International Journal of Pharmaceutics 528 (2017) 202–214

Contents lists available at ScienceDirect

International Journal of Pharmaceutics

journal homepage: www.elsevier.com/locate/ijpharm

Preparation, characterization, and cytotoxicity studies of niclosamide loaded mesoporous drug delivery systems

Vishwas Pardhi[a], Rahul B Chavan[a], Rajesh Thipparaboina[a], Sowjanya Thatikonda[b], VGM Naidu[b], Nalini R Shastri[a,*]

[a] Solid State Pharmaceutical Research Group (SSPRG), Department of Pharmaceutics, National Institute of Pharmaceutical Education and Research (NIPER), Hyderabad, 500037, India
[b] Department of Pharmacology, National Institute of Pharmaceutical Education and Research (NIPER), Hyderabad, 500037, India

ARTICLE INFO

Article history:
Received 14 March 2017
Received in revised form 1 June 2017
Accepted 2 June 2017
Available online 5 June 2017

Keywords:
Silica
Dissolution
Cancer
Repurposed
Porosity

ABSTRACT

Recent reports on the anticancer potential of niclosamide have opened new avenues for anticancer treatment. Niclosamide belongs to the BCS class II, which is indicative of poor solubility and dissolution rate limited absorption. The aim of this study was to improve the dissolution rate of the drug by mesoporous drug delivery system. Porous silica grades (ordered and nonordered) with different pore size, pore volume and surface area were used in the study. The drug was loaded on silica carriers by the solvent evaporation method and characterized by BET surface area analysis, SEM, P-XRD, DSC, and FTIR. A discriminatory dissolution medium was developed for performing the in vitro dissolution of niclosamide. In comparison to the plain drug, all silica based formulations showed improvement in the dissolution rate. Maximum enhancement in the dissolution rate was observed in 1:2 drug:carrier loading ratio when compared to 1:1 ratio. Different properties of mesoporous silica like structural geometry, pore size and microenvironment pH demonstrated a significant impact on drug release from the formulations. Cytotoxicity of the optimized mesoporous formulations of niclosamide was explored in HCT-116, HCT-15, NCI, MDA-MB-231 and A549 cancer cell lines. Nearly 3 fold and 2 fold increase in% cytotoxicity of drug loaded Syloid-244 and Sylysia 350 at 1:2 ratio respectively, were observed when compared to the plain drug.

1. Introduction

Absorption of drugs across the gastrointestinal tract is limited by a number of factors such as adequate solubility and appropriate stability in the gastrointestinal fluids, reasonable intestinal permeability, absence of pre-systemic metabolism etc. During past few decades, the number of new chemical entities (NCE) with poor aqueous solubility has increased enormously. Nearly 90% of the NCEs exhibit low solubility in water, resulting in poor oral bioavailability, lack of dose proportionality and high intra/inter-subject variability. As a result, these compounds tend to display dissolution rate limited absorption (Loftsson and Brewster, 2010; Porter et al., 2008). For improving the dissolution rate, modification of physicochemical properties is a very common approach which includes particle size reduction and salt formation. However, these approaches have their own limitations. The salt formation of neutral compounds is not possible and the synthesis of salts of the weak acid and weak base may not always be feasible. Besides, the salts that are formed may revert back to their original acid or base forms probably leading to aggregation in the gastrointestinal tract (Gursoy and Benita, 2004). To overcome these limitations, various formulation strategies such as complexation (Stella and Rajewski, 1997), lipid based systems, nanocrystals (Shete and Bansal, 2016), amorphous solid dispersions (Kaushal et al., 2004), co amorphous systems (Chavan et al., 2016) and permeation enhancers have been adopted with a considerable degree of success. In recent years, mesoporous drug carriers to improve the oral bioavailability of poorly water soluble drug compounds have gained importance. The adsorption of drug on the

* Corresponding author at: Department of Pharmaceutics, National Institute of Pharmaceutical Education & Research (NIPER), Balanagar, Hyderabad, 500037, India.
E-mail addresses: nalini@niperhyd.ac.in, svcphod@yahoo.co.in, nalini.niperhyd@gov.in (N.R. Shastri).

http://dx.doi.org/10.1016/j.ijpharm.2017.06.007
0378-5173/

silica materials to improve dissolution of a poorly soluble drug is a viable and an attractive strategy. Salient features of the carrier materials such as large surface area, tunable pore volume, controllable structural and textural parameters, and presence of large number of silanol group on the surface which act as a potential site for functionalization in order to control pore size and surface properties, make this delivery system a potential approach to address the issues of poorly soluble drugs (Chavan et al., 2015; Unger et al., 1983).

This study aims towards exploring mesoporous drug delivery system for improving the dissolution rate of a 'brick dust' like antihelminthic drug niclosamide, which was recently repurposed for its activity against colon cancer, lung cancer, prostate cancer etc. in various clinical studies (Li et al., 2014). Niclosamide acts by inhibiting the Wnt/β-catenin, mTORC1, STAT3, NF-κB and Notch signaling pathways. In addition, it targets the mitochondria of cancer cells to induce cell cycle arrest, growth inhibition and apoptosis (Li et al., 2014). To-date very few formulation attempts have been reported to improve the solubility and bioavailability of niclosamide for its repurposed activity. Nanocrystals and submicron emulsions were explored, but due to the insoluble nature of the drug, only minor enhancements in solubility and bioavailability of the drug was observed by these approaches (Ye et al., 2015; Zhang et al., 2015). Existing dissolution medium for niclosamide (Sanphui et al., 2012) comprising of isopropyl alcohol (IPA):water (40% v/v) is unable to discriminate the dissolution profiles, since this media due to the presence of IPA extracts the drug from the carrier and fails to provide reliable results. Hence, an attempt has been made to develop a discriminatory dissolution medium to compare dissolution profiles obtained from various mesoporous formulation. A wide range of mesoporous carriers which were representative of different properties such as orderedness or disorderedness of pore, different pore sizes, surface areas, and porosities were selected to study the role of the mesoporous carriers on drug release. Anticancer activity of the plain drug and drug loaded mesoporous carriers were explored in prostate, colon and lung cancer cell lines.

2. Material and methods

2.1. Materials

Niclosamide, SBA-15 and MCM-41 were purchased from Sigma Aldrich (St. Louis, Missouri, United States). Aeroperl-300 and Aerosil 200 were supplied by Evonik Industries (Essen, Germany) as gift samples. Syloid-244 was received as a gift sample from Grace Pharmaceuticals (Columbia, MD, USA). Sylysia-350 and Sylysia-770 were gift samples from Fuji Sylysia Chemical Limited (Kasugai Aichi, Japan). Neucilin-US2 and Fugicalin-SG were kindly gifted by Fuji Chemicals Industries co., Ltd, Japan. Tween 80 (Polysorbate 80) was purchased from SD Fine Chemicals Limited (Vadodara, India). HPLC (High Performance Liquid Chromatography) grade acetonitrile (ACN) and methanol were purchased from Merck, India. All other chemicals used were of analytical grade. In-house generated distilled water was used in all experiments. Amber colored glassware was used for all experiments and storage. All experiments were conducted in dark conditions.

2.2. Selection of mesoporous silica carriers

Carriers with a varying pore size (2–37 nm), pore volume (0.4–1.8 cm^3/g), particle size (0.012–150 μm) and specific surface area (40–1000 m^2/g) were selected for this study. Additionally, these carriers selected also differed in their structural geometry such as ordered (SBA-15, MCM-41), nonordered (Aeroperl-300, Syloid-244, Sylysia, Neucilin-US2 and Fugicalin) and nonporous silica (Aerosil-200). Properties of the selected mesoporous carriers are depicted in supplementary table S1.

2.3. Dissolution media development

2.3.1. Solubility study

The equilibrium solubility studies of niclosamide in different media (0.1 N HCl, 6.8 pH phosphate buffer, 7.4 pH phosphate buffer, water, 7.4 pH phosphate buffer with 2% Tween 80) were carried out by adding an excess amount of the niclosamide to 3 mL of media in 5 mL screw capped amber colored glass vials. These vials were then shaken mechanically in an orbital shaking incubator (Lab Companion Model SI-300) at 100 rpm maintained at 37 °C. After an equilibration period of 48 h, these samples were centrifuged at 10,000 rpm for 10 min to separate the excess drug. The supernatant of the samples was analyzed by UV–vis spectrophotometer (V-650, Jasco, India) at 333 nm after appropriate dilutions in methanol for determining the concentration of niclosamide. Based on the solubility study, phosphate buffer pH 7.4 with 2% Tween 80 was selected for further studies. The linearity parameters for the developed analytical method are reported in supplementary table S2.

2.3.2. Stability study of drug in selected dissolution media

Niclosamide solution 1 μg/mL was prepared in the selected dissolution media by serial dilution from the methanolic stock solution of 1000 μg/mL. These samples were then analyzed at different time points by HPLC (Waters 2695 HPLC system, Milan, Italy, equipped with a degasser, an autosampler, and a 2998 PDA detector) using water:ACN (20:80 v/v) as mobile phase. The injection volume of 20 μL was analyzed at 333 nm wavelength to assess the stability of the niclosamide in the selected dissolution media. All experiments were performed in triplicates.

2.4. Drug loading in silica carriers

Solvent evaporation method was used for loading niclosamide onto mesoporous carriers. Niclosamide was dissolved in acetone (10 mg/mL) and silica carriers were soaked in this drug solution. This dispersion was subjected to stirring for 12 h in a well closed container. After stirring, the solvent was removed under reduced pressure using a rotary evaporator (Hei-VAP Advantage; Heidolph, Schwabach, Germany) at 45 °C. The product obtained was again dried in a tray dryer (Labfit India Pvt. Ltd, India) at 45 °C for 24 h. The dried products were labeled and stored in well closed amber colored containers in desiccators. Two different ratios of drug:carriers (1:1 and 1:2) were prepared to study and optimize drug loading and dissolution enhancement efficiency.

2.5. Determination of drug loading

Drug loaded mesoporous formulation (equivalent to 5 mg of niclosamide plain drug) was suspended in 10 mL of methanol, vortexed for 30 min and then sonicated for 10 min. The mesoporous silica residue was separated by centrifugation of the suspension at 15000 rpm for 10 min. The concentration of niclosamide was determined by analyzing the supernatant by UV–vis spectrophotometer at 333 nm after suitable dilutions with methanol. The percentage drug loading was calculated by using the following formula,

$$\%\ \ drug\ \ loading = \frac{Amount\ \ of\ \ drug\ \ in\ \ formulation}{Amount\ \ of\ \ formulation\ \ \ taken} * 100$$