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Sodium valproate, a histone deacetylase inhibitor, with praziquantel ameliorates *Schistosoma mansoni*-induced liver fibrosis in mice

Mohamed G. Elsakkar [a], Maha M. Eissa [b], Wafaa A. Hewedy [a,*], Rasha M. Nassra [c], Soha F. Elatrebi [a]

[a] *Department of Clinical Pharmacology, Faculty of Medicine, Alexandria University, Alexandria, Egypt*
[b] *Department of Medical Parasitology, Faculty of Medicine, Alexandria University, Alexandria, Egypt*
[c] *Department of Medical Biochemistry, Faculty of Medicine, Alexandria University, Alexandria, Egypt*

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ABSTRACT

Aims: This study explores the potential antifibrotic effect of sodium valproate (SV), an inhibitor of class I histone deacetylase (HDAC) enzymes, and/or praziquantel (PZQ) on *Schistosoma mansoni (S. mansoni)*-induced liver fibrosis in mice.

Main methods: Male Swiss albino mice were divided into nine groups: group I- normal control (NC); group II- uninfected gum mucilage (GM) treated; group III- uninfected PZQ- treated; group IV- uninfected SV-treated; group V- control *S. mansoni* infected mice; group VI- infected GM-treated; group VII- infected PZQ-treated; group VIII- infected SV-treated; group IX- infected PZQ + SV treated. All SV administrations were 300 mg/kg/day orally and administered for five weeks beginning on the 5th week post infection (WPI). All PZQ administrations were 500 mg/kg/day orally and administered for 2 consecutive days beginning on the 7th WPI. Serum transforming growth factor-beta 1 (TGF-β1), tumor necrosis factor-alpha (TNF-α), hepatic hydroxyproline (Hyp) content, and liver function tests (AST and ALT) were determined. Specimens of the hepatic tissues were examined histologically.

Key findings: Treatment of *S. mansoni*-infected mice with SV significantly decreased the serum levels of ALT, TGF-β1 and TNF-α, and the liver tissue hydroxyproline content compared with the *S. mansoni* infected untreated groups. Histologically, treatment with SV revealed regression of the granulomatous inflammatory reaction. Combined treatment with PZQ and SV produces more favorable biochemical results, and aborted granulomatous reaction compared with either drug alone.

Significance: Sodium valproate is a promising anti-fibrotic agent. It demonstrated an anti-fibrotic effect in early stages of *S. mansoni* infection through downregulation of profibrogenic cytokines, and collagen deposition.

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1. Introduction

Hepatic schistosomiasis is one of the most prevalent forms of chronic liver diseases in the world caused by the blood-dwelling trematodes of the genus *Schistosoma. Schistosoma mansoni (S. mansoni)*, which is responsible for intestinal schistosomiasis, causes symptoms ranging from abdominal pain and bloody diarrhea to hepatosplenomegaly, periportal fibrosis and portal hypertension [1].

Praziquantel (PZQ) is the mainstay treatment for all *Schistosoma* species in most endemic areas. However, PZQ is inactive against juvenile schistosomes and only has a limited effect on already developed liver and spleen lesions. Additionally, there are recent concerns about tolerance and/or resistance to PZQ [2].

Complications of schistosomiasis are mostly due to egg-induced granulomatous lesions. The initiation, development, and regression of granuloma are mediated by cytokines and chemokines leading to recruitment of inflammatory cells against the antigenic stimuli. Granulomatous response evolves from an early type 1 helper (TH1) to a sustained and dominant type 2 helper (TH2) cells, where interleukin (IL)-4 and IL-13 being the main cytokines driving this reaction. Although many studies showed that recovery from advanced fibrosis is possible, drugs to prevent and treat fibrosis are only partially effective [3].

The key pathogenic event in hepatic fibrogenesis is activation of hepatic stellate cells (HSCs) [4]. HSCs undergo a process of transdifferentiation from the quiescent vitamin A-storing cells to myofibroblasts. The myofibroblasts are responsible for production of fibrogenic extracellular matrix (ECM) components within the space of Disse and becomes involved in inflammatory signaling. These responses are tightly controlled by cytokine production both from the HSCs themselves as well as the neighboring cells [5]. Growth factors such as TGF- α or –β, reactive oxygen species and products of lipid peroxidation are triggers that activate HSCs [6].

An important process in transcriptional regulation of HSCs is the modification of histones by histone modifying enzymes (HMEs) [7].

* Corresponding author at: Almoassat Medical Campus, Clinical Pharmacology Department-Faculty of Medicine, Alexandria, Egypt.
E-mail address: wafaa.hewedy@alexmed.edu.eg (W.A. Hewedy).

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HMEs include histone deacetylases (HDACs), histone acetyltransferases, histone methyltransferases and histone demethylases. Acetylation by histone acetyltransferases (HAT) abolish the positive charge of the lysine and reduces chromatin compaction, favoring transcription. Deacetylation by the action of HDACs has the opposite effect [8]. However, histone acetylation also modulates binding of many proteins that regulate gene transcription [9]. Functionally, histone modifications have the potential to influence several biological processes including differentiation and transdifferentiation of HSCs in vitro [10].

HDAC inhibitors (HDACIs) are being investigated as drugs used in a range of diseases, including cancers and infectious diseases such as HIV/AIDS, and several parasitic diseases. Recently, the applicability of HDACIs in treatment of fibrotic disorders has been also explored [11].

Sodium valproate (SV), which has been widely used as an antiepileptic and mood stabilizer drug, is found also to inhibit class I HDAC enzymes. Several types of HDACIs, including SV, are undergoing several clinical trials for HDAC-relevant diseases [12]. Based on available data; we aimed in this study to test the hypothesis that SV will be a novel adjuvant to PZQ, the classical anti-schistosomal drug, in treatment of *S. mansoni*-induced liver fibrosis in mice.

2. Material and methods

2.1. Drugs

Sodium Valproate (Depakine) was purchased from Sanofi-Aventis France S.A. company (Paris, France). Praziquantel (Distocide) was obtained from the Egyptian International Pharmaceutical Industries Company (Eipico, 10th of Ramadan, Egypt).

2.2. Animals

The present study was conducted on seventy-two age-matched male *Swiss* albino mice of body weights ranging from 18 to 30 g. At the start of the study, no significant differences were found between the mean weights of the animals in the different tested groups ($p > 0.05$). The mice were purchased from the animal house of Misr University for Science and Technology (MUST), Egypt. The mice were housed in conditions in accordance with the Helsinki declaration for animals and care. The animals were kept under standard conditions of light and temperature, with free access to food and water ad libitum for one week before the start of the experiment as an acclimatization period. All experimental procedures were approved and performed in compliance with the guidelines of the Local Ethical Committee of the Faculty of Medicine, Alexandria University.

2.3. Parasitic infections

The cercariae of *S. mansoni* were obtained from infected *Biomphalaria alexandrina* snails that have been purchased from the Schistosome Biologic Supply Center, Theodor Bilharz Research Institute, Giza, Egypt. The snails were allowed to shed their cercariae in dechlorinated water, by exposure to direct sun light for 1 h. The cercariae were counted in 0.1 mL of dechlorinated water under dissecting microscope after adding one drop of Lugol's iodine. The cercariae were used for infecting the animals by transferring each mouse to a clean sedimentation flask for caudal immersion in few milliliters of aged dechlorinated water, containing 80 freshly shed cercariae, for 90 min [13].

2.4. Pilot studies

A small dose-finding pilot study, with three animals for each dose, was conducted for SV. The drug dose that provided the best improvement in histological sections of the liver after *S. mansoni* infection was chosen.

A pilot study was conducted to test for the possible schistosomicidal activity of SV by studying its effect on adult worm count in *S. mansoni* infected mice. During this study, 12 mice were infected with *S. mansoni* cercariae and divided into two equal groups. The first group was left untreated as a control group while the second group was treated with SV 300 mg/kg/day for 2 weeks beginning on the 5th week post infection (WPI) [14]. The mice were then sacrificed and the adult *S. mansoni* worms were counted. The statistical analysis of the results revealed no significant antischistosomal effect for SV treatment (data not shown).

2.5. Experimental procedure

Thirty-two mice were randomly divided into 4 groups, with 8 mice in each group: group I- Normal control (NC) uninfected mice received distilled water orally; group II- uninfected mice received 2% gum mucilage (GM) orally; group III-uninfected mice were treated with PZQ; group IV- uninfected mice were treated with SV.

The remaining forty mice were infected with *S. mansoni* cercariae. These animals were randomly divided into five groups, with 8 mice in each group: group V- control infected mice received distilled water orally; group VI- infected mice received 2% GM orally; group VII- infected mice were treated with PZQ; group VIII- infected mice were treated with SV; group IX- infected mice were treated with PZQ and SV in combination.

All SV administrations were 300 mg/kg/day orally, and were dissolved in sterile distilled water at a final concentration of 15 mg/mL and administered for five weeks beginning on the 5th WPI. All PZQ administrations were 500 mg/kg/day orally, and were suspended in 2% gum mucilage at a final concentration of 25 mg/mL and administered for 2 consecutive days beginning on the 7th WPI [15]. The prepared drug solutions and the vehicle were administered to the mice as 20 mL/kg.

2.6. Estimation of the biochemical parameters

At the end of the 10th WPI, and on the last day of the experiment, the animals were fasted for 8 h. The mice were anesthetized, and blood samples were collected from the abdominal aortae of all animals, followed by separation of the sera. The sera were stored at −20 °C until used for biochemical studies. The mice were then killed by decapitation. The abdomen of each animal was opened, and the liver was rapidly excised. Each liver was dissected and divided into two parts; one was fixed in formalin for histopathological examinations. The other part was frozen at −80 °C until be homogenized in distilled water using a polytron homogenizer for determination of hydroxyproline (Hyp) content.

2.6.1. Estimation of serum biomarkers of liver injury

The serum aminotransferases were assayed using colorimetric kits (Spectrum Diagnostics, Cairo, Egypt). The serum alanine aminotransferase (*ALT*) and the serum aspartate aminotransferase (AST) were determined according to the methods described by Reitman and Frankel [16]. The results are expressed as units per liter (U/L).

2.6.2. Estimation of serum markers of liver fibrosis

The serum transforming growth factor beta1 (TGF-β1), and tumor necrosis factor α (TNF-α) were assayed using mouse ELISA kits (eBioscience, Vienna, Austria) [17,18]. The kits employ the quantitative sandwich enzyme immunoassay technique and the absorbance of the products of the reactions was measured at 450 nm. The results are expressed as picograms per milliliter (pg/mL).

2.6.3. Estimation of hepatic hydroxyproline content [19]

In brief, aliquots of standard hydroxyproline samples were hydrolyzed in alkali (sodium hydroxide 2 N final concentration). The hydrolyzed

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