

Preparation, characterization and evaluation of the *in vivo* trypanocidal activity of ursolic acid-loaded solid dispersion with poloxamer 407 and sodium caprate

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Ursolic acid is a promising candidate for treatment of Chagas disease; however it has low aqueous solubility and intestinal absorption, which are both limiting factors for bioavailability. Among the strategies to enhance the solubility and dissolution of lipophilic drugs, solid dispersions are growing in popularity. In this study, we employed a mixture of the surfactants poloxamer 407 with sodium caprate to produce a solid dispersion containing ursolic acid aimed at enhancing both drug dissolution and *in vivo* trypanocidal activity. Compared to the physical mixture, the solid dispersion presented higher bulk density and smaller particle size. Fourier Transform Infrared Spectroscopy results showed hydrogen bonding intermolecular interactions between drug and poloxamer 407. X-ray diffractometry experiments revealed the conversion of the drug from its crystalline form to a more soluble amorphous structure. Consequently, the solubility of ursolic acid in the solid dispersion was increased and the drug dissolved in a fast and complete manner. Taken together with the oral absorption-enhancing property of sodium caprate, these results explained the increase of the *in vivo* trypanocidal activity of ursolic acid in solid dispersion, which also proved to be safe by cytotoxicity evaluation using the LLC-MK2 cell line.

Uniterms: Ursolic acid/trypanocidal activity. Chaga's Disease/treatment. Solid dispersions/dissolution. Poloxamer 407. Sodium caprate.

O ácido ursólico é um candidato promissor para o tratamento da doença de Chagas, contudo este fármaco possui baixa solubilidade aquosa e limitada absorção intestinal, ambos os fatores limitantes da biodisponibilidade. Entre as estratégias para potencializar a solubilidade e a dissolução de fármacos lipofílicos, as dispersões sólidas estão crescendo em popularidade. Neste estudo, empregamos mistura dos tensoativos, poloxamer 407 e caprato de sódio, para produzir dispersão sólida contendo ácido ursólico, com o objetivo de aumentar tanto a dissolução do fármaco quanto a atividade tripanocida *in vivo*. Comparada à mistura física, a dispersão sólida apresentou maior densidade e menor tamanho de partícula. Os resultados da análise de espectroscopia no infravermelho com transformada de Fourier mostraram interações intermoleculares do tipo ligações de hidrogênio entre o fármaco e o poloxamer 407. Os experimentos de difratometria de raio-X revelaram a conversão do fármaco de sua forma cristalina para a forma amorfa, mais solúvel. Consequentemente, a solubilidade do ácido ursólico em dispersão sólida foi aumentada e o fármaco dissolveu-se de maneira mais rápida e completa. Em conjunto com as propriedades promotoras de absorção oral do caprato de sódio, estes resultados explicaram o aumento da atividade tripanocida *in vivo* do ácido ursólico em dispersão sólida, que também se provou segura após avaliação de citotoxicidade empregando a linhagem celular LLC-MK2.

Unitermos: Ácido ursólico/atividade tripanocida. Doença de Chagas/tratamento. Tensoativos. Dispersões sólidas/dissolução. Poloxamer 407. Caprato de sódio.

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INTRODUCTION

Chagas disease, a life-threatening pathology that affects approximately 15 million people, is caused by the parasite *Trypanosoma cruzi* (WHO, 2007). The etiological agent's life cycle involves the mandatory passage through both vertebrate and invertebrate hosts, including man and the hematophagous triatomine bug, respectively (Coura, De Castro, 2002). Because of the bug's abundance in low-income households, the parasite affects mainly the poor people living mostly in Latin America; thus Chagas disease has been broadly neglected by the government, pharmaceutical industry and the scientific community, which do not provide sufficient investment to control or eliminate the disease. The World Health Organization states that tropical neglected diseases, including Chagas disease, affect 1 billion people and traditionally rank low in international health agenda. However, it has been recently recognized that efforts to control and disappear neglected diseases can function as a route to socioeconomic development (Dobbish *et al.*, 2012; WHO, 2010).

Since the end of the 1960s, only two drugs have been used for the treatment of Chagas disease: nifurtimox (Lampit[®]) and benznidazole (LAFEPE[®]). However, despite being effective in treating the acute phase of the infection, these drugs display limited efficacy in the chronic phase of the disease and are associated with several side-effects, including hypersensitivity, dermatitis with cutaneous eruptions, depression of bone marrow and polyneuropathy (Jannin, Villa, 2007; Coura, De Castro, 2002). Therefore, it is urgent to discover new drugs to treat Chagas disease that are highly efficacious in both the acute and chronic phases and are devoid of side-effects (Saraiva *et al.*, 2011).

Ursolic acid (38-hydroxy-urs-12-en-28-oic acid), shown in Figure 1, is a naturally occurring pentacyclic triterpenoid compound that possesses important pharmacological properties including hepatoprotective (Saravanan, Viswanathan, Pugalendi, 2006), antioxidant (Ramachandran, Prasad, 2008), immunomodulatory (Raphael, Kuttan, 2003), antiglycative (Wang *et al.*, 2010), vasorelaxant (Aguirre-Crespo *et al.*, 2006) and antimutagenic (Resende *et al.*, 2006) functions. Furthermore, ursolic acid is a potential candidate for the treatment of Chagas disease due to its activity against *Trypanosoma cruzi* (Cunha *et al.*, 2006; Ferreira *et al.*, 2010).

Despite ursolic acid's high potential as a therapeutic option for Chagas disease, this drug has a very limited bioavailability due to its very low solubility in water (Liu, 2005). To overcome this disadvantage, some alternatives have been proposed, including complexation with

cyclodextrines (Li, 2009) and encapsulation in liposomes (Both *et al.*, 2002); however none of these approaches have been evaluated for the enhancement of the trypanocidal activity. In previous studies, our group incorporated ursolic acid in solid dispersion formulations, which are mixtures of lipophilic drugs and hydrophilic carriers, resulting in improvement of drug solubility and dissolution profile as a consequence of decreased particle size, improved wettability and presence of drug in the more soluble molecular or amorphous state (Eloy *et al.*, 2012a; Eloy, Marchetti, 2014; Vasconcelos, Sarmiento, Costa, 2007). In those studies, we employed the lipid mixture Gelucire 50/13 and the polymer poloxamer 407 as carriers and investigated the physicochemical aspects of the solid dispersion formation and we established the best method for the formulation preparation. Despite the *in vitro* enhancement of the trypanocidal activity of ursolic acid in solid dispersions, unpublished *in vivo* trypanocidal activity results of the formulations were not satisfactory. Moreover, ursolic acid is a class IV drug in Bioavailability Classification System (BCS) and thus it is relevant to develop new formulations containing more potent surfactants and oral absorption enhancers to increase ursolic acid bioavailability and, consequently, its trypanocidal activity.

Herein, we propose a novel formulation to enhance the *in vivo* trypanocidal activity of ursolic acid in solid dispersion containing poloxamer 407, a nonionic polymeric carrier with high surfactant and stabilizing properties already employed in solid dispersions (Goddeeris, Van den Mooter, 2008), and sodium caprate, an ionic oral absorption enhancer fatty acid (Lo, Huang, 2000), that has already been proven to enhance the bioavailability of oleanolic acid, ursolic acid isomer (Tong *et al.*, 2011). To our knowledge, this is the first paper on the association of poloxamer 407 and sodium caprate in the manufacturing of solid dispersions and also this is the first report on the evaluation of the *in vivo* trypanocidal activity of pharmaceutical formulations containing ursolic acid. In the present work, we prepared the formulation and the physical mixture, characterized them by drug content, particle size, bulk density, Fourier infrared spectroscopy, X-ray diffractometry, solubility, drug dissolution profile and we evaluated the cytotoxicity against LLC-MK2 cells and the *in vivo* trypanocidal activity.

MATERIAL AND METHODS

Compounds

Ursolic acid was purchased from IDEALFARMA. Sodium caprate was obtained from SIGMA. Poloxamer

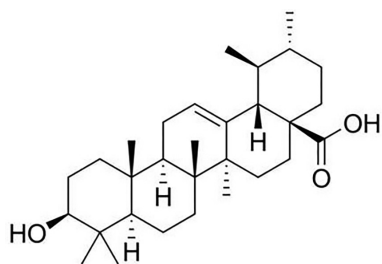


FIGURE 1 - Chemical structure of ursolic acid.

407 was a kind gift from BASF. HPLC-grade acetonitrile and methanol were purchased from MERCK.

Physical mixture and solid dispersion preparation

Solid dispersion was prepared following a solvent evaporation method previously described by our group (Eloy *et al.*, 2012a; Eloy, Marchetti, 2014). Briefly, the drug, poloxamer 407 and sodium caprate (0.2:1:1, w/w/w) were solubilized in methanol (10% w/v), followed by evaporation of the solvent at room temperature with magnetic stirring. Subsequently, the dispersion was stored in an oven at 40 °C for 24 h to allow the complete evaporation of methanol and then pulverized with a mortar and pestle. A physical mixture of the drug and carriers at the same ratio was prepared by simple mixing the drug and carriers and it was used as a formulation control for the experiments.

Particle size

The particle size of the physical mixture and solid dispersion was measured by a laser diffraction size analyzer using a Beckman Coulter LS 13 320.

Bulk density

A volumetric cylinder containing the powder was tapped (500) and the powder density was calculated based upon the weight of the sample and its volume as indicated by the volumetric cylinder before or after tapping (Goddeeris, Van Mooter, 2008).

Fourier infrared transform spectroscopy (FTIR)

FTIR experiments were performed using a Shimadzu IRPrestige-21 instrument. The samples were previously ground and mixed thoroughly with potassium bromide. The potassium bromide discs were prepared by compressing the powders in a hydraulic press. Scans were obtained at a resolution of 2 cm⁻¹ from 4000 to 400 cm⁻¹.

X-ray Diffractometry (XRD)

XRD measurements of the samples were collected on a Siemens/D5005 instrument with a copper anode operated at CuK radiation (1.5406 Å, 40 kV and 30 mA). Patterns were obtained using a step width of 0.05°/s over a range from 2° to 50° at room temperature on a 2θ scale.

Drug content

Determination of drug content in the samples was conducted using a high performance liquid chromatography (HPLC) method previously developed and validated at our laboratory (Eloy *et al.*, 2012b). The method employed a Shimadzu HPLC system consisting of a LC-10ADVP pump, a SPD-10A VP UV detector (operating at 203 nm), a Rheodyne injector and a model CR6-A integrator. An acetonitrile:water (88:12, v/v) system was used as the mobile phase at a flow rate of 1.0 mL/min with a total injection volume of 20 µL. Separation was performed on a C18 reverse-phase column (LiChrospher® (Merck), 250 x 4 mm (5 µm)) at room temperature (25 °C). For the determination, triplicate samples were weighted, diluted with acetonitrile, filtered (0.45 µm) and analyzed.

Solubility determination

The solubility in water of free ursolic acid, in a physical mixture and in solid dispersion was determined by adding excess drug (10 mg) to 10 mL of distilled water under magnetic stirring (300 rpm) at 25 °C in a temperature controlled water bath until equilibrium was achieved (24 h). Triplicate samples were then filtered (0.45 µm), suitably diluted with acetonitrile and analyzed by HPLC.

In vitro drug dissolution

Dissolution studies were performed using an SR8 Plus Hanson Corporation instrument (Chatsworth, CA, USA) by incubating a known amount of sample (equivalent to 7.5 mg drug) in 900 mL of pH 6.8 phosphate buffer (37 °C ± 0.2 °C) with mechanical stirring at 75 rpm with a paddle (Eloy *et al.*, 2012a; Eloy, Marchetti, 2014). At designated time intervals, 1-mL aliquots were withdrawn and filtered (0.45 µm), and then the drug was analyzed by HPLC, following the method already described. The withdrawn volume was replaced with the same amount of fresh dissolution medium. Dissolution was performed under sink conditions in triplicate. Statistical analyses

were carried out with the Prisma 5.0 Software (GraphPad, San Diego, California) using ANOVA with Dunnett's multiple test. Differences were considered statistically significant when $P < 0.05$.

Cytotoxicity

Samples were evaluated for cytotoxicity on LLC-MK2 fibroblasts using the MTT method (Alves *et al.*, 2012). Briefly, LLC-MK2 cells (1×10^6 /mL) were cultured in 96-well plates containing the samples at the following dilutions: 8, 32, 128 and 256 μM . Plates were incubated in a CO_2 incubator at 5% CO_2 and 37 °C for 24 h. Ten μL MTT solution (5 mg/mL) was then added to each well, and plates were incubated for 4 h. Acid isopropyl (100 μL) was then added, followed by incubation at room temperature for 1 h. The plate was read on a spectrophotometer at 595 nm. Statistical analyses were carried out with the Prisma 5.0 Software (GraphPad, San Diego, California) using ANOVA with Dunnett's multiple test.

In vivo Trypanocidal activity

To evaluate the *in vivo* trypanocidal activity, male BALB/C albino mice (weighting, 20-22 g), were infected intraperitoneally with 2×10^4 *T. cruzi* trypomastigotes forms (Y strain), following a method employed by Ferreira *et al.* 2010. The treatment was initiated 48 h after infection and maintained for 14 days. The animals were randomly divided into four groups ($n = 5$), as follows: group I—untreated control (Negative control); group II—animals infected and treated with ursolic acid in physical mixture (UA PM); group III—animals treated with ursolic acid in solid dispersion prepared with sodium caprate and poloxamer 407 (SD CAP/POL); group IV—animals treated with ursolic acid in solid dispersion prepared with poloxamer 407 (SD POL). Oral treatment was undertaken at the concentration of 20 mg/kg daily for 14 days, and the parasitemia was evaluated by counting the number of trypomastigote forms of the parasite per 5 μL of fresh blood, following the method described by Brener, 1962, with blood collected from the animal's tail, starting at the second day of infection. The groups were subjected to their respective treatments in parallel. Statistical analyses were carried out with the Prisma 5.0 Software (GraphPad, San Diego, California) using ANOVA with Dunnett's multiple test. Differences were considered statistically significant when $P < 0.05$. Experiments were approved by the committee on ethics of the College of Pharmaceutical Sciences of Ribeirão Preto (Protocol # 11.1.1029.53.6).

RESULTS AND DISCUSSION

Formulation preparation and characterization

Solid dispersions have been prepared previously by our group using the solvent evaporation and the fusion techniques and the former method was shown to be more suitable, producing particles with increased drug dissolution (Eloy, Marchetti, 2014). For this reason, in the present study we employed the solvent evaporation method for the production of solid dispersion with poloxamer 407 and sodium caprate and this procedure proved to be adequate for yielding a homogenous powder with no appreciable losses, as demonstrated by drug a content value equivalent to $101,82 \pm 0,23$ (%) of the drug amount added to the formulation; this result is comparable to those commonly observed for solid dispersions prepared using solvent evaporation methods, which have been extensively studied and are advantageous because they are inexpensive and industrially feasible (Vasconcelos, Sarmiento, Costa, 2007; Frizon *et al.*, 2013). The particle size of the solid dispersion powder compared the physical mixture was smaller, 64.2 and 110.4 (μm), respectively, which may positively influence the drug solubility, due to increased surface area (Dhirendra *et al.*, 2009). Moreover, the bulk density of the solid dispersion product revealed to be higher than the physical mixture, 0.67 compared to 0.31 g/cm^3 , respectively, which could represent an advantage for the incorporation of the solid dispersion powder in a tablet or a capsule because of the lower volume occupied.

Infrared spectroscopy is usually employed to determine the chemical structure of molecules by detecting the vibration of functional groups in the structure of the analyzed compound and is useful for detecting the occurrence of intermolecular interactions, which cause changes in the peak positions in the spectrum. FTIR spectra provided in Figure 2 show a reduced and broadened ursolic acid carbonyl peak at around 1700 cm^{-1} , highlighted in the figure, for the solid dispersion compared to the sharp peak observed for the pure drug or physical mixture. This difference provides strong evidence of solid dispersion formation (Verheyen *et al.*, 2002). These observations are explained by the fact that the carbonyl group, which is present in the ursolic acid structure (Figure 1), is capable of forming hydrogen bonding with hydroxyls of poloxamer 407 when in a solid dispersion, causing the disappearance or reduction in the intensity of the carbonyl group infrared peak, as has already been observed with solid dispersions formed between poloxamer 407 and ursolic acid (Eloy *et al.*, 2012a; Eloy, Marchetti, 2014) and with ibuprofen (Ali, Willians, Rawlinson, 2010).

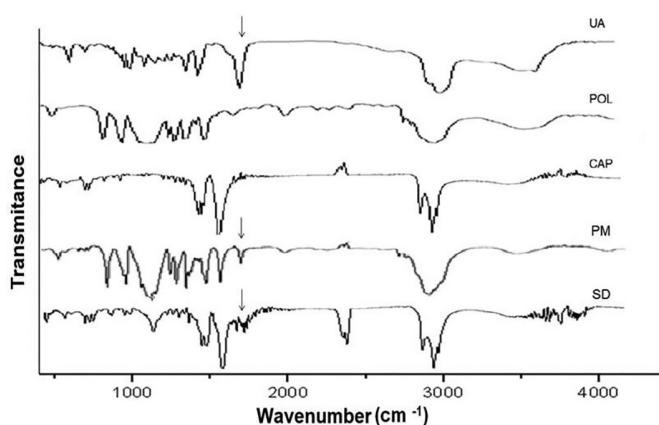


FIGURE 2 - FTIR spectra of ursolic acid (UA), poloxamer 407 (POL), sodium caprate (CAP), physical mixture (PM) and solid dispersion (SD). Arrows indicate the carbonyl peak of ursolic acid.

X-ray diffractometry is another important characterization technique in the study of solid dispersions, because it reveals the physicochemical state of the drug; crystalline structures are associated with defined peaks in the spectrum, whereas amorphous structures do not have diffraction peaks. XRD results, shown in Figure 3, argue for the formation of solid dispersion, due to the reduced intensity of the ursolic acid crystalline peaks present at 6, 8 and 15°, evidenced in the figure, in the solid dispersion spectrum, compared to the pure drug and

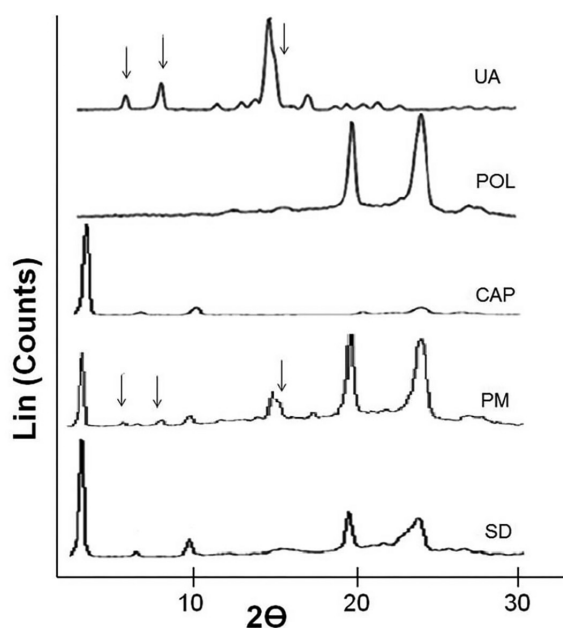


FIGURE 3 - X-ray diffractometry of ursolic acid (UA), poloxamer 407 (POL), sodium caprate (CAP), physical mixture (PM) and solid dispersions (SD). Arrows indicate the diffraction peaks of ursolic acid.

physical mixture spectra, where the same peaks remained sharp. This finding confirmed the drug conversion from its usual crystalline form to the amorphous form, which was previously observed with etoricoxib by Chauhan, Shimpi and Paradkar (2005) and also with ursolic acid in our previous study with other carriers (Eloy, Marchetti, 2014).

Among the factors that influence the solubility of a drug in solid dispersion, the most relevant are hydrogen-bonding intermolecular interactions, the physicochemical nature of the drug (i.e. whether the drug is crystalline or amorphous) and the particle size. The formation of hydrogen-bonding results in an intimate contact between the drug and carrier with consequent improvement of drug solubility influenced by the hydrophilic nature of the polymer, whereas the conversion from the organized crystalline to the disorganized amorphous form means a physicochemical more favorable solubilization process because there is no crystal lattice to be broken in the drug dissolution (Taylor, Sogرافي, 1997). Taken together with the reduced particle size, these factors explained the enhancement in the drug aqueous solubility from 450.09 ± 15.98 to 978.98 ± 110.63 $\mu\text{g/mL}$ for the physical mixture and solid dispersion, respectively, whereas pure ursolic acid solubility was less than 1 $\mu\text{g/mL}$. It should be emphasized that the solubility value achieved for the ursolic acid in solid dispersion described in this paper is 12 times higher than that determined for another solid dispersion prepared with the lipid mixture Gelucire 50/13 with the same drug load (Eloy *et al.*, 2012a), and considerably superior to the solubility of the drug when prepared with poloxamer 407 alone, corresponding to 689.47 $\mu\text{g/mL}$ (Eloy, Marchetti, 2014). The results demonstrate that poloxamer 407 and sodium caprate are a better surfactant carrier system for ursolic acid. Regarding the poloxamer 407 and sodium caprate ratio in the formulation, we prepared other solid dispersion formulations with more sodium caprate, however no further improvement of ursolic acid solubility could be found (data not shown).

In contrast to the physical mixture, the dissolution of ursolic acid from the solid dispersion shown in Figure 4 was complete and fast, equivalent to 100% of the total amount of drug after 15 minutes; this rate hypothetically allows a prompt therapeutic response after administration, which is desirable for the oral treatment of Chagas disease. Because the drug is a weak acid (pKa 5.29) (Du, Chen, 2009), its dissolution is favored in the pH 6.8 phosphate buffer medium, in which the more soluble ursolic acid's ionic form is predominant. Therefore, it is expected that drug will be preferably dissolved in the intestinal lumen (pH 6.8). Better dissolution rate is of paramount

importance to enhance lipophilic drug bioavailability, making it possible to reduce the effective therapeutic dose of the drug, possibly decreasing side effects (Vasconcelos, Sarmiento, Costa, 2007). Improved wettability of particles with decreased size and micellar solubilization caused by self-assembling in the dissolution medium of the amphiphiles, as discussed in the paper by Jones, Leroux (1999), are the most relevant factors that influence the faster dissolution. In this study, the micellar solubilization of the lipophilic drug is certainly caused by the presence of the surfactants poloxamer 407 and sodium caprate.

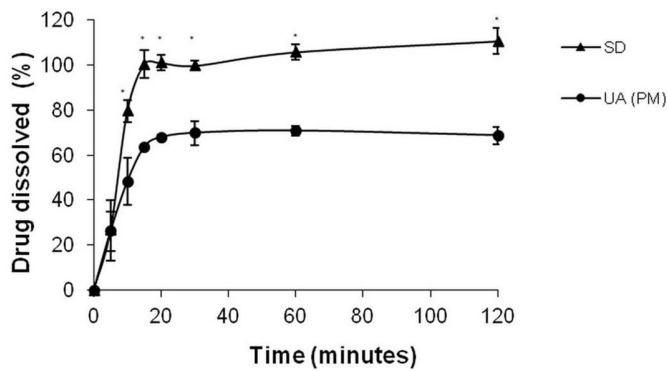


FIGURE 4 - Dissolution of ursolic acid in physical mixture (UA PM) and in solid dispersion (SD) in a phosphate-buffered medium at 37 °C and 75 rpm. *P<0.05.

Cytotoxicity and *in vivo* Trypanocidal activity evaluation

The results in Figure 5 show that the physical mixture and solid dispersion are not cytotoxic to Rhesus monkey kidney epithelial cells (LLC-MK2) even at the highest concentration analyzed, 256 μM, suggesting the safety of the formulations for the mammalian cell model studied. This result is in agreement with other reports on the safety of ursolic acid, which has not been shown to cause acute toxicity as measured by LD50 assays and biochemical parameters (Ferreira *et al.*, 2010). Moreover, our cytotoxicity results based on the MTT assay demonstrated the safety of the surfactant poloxamer 407 used in the formulations up to a concentration of 256 μM. Sodium caprate, naturally found in milk, is an FDA approved food additive and it is regarded as highly safe and has already been used in the formulation of solid dispersions (Maher, 2009; Tong *et al.*, 2011). In our evaluation, 100% cell viability was maintained up to 128 μM.

The therapeutic potential of solid dispersions containing ursolic acid as an option for the treatment of Chagas disease was demonstrated using an *in vivo* trypanocidal activity assay (Figure 06). In this assay,

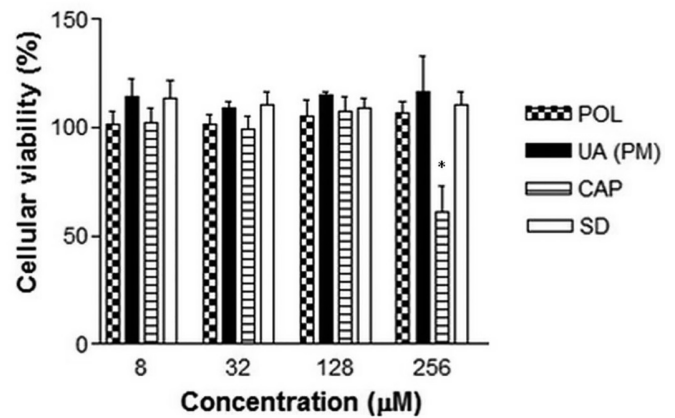


FIGURE 5 - Cellular viability of LLC-MK2 fibroblasts after incubation (24h) with different concentrations of poloxamer 407 (POL), ursolic acid in physical mixture (UA PM) and in solid dispersion (SD). *P<0.05.

the physical mixture (UA PM) showed no difference compared to the negative control (p > 0.05), whereas the drug incorporated in solid dispersion composed of poloxamer 407 and sodium caprate (SD CAP/POL), administered daily at 20 mg/kg/day, was able to induce a statistically significant reduction of the parasitemia at the parasitemic peak (p<0.05), proving the efficacy of the solid dispersion system. This result is in accordance with previous studies by our group, in which we demonstrated that the oral treatment with ursolic acid at 20 mg/kg/day, solubilized in DMSO, tween and ethanol prior to administration, was able to promote a similar decrease in parasitemia (Ferreira *et al.*, 2010). Alternatively, the solid dispersion prepared with poloxamer 407 (SD POL) did not result in reduction in parasitemia, demonstrating that the presence of a potent oral absorption enhancer, such

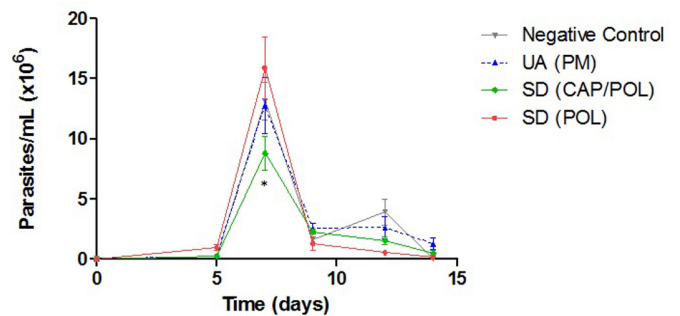


FIGURE 6 - Parasitemic curve of mice infected with 2 × 10⁴ trypomastigotes forms of the Y strain of *T. cruzi* and treated by oral route with: ursolic acid (20 mg/kg/day) in physical mixture (UA PM), in solid dispersion prepared with poloxamer 407 (SD POL), in solid dispersion prepared with poloxamer 407 and sodium caprate (SD CAP/POL) or none (Negative control). *P<0.05.

as sodium caprate, is important to enhance the *in vivo* trypanocidal activity of ursolic acid.

The use of a solid dispersion as a vehicle to deliver ursolic acid was responsible for the enhanced *in vivo* trypanocidal activity. This result can be attributed to the more soluble amorphous ursolic acid performing hydrogen bonding with poloxamer 407, to the enhanced drug release caused by the micellar solubilization of poloxamer 407 and, very importantly, to the oral absorption enhancer property of sodium caprate. There is strong evidence that ursolic acid bioavailability is also limited by the oral absorption because its isomer, oleanolic acid, belongs to class IV in the BCS and its bioavailability was enhanced by the presence of sodium caprate in the solid dispersion containing polyvinylpyrrolidone as a stabilizer (Tong *et al.*, 2011). Thus, the enhancement of drug solubility and the faster dissolution achieved with poloxamer 407 may be insufficient to increase the drug's bioavailability and the trypanocidal activity of ursolic acid. Therefore, employing a potent oral absorption enhancer such as sodium caprate in the formulation, which causes a reversible, mild and transient perturbation of both transcellular and paracellular ways in the intestinal epithelium, as discussed by Maher and collaborators (Maher, 2009), is necessary step in the exploration of ursolic acid's high potential to treat Chagas disease, as demonstrated in this paper. Future studies will be conducted at our laboratory to investigate the pharmacokinetics of ursolic acid after oral administration, not only with solid dispersions but also with other types of formulations.

CONCLUSION

This study was the first report on the incorporation of the lipophilic and antichagasic drug ursolic acid in solid dispersion prepared with the surfactants poloxamer 407 and sodium caprate. We used infrared spectroscopy and x-ray diffractometry to demonstrate that solid dispersion formation occurred through hydrogen bonding between the drug and poloxamer 407 and that the drug was converted from the crystalline to the more soluble amorphous form. Therefore, solid dispersion, presenting smaller particle size and higher bulk density compared to the physical mixture, enabled the marked enhancement of the drug's solubility and its ability to be dissolved in a complete and fast manner, unlike with a physical mixture. The formulation was shown to be safe using a cytotoxicity evaluation with LLC-MK2 mammalian cell line and enhanced the *in vivo* trypanocidal activity of ursolic acid, a consequence of both the potent surfactant poloxamer 407 and the oral absorption enhancer sodium caprate.

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