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Effects of therapeutic dose of ivermectin on plasma nitric oxide and total antioxidant capacity in rabbits

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Abstract. – *Background:* **Ivermectin, an acaricide and anthielmintic drug of the family of avermectins may produce free radicals thus resulting cytotoxic effect on the parasite. Nitric oxide (NO) acts as free radicals and as host defense mechanisms. The antioxidant capacity (TAC) can be described by the analysis of single components in the defense systems against free radicals.**

It was aimed to study the effects of therapeutic doses of ivermectin on the plasma adenosine deaminase (ADA) and gamma glutamyl transpeptidase activities (GGT), total antioxidant capacity (TAC), nitric oxide (NO) and total protein, albumin, globulin levels in rabbits.

Material and Methods: **Twenty healthy New Zealand rabbits were allocated to 2 equal groups. Group I received 0.5 mg/kg and Group II received 1 mg/kg of ivermectin via subcutaneous injection. Blood samples were collected before the experiment, at 24 and 120 hours following the treatments.**

Results: **Ivermectin at therapeutic doses increased plasma NO level at 24 h while decreased TAC at 120 h and did not alter other parameters.**

Conclusion: **These findings may suggest that ivermectin is a safe antiparasitic drug for mammals but to less extent, it may have an effect on the oxidant/antioxidant balance.**

Key Words:

Ivermectin, Nitric oxide, Total antioxidant capacity, Adenosine deaminase, Rabbit.

Introduction

Ivermectin (IVM), an acaricide and anthelmintic drug of a semisynthetic derivative of avermectin B1, produced from *Streptomyces avermitilis* cultures, is a well-tolerated drug with no apparent side effects in mammals at pharmacological doses[1,2]. Ivermectin is recovered from the bloodstream between 4 min and 15 days post-treatment. The mean recovery rate of IVM from plasma is 75%[1,3,4].

Ivermectin functions as an agonist of γ-aminobutyric acid (GABA) receptors and of glutamate-gated Cl-channels, the later restricted to vertebrates[5]. Some Authors suggested that ivermectin may interfere with the gastrointestinal function of target parasites, resulting in starvation of the parasite[6]. It was also suggested that ivermectin may produce free radicals thus leading to cytotoxic effect on the parasite[7,8]. Nitric oxide (NO) acts as free radical and plays role in host defense mechanisms as cytotoxic against microbial agents and tumor cells. This cytotoxicity results from covalent binding of NO to intracellular iron, forming the nitrosyl–iron–sulfur complexes and inhibition of iron-containing enzymes. Thus, NO was shown to inhibit ribonucleotide reductase, which is involved in DNA replication[8]. NO is transferred to blood stream in the form of S- nitrosyl which is formed by binding of NO to free cystein of plasma albumin. NO is present as S-nitroproteins in rabbits at the rate of 95% and of which 80% is in form of S-nitroso-serum albumin[9]. Therefore, any changes in NO concentration may cause alteration in serum protein levels.

Plasma gamma glutamyl transpeptidase (GGT; EC 2.3.2.2) activity is used as indicator of obstructive liver damage as well as cellular immunity[10]. GGT, hydrolyses γ-glutamyl peptide bounds to transfer glutamyl part to an appropriate receiver. The most important substrate of this reaction is glutathione, a well known antioxidant. Another important functional characteristic of GGT is to maintain intracellular glutathione level and to control NO production from GSNO (*S*-ni-

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trosoglutathione) through a regulation of T cells of the immune system[11].

The antioxidant status of tissues can be described by the analysis of the single components in the defense system against free radicals, as well as by the determination of the total antioxidant capacity (TAC)[12]. Plasma or sera concentrations of antioxidants can be measured one by one, but this procedure is time-consuming, labor-intensive and costly, and requires complicated techniques. On the other hand, TAC, whose measurement method has been recently specified and developed, can reflect the total antioxidant status of the plasma[13]. TAC measurement does not represent the sum of activities of antioxidants. It could be used for clinical diagnosis, as it is an easy and less time-consuming procedure[12]

While some studies have demonstrated the immunosuppressive effect of ivermectin in rabbits and rats[14], and lambs[15], an other study[16] demonstrated the immunostimulatory effect in rabbits given 600 μg/kg of ivermectin. It has been reported that ivermectin potentiates the activity of interleukin-2 a lymphokine with immunoregulatory properties. Interleukin-2 is a T-lymphocyte growth factor that controls a variety of T-lymphocyte clones or functions. Adenosine deaminase (ADA; EC 3.5.4.4), that is a purinergic enzyme, controls adenosine and deoxy adenosine levels in tissue. This enzyme is found in lymphatic tissues and distributed to immune system cells especially T-lymphocytes[17]. Determination of plasma or erythrocyte adenosine deaminase activity indicates many pathophysiological events such as oxidative stress, ischemia-reperfusion injury[18] and coronary artery disease[19].

In the light of this information the present study was designed to determine the effect of therapeutic doses of ivermectin, an antiparasitic drug, on plasma ADA, GGT activities, NO, TAC, total proteins, albumin and globulins concentrations in rabbits.

Materials and Methods

Animals

A total of 20 New Zealand rabbits (Laboratory Animal Unit of the University of Kafkas, Kars, Turkey) of both sexes, aged between 9 and 11 months were used. They were kept in cages at room temperature (22-25°C) with a 12:12h light:dark cycle. They were fed a special pelleted rabbit diet *ad libitum*. Rabbits were divided into 2 equal groups. Group I (n=10), and Group II (n=10) received a single dose of 0.5 mg/kg and 1 mg/kg of ivermectin via subcutaneous injection, respectively. Blood samples were taken from the marginal ear vein before the experiment and at 24 and 120 hs after the drug administration into heparinized tubes. Collected blood samples were centrifuged at 3000 rpm for 10 minutes to obtain plasma stored at –20°C until the analysis.

Biochemical Analyses

Determination of NO

Plasma NO levels were measured by the method of Miranda et al[20]. Nitric oxide is known to have very short half life, and it is oxidized to nitrite (NO_2^-) and nitrate (NO_3^-). Level of NO can therefore be determined indirectly by measuring the concentration of nitrite (NO_2^-) and nitrate (NO_3^-). Initially, plasma samples were deproteinized with 10% zinc sulphate. Total NO concentrations (nitrate and nitrite) were determined colorimetrically by the acidic Griess reaction.

Total Antioxidant Capacity

Plasma total antioxidant capacity was colorimetrically determined using a commercial kit (Rel Assay, Gaziantep, Turkey) by the method of Erel[21]. Antioxidants in the sample reduce dark blue-green colored 2,2'-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) radical to colorless reduced ABTS form. The change of absorbance at 660 nm is related with the total antioxidant level of the sample.

Determination of Plasma ADA Activity

ADA activity in plasma was determined at 37 °C according to the method of Giusti and Galanti[22] based on the Bertholet reaction, formation of coloured indophenol complex from ammonia liberated from adenosine, and quantified colorimetrically with spectrophotometer (UV-1201, Shimadzu, Japan). One unit of ADA is defined as the amount of enzyme required to release 1 mmol of ammonia/min from adenosine at standard assay condition. Results were expressed as international unit of enzyme activity.

Plasma total protein, albumin levels and GTT activity were analyzed colorimetrically using commercial kits (bioMérieux, Marcy l'Etoile, France). Globulins were calculated by subtracting the albumin values from total protein values.

Statistical Analysis

Statistical analysis was performed by the statistical package SPSS, version 10.0. Statistical analysis of data was carried out using one-way analysis of variance (ANOVA) followed by Duncan test for within group comparison (0 h as control, 24 h and 120 h). independent samples t-test was used for between group (0,5 mg /kg and 1 mg/kg ivermectin given groups) comparison. Results were expressed as mean ± SE. $P<0.05$ was considered to be significant.

Results

Plasma ADA, and GGT activities and NO, TAC, total proteins, albumin, globulins levels of the corresponding groups are presented in Table I.

Plasma nitric oxide levels were found higher ($p<0.05$) at 24 h than 0 h and 120 h in 1 mg/kg of ivermectin administered group but it was not significantly different in 0.5 mg/kg of ivermectin administred group. Plasma TAC levels were found higher ($p<0.05$) at 0 h and 24 h than 120 h in the 1 mg/kg of ivermectin administered group while this change was not significant in 0.5 mg/kg of ivermectin administered group. Plasma ADA and GGT activities and total proteins, albumin, globulins levels were not statistically significant during the experiment in both groups.

Discussion

Ivermectin has been shown to sustain at higher levels for 13 days in blood of rabbits received subcutaneous by 0.4 mg/kg of ivermectin. Tissue clearance rate of the drug in rabbits has been reported to resemble to that of rat and sheep[23]. Ivermectin is widely used in mammals as it has great safety margin[1] as 1 to 20 mg/kg of oral ivermectin given rats lived for 24 h. Ivermectin had been used subcutaneously at dose rate of 1 mg/kg for the treatment of coccidiosis in rabbits[24].

Table I. Adenosine deaminase (ADA) and gamma glutamyl transpeptidase (GGT) activities and nitric oxide (NO), total antioxidant capacity (TAC), total protein, albumin, globulin levels in plasma of the rabbits (n=10). Data are means ± SE.

Parameters	Time			
	0 h	*24 h*	*120 h*	*P Values*
NO (μM)				
Group I	12.06 ± 2.33	16.73 ± 7.50	11.95 ± 3.86	Ns
Group II	12.06 ± 2.33[b]	21.76 ± 9.11[a]	12.00 ± 1.65[b]	< 0.05
TAC (mmol Trolox Equiv./l)				
Group I	0.91 ± 0.32	0.75 ± 0.27	0.69 ± 0.24	Ns
Group II	0.91 ± 0.32[a]	0.73 ± 0.08[a]	0.63 ± 0.29[b]	< 0.05
ADA (U/l)				
Group I	6.15 ± 1.09	6.42 ± 1.50	6.02 ± 1.87	Ns
Group II	6.15 ± 1.09	7.26 ± 1.85	6.25 ± 0.86	Ns
Total Protein(g/dl)				
Group I	6.63 ± 1.01	6.80 ± 0.61	7.03 ± 0.56	Ns
Group II	6.63 ± 1.01	6.84 ± 0.82	7.06 ± 0.97	Ns
Albumin (g/dl)				
Group I	3.27 ± 0.39	3.51 ± 0.36	3.49 ± 0.30	Ns
Group II	3.27 ± 0.39	3.37± 0.30	3.38 ± 0.37	
GGT (U/l)				
Group I	13.79 ± 4.85	12.88 ± 3.95	12.16 ± 4.51	Ns
Group II	13.79 ± 4.85	13.03 ± 2.82	15.11 ± 4.26	Ns
Globulin (g/dl)				
Group I	3.36 ± 0.80	3.33 ± 0.88	3.57 ± 1.20	Ns
Group II	3.36 ± 0.80	3.43 ± 0.75	3.65 ± 0.80	Ns

[a,b]Shows statistical differences in the same row.

These reports support doses and the duration of the experiment designed in this study[2,23,24].

Ivermectin may produce free radicals and thus results in cytotoxic effect on the parasite[7,8]. NO, produced from l-arginine by nitric oxide synthases (NOSs), is involved in various pathophysiological processes. It acts as free radicals and as host defense mechanisms through cytotoxic effect against microbial agents and tumor cells[8,25]. In this study, NO levels markedly increased at 24 h in the group received 1 mg/kg of ivermectin while NO was not significantly altered in he group received 0.5 mg/kg of ivermectin. This finding disagrees with Zhang et al[25] disagrees with where 2 g/ml and 4 g/ml of ivermectin resulted in decrease in NO by 10% and 30% at 24 h period in lypopolysaccaride treated RAW 264.7 cell culture model. On the other hand, our findings agreed with that of Hsu et al[2] where NO increased in rats orally given 1.5 to 20 mg/kg of ivermectin. However NO increase was evident at 3-6 h and returned to normal at 12 h whereas in our study NO increase was evident at 12 h and returned to normal at 120 h.

Total proteins, albumin and globulins levels were expected to change in parallel to NO that is circulated in blood stream as S-nitrosoprotein[9] but no changes were noted in the present study. Similarly GGT activity which controls synthesis of GSH, an antioxidant playing role in production of NO from *S*-nitrosoglutathione did also not alter.

Ivermectin was reported to counteract against scabies agents by inducing free radicals associated damage and by decreasing antioxidant enzyme activity[7]. Ivermectin associated free radicals production may have been the case in this as TAC level decreased in apparently healthy rabbits. Although free radicals function as defense line against pathogens they also induce harm to host own cells which results in production of antioxidant enzymes and molecules to overcome cellular damage thus antioxidant enzymes and molecules are consumed and therefore decreased in blood. Increased NO level in this study may indicate free radical associated damage due to ivermectin at therapeutic doses and, as result of this, total antioxidant capacity is decreased.

ADA an enzyme of purine metabolism is present in lymphatic tissues like spleen and thymus and T lymphocytes and is therefore considered to play role cellular immune defense[26]. Deficiency of ADA results in severe combined immunodeficiency disease characterised by incompetence of both humoral and cellular immunity[27]. GGT effects immune system by acting as regulator of T lymphocytes[11]. Gammaglobulins and to less extent beta globulins are responsible for immunity, thus are called immunoglobulins[28]. Ivermectin has been shown to play role in immune system as immunostimulator or immunosupressor[14,15,19], but in our study ivermectin did not alter plasma GGT, ADA activities and globulins levels which are considered to be immune system related.

In conclusion, ivermectin at therapeutic doses increased NO level at 24 h while decreased TAC at 120 h and did not alter other parameters of concern. These findings may suggest that ivermectin is a safe antiparasitic drug for mammals but to less extent it may have an effect on oxidant/antioxidant balance.

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