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REGULAR ARTICLES

A study on the status of inflammatory systems in camels naturally infected with *Toxoplasma gondii*

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Abstract *Toxoplasma gondii* is a unique intracellular parasite with a worldwide distribution. This parasite infects a variety of cells in a wide range of animal species such as dromedary camels (*Camelus dromedarius*). In order to evaluate the pattern of possible changes in the blood level of some inflammatory mediators and antioxidant enzymes in camels infected with *T. gondii*, blood samples were taken from a total of 493 dromedary camels and serum concentrations of inflammatory mediators, acute phase proteins and antioxidant enzymes were measured. According to serological data, no seropositivity was found for anti-*T. gondii* IgM in serum samples; however, 49 camels (9.93 %) showed positive titrations for anti-Toxoplasma IgG. The analyses of data in seropositive animals showed significant increases ($P<0.05$) in the serum level of IL-1β and adenosine deaminase activity; however, IFN-γ and TNF-α demonstrated no significant changes in serum samples of the infected camels. In addition, while major acute phase proteins (haptoglobin (Hp) and serum amyloid A (SAA)) were markedly elevated in infected camels, the activity of antioxidant enzymes (SOD and GPX) was remarkably decreased in the blood samples of infected animals. Thus, during the chronic infection in camels, *T. gondii* can promote significant rises in concentrations of some cytokines (such as IL-1β), acute phase proteins and adenosine deaminase.

Keywords *Toxoplasma gondii* · Inflammatory mediators · Adenosine deaminase · Acute phase proteins · Antioxidant enzymes

Introduction

The infection with the intracellular Apicomplexa protozoan, *Toxoplasma gondii*, occurs in most areas of the world. The parasite has been reported to infect most animal species in food chain, including camels (Dubey 2010). Dromedary camels (*Camelus dromedarius*) are important to the economy of many countries (Tajik et al. 2011). The seroprevalence of the infection with *T. gondii* in camels varies widely depending on the localities of the world (Shaapan and Khalil 2008), ranging from 14.57 % in Iran (Hamidinejat et al. 2013) to 90.90 % in Turkey (Utuk et al. 2012), suggesting the variable occurrence and economic importance of toxoplasmosis in Asia.

Camels acquire *T. gondii* infection through ingestion of the oocysts shed by domestic cats or wild felids (Elamin et al. 1992). The parasites then undergo asexual multiplications in various tissues of camels as tachyzoites and bradyzoites. In the initial stages of the infection, IgM is secreted, and this is followed by a switch to IgG secretion during the second week of the infection (Remington and Krahenbuhl 1982). The phase of the infection is generally diagnosed based on the detection of those specific antibodies. Some assays have suggested *Toxoplasma*-specific IgM antibodies as indicators of the acute phase infection (Yasodhara et al. 2001). However, since IgG antibodies to *Toxoplasma* spp. can be detected for a long time after the acute infection (Chronic phase), thus, the IgG avidity determination can be used to distinguish the chronic infections (Cozon et al. 1998).

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Previous works showed that antibody alone does not produce adequate immunity (Johnson et al. 1983) and the activity of phagocytic cells as cell-mediated immunity is also essential (Mauel 1984). This process is regulated by helper and suppressor T lymphocytes which control production and secretion of lymphokines, such as the interferons (IFNs) and interleukins (ILs). The in vivo experiments confirm the crucial role of IFN-γ (Suzuki et al. 1988) and tumour necrosis factor (TNF)-α (Kelly et al. 1989) in inhibition of parasite proliferation. While the parasite possibly activates the cytokines, the relationship between the level of the secreted cytokines and the pathogenesis of the parasite in camels was not clearly described.

Adenosine deaminase (ADA) is a cytoplasmic enzyme which is essential for lymphocytes differentiation and proliferation. Increased ADA is due mainly to increased immune cell numbers (Ungerer et al. 1992). The main biological activity of ADA is to protect lymphocytes from toxic effects of 2-deoxyadenosine and deoxyadenosine triphosphate which depress immune functions (Senesi et al. 1990). In this point of view, changes in ADA activity could likely reveal some aspects of immunity to protect against the parasite during the infection.

The acute phase proteins (APPs) are a group of blood proteins that mainly synthesize in the liver. The APP concentrations can increase (positive APPs) or decrease (negative APPs) in inflammatory diseases (Eckersall 2000). It has been shown that the APP concentrations change in animals subjected to challenges such as infection or stress (Murata et al. 2004). Thus, APP parameters could be used for diagnostic purposes (Gruys et al. 2005). As a result, it seems that APPs should receive more consideration when investigating the epidemiology. On the other hand, it was hypothesized that the alterations in the activity of antioxidant enzymes, particularly superoxide dismutase (SOD), glutathione peroxidase (GPX) (Shiono et al. 2003b; Razavi et al. 2011; Nazifi et al. 2011) and catalase (Grewal et al. 2005) may play a role in the pathogenesis of some protozoans such as *Theileria* in ruminants.

This study was therefore undertaken to evaluate the serum concentrations of the main inflammatory cytokines (IL-1β, IL-6, IFN-γ, TNF-α), ADA, acute phase proteins and antioxidant enzymes activities (SOD and GPX) in camels which are seropositive for toxoplasmosis.

Materials and methods

Collection of blood samples

During a period of 6 months, from the beginning of December 2013 afterwards, different regions were randomly selected from camel-rearing areas located in southern provinces of Iran for sampling. A total of 493 dromedary camels were selected and subjected for blood sampling. Blood samples were drawn from jugular vein into EDTA containing tubes for measuring antioxidant enzyme activities and put into plain tubes without anticoagulant for performing serum assays.

Animal ethics

This research was accomplished under the approval of the state committee on animal ethics, Shiraz University, Shiraz, Iran. Also, we used the recommendations of European Council Directive (86/609/EC) of November 24, 1986, regarding the standards in the protection of animals used for experimental purposes.

Serological tests

The blood specimens in plain tubes were centrifuged at 750*g* for 15 min, and the separated sera were stored at −20 °C until analysis.

The anti-toxoplasma-specific IgM and IgG serum antibodies were analysed by ELISA using commercially available kit (Euroimmun, Germany), incorporating standard positive and negative controls provided in the kits. Antibody levels >10 IU/mL were considered to be positive.

In the present study, the seropositive camels were assigned as the infected group. Also, 50 camels with no anti-toxoplasma-specific IgM and IgG antibodies were randomly selected and referred to as controls.

Inflammatory cytokines

The serum concentration of IL-1β was measured by a quantitative enzyme immunoassay (Sandwich ELISA) kit (Genorise Scientific Inc., Paoli, USA) and presented as pg/mL. The concentrations of IFN-γ and TNF-α were measured by a solid phase Sandwich ELISA (AbC 606 and AbC 607, respectively; Votre fournisseur AbCys S.A. Paris, France) and expressed as pg/mL.

Adenosine deaminase (ADA) activity measurement

ADA activity was assessed by an enzymatic-calorimetric assay kit (Diazyme Laboratories, Gregg Court, USA). This assay is based on the enzymatic deamination of adenosine to inosine which is converted to hypoxanthine by purine nucleoside phosphorylase. Hypoxanthine is then converted to uric acid and hydrogen peroxide (H_2O_2) by xanthine oxidase (XOD). The generated quinone dye was monitored spectrophotometrically and expressed as U/L of serum.

Acute phase proteins

Haptoglobin (Hp) was measured using a commercial kit based on prevention of the peroxidase activity of haemoglobin, which is directly proportional to the amount of Hp. The analytical sensitivity of this test in serum has been determined as 0.0156 mg/mL for Hp by the manufacturer (Tridelta Development Plc, Wicklow, Ireland). Serum amyloid A (SAA) was assayed by a solid phase sandwich ELISA. The analytical sensitivity of this test in serum has been determined as 0.3 μg/mL for SAA by the manufacturer (Tridelta Development Plc, Wicklow, Ireland). Also, the serum samples were analysed for total protein by Biuret method, albumin by the bromocresol green method and the total globulin by the difference of total protein and albumin.

Antioxidant enzymes activities

SOD activity was measured with a commercial kit (RANSOD kit, Randox Com, UK). This method employs xanthine and xanthine oxidase to generate superoxide radicals, which react with 2–(4-iodophenyl)-3–(4-nitrophenol)-5-phenyltetrazolium chloride (INT) to form a red formazan dye. The enzyme activity was then determined by the degree of reaction inhibition, as one unit of SOD corresponded to 50 % inhibition of INT reduction under assay condition. The GPX activity was measured by a commercial kit (RANSEL kit, Randox Com, UK) based on the method of Paglia and Valentine (1967). The values of both enzymes were expressed as units/g of haemoglobin.

Statistical analysis

Student's *t* test was used for comparison of measured parameters between control and infected groups. All values in tables are presented as mean, and standard error of mean (SEM) and $P<0.05$ was considered as statistically significant.

Results

Serologic analyses

Although the serological assays revealed no seropositivity for anti-*T. gondii* IgM in serum samples of camel populations, 49 camels (9.93 %) showed positive titrations for anti-*T. gondii* IgG (diseased group). This result clearly showed that all the serologically infected camels passed through the acute disease and entered in chronic phase of the infection.

Immunological and biochemical findings

The levels of inflammatory cytokines (including IL-1β, IFN-γ and TNF-α), acute phase proteins, ADA and antioxidant enzymes activities in non-infected ($n=50$) and infected animals ($n=49$) are shown in Tables 1 and 2.

In this study, the analyses of data achieved from the inflammatory mediators measurements in infected animals compared to the controls showed significant increase ($P<0.05$) in the serum level of IL-1β. However, interestingly, IFN-γ and TNF-α had no significant changes in serum samples of the infected camels.

The results revealed that ADA had significant increased activity in infected animals compared to control. Furthermore, haptoglobin (Hp) and serum amyloid A (SAA) as the major acute phase proteins were remarkably increased in the infected animals. Similarly, the serum assays revealed marked elevations in total protein, albumin and the total globulin of the infected animals. On the other hand, our results showed that the activity of antioxidant enzymes (SOD and GPX) were remarkably decreased in the blood samples of infected camels.

Discussion

The results obtained in this study clearly demonstrate that the infection with *T. gondii* in camels can induce remarkable changes in the levels of some cytokines (such as IL-1β), APPs, ADA and antioxidant enzyme activities.

In our work, 9.93 % of camels reared in southern Iran showed positive titrations for anti-*T. gondii* IgG. The infected camels could be a potential concern for public health, since camels could be an important source of the infection for other animals and human beings through their milk or meat. The ingestion of uncooked meat was assigned as an important mode of transmission of this parasite, and viable *T. gondii* has been isolated from most animal species in food chain, including camel (Dubey 2010). Consistent with our work, Hamidinejat et al. (2013) found *T. gondii* antibodies in 14.57 % in camels reared in an area in the centre of Iran. Also, using an immunofluorescence antibody test, Sadrebazzaz et al. (2006) showed antibodies against *T. gondii* in 4.16 % of camels reared in the northeastern region of Iran. This difference in results may be attributed to different climate conditions or the existence of infected cats in different areas.

Although the level of IL-1β and ADA activity showed significant increases in *T. gondii*-infected camels, serum concentration of TNF-α and IFN-γ demonstrated no considerable changes in infected animals. These results may disclose that IL-1β, but not TNF-α or IFN-γ, could be assigned as the major cytokine which is produced and protects against

Table 1 The serum concentrations of interleukin-1 beta (IL-1β), gamma interferon (IFN-γ), tumour necrosis factor-alpha (TNF-α) and adenosine deaminase activity (ADA) in infected and control groups (values are expressed as mean±SEM)

Animal status	IL-1β pg/mL	IFN-γ pg/mL	TNF-α pg/mL	ADA U/L
Seropositive camels (n=49)	40.79*±0.44	19.02±0.17	15.73±0.05	103.08*±0.64
Not seropositive camels (n=50)	21.3±0.24	19.05±0.16	15.77±0.05	9.1±0.16

*Shows the significant differences (P<0.05) between infected and non-infected values (P<0.05)

parasites during the chronic phase of toxoplasmosis in camels. In this point of view, the literature on toxoplasmosis shows that our understanding of ruminant immunity to the parasite and also the mechanisms by which TNF, IL-lα and IL-1β exert a protective effect on *Toxoplasma* infection in other species are limited.

Chang et al. (1990) demonstrated that mice treated with TNF, IL-lα, IL-1β, or a combination of TNF plus IL-1α or IL-1β, had a decreased mortality after a lethal challenge with *T. gondii*. However, they stated that TNF was detected in the sera of mice infected with the highly virulent RH strain of *T. gondii* and in the acute phase of the disease not in chronic stage. Using similar models of infection, the experiments using a monoclonal antibody against IFN-γ demonstrated the important role of IFN-γ in defence against *Toxoplasma* infection (Suzuki et al. 1988). Also, it was shown previously that IFN-γ (McCabe et al. 1984) protected a significant number of mice in acute phase of the infection. In contrast with these studies and in line with our data, Pelloux et al. (1992) reported that *T. gondii* alone does not induce secretion of TNF-α by activated human monocytes or macrophages in vitro. The absence of TNF-α induction is a reversible phenomenon because after *Toxoplasma* invasion and addition of positive pooled serum and/or LPS, the infected cells were again able to secrete TNF-α. The lack of TNF-α secretion in our work also agrees with the experiments conducted by Kelly et al. (1989) in which soluble *Toxoplasma* antigen stimulated human monocytes to secrete IL-1 but not TNF-α.

Despite our study not showing evidence of any changes in the value of some major cytokines (i.e., TNF-α and IFN-γ), our data revealed that the acute phase proteins, total protein and globulins maintained their significant levels in chronic phase of toxoplasmosis in camels. In other words, this result indicates that the parasite can induce high degrees of inflammatory responses even in chronic disease. The APPs are a group of blood proteins whose concentrations could change in animals subjected to challenges such as infection, inflammation or stress (Murata et al. 2004; Gruys et al. 2005). Also, SAA and Hp as well as other APPs have been proposed to be markers of stress in cattle and other species (Arthington et al. 2003; Pieiro et al. 2007). In addition, both SAA and Hp are considered among the major positive APPs in cattle and can increase several-fold from baseline levels after tissue injury (Murata et al. 2004); however, the underlying mechanism that causes increase in serum SAA and Hp has not been clearly described. Corroborating our data, some previous studies suggested that the infection of cattle with *Anaplasma* could induce cytokines release such as IL-1 and IL-6 under the effect of which APPs are substantially synthesized in the liver (Latimer et al. 2003; Nazifi et al. 2009).

The significant declines observed in the level of the activity of erythrocytic antioxidant enzymes in the infected camels indicate increased exposure of erythrocytes to oxidative stress. Accordingly, it could be speculated that *T. gondii* can promote significant production of oxidative agents in the infected camels which results in marked effects on key antioxidant defence mechanisms. Consistently, Shiono et al. (2003a) reported that the levels of malondialdehyde (MDA), as a biomarker of lipid peroxidation, began to increase remarkably in proportion to the increase in parasitemia in cattle infected with a haemoprotozoan, *Theileria sergenti*. The parasite may induce hydrogen peroxide (H_2O_2) from peripheral blood phagocytes, and thus, the oxidative damage to the erythrocytes might play an important role in the pathogenesis of anaemia in those animals.

Table 2 The serum values for haptoglobin (Hp), serum amyloid A (SAA), total protein, albumin, globulin and antioxidant enzymes activities (superoxide dismutase and glutathione peroxidase) in infected and control groups (values are expressed as mean±SEM)

Animal status	Hp g/L	SAA μg/mol	Total protein g/dL	Albumin g/dL	Globulin g/dL	SOD U/gHb	GPX U/gHb
Seropositive camels (n=49)	0.54*±0.011	21.78*±0.33	5.63*±0.04	3.528*±0.01	2.11*±0.04	1298.76*±1.86	196.35*±0.92
Not seropositive camels (n=50)	0.27±0.005	9.14±0.15	5.24±0.05	3.81±0.01	1.4±0.05	1387.82±2.17	216.26±0.95

*Shows the significant differences (P<0.05) between infected and non-infected values (P<0.05)

GPX activity is a major mechanism for intracellular decomposition of lipid peroxides (Flohe 1971) and prevents cell membranes from peroxide damage induced by these metabolites (Hafeman et al. 1974). On the other side, it appears that SOD activity, in order to dismutate superoxide radicals, plays a crucial role to protect the cells against oxidative damage (Flohe 1971). Thus, it can be postulated that *T. gondii* may induce a significant oxidative injury in different cell types, at least erythrocytes, even in non-acute phase of the disease.

Recently, several studies on some haemoprotozoan parasites of ruminants have disclosed that the parasitemia causes antioxidant enzymes activities of erythrocytes to be remarkably decreased; as in animals with the higher parasitemia levels, significant declines were evident in the activities of SOD and GPX (Nazifi et al. 2011; Razavi et al. 2011). They stated that the cited enzymes are consumed to scavenge H_2O_2 to protect erythrocytes against oxidative injuries and thus declined in infected animals.

In conclusion, seroepidemiological assays revealed that the prevalence of the infection with *T. gondii* is moderate (9.93 %) in camels reared in southern Iran. Thus, camels could be a potential concern for public health. In addition, this study disclosed that during the chronic infection, *T. gondii* can result in remarkable increases in the levels of some cytokines (such as IL-1β), acute phase proteins and adenosine deaminase in camels. On the contrary, the parasite induces a significant modulatory effect on the antioxidant enzymes activities; however, our data proved that *T. gondii* may not promote any significant effects on concentrations of TNF-α and IFN-γ at chronic phase of the disease.

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Conflict of interest statement We declare that we have no conflict of interest.

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