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EXPERIMENTAL STUDY

Plasma leptin levels in rats with induced Toxoplasma gondii infection

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Abstract: *Background:* To explore the changes in plasma leptin levels of rats with induced Toxoplasma gondii infection.
Methods: The study was conducted on 20 Spraque-Dawley type adult male rats, which were equally divided into two groups. Group 1, general control group. Group 2, infection group (rats in this group were infected with live Toxoplasma gondii parasite, which was injected in 0.5 ml serum physiologic through intraperitoneal route, so that 10–12 parasites were seen in the area under a light microscope). Blood samples collected from all animals 4 weeks after the infection were analyzed to determine plasma leptin levels (RIA).
Results: There was no significant difference between the body weights of groups 1 and 2 at the end of the study. Plasma leptin levels in the infection group (group 2) were significantly higher than those in group 1 ($p<0.01$).
Conclusion: Toxoplasma gondii infection can cause an increase in leptin secretion without changing body weight in a period of 4 weeks in rats *(Tab. 1, Ref. 25)*. Full Text in PDF *www.elis.sk.*
Key words: Toxoplasma gondii, leptin, rat.

Leptin is the 167-amino acid hormonal protein product of the obesity gene, which has been widely researched after being defined by Zhang et al (1). Leptin, which was initially described in relation to satiety and energy balance, was then claimed to be an anti-obesity factor acting through a feedback effect from adipocytes to hypothalamus. There has been a growing body of evidence emphasizing leptin as a critical hormone in the regulation of body weight and food intake in both animals and humans (2–5). Results of research also suggest that leptin is involved in metabolic regulation (6, 7), sexual maturation (8, 9), reproduction (10), hematopoiesis (11), gastrointestinal functions (12), sympathetic activation (13) and angiogenesis (14).

Leptin may be classified as a cytokine due to the similarity between the structure of leptin and its receptors and cytokines (15). Leptin structurally resembles IL-2 in particular and is a crucial T-cell growth factor (15). Circulating concentration of leptin is proportionate to the size of fat mass (16). Decrease in body weight or undernourishment results in hypoleptinemia (16), while decreased leptin secretion leads to an increase in immune defects and infections (17). Reports of the studies exploring the relation between leptin and the immune system put forward the hypothesis that low concentrations of serum leptin increase predisposition to infections by reducing T-helper (Th) cells and directly affecting thymic functions (17, 18). Presence of leptin receptors in CD^+_4 and CD^+_8 T lymphocytes also substantiates the correlation between leptin and immune functions.

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Leptin has a stimulating effect on Th1 cells and an inhibiting effect on Th2 cells. In the context of the cellular immune response to infections, leptin plays an important role in Th1 cell activation and the elevated levels of IL-2, IFN-y and TNF-α, which are products of Th1. It exercises a strong stimulating effect on cytokine production of Th1. The fact that NK cell activation responds to leptin stimulation indicates that leptin also has a major part in NK cell activation (19, 20).

Cellular immunity is the main control mechanism in T. gondii infections. It has been revealed that T-cells mediate the major resistance mechanism against this parasite in laboratory animals and humans (21). As the immunological role of T_8-lymphocyte cell functions has not been fully understood in parasitic infections, studies have concentrated more on the protective functions of T_4-cells (22). Until recently, it has been accepted that T_4-lymphocytes are the principal T-cells that most effectively produced both immunopathological and protective responses, while T_8-lymphocytes basically act as regulators (21, 22). However, recent studies have demonstrated the importance of T_8-cell functions in immunity against T. gondii. The current conviction is that T_4-lymphocytes assume an initial, protective role against T. gondii, whereas T_8-cells have a more critical function in actual cellular defence (21, 22). The objective of the present study is to explore the changes in plasma leptin levels of rats with induced toxoplasma gondii infection.

Methods

The study was conducted at S.U. Experimental Medicine Research and Application Centre on the rats obtained thereof. The study included a total of 20 6-month-old Spraque-Dawley adult male rats, which were grouped as follows:

Group 1, (n: 10) Normal Control Group: The group not subjected to any procedure and on a normal diet.

Group 2, (n: 10) Infected Control Group: The group infected with toxoplasma gondii parasite and on a normal diet.

Experimental animals (except for those in the normal control group) were infected with live toxoplasma gondii parasite, which was obtained from Ankara University School of Medicine Parasitology Department Laboratory and which was injected in 0.5 ml serum physiologic through intraperitoneal route, so that 10–12 parasites were seen in the area under a light microscope. Four weeks after the infection, blood samples were collected from the animals by decapitation and analyzed to determine plasma leptin levels.

Plasma leptin determination

Serum leptin analysis was carried out using Rat Leptin RIA test kit (Linco trademark catalogue no: RL-83K). Limit sensitivity of the rat leptin analysis is 0.5 ng/ml and limit linearity is 50 ng/ml. The results were expressed as ng/ml.

Statistical analyses

SPSS 13.0 statistics software was used in the calculation of data. Paired Samples t test was employed in the evaluation within the group and Student t test in the evaluations between groups.

Results

No significant difference was found between groups 1 and 2 in terms of body weight at the end of the study. Plasma leptin levels in the infection group (group 2) were significantly higher than those in group 1 ($p<0.01$) (Tab. 1).

Tab. 1. Body weights and plasma leptin levels of experimental animals.

Groups	Body weight before study (g)	Body weight after study (g)	Leptin (ng/ml)
Control	266.00±32.81	270.50±33.70	4.09±1.15B
Infected	263.50±44.16	269.50±42.78	7.53± 1.55A
P			0.01

* Means with different superscripted letters in the same column are statistically significant ($p<0.1$).

Discussion

There was no significant difference between body weights of groups 1 and 2 at the end of the study. Plasma leptin levels of the infection group (group 2) were significantly higher, when compared to the levels in group 1. Leptin is sometimes classified as a cytokine due to the similarity between the structure of leptin and its receptors, and cytokines (15). The structure of leptin resembles those of interleukin (IL) 6 and IL-11, while leptin receptor is homologous to IL-6 receptor (15). Besides having a stimulating effect on leukocyte synthesis, leptin has been shown to reinforce the stimulating effect of erythropoietin on erythrocytes. Therefore, leptin deficiency causes problems in haematopoiesis (23). Like bacterial antigens, leptin activates macrophages, increases their phagocytic activity and stimulates the secretion of pro-inflammatory and anti-inflammatory cytokines from the macrophages (11). Leptin accelerates neovascularisation and wound healing. Furthermore, leptin deficiency increases predisposition to infection and inflammation, and this increase is associated with an impairment of cytokine production (19). Leptin is known to have a substantial part in natural and acquired immunity. As leptin levels are elevated during infection/inflammation, it has been argued that leptin is a crucial factor in the host's response to inflammation (24). Anorexia, which is seen in the course of infections, is believed to be the host's acute phase response to infection. However, although anorexia has its uses in the onset, extended anorexia is known to exercise some harmful effects by delaying healing (25). Bacterial cell wall components (like lipopolysaccharides and peptidoglycanes), microbial nucleic acids and viral glycoproteins trigger acute phase reaction, and thus anorexia (25). Bacterial/viral products stimulate the production of proinflammatory cytokines (ILs, tumour necrosis factor alpha, TNF-α, interferons) (19). Cytokines, in turn, increase leptin expression in adipose tissue. Both microbial products and the resulting cytokines, as well as leptin, reduce food intake. Therefore, it is speculated that TNF-α, IL-1 and IL-6 are responsible for the anorexia that develops during inflammation and infection and that leptin partially mediates these effects of cytokines (20). Altogether, these data suggest that there is an inevitable relation between cellular immunity and leptin. Elevated leptin levels were established in rats with induced T. gondii infection and may be seen as an expected result. Examination of how various cytokines are affected leptin and T. gondii infection may provide us with new data on this topic.

Conclusion

Toxoplasma gondii infection can cause an increase in leptin secretion without changing the body weight within a period of 4 week in rats.

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