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ARTICLE

Development of a brazilian nanoencapsulated drug for schistosomiasis treatment

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ABSTRACT

Schistosomiasis is a parasitic disease that, according to the World Health Organization, constitutes a major public health problem associated with severe morbidity, mostly children in preschool age. The administration of drugs in children always constitutes a difficult task, especially when formulations are not developed specifically for pediatric use, when high doses of drug are required and the drug has a bitter taste, as in the case of praziquantel. Polymer nanoparticles are promising systems for development of encapsulated drugs with low water solubility and bitter taste, due to the good physical and chemical stability, adequate biocompatibility and simple manufacturing processes. Moreover, they can enhance the bioavailability and reduce variability of treatment among patients. Poly (methyl methacrylate) doped with praziquantel was produced through a miniemulsion polymerization process to compose a pediatric pharmaceutical suspension. Nanoparticles were characterized in terms of physico-chemical properties, toxicological properties and biological activity in mice, being concluded that obtained results were satisfactory. The results were encapsulation rate around 90%, absence of chemical interaction drug - polymer and the presence of biological activity. A collaborative approach was used for this development, involving national partnerships and independent funding mechanisms, a powerful pathway for development of drugs for neglected diseases.

KEYWORDS: Nanoparticles; Praziquantel; Miniemulsion Polymerization; Development Partnerships; Schistosomiasis

Introduction

In many countries including Brazil, drug development has been growing steadily and gaining space on the government agenda, and this trend is justified by the increase in technological development in Brazil, among other factors[1].

At present, the 10 largest pharmaceutical companies account for approximately 40.4% of the global market, and approximately 75% of this production is directed to the United States, Japan, Germany, France, Italy, and the UK. Thus, investments in research and development (R&D) are primarily focused on chronic diseases, depression, and other diseases common to these populations[1,2,3]. Therefore, adequate drugs for the treatment of the so-called neglected diseases have not been developed (or are developed rather slowly) because of the lack of interest of the major pharmaceutical companies[4].

Schistosomiasis is a neglected parasitic disease and a major public health concern in developing countries. Brazil is the most affected country in the American continent[5,6,7,8]. According to the Unified Public Health System [Sistema Único de Saúde (SUS)], 64,811 new cases and 524 deaths caused by schistosomiasis were registered in Brazil in 2011[9]. This disease is present in 74 countries in Africa, Asia, and South America, and millions of people are infected every year[8,10,11,12]. According to the World Health Organization (WHO), in tropical and subtropical areas, schistosomiasis is the second most important disease from a socio-economic and public health perspective, surpassed only by malaria. Thus, the use of chemotherapy is warranted to implement an efficient transmission control method, in addition to adequate sanitary measures[10].

Praziquantel (PZQ) is a broad-spectrum anthelmintic that is recommended by the WHO for the oral treatment of schistosomiasis[10]. This drug has low solubility in water and a very bitter taste[12]. The current treatment comprises daily oral intake of doses of 40–60 mg/kg[13]. At present, PZQ is marketed in the form of tablets with doses of 150, 500, and 600 mg. The target population for treatment is generally children who are particularly vulnerable to schistosomiasis. When infected during school age, children are often psychologically and intellectually impaired[14]. The current pediatric treatment has been adapted from the adult formulation and is not entirely adequate because the suspension formulations may not have the appropriate organoleptic characteristics and the dosage may also be inadequate on account of breaking of the tablets[15]. Furthermore, whenever children are the target population, medications must be palatable to improve treatment adherence[16].

The only specific liquid formulation of PZQ for pediatric use that has been cited in the literature is Epiquantel (120 mg/mL suspension), which is produced by the Egyptian International Pharmaceutical Industries Co. (EIPICO)[17]. The formulation is sold in 15 mL vials with a shelf life of 3 years at room temperature, although it is not distributed by SUS in Brazil or by the WHO in the rest of the world[18,19]. Moreover, data on product taste have not been reported in the literature for unknown reasons. Therefore, a pediatric formulation of PZQ with good acceptance and adequate stability is still lacking, perhaps because of the difficulty in masking its extremely bitter taste.

Drug development comprises several steps including survey of active molecules, development of the formulation, testing of safety and effectiveness, and registration of the product. It is estimated that approximately 40% of new drugs fail during the development process because of poor bioavailability, generally associated with low solubility in water[20,21]. This is one of the biggest challenges that the pharmaceutical industry has faced in recent times with regard to the formulation stage. Thus, several alternatives have been considered in an attempt to increase drug solubility, including preparation of microemulsions, encapsulation in liposomes, formation of salts, reduction of the size of solid particles, formation of solid dispersions, complexation, and encapsulation in polymeric nanoparticles.

Pharmaceutical companies have been investing heavily in nanotechnology because of several reasons: potential for developing drug delivery systems, stabilization of drugs, protection of proteins and recombinant DNA against pH and light, prevention of toxicity, increased absorption rates of the drug and bioavailability, masking taste and other organoleptic characteristics, and as a tool for modulating drug release[22,23,24,25,26,27].

Collaborative networks involving universities, government institutions, and non-governmental organizations (NGOs) have played a pivotal role in research conducted by multidisciplinary teams focused on developing new products for neglected diseases[28]. This network structure allows, at least in principle, the collaboration of highly trained professionals working in the R&D environment to solve a particular problem. In this sense, the authors and institutions involved in this study have collaborated as a research team for the development of solutions for the treatment of neglected diseases.

The aim of this study is to present the results and discuss the difficulties and the importance of interdisciplinarity in the development of a nanoencapsulated drug for the treatment of schistosomiasis, with focus on product registration in Brazil.

Method

Production of nanoparticles

The poly (methyl methacrylate) (PMMA) polymer doped with encapsulated PZQ was produced by one-step miniemulsion polymerization (*in situ*)[22]. This technique has been widely used in R&D of new drugs because it is inexpensive, allows adequate control of particle size distribution, and allows the scale up. It's an important point that often overlooked by some researches grups wich could incrase in fail projects because of the difficulties in the industrialization process[29,30].

The production steps were optimized according to equipment availability and were developed in the Laboratory of Polymer Engineering (EngePol) at the Alberto Luiz Coimbra Institute for Graduate Studies and Research in Engineering

(COPPE) of the Federal University of Rio de Janeiro (UFRJ) and in the R&D laboratories at the Institute of Pharmaceutical Technology (Farmanguinhos) of the Oswaldo Cruz Foundation (Fiocruz), all located in Rio de Janeiro.

A miniemulsion was prepared by mixing an organic phase comprising the monomer, mineral oil, PZQ, Eudragit® E100, and the crosslinking agent ethylene glycol dimethacrylate (EGDMA) with an aqueous phase comprising water, sodium lauryl sulfate, and sodium bicarbonate. Both phases were mixed in a high-pressure homogenizer. The miniemulsion was then added to a jacketed reactor where the polymerization reaction was initiated by the addition of an initiator, as described by Fonseca et al.[22]. After establishing the reaction conditions, 3 reactions were performed with variations in the organic phase: NPa, comprising the monomer, mineral oil, Eudragit® E100, and crosslinker; NPb, consisting of the monomer, Eudragit® E100, and crosslinker and; NPc, comprising the monomer, mineral oil, and crosslinker.

The PMMA polymer presents good thermal performance, good physical and chemical resistance to external factors (such as light and humidity), transparency, and excellent dimensional stability. It is biocompatible but not biodegradable. PMMA was the first acrylic polymer to be used as a biomaterial and has been successfully used in a wide range of medical and pharmaceutical applications, such as in dental cement, bone prostheses, and drug coating for oral administration[31].

Characterization of nanoparticles

The nanoparticles were characterized in terms of particle size distribution by laser diffraction, morphology by scanning electron microscopy, and residual monomer content by gas chromatography (this indirectly determines the conversion of the monomer to polymer). The encapsulation efficiency, i.e., the amount of PZQ in the nanoparticles, was determined by high-performance liquid chromatography. Moreover, potential chemical interactions between the drug and the polymer matrix were assessed by infrared and thermal analysis. Furthermore, the release/dissolution profile of PZQ from the polymer matrix was investigated in the conditions of simulated gastric medium at pH 1.2 and simulated enteric medium at pH 6.8 to evaluate the release of PZQ under both extremes of the pH range of the gastrointestinal tract.

The analytical steps were optimized according to equipment availability and were performed in the same production facilities where the nanoparticles were developed. Depending on the specific instrumental demands for nanoparticle characterization, other UFRJ departments were also involved, including the School of Chemistry (EQ) and the Institute of Macromolecules Professor Eloisa Mano (IMA).

Studies of *in vitro* and *in vivo* activities

All new products under development must be subjected to efficacy and safety tests. In the present study, these tests were performed at the Laboratory of Schistosomiasis at the René Rachou Research Center (CPqRR), Fiocruz, Belo Horizonte, after approval by the Ethics Committee on Animal Use (CEUA) (License #L0118/09).

The *in vitro* activity test was performed by a direct assay using worms that had been removed from mice at 45 days after infection.

The *in vivo* studies were conducted in mice at 45 days after infection and with 100 cercariae of *Schistosoma mansoni*. The nanoencapsulated material with and without PZQ and the tablet were administered by gavage. After treatment of 78 animals, the control group was sacrificed immediately and the other groups were sacrificed 30 days after initiation of treatment.

Toxicity studies

Toxicity studies involved cytotoxicity and genotoxicity assays (comet assay) in blood cells conducted at the National Institute of Quality Control in Health (INCQS), Fiocruz, Rio de Janeiro.

Formulation development

The pharmaceutical form in the preliminary study was extemporaneous suspension formulation, which is widely used in pediatrics because of its ease of administration and dose adjustment as a function of corporal mass. Moreover, the packaging of the product in sachets favors the distribution of the drug in geographical areas that are difficult to access.

The direct mixture of the formulation with pharmaceutical grade excipients was performed at the Laboratory of Pharmaceutical Technology and other research laboratories at Farmanguinhos, Rio de Janeiro. Physical and physico-chemical tests were performed to assess product quality. Sensory analysis was performed by trained staff comprising the researchers involved in this work at 2 time periods: immediately after product reconstitution and 10 min after reconstitution. The samples were not swallowed and were expectorated into specific containers. The participants rinsed their mouth with water after each tasting.

Results and discussion

Technical aspects

Several reactions were performed according to an appropriate experimental design. Particle size ranged from 60 to 105 nm, with a unimodal and narrow distribution (the polydispersity index was <1.1). The conversion from monomer to polymer was greater than 96%, with negligible residual monomer levels (<1 ppm) after lyophilization. The efficiency of encapsulation was approximately 90%. Analysis by differential scanning calorimetry and infrared indicated that PZQ formed a solution with the polymer matrix, with no chemical interaction between the drug and the polymer[22].

As shown in Figure 1, PZQ could be quickly released from the nanoparticles at pH 1.2 (as in the stomach) and pH 6.8 (as in the intestine), reaching levels comparable to those obtai-

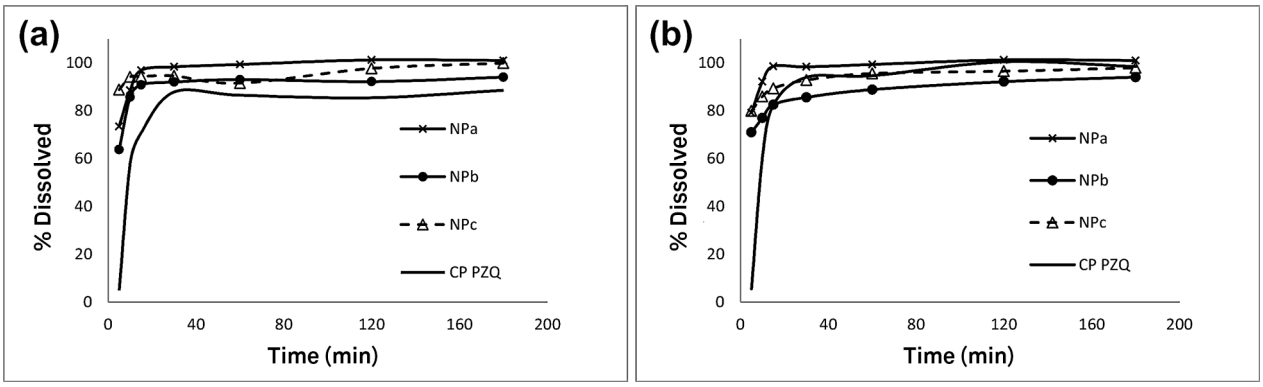


Figure 1. Results of the release of PZQ from NPs at (a) pH 1.2 and (b) pH 6.8 compared with that from CP PZQ (commercially available 600 mg PZQ tablets). Composition of the organic phase: NPa (monomer, PZQ, mineral oil, Eudragit® E100, and EGDMA); NPb (monomer, PZQ, Eudragit® E100, and EGDMA); and NPc (monomer, PZQ, mineral oil, and EGDMA). The aqueous phase in all three reactions comprised water, sodium lauryl sulfate, and sodium bicarbonate.

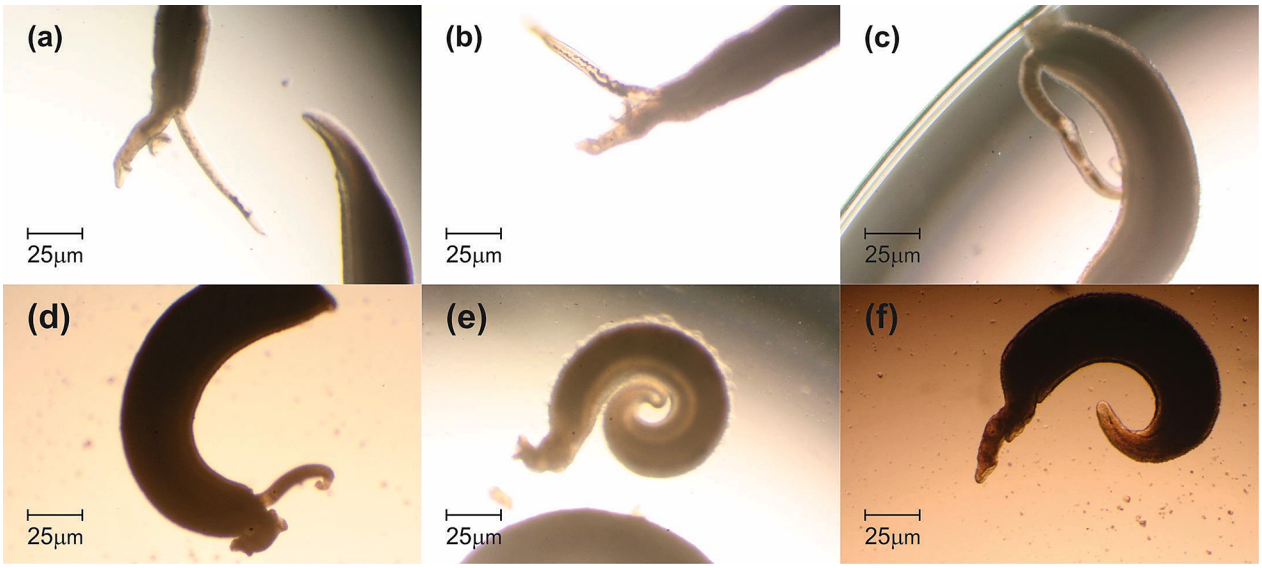
ned with 600 mg tablets of PZQ from a national manufacturer. Moreover, we did not observe any significant difference between the tested nanoparticle compositions (NPa, NPb, and NPc). These results were considered satisfactory because the medication is intended for oral use.

Figure 1. Results of the release of PZQ from NPs at (a) pH 1.2 and (b) pH 6.8 compared with that from CP PZQ (commercially available 600 mg PZQ tablets). Composition of the organic phase: NPa (monomer, PZQ, mineral oil, Eudragit® E100, and EGDMA); NPb (monomer, PZQ, Eudragit® E100, and EGDMA); and NPc (monomer, PZQ, mineral oil, and EGDMA). The aqueous phase in all three reactions comprised water, sodium lauryl sulfate, and sodium bicarbonate.

In the *in vitro* activity assays (Figure 2) contraction of the worms was observed 1 h after contact with the nanoparticles doped with PZQ. Same results were also observed in case of worms that received PZQ in the form of a tablet. In contrast, worms treated with the culture medium alone remained active and were able to lay eggs.

Figure 2. Worm morphology 1 h after treatment in a specific culture medium. (a) Control, (b) NPs (2.0 µg/mL) without PZQ, (c) NPs (1.0 µg/mL) with 600 mg PZQ tablets, (d) NPc (0.5 µg/mL), (e) NPc (1.0 µg/mL), and (f) NPc (2.0 µg/mL).

These assays lasted for 7 days and it became evident that the worms treated with samples containing PZQ did not recover their structure or motility, and most of them died. Worms that received the sample nanoparticles without PZQ showed decrease oviposition rates and hence impaired development compared with controls, and this effect of nanoparticles were important for worm control. These results corroborate the



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anthelmintic activity of PZQ even when it is encapsulated in PMMA nanoparticles.

In the *in vivo* activity assay, an extremely high mortality rate was observed and the mean number of worms recovered in the control group was 10% to 30% above normal, indicating a high infection worm burden[32,33,34]. Furthermore, the incorporation of PZQ in the nanoparticles was not an easy task because of the high solid content necessary to achieve a dose similar to tablets. Also, the gavage needle clogged several times, which may have caused fluctuations in the administered dose. This procedure will be reassessed in future studies.

The toxicity of nanoencapsulated materials depends on a number of factors, including size, concentration of drug and nanoparticles, exposure time, health status, and individual characteristics of the target organism. The mechanisms of toxicity of nanoparticles are not fully understood, which hinders the prediction of their potential effects on the health and environment. Thus, studies on the toxicity of new nanotechnology materials are highly essential[35].

In the present study, the nanoparticles did not induce a decrease in cell viability compared with controls, suggesting that the nanoparticles had no cytotoxic potential. The comet assay also demonstrated the absence of DNA damage. Overall, these results were considered satisfactory, although further studies are necessary to confirm the absence of cytotoxicity and genotoxicity.

The encapsulation process was efficient at masking the bitter taste of PZQ, considering that no bitter taste was experienced during the sensory evaluation and also because the formulation had a high content of sweeteners. However, the bitter taste was felt 10 min after dispersion of the lyophilized solid in water, indicating the rapid release of PZQ. This finding is consistent with the data presented in Figure 1, which indicates the rapid release of the drug. In the future, it should be possible to extend the release time and hence prevent the development of bitter taste in the mouth after drug exposure. Moreover, during the test, the participants felt some sandiness in the tongue, probably because of the solid content present in the formulation (25%). Sandiness may be associated with particle agglomeration during the lyophilization process and will be further explored in the near future.

Some additional steps are still necessary to effectively turn this drug formulation into a marketable pharmaceutical product. Thus, after construction of the Engepol Polymers Pilot Plant, we initiated the process for obtaining the certification for good manufacturing practices (GMP). The objective of GMP, within its scope of implementing the concept of quality assurance in the industry, will be to produce batches of encapsulated PZQ in accordance with the rules of the Brazilian Health Surveillance Agency (ANVISA), which is responsible for conducting toxicological and *in vivo* tests in animals and humans.

Figure 3. Illustrates the steps being developed for the production of the new nanoencapsulated drug for the treatment

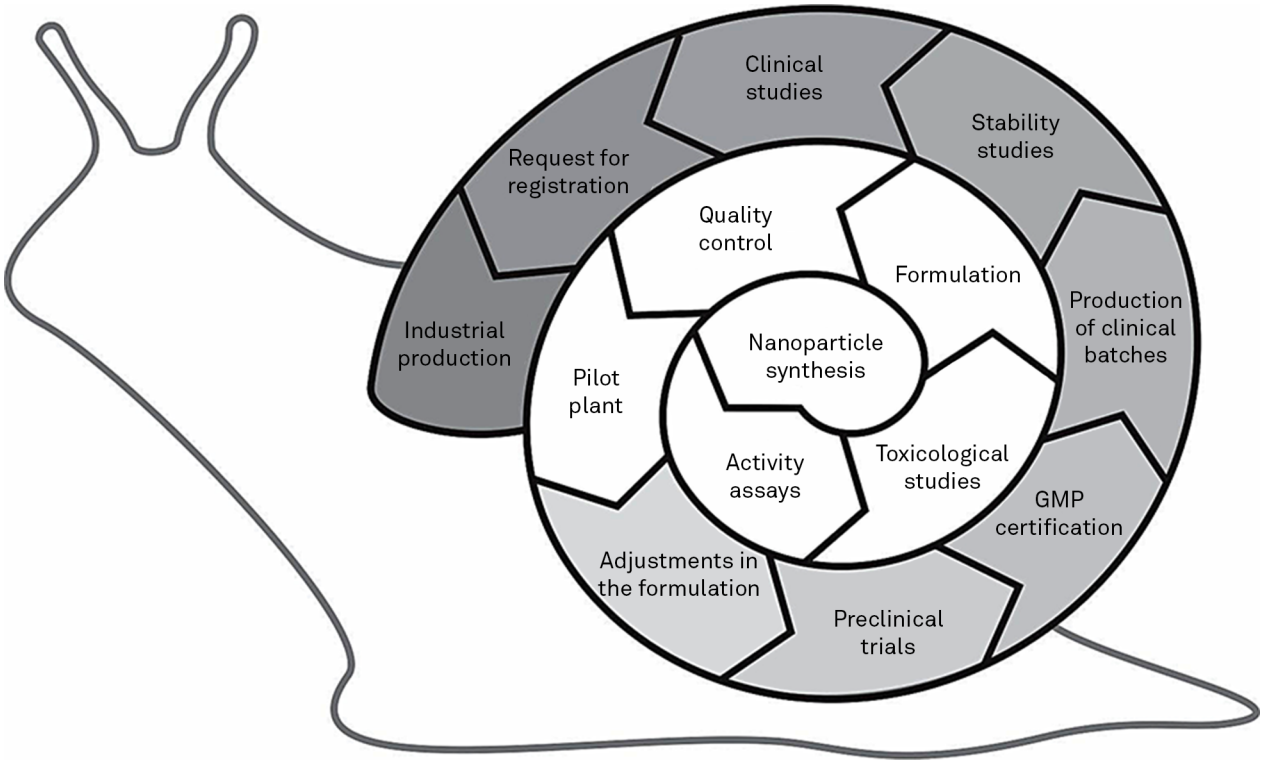

Figure 3. Steps in the development of a novel nanoencapsulated drug for the treatment of schistosomiasis. Steps that are under development are indicated with white backgrounds, whereas future steps are indicated with gray backgrounds.

of schistosomiasis (white background), as well as the steps that are yet to be performed (gray background), with the view of planning the actions necessary to create the technical dossier for the registration of the new pediatric product for the treatment of schistosomiasis.

Conclusions

In situ incorporation of PZQ in PMMA nanoparticles by miniemulsion polymerization was successful. There was an adequate control of particle size and no chemical interaction was observed between the drug and the polymer. The *in vivo* activity of the nanoparticles doped with encapsulated PZQ was comparable to that of the usual oral treatment. Moreover, these nanoparticles showed no cytotoxicity or genotoxicity. Thus, these nanoparticles are promising candidates for the development of new drug delivery systems.

It can be concluded that the developed nanoparticles have great potential to overcome the limitations associated with products currently available in the market for the treatment of schistosomiasis because of their ability to modulate drug release with the highest rate of absorption into the body and to mask the unpleasant taste of the drug, which are considered important steps for increasing the efficiency of pediatric treatments.

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