MINIREVIEW

First Century of Chagas' Disease: An Overview on Novel Approaches to Nifurtimox and Benznidazole Delivery Systems

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ABSTRACT: Hundred years after the discovery of Chagas' disease, there is a lack of effective treatment to control this neglected disease caused by the parasite *Trypanosoma cruzi*. The transmission is primarily through vector-borne blood transfusion or during pregnancy, producing high mortality and morbidity among poor people in many countries of Latin America. In the last decades, the efforts have been focused mainly on the elimination of vectors. At the same time, screening of blood donors in order to avoid transfusional transmission has been improved all over the world. However, Chagas' disease is still a major public health problem, with estimates of nearly 90 million people at risk of infection and more than eight million infected in 18 endemic countries. Despite the high incidence in endemic regions and the dissemination of neglected diseases in North America and Europe, to date, there are only two drugs developedn and prescribed for the treatment of Chagas' disease, nifurtimox (tablets of 120 mg) and benznidazole (tablets of 100 mg). In this review, different approaches carried out in the last decades for developing novel pharmaceutical formulations for the delivery of nifurtimox and benznidazole are discussed. © 2011 Wiley Periodicals, Inc. and the American Pharmacists Association J Pharm Sci 101:888–894, 2012

Keywords: Chagas' disease; nifurtimox; benznidazole; dissolution rate; microparticles; nanoparticles; liposomes; oral drug delivery

INTRODUCTION

Chagas' disease or American tripanosomiasis is a zoonosis discovered by Dr. Carlos Chagas, a Brazilian physician, who first identified the infection caused by *Trypanosoma cruzi* in 1909.[1] His valuable study included description of the entire lifecycle of the parasite, vectors, and animals and humans that act as reservoir hosts. Although the vectorial transmission has been reduced, Chagas' disease is still a major public health problem and one of the leading causes of morbidity, long-term disability, and mortality in Latin America, with estimates of nearly 90 million people at risk of infection and more than eight million infected in 18 endemic countries.[2,3] Furthermore, the number of yearly new cases due to vectorial transmission was around 42,000 people, and the infected neonates by congenital Chagas' disease per year were nearly

15,000.[4] Additional to human morbidity and mortality, this parasitic infection places a substantial burden in poorer, developing countries, increasing both the poverty and vulnerability of those people.

Chagas' disease is transmitted primarily by insects, known as "kissing bugs," through the bite wound or mucous membranes. Moreover, the dissemination of this parasitic infection may occur by transfusion of blood from persons infected with *T. cruzi* and/or organ transplantation. Also, infected pregnant women may transmit *T. cruzi* to her newborn, resulting in congenital Chagas' disease. A noncommon route of infection is through ingesting food and drink contaminated with the faeces of a triatomine carrying the parasite. The disease is characterized by three clinical forms, namely, acute, indeterminate, and chronic. Acute phase is usually a mild illness and often unrecognized, but may present symptoms of fever, hepatosplenomegaly, meningoencephalitis, and myocarditis with a case fatality rate of less than 5%. The indeterminate phase, generally asymptomatic, comprises a period that may last 10–20 years between the acute and chronic phases. Then, nearly 30% of

those infected patients will develop the chronic form of the disease, resulting in different pathologies in the gastrointestinal tract, disorders of the central and peripheral nervous system, and severe cardiac events.[5] This phase, whether indeterminate or symptomatic, is the most common form of presentation in nonendemic regions. Lately, and due to the great dissemination from endemic areas in Latin America to the United States, Canada, and several countries of the European Community, Chagas' disease has become a serious concern for the nonresident tropical population.[6] Nearly 300,000 people live with Chagas' disease in the United States, and more than 80,000 cases are being reported in Spain.[7] In North America, as recently stated, this parasitic infection is becoming a serious concern, which can have severe consequences for public health, principally because of its potential to contaminate the blood transfusion supply as well as its dissemination through surgical implantation of infected donor organs to patients in nonendemic areas.[8,9] Particularly, Chagas' disease has significant public health implications in Southern California, where there is a large immigrant population from countries where this neglected disease is endemic. Therefore, there is an urgent need to implement additional controls for blood banks and organ transplantation.[10]

CHAGAS' DISEASE CHEMOTHERAPY

Although some progress focused in the process of drug discovery have been made in recent years, the significant cost of these projects and the lack of assurances that they can make a return on their investment discouraged pharmaceutical companies from investing in research and development programs against Chagas' disease. As already reported, 1393 new medicines were approved between 1975 and 1999. Unfortunately, only 13 medicines out of the total were for the treatment of neglected diseases, but none of them were related with Chagas' disease.[11] As a consequence, to date, there are only two therapeutic agents applied to the treatment of Chagas' disease (Fig. 1). One of them is nifurtimox, manufactured as Lampit®. It is available only as 120 mg tablets prescribed at dosages of 8–10 mg/(kg day) for 90–120 days.[12] Its production was interrupted in 1997, but because of some clinical trials in combination with other ac-

tive compounds to treat African sleeping sickness, the production was restarted in 2000.[13] The other drug, benznidazole, is manufactured as Rochagan® (Brazil) or Radanil® (Argentina). It is available as a unique presentation of 100 mg tablets, and the usual dosage is 5–7 mg/(kg day) for 30–60 days.[14] Also, a prophylaxis treatment at a dose of 5 mg/kg three times per week is recommended for chronic patients with human immunodeficiency virus.[15] Both antiparasite agents are used in the acute phase with cure rates up to 80% after 2 months of treatment. These medicines are considered less effective in the chronic phase, although there are some controversies between the scientific experts about the efficacy in that period. Unfortunately, one the major drawbacks of these active compounds are the high rate of adverse effects. Particularly, the patients treated with nifurtimox may suffer from serious digestive disorders, whereas benznidazole may produce dermatitis with cutaneous eruptions, myalgias, polyneuropathy, polyneuritis, and bone marrow disorders.[16] Benznidazole is better tolerated than nifurtimox and, therefore, it is considered the first-choice treatment. The availability of benznidazole is guaranteed by Roche, even though the technology is being transferred to Lafepe (Pernambuco, Brazil).[13]

Although both active compounds are included in the World Health Organization project "Better medicines for children," an effective chemotherapy for paediatric population infected with Chagas' disease is still lacking.[17] Because both nifurtimox and benznidazole are formulated only into tablets, the pediatric population is treated by fractioning the solid dosage form in pieces according to the body weight of each patient.[18] This quite primitive and nonrecommended therapy may cause undesirable side effects due to the potential erroneous dosage.[19,20] In addition, because paediatric patients include neonates (0–28 days), infants (1–12 months), and children (1–12 years), another drawback for the availability of pediatric dosage forms is the particular dosing regime in each age group. In this context, a potential solution might be the design of liquid formulation as syrups, suspensions, and/or drops. In the case of liquid preparations, the dose is easy to adapt to bodyweight and there are fewer problems with swallowing. Nevertheless, the taste and/or flavor of the drugs are crucial to achieve good compliance, especially in the case of Chagas' disease where the treatment is between 30 and 60 days.

Figure 1. Molecular structures of nifurtimox and benznidazole.

NIFURTIMOX NANOPARTICLES

Encapsulation of active compounds into polymeric carriers provides several benefits over the conventional dosage forms. Particularly, the incorporation of drugs into nanocarrier systems increases their

therapeutic potential by improving intracellular delivery and retention time inside the cell. In 1998, González-Martin and coworkers described the development of nanoparticles of polyalkylcyanoacrylate as a carrier for nifurtimox. Ethylcyanoacrylate nanoparticulate systems were prepared through an emulsion polymerization technique by using variable amounts of the drug, polyethylcyanoacrylates, and surfactants. Nifurtimox nanoparticles obtained were less than 200 nm in size, and the drug content uptake into the nanoparticles was 33.4%. The highest release of nifurtimox from the nanoparticles was around 65% at a physiological pH value of 7.4. The studies *in vitro* showed an improved trypanocidal activity over a standard solution of nifurtimox. Nanoparticles loaded with 2 µg/mL of nifurtimox presented a 100% inhibition of the epimastigotes, whereas the free drug and the unloaded carrier produced an inhibition of only 50%. When the nanosystem was prepared using 5 µg/mL of the drug, the parasitic infection was reduced nearly 90%, similar to empty ethylcyanoacrylate particles and free nonencapsulated nifurtimox. The assay carried out using metacyclic form-infected Vero cells gave as result a similar biological activity between the loaded and unloaded nanoparticles and the free drug. Although it was demonstrated that unloaded nanoparticles have trypanocide activity similar to that of nifurtimox single solution, no mechanism was postulated in order to explain the unexpected biological activity.[21] In other work, the same research group reported that nifurtimox-loaded nanoparticles clearly displayed a higher trypanocide activity on both trypomastigote and intracellular amastigote in comparison with unloaded nanoparticles and nifurtimox in solution. When used at high concentrations, both the loaded and unloaded nifurtimox nanoparticles produced a considerable cellular lysis, probably due to the degradation of the polymeric chain and the generation of toxic side products.[22] To the best of our knowledge, no further research work was carried out with the aim of developing novel nifurtimox delivery systems.

BENZNIDAZOLE LIPOSOMES

Regarding benznidazole, the literature data show recent advances in the area of nanoparticle formulations. Liposomes are microscopic spherical particles in which the membranes are constituted by naturally derived phospholipids mixed with other lipid derivatives such as cholesterol and glycolipids among others. Phospholipid derivatives are able to form micelles or are organized as lipid bilayers with the hydrophobic tails lined up against one another and the hydrophilic head group facing the water on both sides. Liposomes show great potentials to encapsulate hydrophilic drugs in the aqueous cavity or introduce hydrophobic drugs into the membrane as a component. They are considered versatile drug carriers to deliver drugs to the site of action and controlling its release at predetermined rates.[23] In 2002, liposomal carriers were developed by Morilla et al. with the aim of improving the pharmacokinetics and bioavailability of benznidazole, particularly in the acute phase of the disease. As showed by the authors, because of the behavior of the drug with the lipid components of the liposomal formulation, it was decided to prepare multilamellar liposomes instead unilamellar ones by applying thin-film method, developed earlier as a convenient way to add low water-soluble drugs to membrane-forming lipid derivatives. The best performance of the lipid matrix as drug carrier was obtained using a mixture solution of hydrogenated phosphatidylcholine from soybean, distearoyl–phosphatidylglycerol, and cholesterol in chloroform–methanol.[24] Later, the same research group described an interesting *in vivo* study in which a single bolus of benznidazole included in liposomes was administered by three parenteral routes: intramuscular, subcutaneous, and intravenous(ly) (i.v.). After i.v. administration of 0.2 mg drug/kg, an increased liposomal benznidazole in liver was detected in comparison with the nonencapsulated drug. However, the parasitic infection in mice did not diminish. It was postulated that intracellular pathways after benznidazole liposome uptake might be directly related with the resulting therapeutic action.[25]

BENZNIDAZOLE MICROPARTICLES

In general, conventional drug delivery produces sharp increases of drug concentration at potentially toxic levels. With the aim of modulating the release of bioactive agents, in the past decades, there has been a substantial interest focused in the application of biodegradable polymers to the design of microparticulate delivery systems as carriers for drugs, proteins, and microorganisms.[26] In particular, microparticles for the oral route of administration have several advantages such as uniform distribution in the gastrointestinal tract, protection of labile therapeutic agents in acidic environments, and a higher accuracy of the doses.[27] Chitosan, a linear polyamine with a high ratio of glucosamine to acetylglucosamine units, is a natural polymer obtained by deacetylation of chitin.[28] It is a biologically safe, nontoxic, biocompatible, and biodegradable polysaccharide widely used as carrier for the development of different pharmaceutical formulations.[29,30] However, the unique application of chitosan for the delivery of antichagasic compounds was proposed by our group. The preparation and optimization of chitosan

microparticulate systems prepared by coacervation method and analyzed employing artificial neural network (ANN) was carried out. Chitosan microparticles showed an enhanced dissolution rate for benznidazole in comparison with the free drug, confirming that the polymeric microparticles conferred improved the dissolution properties of the drug. This methodology was proved to be very efficient in decreasing the particle size and increasing the encapsulation efficiency. The influence of several factors in the microparticle formulation was evaluated in order to distinguish those which have a significant effect on four responses: yield, dissolution rate, encapsulation efficiency, and size of the microparticles. The analysis was composed of the following four phases: (i) screening the influential factors with a Plackett–Burman design, (ii) modeling the responses using ANNs, (iii) finding the optimal conditions through desirability considerations, and (iv) verifying the optimal formulation. The optimal combination of the microencapsulating material was found to be 1.5% (w/v) polymer concentration, 6.0% (w/v) NaOH solution, 1400 rpm stirring rate, and 5 mL/min spraying rate.[31]

BENZNIDAZOLE–METAL-BASED COMPLEXATION

In the last decades, there has been a growing interest in the coordination chemistry applied to drug research and development, particularly since the discovery and further successful application of platinum complexes as antitumor agents. It is known that one of the key steps on coordination chemistry toward the development of novel medicines is the selection of metal ligands, because it may provide a particular reactivity to the coordination with the corresponding bioactive molecule. Although metal-based medical applications were focused mainly on the treatment of cancer, recently, the rational design of metal-based therapeutic agents was found to be a suitable pathway for the treatment of parasitic infections because they can show a pronounced selectivity for selected parasites. Thus, several reports described the formation of complexes by means of ruthenium, gold, and iridium with antimalarial compounds. Also, nitric oxide donor compounds are a convenient and effective approach for killing the *T. cruzi*, due to different mechanisms.[32,33] In this context, the design of metal-based benznidazole complexes as nitric oxide donor was proposed by Silva et al.[34] Ruthenium coordination to benznidazole originated *trans*-[Ru(Bz)(NH$_3$)$_4$-SO$_2$](CF$_3$SO$_3$)$_2$ complex, which was prepared following a general procedure already published. It was obtained in 55% yield and fully characterized by spectroscopic techniques (^{1}H and ^{13}C nuclear magnetic resonance, infrared, and ultraviolet–visible) and density functional theory calculations. Attaching a

ruthenium-containing fragment to benznidazole resulted in a strong modification of the electronic and hydrophilic properties. The molecular properties (lowest unoccupied molecular orbital and GAP energies and natural bond orbital charge analysis) showed that the nitro group moiety exhibited a higher electrophilic character in the metal complex than in the noncomplexed drug. It was postulated that the coordination of benznidazole to the [Ru(NH$_3$)$_4$SO$_2$]$_2^+$ moiety will activate the nitro group toward reduction favoring nitro radical anion formation. Regarding the hydrophilic behavior of the complex, a remarkable increase was observed in its aqueous solubility in comparison with the parent drug, probably due to the presence of NH$_3$ and SO$_2$ ligands. Cytotoxicity evaluation *in vitro* using macrophages or spleen cells did not show any reduction in the cells' viability when assayed at a range concentration of 10 nM–1 mM. *In vitro* antiproliferative activity on epimastigotes showed that ruthenium–benznidazole complex exhibited greater antiproliferative activity than the corresponding drug. In addition, *in vivo* trypanocidal activity (acute model) in mice was carried out following different protocols. Mice were orally treated with the ruthenium complex at 100 nmol/kg, and a 60% survival of the treated animals was observed. When the same treatment was carried out using 385 nmol/kg, 100% of the mice were protected against death. Therefore, the higher trypanocidal activity for the metal complex in comparison with benznidazole could be due to the coordination of the drug to the *trans*-[Ru(NH$_3$)$_4$-L]$_2^{+/+/0}$ moiety. Following this article, the same researcher synthesized a series of ruthenium nitrosyls, *trans*-[Ru(NO$^+$)(NH$_3$)$_4$L]X$_3$ (L = imidazole) and [Ru(NO$^+$)(HEDTA)] against the Y strain of the parasite.[35] The antiproliferative analysis was carried out using sodium nitroprusside (SNP) as the donor of nitric oxide reference. Most of the ruthenium complexes' nitric oxide donors exhibited higher antiproliferative activity than SNP. In addition, Silva et al.[34,35] found that release of nitric oxide from synthesized metallic complexes produced an interesting trypanocidal activity, whereas the ruthenium complexes, which were not able to release nitric oxide, did not show any activity. In addition, a correlation between the antiproliferative activity and the reduction potential of the fragment RuNO$^+$/RuNO^0 was observed; when the reduction potential of the NO$^+$/NO^0 couple became more positive, the antiproliferative activity increased as well. Evaluation of the histological studies of cardiac tissue sections of mice infected with *T. cruzi* and treated with selected Ru(NO) derivatives for 15 consecutive days indicated the elimination of extracellular and intracellular forms of the Y strain of the parasite. It could be due to the action of the nitric oxide molecules released from those ruthenium complexes despite its short half-life. Although the

production of NO should be controlled to avoid cytotoxic damage to the host, these metal-based compounds may represent a novel strategy for the synthesis of promising anti-*T. cruzi* compounds.

BENZNIDAZOLE LIQUID SYSTEMS

It is important to note that both nifurtimox and benznidazole tablets are used frequently in infants and children. However, it is well known that tablets and capsules are usually unsuitable for children aged less than 4 years, and a suitable strength may not be available for use in older children.[36] The default option for the lack of commercially available liquid formulations may be the extemporaneously prepared formulations.[37] Unfortunately, the production of extemporaneous formulations for Chagas' disease is lacking resources in endemic regions. In agreement with the biopharmaceutics classification system, benznidazole is a class III drug, which indicates a high intestinal permeability and high water solubility;[38] however, some scientific publications described the drug as poorly soluble in water.[39,40] As a consequence of it, a low and erratic bioavailability is usually expected. Because the enhancement of solubility of benznidazole did not receive much attention so far, in 2006, our group described the application of cosolvency technique to improve their solubility. Several pharmaceutically accepted solvents such as ethanol, propylene glycol, polyethylene glycol (PEG), benzyl alcohol, diethyleneglycolmonoethyl ether, and surfactants were assayed in order to develop a liquid trypanocidal medicine. Among all the combinations of the tested solvents, those containing a mixture of PEG 400, ethanol, water, and buffer solution (pH 2.5) were able to increase the benznidazole solubility from 0.4 mg/mL to 10 mg/mL. The physical stability study showed that the PEG 400-based formulations remained unchanged with respect to color stability, and no turbidity or precipitate formation was detected at the storage conditions. In the particular case of cosolvent systems using Transcutol, it was revealed to be toxic per se against parasite, without the inclusion of the drug. Trypanocide activity exhibited by benznidazole forrmulations in PEG 400 was similar as compared to the activity obtained from benznidazole in dimethyl sulfoxide. Regarding all the systems proposed, it is worth noting that the antiparasite compound in PEG 400–potassium biphthalate buffer (7:3) showed the highest activity as a trypanocide when assayed over two strains of *T. cruzi*, Tul 2, and CL Brener. These solvent systems (placebo) also exhibited low toxicity against a mammal cell line, thus increasing interest in them for testing on an animal infection model. Furthermore, these simple, effective, and novel formulations may also provide a better pharmacokinetic profile because the drug is completely dissolved, which would improve the absorption.[41]

BENZNIDAZOLE MICROCRYSTALS

As previously described, benznidazole is poorly soluble in water, which is associated with poor dissolution properties. As a consequence, an erratic and/or low bioavailability after oral administration may be observed. It is well known that drug particle size reduction, which increases the total surface area, has often been used to enhance dissolution and further bioavailability of poorly water-soluble compounds.[42,43] Micronization is one of the most used methods to obtain particles of reduced size. However, there are several disadvantages associated with it, such as particle agglomeration, physical or chemical instability of the obtained crystals, and improper flow characteristics of the micronized powders.[44,45] Therefore, an *in situ* micronization technique without any mechanical process was developed recently by means of solvent precipitation with the addition of polymeric stabilizers in order to avoid the agglomerates.[46] Maximiano et al.[47] described the preparation of benznidazole microcrystals by solvent change precipitation and its inclusion into tablets as an alternative solid dosage form, which is capable of overcoming that limitation and delivers the drug at optimal concentration for the desired therapeutic effect. The formation of crystals of reduced sized was carried out by means of a saturated solution of benznidazole prepared with selected organic solvents and water. In addition, several polymeric excipients such as hydroxypropylmethylcellulose, hydroxyethylcellulose, pregelatinized starch, and PEG 400 were used as stabilizing agents in order to prevent the molecular association and to avoid crystal growth.[48] It was found that acetone–water was the most effective mixture for the precipitation of drug microcrystals. In addition, the drug dissolution rate increased in the presence of the stabilizing polymers in comparison with benznidazole alone. Particularly, hydroxyethylcellulose and PEG 400 were able to increase the drug dissolution up to 90% in 10 min. Benznidazole microparticles obtained in this study were homogeneous acicular crystals similar in shape to the original crystals, but differing significantly in terms of size and thickness. According to thermal analysis, it was observed that drug microcrystals and untreated drug particles displayed similar characteristics, without changes in the melting peak of the drug. The diffraction patterns of benznidazole microcrystals were similar to those of the pure drug, indicating that the crystallinity after the precipitation did not essentially change. Following a trend observed in the dissolution assay of micronized powders,

there was a significant enhancement in the dissolution rate of benznidazole microcrystals from tablets. Drug dissolution rate was faster for tablets containing benznidazole microcrystals, with 85% released within 10 min as compared with 30% determined for the available medicine. Regarding the *in vivo* evaluation against *T. cruzi*, each group treated with a dose of 100 and 50 mg/kg had a significant decrease in the parasitemia peak in comparison with the control group, being more pronounced in the case of micronized formulations. Thus, the *in vivo* studies confirmed the utility of benznidazole microcrystals as a novel and effective alternative to improve the treatment of Chagas' disease.

CONCLUSION

Because of several national and international activities, promoted by several public health organizations and private foundations, the transmission of *T. cruzi* has been effectively diminished in many regions of Latin America. As previously stated, up to date, nifurtimox and benznidazole are the only two active compounds used to treat Chagas' disease. Therefore, it is required to increase the efforts in order to ensure continuous synthesis and production of those medicines as well as to support the research and development oriented to paediatric drug formulations. On the contrary, further efforts are required to determine the optimal doses, duration of treatment, and dosing schedules for the existing medicines for infected patients in order to improve the treatment of neglected diseases. In conclusion, a better and more efficient control of this neglected disease will be achieved if there is a continuous participation of the public sector and the private investors for supporting the research in the academy and pharmaceutical companies for the discovery of more effective trypanocidal compounds and the optimization of current drug formulations.

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