Հայաստանի Գիտությունների Ազգային Ակադեմիա
Национальная Академия Наук Армении
National Academy of Sciences of Armenia

Հայաստանի Կենսաբանական Հանդես
Биологический Журнал Армении
Biological Journal of Armenia

• Փորձարարական և տեսական հոդվածներ • Экспериментальные и теоретические статьи •
• Experimental and theoretical articles •

Biolog. Journal of Armenia, Supplement 1 (66), 2014

ANTI-INFLAMMATORY EFFECTS OF HYPOTHALAMIC PROLINE RICH POLYPEPTIDE GALARMIN (PRP-1) IN LPS-INDUCED ENDOTOXIC SHOCK

M.B. MATEVOSYAN, T.P. HARUTYUNYAN, A.A. DURGARYAN

H. Buniatian Institute of Biochemistry NAS RA
mara_matevosyan@hotmail.com

В работе обобщены результаты исследований протекторного и противовоспалительного влияния богатого пролином полипептида - галармина при эндотоксическом шоке, индуцированном липополисахаридом (ЛПС) в условиях *in vivo*. Показано, что галармин значительно снижает уровни противовоспалительных цитокинов и повышает выживаемосать зараженных животных.

Богатые пролином полипептиды - галармин - липополисахарид - цитокины - выживаемость

Ներկայացված են հիպոթալամուսի պրոլինով հարուստ պոլիպեպտիդ գալարմինի (PRP-1) հակաբորբոքային ազդեցությունը ԼՊՍ-ով հարուցված էնդոտոքսիկ շոկի դեպքում *in vivo* պայմաններում:

Ստացված արդյունքները ցույց են տալիս, որ գալարմինը զգալիորեն նվազեցնում է ցիտոկինների հակաբորբոքային մակարդակները և բարձրացնում է վարակված կենդանիների կենսունակությունը:

Պրոլինով հարուստ պոլիպեպտիդ - գալարմին - լիպոպոլիսախարիդ (ԼՊՍ) - ցիտոկիններ - կենսունակություն

In the present work are summarized received data on protective and anti-inflammatory effect of hypothalamic proline rich polypeptide Galarmin during LPS-induced endotoxic shock. Received results indicate that the administration of Galarmin significantly increased the survival of infected mice and had a pronounced anti-inflammatory effect effect.

Proline rich polypeptides - Galarmin - LPS - cytokines - survival

The accumulated literature data on central nervous system (CNS) interaction with the immune system, expression of some cytokines and their receptors in cultures of human neurons, in astrocytes and microglia, testify to the existence of the brain immune system. Data received by A. Galoyan on the biosynthesis of a number of known interleukins and novel proline-rich polypeptides (PRPs) in nerosecretory neurons of the hypothalamus (*N. paraventricularis* and *N. supraopticus*) demonstrate that neuroendocrine nuclei of the hypothalamus are possibly a center of the neuro-endocrine-immune system of brain [1,2]. These peptides originate by proteolysis of the C-terminal glycoprotein neurophysin II and along with vasopressin and oxytocin, are transferred from the hypothalamus to the neurohypophysis by axonal transport [1]. It has been shown that one of them, PRP-1 or

galarmin (AGAPEPAEPAQPGVY), is a regulator of humoral and cellular immunity, thymocyte differentiation, and myelopoiesis. Studies have shown that galarmin stimulates the antigen-presenting function of macrophages, expression and release of human growth factor by transformed BALB/c mice fibroblasts, enhances spontaneous or fMLP- and PMA-induced oxidative burst, as well as the intracellular killing of *S. aureus* by human neutrophils and monocytes [3-7]. The antibacterial activity of galarmin was shown in vivo against different strains of Gram-negative and Gram-positive bacteria, including *Salmonella* (typhimurium, choleraesuis, typhi), *Escherichia coli, Cholerae suis, Shigella* (flexneri, sonnei), *Streptococcus pneumonia*, etc. [8]. In our previous experiments, we performed a systemic infection with *Staphylococcus aureus* and MRSA in mice showing a remarkable protective effect. Thus, galarmin injected intramuscularly (i.m.) 1μg/mice 24 hours before infection could fully protect mice against lethal methicillin-sensitive and methicillin-resistant *S.aureus* (MRSA) infection (100% of survival vs. 0% in the untreated group) when the peptide itself is devoid of direct inhibitory activity on bacterial [9]. However it was not clear whether galarmin protects from specific bacterial strain or its effects should be attributed to the capacity either to boost or modulate the immune system or to induce an environment that would prevent the deleterious effects of an overzealous inflammatory response. As one of the possible mechanisms of galarmin protective activity one can assume it's possible role to interfere with the exacerbated inflammatory response initiates by pathogen-associated molecular patterns. To better understand how galarmin can affect the cytokines and hormonal regulation during severe inflammatory background, we used LPS injected mouse model, which result s in a dramatic increase in the expression of pro-inflammatory cytokines. Furthermore, we demonstrate that the specific restoration of cytokines and cortisol levels in mice by galarmin leads to the restoration of normal phenotype. Our data demonstrate for the first time that galarmin promote the regulation of LPS induced inflammation by controlling the expression of main inflammatory cytokines and cortisol.

Material and Methods.

Animals used. Young (4- to 5-week-old) male pathogen-free BALB/c mice with body weight of 16-18 g, received from the Institute animal house were used at the study. Animal housing and care were performed according to the US National Research Council's "Guide for the Care and Use of Laboratory Animals".

Proline-rich polypeptides: 24h before LPS treatment mice were intraperitoneally (i.p.) injected with 1 μg/mouse of galarmin (AGAPEPAEPAQPGVY) in a volume of 200 μl of pyrogen-free saline or with equal amount of saline vehicle alone (as a control). Prior to the testing *in vivo,* peptides were ensured for not contamination with lypopolysacharide (LPS) using highly sensitive the Limulus amebocyte lysate test (LAL-test).

LPS-induced inflammation and lethality. LPS (*Escherichia coli* 055:B5; Sigma-Aldrich) was diluted in pyrogen-free saline and given 5 mg/kg i.p. Cumulative mortality was then monitored over the next 7 days. All surviving animals were euthanized by cervical dislocation.

Levels of cytokines and corticosterone in serum. Blood samples were collected by cardiac puncture 2h after the LPS challenge. The blood was allowed to clot at 4C for 2h and centrifuged to obtain serum samples that were stored in aliquots at -20C before assaying them for cytokines and cortisol. Quantification of the mouse serum levels of IL-1, tumor necrosis factor- alpha (TNF-a), cortisol and immunoglobulins of the main classes (IgA, IgM, IgG) were determined using enzyme-linked immunosorbent assays kits (ELISA) per the manufacturer's instructions (Vector Best, Russia), and the wells were read at 450 nm on an optical plate reader StatFax 303plus. Standard curves were prepared using purified cytokine standards. Each experimental sample was run in duplicate.

*Statistics.*The data are expressed as the mean with the standard error of the mean. The statistical significances were determined using SPSS 11.0 software (SPSS, USA). Comparative analysis was performed using parametric univariate analysis of variance (ANOVA), and multiple

comparisons were performed using Scheffé's test for linear contrasts and paired Student's t-test. The level of significance was defined at $P \leq 0.05$.

Results.

The results shown in Fig. 1 reflect the lethality of LPS in mice challenged with lethal dose of LPS. LPS-treated mice were pre-injected with galarmin, and the number of surviving mice was determined. Galarmin significantly enhanced the survival of mice, and had a pronounced protective effect during the period of development of the inflammation that is reflected in weight loss recovery. As shown in Fig. 1a, the weight loss was weakly more severe among LPS treated mice than in galarmin group and total recovery was obtained within one week. In particular, when mice were injected with 5 mg/kg LD_{100} of LPS, survival in galarmin-treated mice was 60%, whereas no survivors were observed among control mice given vehicle alone (Fig. 1b).

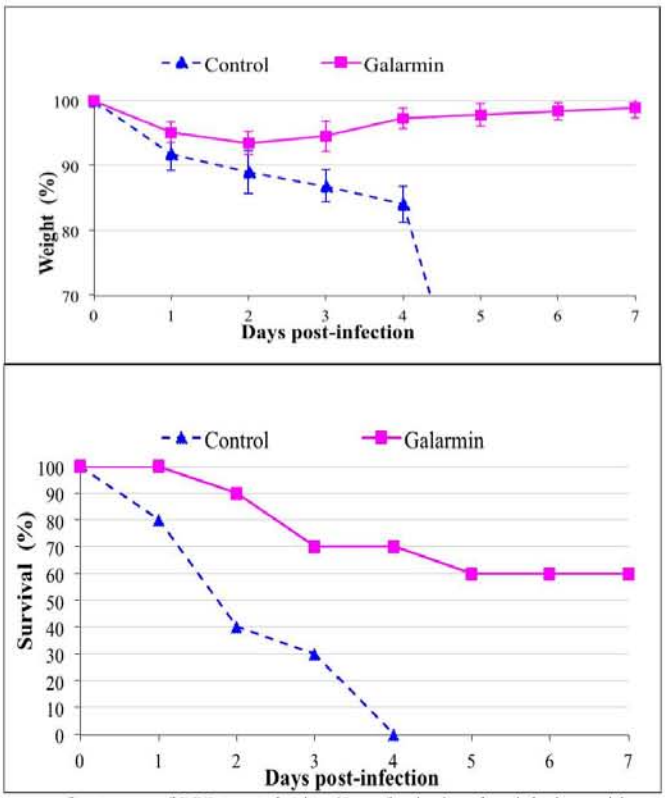

Fig. 1. Survey of outcome of LPS-treated mice (5 mg/kg i.p.) and weight loss without (control) and with galarmin pre-treatment (1 μg/mouse) 24h before LPS challenge. Data is expressed as mean ± SD (n = 10/group).

In agreement with our experimental model, we analyzed mice one day galarmin treatment for the serum level of pro-inflammatory cytokines, immunoglobulins and cortisol levels. Levels in serum of TNF- α and IL-1 increased after the administration of LPS. However, our data showed that at 2h after LPS treatment, the standard time of most research studies [10], the level of pro-inflammatory cytokines (IL-1, TNFα) was significantly lower following galarmin treatment, and the apparent level of cortisol was considerably higher than that of galarmin-untreated groups, while there was no any changes in immunoglobulins levels. Two hours after LPS injection, the serum showed a

significant increase in TNFα in the positive control group (183.8 pg/ml) group when compared with the negative and Galarmin+LPS groups (43 pg/ml and 94.45 pg/ml, respectively, $P \leq 0.05$) (Fig. 2a). These changes were similar and even more expressed in IL-1 with high increase over the negative control following LPS treatment (488.2 pg/ml vs 135.5 pg/ml) and up to two-fold normalization to the control level in Galarmin-LPS group (207.6 pg/ml) (Fig. 2b). The expression of cortisol in the LPS group (1200 pg/ml) was significantly lower than in the groups (control, Galarmin and Galarmin+LPS: 2622 pg/ ml, 2573.75 pg/ml and 2031.25 pg/ml respectively) (Fig. 2c). The levels of IgG, IgM and IgA were not significantly different among the experimental groups (Fig. 2d-f).

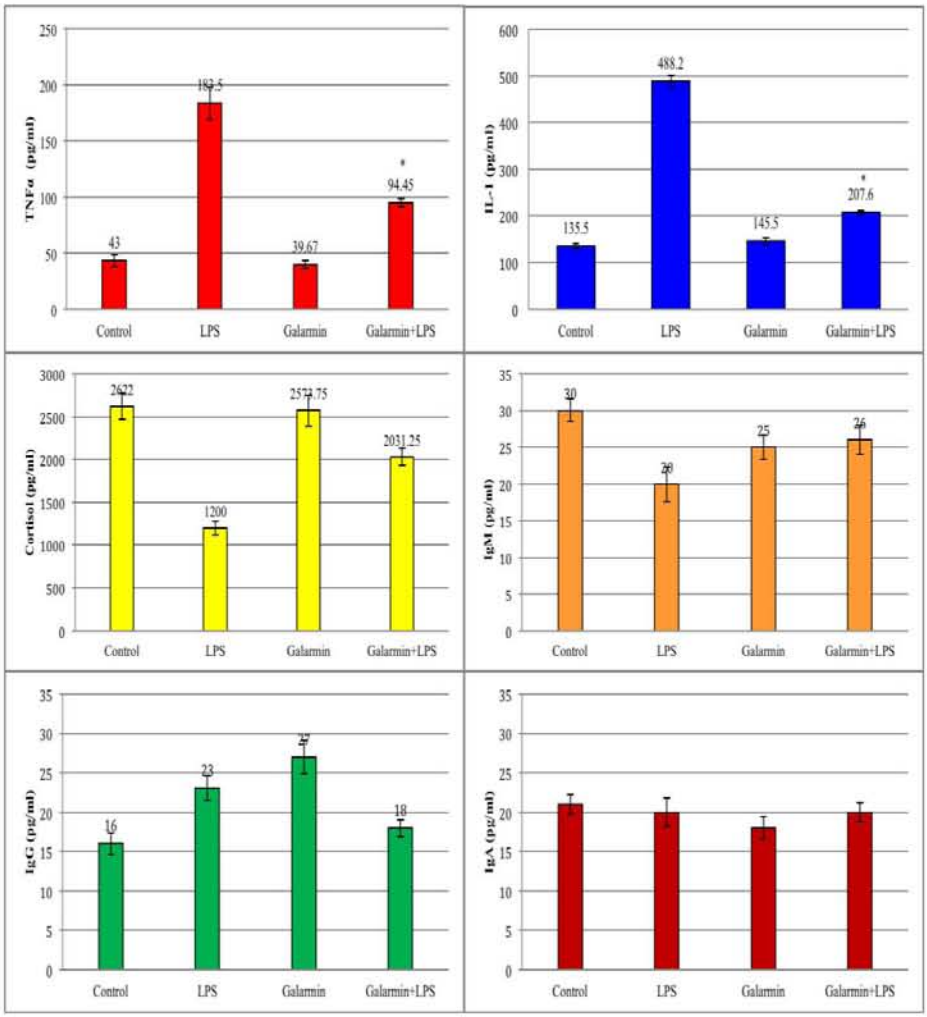

Fig.2. Levels in sera of TNFα (a), IL-1 (b), cortisol (c), and immunoglobulins (d-f) in each group 2h after administration of LPS (5 mg/kg) and galarmin (1 μg/mouse) to BALB/c mice *$P \leq 0.05$, n=10.

Discussion.

The effect of hypothalamic proline-rich polypeptide galarmin against LPS induced inflammation in BALB/c mice was investigated. Mice were treated with galarmin and were then injected with LPS. The administration of galarmin significantly increased the

survival of infected mice and had a pronounced protective effect during the period of development of the inflammation. The level of pro-inflammatory cytokines such as TNF**α** and IL-1 were significantly lower following PRP-1 treatment while there was no changes in the main classes immunoglobulin's serum levels (IgG, IgM, IgA). Altough galarmin favored glucocorticoid (cortisol) production that could offer an explanation for such an observation associated better control of the inflammatory response involved in anti-inflammatory background of galarmin. As one of the possible mechanisms of galarmin protective activity following data showed in this paper one can assume it's possible role to interfere with the exacerbated inflammatory response initiates by pathogen-associated molecular patterns (PAMPs) such LPS.

Our results indicate increase in cortisol levels following galarmin challenge. It has been reported that glucocorticoids inhibit TNF production *in vitro* and *in vivo* if administered prior to LPS stimulation but are rather less effective when applied after the induction of TNF synthesis [10, 11]. It is possible that the regulation of cortisol and possibly other corticosteroids is the underlying mechanism of galarmin protective effects as a regulator of immune and inflammatory reactions. It is quite likely that the cytokine network is irreversibly engaged in producing a state of shock just after stimulation with LPS, while the down-regulating factors, released during these processes, may not efficiently control cytokine generation in the acute phase and galarmin plays it beneficial role by triggering the compensatory mechanisms. These observations are in agreement with our previous reports of the galarmin-related increase in IL-10 production [9], an important anti-inflammatory cytokines.

In conclusion, the major finding of this study is the apparent protective effect of hypothalamic proline rich peptide galarmin against the lethal effect of LPS. Our results indicate the ability of galarmin to bring about rapid recovery of immunoactivated state. Galarmin may be a potential useful adjunct in clinical medicine for the protection of autoimmune and immunocompromised hosts, however the exact molecular mechanisms of galarmin action have to be further investigated.

Acknowledgments.
This research has been undertaken with financial support of ANSEF grant microbio #3244.

REFERENCES.

1. *Galoyan A.A.* Concepts of Neuroendocrine Cardiology and Neuroendocrine Immunology, Chemistry and Biology of Signal Molecules. Neurochem. Res *35*(2): 2001-2027, 2010.
2. *Galoyan A*. The brain immune system: chemistry and biology of the signal molecules. In: Lajtha A (ed.), Galoyan AA, Besedovsky HO (eds) Handbook of neurochemistry and molecular neurobiology, v. Neuroimmunology, 3rd ed. Springer, New York, 2008.
3. *Galoyan A.A., Korochkin L.I., Rybalkina E.J., et al.* Hypothalamic proline-rich polypeptide enhances bone marrow colony-forming cell proliferation and stromal progenitor cell differentiation. Cell Transplantation *17*(9): 1061-1066, 2008.
4. *Galoyan A.A., Aprikyan V.S.* A new hypothalamic polypeptide is a regulator of myelopoiesis. Neurochem Res *27*(4): 305-312, 2002.
5. *Aprikyan V.S., Galoyan A.A.* New hypothalamic peptides in regulation of thymocytes differentiation. Neurokhimiya RAS & NAS RA *19*: 227-231, 2002.
6. *Bezirganyan KB, Davtyan TK, Galoyan AA* Hypothalamic Proline Rich Polypeptide Regulates Hematopoiesis. Neurochem. Res *35*(6): 917-924, 2010.

7. *Davtyan T.K., Manukyan H.A., Mkrtchyan N.R., Avetisyan S.A., Galoyan A.A.* Hypothalamic Proline-Rich polypeptide is a regulator of oxidative burst in human neurotrophils and monocytes. Neuroimmunomodulation *12*: 270-284, 2005.
8. *Galoyan A.A.* Brain Neurosecretory Cytokines: Immune Response and Neuronal Survival. Kluewer Academic/Plenum Publishers, New York, 2004.
9. *Durgaryan A.A., Matevosyan M.B., Seferyan T.Y., Sargsyan M.A., Grigoryan S.L., Galoian K.A., Galoyan A.A.* The protective and immunomodulatory effects of hypothalamic proline-rich polypeptide galarmin against methicillin-resistant Staphylococcus aureus infection in mice. Eur J Clin Microbiol Infect Dis *31*: 2153-2165, 2012.
10. *Shemi D., Azab A.N., Kaplanski J.* Time-dependent effect of LPS on PGE2 and TNF-alpha production by rat glial brain culture: influence of COX and cytokine inhibitors. J Endotoxin Res *6*(5): 377-81, 2000.
11. *Håkansson H.F., Smailagic A., Brunmark C., Miller-Larsson A., Lal H.* Altered lung function relates to inflammation in an acute LPS mouse model. Pulm Pharmacol Ther. *25*(5): 399-406, 2012.
12. *DiDonato J.A., Mercurio F., Karin M.* NF-κB and the link between inflammation and cancer. Immunol Rev. *246(1)*: 379-400, 2012.
13. *Schmidt-Ullrich R., Me´met J., Lilienbaum A., Feuillard J, Raphael M, Israel A* NF-kB activity in transgenic mice: developmental regulation and tissue specificity. Development *122*: 2117–2128, 1996.
14. *Black P.H.* Immune system-central nervous system interactions: effect and immunomodulatory consequences of immune system mediators on the brain. Antimicrob. Agents Chemother. *38*: 7–12. 1994.
15. *Reichlin S.* Neuroendocrine-immune interactions. N. Engl. J. Med. *329*: 1246–12531993.
16. *Parant M., Contel C.L., Parant F., Chedid L.* Influence of endogenous glucocorticoid on endotoxin-induced production of circulating TNF-alpha. Lymphokine Cytokine Res. *10*: 265–271, 1991.
17. *Miller-Larsson A., Runström A., Brattsand R.* Adrenalectomy Permits a late, local TNF-alpha release in LPS-challenged rat airways. Eur Respir J. *13*(6): 1310-7, 1999.