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Research paper

A comparative study on the anti-schistosomal and hepatoprotective effects of vinpocetine and isosorbide-5-mononitrate on *Schistosoma mansoni*-infected mice

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ABSTRACT

Schistosomiasis is a remarkable public health problem in developing countries. Presently, praziquantel is the optional drug for all human schistosomiasis. Owing to the increased praziquantel resistance, there is an urgent need to develop new alternatives. This study aims at determining the anti-schistosomal and/or the hepatoprotective effects of the anti-inflammatory drug; vinpocetine, and the vasodilator and the nitric oxide donor; isosorbide-5-mononitrate, in comparison to praziquantel. In the present research, the therapeutic efficacies of these drugs were assessed in Swiss albino female mice (CD-I strain) experimentally infected with an Egyptian strain of *Schistosoma mansoni*, using some general, parasitological, and histopathological parameters. In this work, praziquantel significantly reduced worm burden and hepatic egg load, increased the percentage of dead eggs in the small intestine and decreased granuloma count, but did not reduce granuloma diameter. While, either vinpocetine or isosorbide-5-mononitrate monotherapy did not induce significant reduction in the worm count, hepatic egg load or shift in the oogram pattern, but significantly reduced granuloma count and diameter. Moreover, isosorbide-5-mononitrate significantly reduced hepatic inflammation and necrosis. The best results were obtained in the mice groups treated with isosorbide-5-mononitrate combined with praziquantel or vinpocetine. Our results point to vinpocetine and isosorbide-5-mononitrate as a convenient and promising adjuvant to praziquantel for ameliorating schistosomal liver pathology. Further studies are recommended to reveal the actual pathways responsible for the different activities of vinpocetine and isosorbide-5-mononitrate.

1. Introduction

Schistosomiasis is a major parasitic disease caused by infection with blood dwelling flukes of the genus *Schistosoma*. Schistosomiasis is considered the most frequent cause of liver fibrosis, affecting about 240 million people worldwide (Richter et al., 2015), and causing approximately 4.5 million disability-adjusted life years loss annually (World Health Organization, 2016).

The main lesion in chronic schistosomiasis represents a complex delayed-type hypersensitivity response to sequestered ova trapped in tissues during the peri-intestinal migration or after embolization in the liver. Soluble egg antigen and proteolytic enzymes released by ova provoke granulomatous cell-mediated immune response, with recruitment of eosinophils, granuloma formation and liver fibrosis (Wynn et al., 2004).

Liver fibrosis can be considered as a dynamic and highly integrated cellular response to chronic liver injury. Whatever the aetiology, the evolution of chronic liver disease is characterized by continuation of parenchymal necrosis, chronic hepatitis and qualitative as well as quantitative changes in extracellular matrix (ECM) composition. Activation of hepatic stellate cells (HSCs), their transformation into myofibroblasts, and involvement of macrophages and Kupffer cells prevail at cellular level (Gutiérrez-Ruiz and Gómez-Quiroz, 2007). At the molecular level, growth factors, cytokines and chemokines, changes in ECM organization and composition as well as reactive molecules provoked by oxidative stress have been suggested to play a pathogenic role (Friedman, 2000). It is worth noting that progressive liver fibrogenesis is principally maintained by chronic activation of the wound healing response and oxidative stress (Parola et al., 2008).

Vinpocetine (Fig. 1A) is derived from the alkaline vincamine molecule, extracted from the periwinkle plant, *Vinca minor*. It is a well-established anti-inflammatory drug used to improve cerebral

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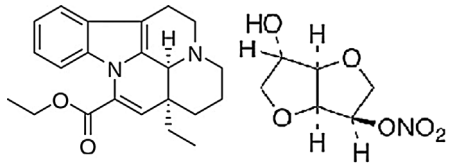

A. Vinpocetine B. Isosorbide-5-mononitrate

Fig. 1. (A and B) Chemical formulae of vinpocetine and isosorbide-5-mononitrate, respectively.

circulation and cognitive function (Jeon et al., 2010). Vinpocetine dilates blood vessels, hence improves circulation in the brain. Also, it has a neuronal protective effect that increases the resistance of the brain to hypoxia and ischaemic injury (Adám-Vizi, 2000). Additionally, vinpocetine may be a promising remedy in the treatment of liver diseases, as it reduces serum levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase, and causes significant decrease in hepatic necrosis in carbon tetrachloride (CCl4)-treated rats (Abdel Salam et al., 2007). Moreover, vinpocetine inhibits lipopolysaccharide (LPS) and tumour necrosis factor- α (TNF-α)-induced inflammatory responses in liver, hence preventing the up-regulation of nuclear factor-kappa B (NF-kB) in vascular smooth cells of blood vessels (Lindström and Bennett, 2005). It also decreases the TNF-α-induced expression of the mRNA of pro-inflammatory molecules as interleukin-1 β (IL-1β), monocyte chemoattractant protein-1 (MCP-1), and vascular cell adhesion molecule-1 (VCAM-1) (Joen et al., 2010).

To exert their pharmacologic activities, nitro compounds as isosorbide-5-mononitrate (IS-5-MN) (Fig. 1B) must be enzymatically metabolized to release nitric oxide (NO). It has been shown that NO inhibits smooth muscle cell proliferation (Nakaki et al., 1990), platelet adhesion (Radomski et al., 1987), leukocyte adhesion (Kubes et al., 1991), and endothelin generation (Boulanger and Lüscher, 1990). It is well known that IS-5-MN acts on a specific nitrate receptor in the smooth muscle cell membrane (Needleman et al., 1973), mediating vascular smooth muscle relaxation, and causing hyperpolarization of the cell membrane (Miyagi et al., 1996). Moreover, anti-oxidants as IS-5-MN act through altering the activity of the oxidation-reduction system, thereby reducing inflammation (Grimble, 1994). It is interested to know that NO is involved in the modulation of hepatic microcirculatory perfusion (Lhuillier et al., 2006). Additionally, NO may have anti-fibrotic effect by inhibiting the proliferation, motility and contractility of HSCs, besides decreasing the fibrillar ECM accumulation (Failli et al., 2000). Fiorucci et al. (2004) demonstrated that NO donation also protects the liver from acute failure, and suppresses pro-inflammatory cytokines such as interferon- γ (IFN-γ) and tumour necrosis factor-α (TNF-α). Recently, delivery of NO to the liver was reported to decrease hepatic inflammation in *Schistosoma mansoni*-infected mice, and to diminish the granulomas size (Sombetzki et al., 2015).

To the best of our knowledge, no data have been published on the use of vinpocetine and/or isosorbide-5-mononitrate in treatment of schistosomiasis, despite the well-known anti-inflammatory influence of vinpocetine (Jeon et al., 2010) as well as the vasodilator and anti-oxidant effects of isosorbide-5-mononitrate (Grimble, 1994; Miyagi et al., 1996). Therefore, this work aimed to investigate the possible anti-schistosomal and/or hepatoprotective activity of these drugs used as a mono- or combined-therapy compared with praziquantel in *S. mansoni*-infected mice based on some general, parasitological and histopathological criteria.

2. Materials and methods

2.1. Drugs

Vinpocetine (Vinporal, Amriya Pharmaceutical Industries, Egypt), isosorbide-5-mononitrate (Effox, Minapharm, Egypt), and praziquantel (Biltricide, Alexandria Co. for Pharmaceuticals & Chemical Industries, Egypt) were used in the study. Vinpocetine and IS-5-MN tablets were ground and used as a freshly prepared aqueous suspension by vortexing. PZQ was ground and used as a freshly prepared aqueous suspension in 2% Cremophor El (Sigma Chemical Co., St. Louis, USA) by vortexing.

2.2. Animals, parasites and infection

All animal studies presented here were approved by the Mansoura Faculty of Medicine-Institutional Research Board (MFM-IRB, #R/16.06.112) and the Medical Experimental Research Center (MERC), Faculty of Medicine, Mansoura University, Mansoura, Egypt, based on the institutional and national regulations for animal experimentation. A total of 110 Swiss albino female mice of CD-I strain (aged 6–8 weeks, and weighing 20–25 gm) were purchased from the Schistosome Biological Supply center (SBSC), Theodor Bilharz Research Institute (TBRI), Imbaba, Giza, Egypt.

Mice were weighed and then infected with *S. mansoni* cercariae Egyptian strain, freshly shed from infected *Biomphalaria alexandrina* snails, purchased from the SBSC, TBRI, after exposure to light for 30 min. Each mouse was subcutaneously infected with 60 ± 10 cercariae (Smithers and Terry, 1965).

2.3. Animal groups

Mice were randomly allocated into eight groups, each of 10–15 mice at the beginning of the experiment:

Group I: Normal non-infected, control (n = 10).
Group II: Infected non-treated (n = 14).
Group III: Infected and treated with PZQ (n = 13).
Group IV: Infected and treated with vinpocetine (n = 13).
Group V: Infected and treated with vinpocetine and PZQ (n = 15).
Group VI: Infected and treated with IS-5-MN (n = 15).
Group VII: Infected and treated with IS-5-MN and PZQ (n = 15).
Group VIII: Infected and treated with vinpocetine and IS-5-MN (n = 15).

Mice were kept at the MERC, Faculty of Medicine, Mansoura University, Mansoura, Egypt, in an air-conditioned animal house, at 20–22 °C, with 12 h light and 12 h dark cycle, and maintained on a standard commercial pellet diet, and normal drinking water *ad libitum*.

The doses of vinpocetine and IS-5-MN used were equivalent to the highest doses, which induced improvement in rat models of CCl4-induced liver injury; 8.4 mg/kg and 7.2 mg/kg, respectively (Abdel Salam et al., 2007, 2010). According to Paget and Barnes (1964) drug conversion tables, doses used for mice were calculated to be 11.76 mg/kg and 10.08 mg/kg for vinpocetine and IS-5-MN, respectively. The difference in drug dosing conversion between species is not only due to the variations in the body weight, but also the body surface area, as well as the biochemical and functional systems which in turn alter pharmacokinetics (Nair and Jacob, 2016). Both drugs were administered from the fourth to the tenth weeks post infection (WPI), 5 days/week. PZQ was given 6 WPI in a dose of 500 mg/kg/day for two successive days (Gönnert and Andrews, 1977). Drugs were administered by oral gavage (after being dissolved in water or 2% Cremophor El), using a mouse feeding needle (Kent Scientific Corporation), in a volume of 200 µl/mouse.

Mice in all groups were weighed before euthanasia at the end of the study, 10 WPI by i.p. injection of thiopental sodium (Egyptian International Pharmaceutical Industries Co., E.I.P.I.C.O) in a dose of 100 mg/kg. Then the spleen weight/mouse was measured.

2.4. Assessment of parasitological criteria

After euthanasia, hepatic and porto-mesenteric vessels were