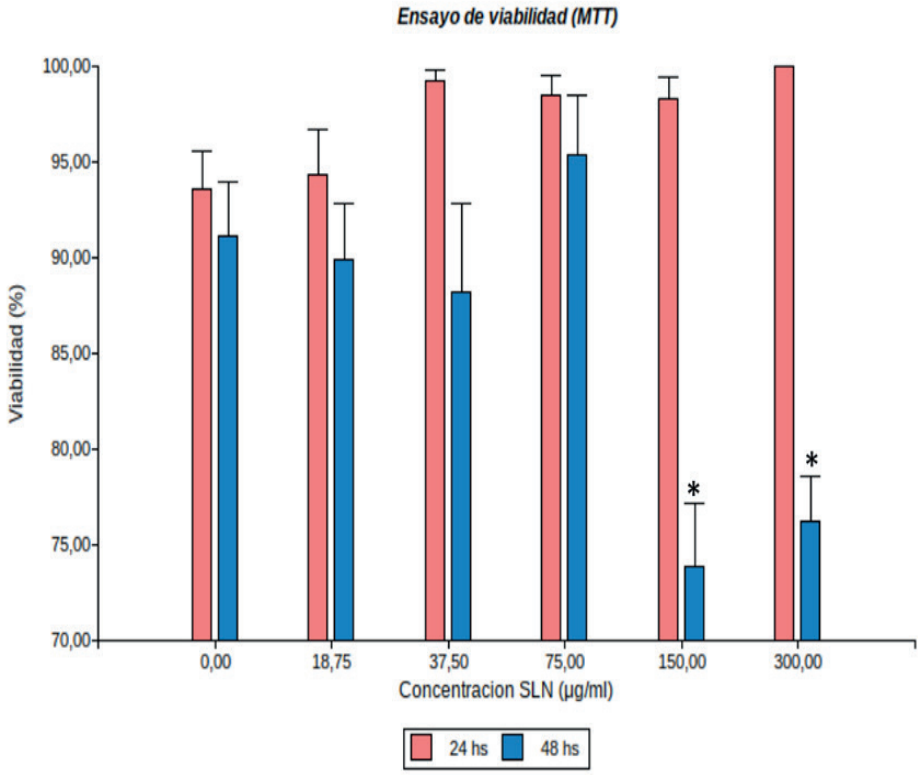

Figure 1. Viability expressed as a percentage (%) of human lymphocytes exposed to different concentrations of MM SLNs (μg/ml). The red bars correspond to 24 hours of exposure of lymphocytes to the different concentrations of MM SLNs, and the blue color corresponds to 48 hours of exposure of lymphocytes to the different concentrations of MM SLNs. *=p≤0,05.

Since cells exposed for 24 hours showed the highest levels of viability, the Comet assay was decided to be performed exclusively under these experimental conditions.

Quantification of Thiobarbituric Acid Reactive Substances (TBARs)

The results of the TBARs quantification assay are presented in figure 2. No statistically significant differences were detected between the different concentrations tested, suggesting that exposure to MM SLNs did not induce relevant changes in lipid peroxidation levels in the treated lymphocytes.

Assessment of DNA damage by the Cometa assay

Figure 3 shows the Comet assay results for quantifying DNA damage in human lymphocytes exposed to different concentrations of MM SLNs. No statistically significant differences in Tail Moment values were observed, indicating that exposure to these nanoparticles did not generate a detectable increase in genotoxic damage under the experimental conditions.

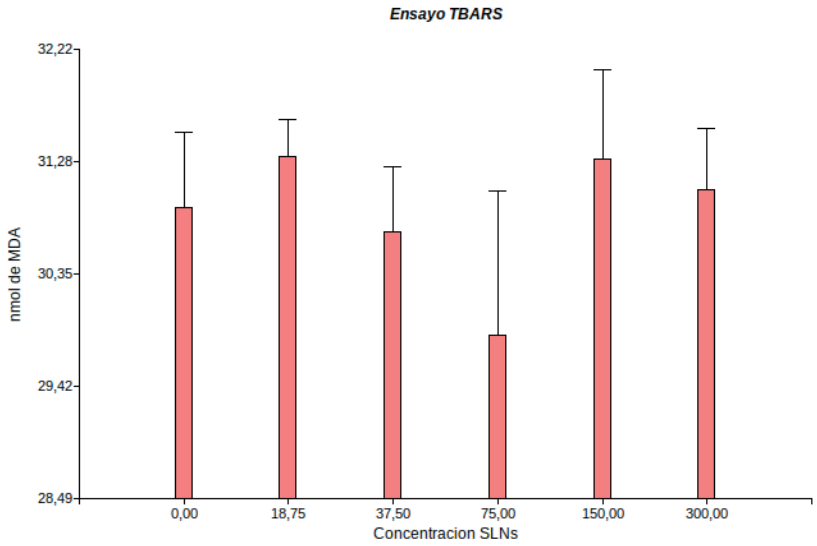

Figure 2. MDA nanomoles in blood cells exposed to different concentrations of MM SLNs (µg/ml) for 4 hours

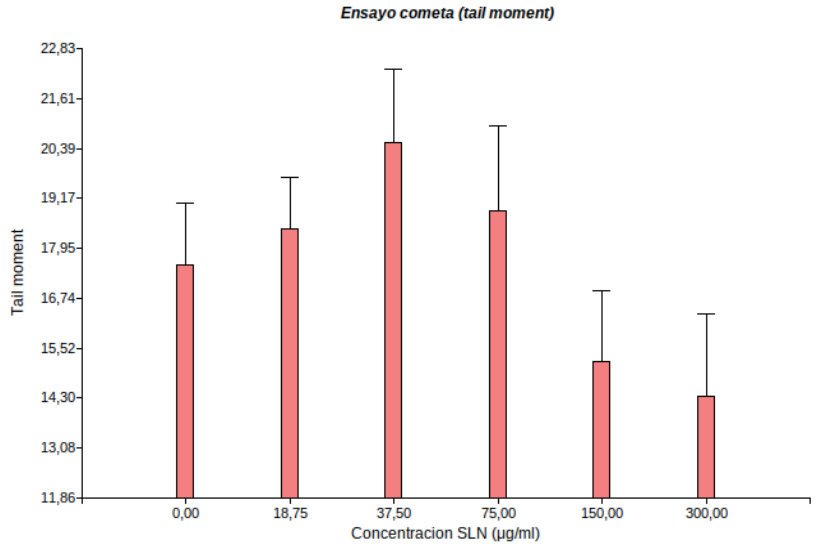

Figure 3. Tail moment in lymphocytes exposed to different concentrations of MM SLNs (µg/ml)

DISCUSSION

Solid lipid nanoparticles (SLNs) have gained great interest in the pharmaceutical industry as drug delivery systems, offering advantages over other nano vehicles such as emulsions, liposomes, and polymers.[18] In this study, the biocompatibility of a specific MM SLN system in human lymphocytes was evaluated by in vitro assays.

Cell viability is one of the most commonly used assays to determine the biocompatibility of SLNs.[14] In our study, no statistically significant differences in cell viability were observed after 24 hours of exposure to different concentrations of MM SLNs (18,75-300,00 µg/ml) compared to the control group. However, after 48 hours, the highest concentrations (150 and 300 µg/ml) showed a decrease in viability below 85 %, suggesting a possible time- and dose-dependent effect.

These results are consistent with those reported by Ridolfi et al.[19], who evaluated the cytotoxicity of MM SLNs in 3T3 and HaCaT cell lines. They observed viability between 70 % and 100 % after 24 hours of exposure, with a marked reduction at concentrations of 500 µg/ml.

Cell viability was above 85 % at 24 hours, so the Comet assay was performed exclusively under these experimental conditions. The results showed no statistically significant differences in DNA damage levels at any of the concentrations tested.

These results also agree with those reported by those who evaluated the genotoxicity of different formulations of cationic solid lipid nanoparticles (cSLN) in HepG2 and Caco-2 cells using the Comet assay. The authors observed that, at concentrations below 1 mg/ml, cSLNs did not induce a significant increase in DNA damage. However, an increase in genotoxicity was evident at cytotoxic concentrations, suggesting that DNA fragmentation may be

related to cell death processes rather than direct genotoxic damage.

The findings obtained in this study are also consistent with recent reviews on the genotoxicity of nanomaterials. They noted that multiple Comet assay studies have not detected significant genetic damage in cells exposed to nanoparticles at non-cytotoxic concentrations. They also pointed out that the toxicity of nanoparticles depends on their composition and the type of coating, with biocompatible formulations such as SLNs being less likely to induce DNA damage.

The thiobarbituric acid reactive substances (TBARs) assay showed no statistically significant differences between the concentrations tested, suggesting that MM SLNs do not induce increased lipid peroxidation in human lymphocytes. However, several studies have indicated lipid nanoparticles may modulate cellular oxidative status.

For example, they reported that HaCaT cells exposed to idebenone-SLNs showed almost 100 % viability at 1-10 µM concentrations. However, this was reduced to 80 % when the cells were treated with unloaded SLNs. It has been reported that cationic SLNs can induce severe oxidative stress in HepG2 cells, an effect attributed to the presence of CTAB, a cationic surfactant that can generate oxidative damage.[23] To reduce this effect, using anionic surfactants or including antioxidants in the formulation has been proposed, which could prevent the intracellular accumulation of ROS and their detrimental effects on cellular macromolecules such as DNA, proteins, and membrane lipids.

Nanoparticles can generate different biological effects depending on their ability to release ions, their catalytic activity, and their redox potential, which are linked to their potential toxicity.[24] It has been shown that silver nanoparticles, for example, can induce toxicity in a size-dependent manner due to the release of Ag+ ions, which can cause cell damage and oxidative stress, affecting mitochondrial functions and promoting inflammation. Strategies such as surface functionalization with lipid coatings have been shown to reduce the release of metal ions and thereby mitigate their toxicity.

The results suggest that MM's SLN formulation exhibits high biocompatibility with human lymphocytes. This characteristic is due to both the nature of the lipids used and the use of non-ionic surfactants (TWEEN 80), which minimize cell toxicity. However, it is essential to consider that the biocompatibility of SLNs may vary depending on their lipid composition and type of surfactant, highlighting the need for formulation-specific studies. Given the increasing development of nanomedicines, designing safe formulations for clinical application is crucial, ensuring their components are biologically non-toxic and minimize environmental impact.

CONCLUSIONS

From the analysis of the results obtained in this study, it is concluded that solid lipid nanoparticles of myristyl myristate (SLNs of MM) show high biocompatibility with eukaryotic cells in an in vitro model, at least in exposures of up to 24 hours. These findings suggest that, under the conditions evaluated, MM SLNs do not pose a significant risk for biomedical application. The primary evidence supporting this conclusion is:

Cell viability: no significant reduction in human lymphocyte viability was observed after 24 hours of exposure to concentrations of 18,75, 37,50, 75,00, 150,00, and 300,00 µg/ml compared to the control group (0,00 µg/ml). At 48 hours, 150,00 and 300,00 µg/ml concentrations decreased cell viability to below 85 %, an effect similar to that reported in previous studies with uncharged nanoparticles and non-cationic surfactants.

Genotoxicity: According to the results of the Comet assay, no significant genetic damage was detected in human lymphocytes exposed to concentrations of 18,75, 37,50, 75,00, 150,00, and 300,00 µg/ml for 24 hours. Although Garcia et al. conclude that SLNs have a low genotoxic risk, most previous studies did not use fluorescent alkaline electrophoresis (Cometa assay), a highly sensitive technique for quantifying genetic damage.

These results reinforce the feasibility of MM SLNs as potential drug delivery systems. However, future studies must assess their safety in prolonged exposures and in vivo models, considering factors such as accumulation, metabolization, and elimination of the nanoparticles in whole organisms.

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CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

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