Communication

Contribution to the improvement of an oral formulation of niclosamide, an antihelmintic drug candidate for repurposing in SARS-CoV-2 and other viruses

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ABSTRACT

Niclosamide (NCL) is an effective anthelmintic agent that has been shown to possess broad-spectrum antiviral activity, including against SARS-CoV-2. Due to its poor solubility in aqueous medium, however, the commercially available NCL formulations can act only locally in gastrointestinal worms and are not suitable to achieve plasmatic levels to treat systemic diseases. Consequently, the repurposing of this drug represents a challenge for formulation development with serious risks to the biological availability and can compromise preclinical and clinical outcomes. Herein, we report possible formulation, through the research and development, of stable amorphous solid dispersions to improve its solubility. The results of exploratory screening of NCL-polymer dispersions (performed through X-ray powder diffraction and kinetic solubility studies) indicate that soluplus-niclosamide dispersions can increase its aqueous solubility and, consequently, have the potential to enhance NCL bioavailability. This outcome can be used for the development of oral dosage forms for clinical trials in SARS-CoV-2 and other viruses.

INTRODUCTION

Niclosamide [N-(2′-chloro-4′-nitrophenyl)-5-chlorosalicylamide] (Figure 1) is an anthelmintic drug developed to treat tapeworm infections in early 1960´s[1] and it is listed in the WHO list of essential medicines. Niclosamide has been evaluated in clinical studies for cancer treatment and, also several *in vitro* studies indicate that NCL has great potential to act as a broad-spectrum viral replication inhibitor, including major infections such as zika, hepatitis C, adenovirus, chikungunya, dengue, among others [2–5]. Recently it has been identified as a potential candidate to treat SARS-CoV-2 infections[5,6], which can be a solution in part to the ongoing pandemic. However, NCL is classified according to the Biopharmaceutical Classification System as class II showing very limited oral bioavailability due to its poor solubility in water (5-8 mg/L)[7,8]. Consequently, its action is local in the intestine and does not reach suitable plasma levels for systemic therapeutic applications. Most of experiments showing *in vitro* activity are conducted by using DMSO solutions, which obviously cannot be used for systemic use in humans. Barbosa and collaborators (2019) reinforce that one of the biggest challenges for the use of niclosamide in systemic therapies (e.g. tumors or infections) is its low solubility in water, since its crystalline structure and high lipophilicity affect oral absorption[9]. Thus, these undesirable characteristics can compromise its clinical efficacy, since they result in the need for administration of high doses to patients, which causes unwanted adverse effects such as, for example, colitis, diarrhea and gastrointestinal irritation, in addition to not reaching levels plasma levels for the desired therapeutic activity.

Some alternatives have been reported to advance in the state of the art and improve the solubility of niclosamide; however, the majority have clear limitations for the production of tablets and capsules for mass administration, due to the great challenge that this drug represents. In the present communication, we report the result of the screening of orally approved pharmaceutical polymers to form a relatively stable amorphous solid dispersion, which shows potential use in the reformulation of this drug in solid dosage forms aiming to improve its solubility and, consequently, its oral bioavailability.

MATERIAL AND METHODS

Materials

Niclosamide (purity >99%) was obtained from Cayman Chemical (USA). Polymers tested were poloxamers Kolliphor ® P188 and Kolliphor ® P407 (polyethylene-propylene glycol copolymers) and Soluplus ® (polyvinyl caprolactam-polyvinyl acetate-polyethylene glycol graft copolymer), obtained from BASF (Brazil); Plasdone™ S-630 (poly(1-vinylpyrrolidone-co-vinyl acetate)) and Klucel™ (hydroxypropyl cellulose), obtained from Ashland (Brazil); Povacoat ® type F (polyvinyl alcohol/acrylic acid/methyl methacrylate copolymer) obtained from Daido Chemical Co. (Japan); hydroxypropylmethyl cellulose phtalate (HPMCP) derivatives HPMCP 50, HPMCP 55 and HPMCP 55S, obtained from Shin-Etsu (Japan). Ethanol and acetone (Sigma Aldrich) were used as solvents. All samples were used as acquired.

Preparation of the solid dispersions

Drug-polymer solutions were prepared varying w/w proportion, to identify the highest possible drug concentration in the solid dispersion. Drug-polymer proportions in the mixtures were 1:3, 1:1 and 3:1, for a total of 400 mg of formulation, which were dissolved in 50 mL of an ethanol:acetone mixture (50:50 w/w). Solutions were rotary evaporated in a Buchi Rotavapor® R-215 equipment. Bath temperature was set to 50 ºC, and evaporation process lasted 1 hour. Samples were dried at room temperature (25 ºC).

X-Ray Powder Diffraction (XRPD) characterization

The crystallinity of the solid dispersions was evaluated by XRPD. Diffraction measurements were performed in a Bruker difractometer (D8 Advance), using CuKα radiation (λ = 1.5418 Å). Scanning was performed in the 2-40º range (2Θ), with a step size of 0.02º (2Θ) and a 1 sec/step scanning speed. Data were collected in reflection mode.

Accelerated stability study

Amorphous solid dispersions were exposure to 40 ºC/75% RH conditions for 5 days. Formulations were disposed in watch glass without covering it. Afterward, the samples were then evaluated by XRPD.

Kinetic solubility studies

Kinetic solubility exploratory studies of the most stable dispersions were performed by using a spectrophotometer (SI Photonics, USA) with a 0.5 cm probe in 40 mL of phosphate buffer (pH 6.8) with 1% SDS at temperature of 37 ° C +/- 2 under magnetic stirrer with a rotation of 300 rpm.

RESULTS AND DISCUSSION

The polymers plasdone^{TM} S-630 (copovidone) and soluplus showed an excellent and equivalent capacity to amorphize niclosamide; this fact was confirmed by two techniques: DSC (not shown) and XRPD (Figure 2). Thus, both were expected to have the same ability to promote an increased the solubility of niclosamide compared to other polymers. Unexpectedly, the solid dispersion with soluplus® provided the best result, much higher than the dispersion with plasdone, reaching an average concentration of niclosamide of 26 µg / mL. This value represents approximately a 73% increase compared to pure crystalline niclosamide (15 µg/mL) (Figure 3) and a 100% increase in comparison with the most soluble anhydrous form reported in literature (13 µg/mL)[10]. The

improvement observed was also superior to the increase in solubility promoted by the simple physical mixture between niclosamide and soluplus®, proving that this unexpected positive effect occurred not only due to the ability of polymer to solubilize, but also due to amorphization. The 3:1 proportion tested crystallized (data not shown).

CONCLUSION

The amorphous solid dispersions based on niclosamide-soluplus combinations have the potential for the development of oral solid dosage forms with improved solubility properties. Further improvement in niclosamide solubility is still necessary; however, this outcome can contribute to reducing the development time of improved oral dosage forms for preclinical and clinical trials in SARS-CoV-2 and other viruses.

ACKNOWLEDGEMENT

The authors acknowledge the São Paulo Research Foundation (FAPESP) for financial support [grant #2018/00273-0, São Paulo Research Foundation (FAPESP), SP, Brazil]. This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) - Finance Code 001.

CAPTIONS FOR FIGURES

Figure 1 – Molecular structure of niclosamide

Figure 2. X-ray powder diffraction patterns of 1:3 niclosamide:Polymer dispersions after stability studies at 40 °C/ 75%RH for five days.

Figure 3. Kinetic solubility of pure crystalline niclosamide and niclosamide-polymers amorphous dispersions (1:1) in phosphate buffer (pH 6.8) containing 1.0% SDS at 37±0.5 °C.

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