International Immunopharmacology 25 (2015) 440–449

Contents lists available at ScienceDirect

International Immunopharmacology

journal homepage: www.elsevier.com/locate/intimp

# The impact of immunomodulator compound from the group of substituted thiadiazines on the course of stress reaction

P.A. Sarapultsev [a], O.N. Chupakhin [b,c], S.U. Medvedeva [a,c], E.A. Mukhlynina [a,c], S.A. Brilliant [a], L.P. Sidorova [c], I.G. Danilova [a,c], A.P. Sarapultsev [a,c,*]

[a] Institute of Immunology and Physiology of the Ural Branch of the RAS, Russia
[b] The IJ Postovsky Institute of Organic Synthesis of the Ural Branch of the RAS, Russia
[c] Federal State Autonomous Educational Institution of Higher Professional Education, Ural Federal University named after the first President of Russia B. N. Yeltsin, Russia

ARTICLE INFO

*Article history:*
Received 20 August 2014
Received in revised form 12 February 2015
Accepted 12 February 2015
Available online 1 March 2015

*Keywords:*
Stress
L-17 compound
Thiadiazines
6H-1,3,4-Thiadiazine
Anti-stress agents

ABSTRACT

A significant role of the stress response to many different diseases prompted a search for new specialized and non-specialized anti-stress agents. This study examines the effect of the compound L17 from the group of 5-phenyl substituted-6H-1,3,4-thiadiazine-2-amines, on the manifestations of the stress response. The authors used a standard model of immobilization stress, in which an animal was immobilized on its back for 6 h a day. Parameters of the morphological and functional states of the organs studied were measured and biochemical and enzyme-immunoassays were carried out on the first and second days. This study reveals that the main mechanism by which the L17 compound mediates of its anti-stress was by activation of macrophages on the second day of the experiments and the inhibition of apoptosis in the thymus. The results enable us to suggest that the compound L17 does not improve resistance to stress; however, it does lower the reaction to stress.



## 1. Introduction

### 1.1. The concept of stress

The modern concept of stress is based on Hans Selye's fundamental studies of the stress response and its effect on physiological functions and homeostasis of organisms [1–3]. However, the term "stress" is now often used as a generic term, which suggests the response of an organism to different psychosocial, physical, and ecological factors and their effects on physical and psychic well-being [4]. In any case, the response of vertebrate animals to new irritants (stress) is mediated *via* the activation of the hypothalamic–pituitary–adrenal gland (HPA) axis and/or the sympathoadrenal system [5–8], which define the nature and intensity of stress response effects.

### 1.2. Products of the sympathoadrenal system

One of the main products of the sympathoadrenal system is catecholamines. Catecholamines modulate a range of immune functions such as proliferation, production of cytokines and antibodies, cytolytic activity, and migration of immune cells [9,10]. The effects of catecholamines are based on the presence of adrenoreceptors (ARs), especially α2-AR, which is expressed on practically every cell participating in the immune response. The stimulation of α2-AR inhibits cell-mediated immunity, while stimulating humoral immunity [11,12]. Thus, the stimulation of α2-AR on monocytes and macrophages reduces the generation of inflammatory cytokines such as IL-1, TNF-α, IL-6, and IL-8 [13–19]. Experiments with long-term infusion of α-receptor agonists showed not only a considerable reduction in TNF-α production but also increased production of IL-10 [20]. Moreover, activation of α2-AR reduces superoxide production by neutrophils; superoxide plays an important role in bactericidal activity by inhibiting chemotaxis [21], inhibiting chemotaxis [22–24], and inducing apoptosis [25].

### 1.3. Products of the hypothalamic–pituitary axis

The hypothalamic–pituitary axis (HPA) produces glucocorticoids (GC). GCs have both direct and indirect effects on the functioning of the immune system on both the molecular and cellular level. On the cellular level, GCs inhibit the access of leukocytes to inflamed areas, ablate the function of leukocytes, endothelial cells and fibroblasts, and inhibit the formation and function antibodies involved in an inflammatory response [26]. On the molecular level, GCs directly bind to glucocorticoid reaction elements (GRE) on glucocorticoid-responsive genes, which

* Corresponding author at: Institute of Immunology and Physiology (IIP) of the Ural Division of Russian Academy of Sciences, 106 Pervomaiskaya str, 620219 Ekaterinburg, Russia. Fax: +7 343 374 0070.
*E-mail address:* a.sarapultsev@gmail.com (A.P. Sarapultsev).

http://dx.doi.org/10.1016/j.intimp.2015.02.024
1567-5769/

amplify the transcription of the genes that encode anti-inflammatory albumins including lipocortin-1, IL-10, the antagonist of the IL-1 (IL-IRA) receptor, and the inhibitor of the neutral endopeptidase (NEP) [27]. The indirect impact of GCs is manifested through their ability to inhibit synthesis of almost all known cytokines and some cell surface molecules by decreasing production of transcription factors such as NK-κB or AP-1 [27,28].

It has been demonstrated that stress can alter immune regulation by increasing the activity of interleukins, in particular IL-6 [29,30], which is an important mediator of several types of inflammation [31]. On the other hand, as has been shown by *in vitro* and *in vivo* experiments, GCs inhibit the expression of IL-6 in humans and animals through an NF-kappaB-dependent nuclear mechanism. In a similar manner, GCs can also reduce the concentration of other interleukins, such as IL-1α and IL-1β [32]. Under normal conditions, when the GC concentration is within the physiological range, the production of IL-2 is favored while the synthesis of IL-4 is inhibited [33]; however, as the concentration of GCs increases, GCs inhibit the expression of IL-4 and IL-5 in mast cells, thus inhibiting the production of IgE and preventing the development of eosinophilia [34]. Moreover, according to P.H. Hart et al., GCs inhibit the production of TNFα and PGE2 by activated monocytes/macrophages [35].

Activation products of HPA also interact with cell effectors of the immune system. Stress response causes typical changes in peripheral blood [36]. Corticosteroids increase hemoglobin and erythrocyte concentrations in blood, probably by inhibiting erythrophagia [37]. Neutrophilia, caused by acute or chronic stress (introduction of corticosteroids), is caused by accelerated removal of polymorphonuclear leucocytes from the bone marrow as well as by their return into the cell circulation from the walls of blood vessels [38–40]. Unlike the numbers of neutrophils, the numbers of lymphocytes, eosinophils, monocytes, and basophils starts to decrease after 4–6 h after onset of stress reaction, which is caused by cell redistribution and activation of GC-induced apoptosis [37].

### 1.4. Action of stress on immune cells

Stress selectively inhibits Th1 cell function and results in a transition towards the formation of the Th2-phenotype, with generation of appropriate cytokines [11,41]; furthermore, the stronger the stressor, the more obvious is the effect [42]. Stress mediates this transition through the activity of GCs and catecholamines. While GC, noradrenaline, and adrenalin inhibit formation of IL-12, which is one of the key inductors of differentiation of immature cells to the Th1 phenotype, noradrenaline and adrenalin also increase the production of IL-10, which inhibits Th-1 activity and increase the function of Th2 cells [11]. Moreover, it was shown that GCs are capable of modulating the activation pattern of Th cells (mainly $CD4^+$ cells), which shifts activity of the immune system from a cellular response to a humoral response [43].

*In vitro* studies have demonstrated that corticosteroids have different effects on B-cells depending on the B-cell's stage of differentiation. Early events such as activation and proliferation of stimulated B cells are profoundly suppressed by the presence of *in vitro* CSs. Later events in the B cell cycle such as the proliferative response to B cell growth factor after either *in vivo* or *in vitro* activation are less sensitive to the suppressive effects of *in vitro* CSs. The final events in the B cell cycle; namely, the differentiation to the immunoglobulin-producing state, is not suppressed by *in vitro* CSs [44].

The decreased activity of natural killer cells and reduced spontaneous cytotoxicity due to corticosteroids can be explained by the inhibition of adhesion between NK cells and target cells [45]. Specifically, corticosterone-induced suppression of NK cell cytotoxicity occurs only under prolonged, but not short exposure to stress, and has smaller than the prominent impact of epinephrine [46].

An important aspect of the GC anti-inflammatory effect is the significant influence GCs have on adhesion between neutrophils and endothelial cells due to the inhibition of mRNA for ELAM-1 (endothelial-leukocyte adhesion molecule-1) as well as on ELAM-1 and ICAM-1 (intercellular adhesion molecule-1) expression [47]. GCs not only weaken adhesion of polymorphonuclear neutrophils to the endothelial surface [48], as they also lengthen the transmigration of cells, in which diapedesis has started, by a factor of 3–4 [49].

The stress response affects phagocytosis as well. Changes in neutrophilic phagocytosis parameters under stress are usually of a two-phase nature [50]. The first phase is characterized by a decrease in the relative parameters and an increase of the absolute parameters of neutrophilic phagocytosis, while the second phase (24 h under stress), by the increase of both kinds of phagocytosis parameters. The depressed state of the relative parameters during the first phase can be attributed to the relatively immature state of neutrophils leaving the bone marrow [50]. High concentrations of catecholamines, typical of the stress response, also contribute to the suppression of macrophage phagocytosis, while low concentrations of catecholamines are a strong stimulator of phagocytosis. This suggests the concentration-related two different pathways of catecholamine impact on phagocytosis, classical non-genomic at high concentration while genomic pathway at low concentration [51].

In accordance with the data described above, the stress response has a considerable effect on practically every parameter of the immune system. Moreover, the duration and peculiarities of the stress response are the main factors defining the nature and characteristics of immune system changes [30]. The role played by stress in the early stages and later development of cardiovascular disease has been extensively described in a large number of studies [52]. It was shown that the stress response can trigger cardiovascular problems both directly by triggering of neuroendocrine mechanisms and by increased activity of the autonomous nervous system [53], and indirectly through stress-induced behavioral changes [54]. In addition to this, the stress response can infringe on wound healing by affecting the inflammation stage of the healing process [55]. From a clinical point of view, the stress response can result in longer hospitalization periods and a higher incidence of complications in surgical patients [56]. Stress also affects the function of T- and B-lymphocytes, which results in the increased risk of rhinovirus infection in patients, worsening of virus infections, and even in rapid HIV progression [57].

### 1.5. Anti-stress agents

A significant contribution of the stress response to pathogenesis of diverse medical problems triggered a search for new specialized and non-specialized anti-stress agents [58]. Some of these compounds confer anti-anxiety and anti-inflammatory [59,60] effects in addition to anti-stress properties, while some of them are characterized by a combination of anti-stress, antioxidant, and antibacterial properties [61].

Our group demonstrated in earlier studies that the compound L-17 from the group of 5-phenyl substituted-6H-1,3,4-thiadiazine-2-amines, has an effect on the course and severity of experimental myocardial infarction [62,63] and acute pancreatitis complicated with systemic inflammation syndrome [64,65]. Compound L-17 reduced the severity of stress response manifestations that accompanied systemic inflammation; L-17 reduced levels of IL-6 and IL-10, reduced the severity of neutrophilia, increased levels of monocytes and leucocytes in blood [65], and reduced the flow of neutrophils while increasing the flow of monocytes/macrophages to areas of inflammation [62]. These data suggest that compound L-17 might possess anti-stress properties. In order to eliminate the possibility of the effect of inflammation on the response characteristics, we decided to examine the effect of compound L-17 on immobilization stress.

The aim of the study was the revealing the possible mechanisms of action of the compound L-17 from the group of 5-phenyl substituted-6H-1,3,4-thiadiazine-2-amines on the course of stress reaction.