Clinical Nutrition 32 (2013) 592–598

Contents lists available at SciVerse ScienceDirect

Clinical Nutrition

journal homepage: http://www.elsevier.com/locate/clnu

Original article

Zinc and pregnancy: Marked changes on the immune response following zinc therapy for pregnant females challenged with *Trypanosoma cruzi*

Cássia Mariana Bronzon da Costa [a], Vânia Brazão [a], Christian Collins Kuehn [a], Luiz Gustavo Rodrigues Oliveira [a], José Clóvis do Prado Júnior [a], Miguel Angel Sala [b], Ana Amélia Carraro Abrahão [a,*]

[a] *Laboratório de Parasitologia, Departamento de Análises Clínicas, Toxicológicas e Bromatológicas, Faculdade de Ciências Farmacêuticas de Ribeirão Preto FCFRP-USP, Universidade de São Paulo, Avenida do Café s/n°, 14040-903 Ribeirão Preto, SP, Brazil*
[b] *Laboratório de Histopatologia Departamento de Morfologia, Estomatologia e Fisiologia, Faculdade de Odontologia de Ribeirão Preto, Universidade de São Paulo, Avenida do Café s/n, 14030-610 Ribeirão Preto, SP, Brazil*

ARTICLE INFO

Article history:
Received 1 December 2011
Accepted 21 October 2012

Keywords:
Zinc
Pregnancy
Trypanosoma cruzi
Immune response

SUMMARY

Background & aims: The occurrence of infectious disease processes during pregnancy has significant effects on maternal health and can lead to adverse pregnancy outcomes. The aim of the present study was to examine the potential role of zinc treatment during *Trypanosoma cruzi* infection in pregnant animals.
Methods: Female Wistar rats weighing 180–200 g were used in all experiments. Production of nitric oxide, peritoneal macrophages counts, and concentrations of IFN-γ and TNF-α were measured, and the potential protective effects of zinc on fetal development were assessed at 14-day post-infection.
Results: Nitric oxide concentrations were higher in pregnant zinc-treated animals than in their untreated counterparts, despite similar levels of the macrophages, IFN-γ and TNF-α. Zinc therapy was associated with a significant reduction in parasitemia and cardiac parasite burden. Higher placental and birth weights were observed in animals given prenatal zinc supplementation compared to untreated animals.
Conclusions: These data confirm the critical importance of adequate zinc intake during the peri-conceptional period and indicate that zinc has an effective role in preventing adverse outcomes of pregnancy and reducing the risk of common infections such as Chagas' disease.

1. Introduction

During pregnancy, levels of various hormones and serum factors that may modulate lymphocyte or macrophage synthesis, activation, and/or function shift considerably to support a healthy pregnancy.[1] This adaptation plays a protective role in the feto-maternal relationship, protecting the fetus from rejection by the maternal immune system while at the same time maintaining adequate maternal host defense mechanisms to fight infection.

A wealth of new information on how the immune system develops and operates has provided new insights into the complex and multifaceted host immune response to *Trypanosoma cruzi*. Because *in vivo* models may provide data with clearer clinical relevance, several different experimental models have been used to mimic human infection, including sylvatic rodents,[2,3] mice[4] and rats.[5,6]

Growing evidence indicates that infectious processes during pregnancy have significant effects on maternal health and can lead to adverse pregnancy outcomes, including spontaneous abortion, pre-term labor, preeclampsia, and intrauterine growth restriction.[7] Moreover, emerging evidence supports a bi-directional interaction between nutrition status and incidence of infectious disease, such that the consequences on target tissues of either one are moderated by the other. A functional decline in plasma zinc levels has been reported during the acute phase of infections. This decline is likely due to an increased zinc requirement in the host because pro-inflammatory cytokines mediate changes in hepatic zinc homeostasis, leading to the sequestration of zinc into liver cells and subsequently to hypozincemia.[8]

Nutritional zinc requirements are difficult to determine because many dietary factors affect the bioavailability of zinc, and

* Corresponding author. Tel.: +55 16 602 4153; fax: +55 16 602 4163.
E-mail address: acarraro@fcfrp.usp.br (A.A. Carraro Abrahão).

0261-5614/$ – see front matter © 2012 Elsevier Ltd and European Society for Clinical Nutrition and Metabolism. All rights reserved.
http://dx.doi.org/10.1016/j.clnu.2012.10.012

physiological requirements of zinc vary greatly between different conditions and age groups. Different intervention trials have been tested and have found that supplementation with daily intakes of 10–30 mg of zinc can be considered an important adjuvant therapy for treating infectious diseases in children in developing countries.[9]

Physiologically, zinc serum concentrations decline during pregnancy, primarily due to hemodilution and decreased albumin levels.[10,11] While 10.5 mM is considered to be the lower limit of serum zinc during the first and second trimester, zinc levels decline to 9.5 mM in the third trimester.[11] Considering that the estimated gestation period in rats is 21 days,[12] we hypothesize that zinc levels will sharply decrease during both pregnancy and *T. cruzi* infection.

Previous studies performed by our group have provided evidence for the involvement of zinc in up-regulating the host's immune response in cases of Chagas' disease, protecting animals against the harmful actions of *T. cruzi* infection.[13,14] During pregnancy and in cases of *T. cruzi* infection, nitric oxide (NO) has complex and diverse functions in physiological and pathological events. Among its many biological functions, NO is a potent vasodilator and immune modulator; in addition, NO concentrations are generally elevated during pregnancy.[15]

NO is a small lipophilic molecule enzymatically originated from cleavage of the terminal guanidino nitrogen of L-arginine by a family of NOS.[16] When activated, the NO system triggers enhanced macrophage cytotoxicity, which is the first line of defense against invading pathogens, as it elicits cellular apoptosis in different systems.[17]

It has been shown by several investigators that IFN-γ, TNF-α, and chemokines are strong inducers of NOS_2 and that these molecules are produced in high concentrations during acute *T. cruzi* infection.[18–20] Conversely, TNF-α and IL-10 negatively regulate NO production.[20]

As previously described, zinc deficiency leads to impaired function of the specific and non-specific immune response, which can lead to an increased susceptibility to bacterial, viral and parasitic infections.[21] Notably, a lack of zinc can impair the development of the immune system, thereby emphasizing the importance of a balanced zinc intake during pregnancy.[22] In animals, gestational zinc deficiency induces thymic and splenic involution, consequently impairing active and passive immunity.[22]

Based on the immunobiology of zinc previously described, our hypothesis was that therapy with this oligoelement can have a protective effect on fetal development and maintenance of the immune response in the infected mother during gestation. To test this hypothesis, we evaluated NO, IFN-γ and TNF-α concentrations and mobilization of peritoneal macrophages during the acute phase of *T. cruzi* infection.

2. Material and methods

2.1. Pregnancy

Female Wistar rats weighing 180–200 g were used in all experiments. Rats were obtained from the Facility House of the Universitary Campus of Ribeirão Preto. Animals were randomized into the following groups: pregnant control (PC), pregnant control treated with zinc (PCZ), pregnant infected (PI), pregnant infected treated with zinc (PIZ). Each experimental group consisted of 5 animals. The females were housed two to a cage. One male Wistar rat was introduced into each cage and was allowed to mate with the females. The time of appearance of a vaginal plug was designated as day 1 of gestation. Mated females were removed from males and housed in groups of five females per cage. Commercial rodent diet and water were available *ad libitum*. Rat bedding was changed 3 times/week to avoid concentration of ammonia from urine. The protocol of this study was approved by the local Ethics Committee protocol number 01.193.53.4.

2.2. Parasites and experimental infection

Soon after mating and confirmation of pregnancy, rats were intraperitoneally (i.p.) inoculated with 1×10^5 blood tripomastigotes of the Y strain of *T. cruzi*.[23] Parasitemia was determined by Brener's method.

2.3. Treatment scheme

Rats were orally treated with zinc sulfate (Sigma Chemical Co. MO, USA) dissolved in 0.1 ml of distilled water at a dose of 20 mg/kg body weight[6] once a day at the same time, starting the day after the inoculum and every day until the end of the experiment.

2.4. Euthanasia

On day 18 of pregnancy, which corresponded to 14 days after infection, animals were euthanized with anesthesia using tribromoethanol (2.5%) administered intraperitoneally at a dose of 0.1 ml per 10 g of body weight.

2.5. Quantification of peritoneal macrophages

Peritoneal cells were harvested with an injection of 5 ml of cold RPMI 1640 medium into the peritoneal cavity. The cells were centrifuged at 410 *G* for 15 min and the pellet re-suspended in 1 ml of RPMI 1640 medium and diluted (1:10) with Turk's solution (15 ml glacial acetic acid; 0.2 ml gentian violet 2%; 500 ml distilled water). Macrophages were counted in a Neubauer chamber.

2.6. Measurement of NO production

NO production was measured according to the method used by Brazão et al. (2010),[24] who measured accumulated supernatant nitrite (a stable breakdown product of NO) in pregnant and virgin animals using the Griess reaction. Cells were collected from the peritoneal cavity and re-suspended in RPMI 1640 medium (LGC). The cells were centrifuged for 15 min and the pellet was re-suspended in ice cold RPMI 1640 medium. The cells were adjusted to 2×10^6 cells/mL. Volumes (100 μL) of cell suspensions were plated onto each well of 96-well flat-bottom culture plates (Corning) with or without LPS (10 μg/mL). Plates were incubated at 37 °C for 48 h in 5% CO_2 atmosphere. Subsequently, 100 μL of the supernatant was collected and transferred to new 96-well flat-bottom culture plates. The supernatants were incubated with 100 μL of Griess reagent (50 μL of 1% sulfanilamide (Sigma) plus 50 μL of 0.1% N-1-naphthylethylenediamine (Sigma) in 5% phosphoric acid solution) at room temperature for 5 min. The absorbance was determined in triplicate at 540 nm and expressed in micromoles.

2.7. Cytokine assays

Serum obtained 18 days post pregnancy was used for cytokine assays. Concentrations of IFN-γ were measured by specific two-site enzyme-linked immunosorbent assay (ELISA) according to the manufacturer's specifications with reference standard curves, using known amounts of the relevant rat recombinant cytokines. All samples were processed individually and assayed in duplicate, with plates read at 450 nm. R&D Systems