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Biochemical Toxicity of Ivermectin in Wistar Albino Rats

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Abstract: Ivermectin, a macrocyclic lactone, is commonly used against parasitic diseases in both animals and human beings. Although, its anti-parasitic activity is well known, there is dearth of literature on its effect on the host. The present study on biochemical effects of ivermectin in Wistar albino rats was undertaken to evaluate if it is toxic to the host ?. This investigation evaluated the effect of ivermectin on the plasma concentrations of AST, ALT, triglycerides, cholesterol, LDH and the hepatic levels of nucleic acids, proteins, malondialdehde (MDA) and non-protein sulfhydryl groups (NP-SH) in Wistar albino rats. The animals were intraperitoneally (ip) treated with different doses (5, 10 and 15 mg/kg., body weight) of ivermectin as a single dose and sacrificed after 24 hrs. The treatment induced significant biochemical changes in both plasma and the hepatic cells. The observed toxicity may be due to the disruption of chlorides and calcium in the cellular equilibrium, stimulation of catecholamines, GABA receptors and the genesis of cAMP caused by the oxidant nature of certain neurotransmitter metabolites triggered by ivermectin. The present investigation suggests that ivermectin-induced biochemical changes in the host animal may be few of the probable mechanisms of its antiparasitic activity.

Key words: Ivermectin · Rats · Plasma · Hepatic Cells · Biochemical Toxicity

INTRODUCTION

Ivermectin (22, 23- dihydroavermectin B_{1a}) is a macrocyclic lactone isolated from *Streptomyces avermitilis* and is commonly used against parasitic diseases in domestic and wild animals caused by parasitic nematodes and arthropods [1]. In human, it is considered the first drug of choice for the treatment of onchocerciasis, strongyloidiasis, ascariasis, trichuriasis and enterobiasis [2-5]. The antiparasitic action of ivermectin is reported to be due to its interaction with glutamate and gama-aminobutyric acid (GABA)-gated chloride channels, which cause influx of chloride ions across the cell membranes [6] and cause paralysis in many types of parasites [7]. Nevertheless, the efficacy of these drugs depends on the toxic concentrations presented to the parasite for certain duration to cause an irreversible damage that may cause harm to the host as well [8]. Although a large number of papers have been published on the antiparasitic activity of ivermectin, little is known about its toxicity to the host. The exceptions are a few reports on its adverse effects in mammals, such as abdominal pain, nausea, tachycardia, asthma, edematous swellings, pruritus, back pain and ocular troubles, cardiac dysfunction, liver disease [9, 10]. Ivermectin-induced adverse reactions have highlighted its oxidative nature with increasing load of toxic oxygen intermediates causing activation of neutrophils [11] and burst of eiosinophilic granulocytes [12].

Therefore whilst the antiparasitic action of ivermectin is generally considered to be due to its potentiation of the release and binding of GABA at certain synapses, the possibility than an increase in reactive oxygen intermediate products may be involved in toxic manifestations to the host. In view of the clinical importance of ivermectin both in animals and humans and the dearth of literature on the host toxicity, it was considered worthwhile to study the biochemical effects of ivermectin on blood plasma and hepatic tissue after acute treatment in rats.

MATERIALS AND METHODS

Chemicals: Ivermectin (22, 23- dihydroavermectin B_{1a}) was obtained from Merck Sharp and Dohme B.V., Haarlem, Netherlands. All the laboratory reagents were purchased

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from Sigma Chemical Company, St. Louis, MO, USA. The biochemical estimations on GOT, GPT, triglycerides, cholesterol and LDH were carried out by using the specific commercial kits (Randox diagnostic reagents, Randox Laboratories, USA).

Experimental Animals: Male Wistar albino rats, bred at College of Pharmacy, Riyadh, Saudi Arabia, aged 6-7 weeks and weighing 225-250 g were used. The animals were maintained under standard conditions of humidity, temperature and light (12 h dark, 12 h light); they were provided with Purina chow diet and had free access to water. The conduct of experiments and the procedure of sacrifice (using ether) were approved by the Ethics Committee of the College of Pharmacy, King Saud University, Riyadh, Saudi Arabia.

Dose, Route and Duration of Treatment: The doses of Ivermectin selected were 5, 10 and 15 mg/kg., body weight. The highest dose was close to ¼ LD_{50} (55 mg/kg b.wt..) intra peritoneal dose for rats [13-15]. Suspension of ivermectin in saline was administered by intraperitoneal (ip) route as a single dose.

Experimental Groups: The animals were randomly assigned to different control and treatment groups (5 animals in each group). The experimental groups consisted of: (1) Control (Saline); (2) Ivermectin, 5 mg/kg., body weight; (3) Ivermectin, 10 mg/kg., body weight and (4) Ivermectin, 15 mg/kg., body weight. The animals were sacrificed 24 hrs after the treatment, using anesthetic ether. Blood was collected by cardiac puncture; the plasma was separated and refrigerated. From the same animals liver tissues were excised. The plasma and liver tissues were stored at -70°C until used in the biochemical analysis.

Biochemical Procedures: Enzymes (AST, ALT, triglycerides, cholesterol and LDH) were determined in the plasma and the levels of nucleic acids, proteins, malondialdehyde and glutathione (non-protein sulfhydryl groups) were estimated in the hepatic tissue [16-20].

Enzyme Analysis: The enzyme analysis in plasma was done by using a Dimension Clinical Chemistry System, Dupont, USA [16].

Estimation of Proteins and Nucleic Acids: Total proteins were estimated by the modified Lowry method of Schacterle and Pollack [17]. Bovine serum was used as standard. The method described by Bregman [18] was used to determine the levels of nucleic acids. Tissues were homogenized the homogenate was suspended in ice-cold trichloroacetic acid (TCA). After centrifugation, the pellet was extracted with ethanol. The levels of DNA were determined by treating the nucleic acid extract with diphenylamine reagent and reading the intensity of blue color at 600 nm. For quantification of RNA, the nucleic acid extract was treated with orcinol reagent and the green color was read at 660 nm. Standard curves were used to determine the amounts of nucleic acids present.

Determination of MDA Concentrations: The method described by Ohkawa *et al*. [19] was used to determine the concentrations of MDA, an indicator of lipid peroxidation. The tissue was homogenized in TCA solution and the homogenate suspended in thiobarbituric acid. After centrifugation the optical density of the clear pink supernatant was read at 532 nm. Malondialdehydebis (dimethyl acetal) was used as an external standard.

Quantification of GSH Levels: The concentration of GSH in liver tissue was measured as NP-SH as described by Sedlak and Lindsay [20]. The thawed tissues were homogenized in ice cold 0.02 M ethylenediaminetetraacetic acid disodium. Aliquots of tissue homogenate were treated with 50% w/v trichloroacetic acid and centrifuged. Supernatant fractions were mixed with Tris buffer and 5-5”-dithiobis-(2-nitrobenzoic acid) (DTNB) was added. After shaking the contents, the absorbance was measured within 5 min after the addition of DTNB against reagent blank with no homogenate.

Statistical Analysis: The different studies undertaken were statistically analyzed by One way Analysis of Variance and Post hoc Tukey-Kramer multiple comparison test.

RESULTS

The results obtained in the present study are summarized in Tables 1-3. Ivermectin treatment was found to induce a significant increase in the plasma levels of AST (5 mg/kg, $P<0.05$; 10 mg/kg, $P<0.01$; 15 mg/kg, $P<0.001$), ALT (10 and 15 mg/kg, $P<0.05$), triglycerides (10 and 15 mg/kg, $P<0.05$), cholesterol (10 and 15 mg/kg, $P<0.05$) and LDH (10 and 15 mg/kg, $P<0.05$) as compared to the values obtained in the control (Table 1).

Table 1: Effect of ivermectin on biochemical changes in plasma of rats

Sl No.	Treatment and dose (mg/kg., body weight)	AST(μ/L)	ALT(μ/L)	Triglycerides (m mol/L)	Cholesterol (m mol/L	LDH (μ/L)
1	Control (saline)	107.00 ± 04.05	66.3 ±2.18	1.40 ± 0.18	1.40 ± 0.10	376.0 ± 48.28
2	Ivermectin (5)	137.67 ± 12.68*	67.0 ± 3.00	1.77 ± 0.15	1.38 ± 0.06	404.5 ± 14.45
3	Ivermectin (10)	145.67 ± 07.69**	72.0 ± 1.00*	1.83 ± 0.12*	1.68 ± 0.09*	571.3 ± 52.57*
4	Ivermectin (15)	147.59 ± 06.92***	73.6 ± 0.98*	2.12 ± 0.14*	1.70 ± 0.08*	568.0 ± 49.34*

Five rats were used in each group. Values are Mean ± S.E.

*P<0.05; **P<0.01; ***P<0.001 (One way Analysis of Variance and Post hoc Tukey-Kramer multiple comparison test)

Table 2: Effect of Ivermectin on hepatic levels of protein and nucleic acids in rats

Sl. No.	Treatment and dose (mg/kg., body weight)	DNA (μg/100 mg)	RNA (μg/100 mg)	Total proteins (mg/100 mg)
1	Control (saline)	181.32 ± 2.89	720.63 ± 31.16	16.82 ± 0.17
2	Ivermectin (5)	177.38 ± 2.50	718.08 ± 44.75	16.41 ± 0.16
3	Ivermectin (10)	170.97 ± 3.21*	630.81 ± 23.13*	16.00 ± 0.12
4	Ivermectin (15)	163.35 ± 3.89**	623.72 ± 26.23*	15.23 ± 0.59

Five rats were used in each group. Values are Mean ± S.E.

*P<0.05; **P<0.01 (One way Analysis of Variance and Post hoc Tukey-Kramer multiple comparison test).

Table 3: Effect of Ivermectin on Glutathione and malondialdehyde concentrations in hepatic cells of rats

Sl. No.	Treatment and dose (mg/kg., body weight)	Glutathione (NP-SH) concentration	Malondialdehyde concentrations (n moles/g wet tissue)
1	Control (saline)	95.49 ± 2.80	325.40 ± 14.13
2	Ivermectin (5)	74.19 ± 5.83**	335.25 ± 11.45
3	Ivermectin (10)	68.67 ± 3.67***	397.20 ± 09.91**
4	Ivermectin (15)	61.45 ± 4.85***	410.38 ± 11.23**

Five rats were used in each group. Values are Mean ± S.E.

**P<0.01; ***P<0.001 (One way Analysis of Variance and Post hoc Tukey-Kramer multiple comparison test)

The hepatic concentrations of DNA were significantly reduced by the treatment of ivermectin at the higher doses (10 mg/kg, P<0.05; 15 mg/kg, P<0.01). The RNA levels were also found to decrease (10 and 15 mg/kg, P<0.05), whereas the protein contents were not affected as compared to the values obtained in the control (Table 2). Ivermectin treatment caused a significant reduction in the hepatic levels of NP-SH at all the three doses (5 mg/kg, P<0.01; 10 and 15 mg/kg, P<0.001). The MDA concentrations were significantly increased at the higher doses (10 and 15 mg/kg, P<0.01) as compared to control (Table 3).

DISCUSSION

The results of this study show that the treatment with ivermectin caused a significant increase in the plasma activities of AST, ALT and LDH and the levels of triglycerides and cholesterol. These data are supported by a reciprocal change in the concentrations of NP-SH and MDA observed in the same study. Literature reports have also shown that treatment with ivermectin caused an activation of neutrophils and an increase in elastase levels in plasma of patients and normal subjects. It also has a modulatory effect on the oxidative burst of eiosinophilic granulocytes and increases the production of reactive oxygen metabolites [11, 12].

The mode of action of ivermectin-induced oxidative damage is not known. Nevertheless, the disturbance in intracellular chlorides, disruption of chloride ionic status [21] and loss of intracellular concentration of Ca_2+ [22] may be responsible for the observed oxidative change [23] as a result of the interactions between ivermectin and GABA-gated and insensitive channels. Previous studies [24, 25] have also demonstrated interactions between ivermectin and GABA-gated and insensitive channels to cause imbalance in cellular equilibrium of chlorides due to reduced membrane resistance. Thus the electrophilic and oxidant nature of ivermectin might be responsible for the observed biochemical changes.

The data on nucleic acids showed ivermectin to inhibit the hepatic contents of DNA and RNA. These results confirm the ivermectin and ivomec-induced genotoxicity and cytotoxicity in Chinese hamster ovary cells *in vitro* [26, 27]. The observed effect of ivermectin on nucleic acids is attributed to the production of cAMP caused by the stimulation of catecholamines and GABA receptors [28]. cAMP has been suggested to mediate in the reduction of intra-hepatic free glutathione/mixed disulfide ratio [29]. Previous reports have also established the adverse effects of cAMP on DNA synthesis, repair and integrity [30]. Furthermore, the observed inhibition of nucleic acids may also be due to the genesis of free radicals caused by ivermectin-related disturbed chloride and Ca_2+ homeostasis [21-23]. These oxidative changes might have influenced topoisomerases and endonucleases, thereby causing an increase in DNA strand breaks and cleavage complexes [31].

In summary, the results of the present investigation have clearly demonstrated the detrimental effects of ivermectin on different biochemical indices observed in the present study. Although, it is difficult to explain the exact mechanism of action, the influence of ivermectin on the reciprocal changes in MDA and NP-SH suggest a role of free radicals in causing adverse changes in hepatic levels of nucleic acids and different biochemical indices in plasma. The present investigation suggest that ivermectin-induced biochemical changes in the host animal may be few of the probable mechanisms of its antiparasitic activity.

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