The effect of pinealectomy and zinc deficiency on nitric oxide levels in rats with induced Toxoplasma gondii infection

Abdulkerim Kasim Baltaci, Rasim Mogulkoc, Yusuf Turkoz, Cem Seref Bediz, Fikret Ozugur

a Selcuk University, Meram Medical School, Department of Physiology, Konya, Turkey
b Inonu University, Medical School, Department of Biochemistry, Malatya, Turkey
c Dokuz Eylul University, Medical School, Department of Physiology, Izmir, Turkey

Summary

Principles: This study aims at investigating how zinc deficiency and pinealectomy affect nitric oxide levels in rats infected by Toxoplasma gondii.

Methods: The study was conducted on a total of 50 adult, male rats of Sprague-Dawley species. The study groups were as follows: General, intact control group (Group I, n = 10), infected control group (Group II, n = 10), infected and zinc-deficient group (Group III, n = 10), infected and pinealectomized group (Group IV, n = 10), infected, zinc-deficient and pinealectomized group (Group V, n = 10). After the experiment the rats were decapitated and levels of zinc, melatonin and total nitrite were identified in the blood samples collected.

Results: The total nitrite levels in groups IV and V were more than those in all other groups (p <0.01). The total nitrite levels in Group II were also higher than those in Groups I and III (p <0.01). Plasma zinc levels in the zinc-deficient group and zinc-deficient and pinealectomized group were lower than those in all other groups, while melatonin levels were lower in infected pinealectomized group (Group IV) and infected, zinc-deficient and pinealectomized group (Group V) than all others (p <0.01).

Conclusions: The present study shows that plasma nitric oxide levels increase during Toxoplasma gondii infection, but this increase becomes more apparent in the presence of melatonin deficiency and is inhibited by zinc deficiency.

Key words: Toxoplasma gondii; zinc deficiency; pinealectomy; nitric oxide

Introduction

Nitric oxide (NO) is a biologically active and unstable agent formed from L-arginine amino acid via a reaction catalysed by nitric oxide synthetase (NOS) [1]. NO may enter a reaction with superoxide anion (O2) and this may lead to the formation of peroxynitrite (ONOO). This newly formed molecule oxidizes sulfhydryl groups and forms hydroxy radicals [2]. Since nitric oxide is a biologically active agent, it takes part in several biological functions. Therefore, release and inhibition of nitric oxide vary depending on different factors. Inhibition of nitric oxide by superoxide and peroxynitrite [3] and nitric oxide’s causing zinc release from presynaptic nerve ends [4] are examples of this variation.

It is reported that nitric oxide is released at various levels during the development of such parasitic infections as Toxoplasma gondii and Trypanosoma cruzi, depending on the course of the infection [5, 6]. Parallel to this, it is also stated that nitric oxide is released in response to the infection during the course of Toxoplasma gondii [7].

Melatonin, secreted from pineal gland, also has an active role in clearing hydroxyl and peroxyl radicals. Besides, melatonin stimulates several oxidative enzymes like glutathione peroxidase, glutathione reductase and superoxide dismutase [8]. It is also reported in some studies that melatonin inhibits iNOS activity and reduces nitrite levels [9–11].

It has also been reported that zinc, which is a trace element important for normal function of the body, plays an antioxidant role in the protection of cells and that there is a significant correlation between plasma zinc levels and lipid peroxides [12]. It has also been put forward in other studies that zinc suppresses iNOS expression in keratinocytes stimulated by TNF-α and INF-γ [13] and that a zinc-deficient diet causes high iNOS expression in the intestines after subcutaneous IL-1α injection [14].

When all this information is evaluated together it seems that there is no study investigating
the relations among *Toxoplasma gondii*, melatonin, zinc and nitric oxide. The aim of the present study was to find out how zinc and melatonin deficiency affect, either individually or in combination, plasma nitric oxide levels in rats infected with *Toxoplasma gondii*.

### Material and methods

This study was carried out at the Selcuk University Experimental Medicine Research and Application Center (SUDAM). All experiments were subject to approval by the SUDAM Ethics Committee. Male, adult Spraque-Dawley rats (weighing 210–260 g) were used in the study. Rats were housed in plastic non-galvanised cages and fed with standard pellet food and tap water (except for zinc-deficient rats). The animals were kept in 12 h light/12 h dark cycle (light from 07:00 to 19:00) at constant temperature and humidity (21°C and 50%, respectively).

A total of 50 rats was divided into five groups as 10 rats for each group.

_T. gondii infection:_ Experimental animals (except for those in the normal control group) were infected with RH strain of toxoplasma gondii parasite by intraperitoneal injection in 0.5 ml serum physiologic so that 10–12 parasites would be seen in the area by light microscope.

**Group I (Intact controls, n = 10):** These rats were non-infected, non-pinealectomized and fed with normal diet which including 97 mg zinc in each kg of diet for 4 weeks.

**Group II (Infected controls, n = 10):** These rats were infected and fed with normal diet for 4 weeks.

**Group III (Zinc deficient and infected rats, n = 10):** These rats were infected with *T. gondii* and fed with zinc deficient pellets that included 0.65 ppm Zn in each gram of food and bi-distilled water for 4 weeks in order to prevent uncontrollable zinc intake [15, 16].

**Group IV (Melatonin deficient and infected, n = 10):** These rats were infected with *T. gondii* one week after pinealectomy. Pinealectomy was done as described by Kuszak and Rodin [17] under general anesthesia (Rompun and Ketamin Hydrochlorur) and fed normal rat diet, including 97 mg Zn in each kg of diet for 4 weeks.

**Group V (Zinc and Melatonin deficient infected, n = 10):** Animals in this group were infected one week after pinealectomy and fed on a zinc-deficient diet and given bi-distilled water for 4 weeks.

After the 4-week experimental period all animals were sacrificed between 9.00–10.00 am and blood samples were collected to determine plasma zinc, melatonin, and NO levels.

#### Assays

**Zinc measurement:** Blood samples were centrifuged and the plasma was kept at ~20°C until analysis. Zinc was determined by atomic absorption spectrophotometers (Shimatsu ASC-600).

**Melatonin measurement:** Blood samples were centrifuged for 10 minutes at 2700 rpm at 4°C. Plasma melatonin analyses were made by RIA (Melatonin J-125 RIS, catalogue no: MEL 180). This method reliably detects melatonin concentrations as low as 2 pg/ml. Cross reactivity for melatonin is 100 percent.

**NO measurement:** Serum nitric oxide levels were measured as total nitrite with the spectrophotometric Greiss reaction. This procedure was partly adapted from the method described by Davidge et al. [18]. It was shown that total nitrite is an index of endogenous nitric oxide production [19, 20].

#### Statistics

The statistical analysis was performed using SPSS statistical program. The results are expressed as mean ± standard deviation. Kruskall-Wallis variance analysis was used for comparison between groups and Mann Whitney U test was applied to those with p <0.05. The level of statistical significance was set at p <0.01.

### Results

Plasma zinc, melatonin and total nitrite levels of the groups are presented in Table I. Plasma zinc levels were significantly lower in the infected, zinc-deficient group (Group III) and the infected, pinealectomized, zinc-deficient group (Group V) than all other groups (p <0.01). There was no significant difference between Groups III and V in terms of zinc levels. Plasma zinc levels of the infected, pinealectomized group (Group IV) were lower than those of the intact controls (Group I) and infected controls (Group II) (p <0.01). Zinc levels in Groups I and II were not different.

Plasma melatonin levels were lower in the infected, pinealectomized group (Group IV) and the
infected, pinealectomized, zinc-deficient group (Group V) than all other groups (p < 0.01). There was no significant difference between melatonin levels of Groups IV and V. Plasma melatonin levels in the infected, zinc-deficient group (Group III) were lower than those in intact controls (Group I) and infected controls (Group II), but there was no difference between melatonin levels of the two.

As for total nitrite levels, these were higher in the infected pinealectomized group (Group IV) and the infected, pinealectomized and zinc-deficient group (Group V) than all others (p < 0.01). Total nitrite levels of Groups IV and V were not different. Total nitrite levels were higher in the infected control group (Group II) than Groups I and III (p < 0.01) and in the infected, zinc-deficient group (Group III) than Group I (p < 0.01).

**Discussion**

When the findings obtained at the end of the experiment are evaluated with particular regard to nitric oxide (NO), it is seen that total nitrite levels in the infected controls (Group II) were higher than in the intact control group where no application was made (Group I) as well as in the infected, zinc-deficient group (Group III). This finding demonstrates that *Toxoplasma gondii* infection causes an increase in nitric oxide levels. Results of studies investigating how nitric oxide levels are affected in *T. gondii* infection are inconsistent [5, 6]. It was reported in a study that nitric oxide levels decreased in *T. gondii* infection [6]. Similarly, Seabra et al. [21] mentioned a partial inhibition in nitric oxide produced by active macrophages in *Toxoplasma gondii* infection. It is claimed in the concerned study that the decrease in nitric oxide resulted from the deactivation of macrophages. The increased levels of nitric oxide in *T. gondii* infection, arrived at in our study, are inconsistent with the findings of the mentioned researchers. However, Brunet [22] stated that nitric oxide levels increase in *Toxoplasma* infection and this increase is accepted as a physiological result of the immune response to *Toxoplasma* infection. The protective role of the increase in nitric oxide levels is noted in taking *Toxoplasma* infection under control, particularly in the chronic phase of the infection [23]. It is reported that the increase in nitric oxide against intra-cellular infection is necessary to control the host in toxoplasmosis [24]. Similarly, many researchers reported an increase in nitric oxide levels in *T. gondii* infection [7, 25–27]. Results obtained by the above-mentioned researchers are parallel to the increased nitric oxide levels we obtained in the infected control group.

Nitric oxide levels in the infected and zinc-deficient group (Group III) were higher than those in the control group, but lower than all other infected groups in our study. Our *medline* search did not reveal any study that addressed zinc, nitric oxide and *toxoplasma* infection together. However, there were studies investigating the relations between zinc and nitric oxide irrespective of infection and the results of these studies pointed to a positive relation between zinc and nitric oxide [28, 29]. In fact it is possible that there is a mutual interaction between zinc and nitric oxide. An important reason that creates this possibility is that while there is a decrease in nitric oxide activity as a result of zinc deficiency [30], nitric oxide leads to zinc release from presynaptic nerve endings and the zinc that is released affects nitric oxide activity [4]. As a result, the finding we obtained shows that a zinc-deficient diet inhibited the nitric oxide production that is induced by *T. gondii* infection. It was shown that deficiency of zinc, an important trace element, in the diet caused reductions in body weight [31]. Likewise, we compared weight changes of experimental animals in the part of this study that has not been published yet. Here we observed that zinc deficiency led to a significant weight loss in animals. Weight loss brought about by zinc deficiency in animals will affect the immune system negatively.

There was a significant increase in nitric oxide levels in the infected, pinealectomized group (Group IV). Likewise it was reported in studies investigating the relation between melatonin and nitric oxide that nitric oxide production was inhibited depending on melatonin administration at physiological concentrations [32] and that this effect caused by melatonin was seen not only in *in vivo* studies but also in *in vitro* studies [33]. It is stated that this fall in nitric oxide levels affected by melatonin application results from the inhibition of iNOS expression [9, 34–36]. These results obtained by other researchers are supportive of the results we obtained. In addition, nitric oxide levels in this group (Group IV) were significantly higher than those in the infected control group (Group II) and infected, zinc-deficient group (Group III). These results demonstrate that the increase in nitric oxide levels seen in *T. gondii* infection is further intensified by melatonin deficiency.

Nitric oxide levels of the infected, pinealectomized and zinc-deficient group (Group V) were higher than those in Groups I, II and III, but not different than those in Group IV. These findings suggest that zinc deficiency cannot inhibit the increased nitric oxide production caused by pinealectomy in addition to infection. Zinc deficiency in the diet leads to a decrease in T cells, particularly in TH1 functions and thereby in the production of IFN-gamma and IL-2, products of TH1. Thus, it unfavourably influences cell-mediated immunity and lytic activity of NK cells [37]. Melatonin, the major neuro-hormone secreted by the
pineal gland, affects cellular immunity both directly and indirectly [38]. Following pinealectomy, significant decreases are found in plasma zinc, zinc-dependent hormone thymlnine, IL-2, IFN-gamma, T cell count and NK cytotoxic cell activity and these are corrected with pharmacological doses of melatonin administration [39, 40]. Melatonin also controls secretion of gamma interferon, which plays a key role in immune system activity, and IL-2, which is secreted from TH1 lymphocytes [40]. It seems that zinc is an essential media-
tor in all effects that melatonin exercises on the immu-

When the findings of the study are assessed as a whole, it is seen that T. gondii infection and mela-
tonin deficiency together with T. gondii infection lead to an increase in nitric oxide production, but pinealectomy applied parallel to the infection is more effective in this increase. Another important finding of our study is that increased nitric oxide production in T. gondii infection is inhibited by a zinc-deficient diet.

Correspondence:
Dr. Abdulkerim Kasim Baltaci
Selcuk University, Meram Medical School,
Department of Physiology, Konya, TURKEY
E-Mail: baltaci@seluk.edu.tr

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Erratum

In the following article the name of the second author J. Al Maiwenn has not been correctly printed:


The name should be printed as Maiwenn J Al. (instead of J. Al Maiwenn). We apologize for the mistake.
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