PHARMACOLOGY AND TOXICOLOGY

Effect of Ladasten on the Content of Cytokine Markers of Inflammation and Behavior of Mice with Experimental Depression-Like Syndrome

A. V. Tallerova, L. P. Kovalenko, A. D. Durnev, and S. B. Seredenin

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 152, No. 7, pp. 65-67, July, 2011
Original article submitted December 27, 2010

The effects of ladasten and reference product imipramine (10 mg/kg) on the content of cytokines TNF-α, IL-6, IL-10, IL-1α, IL-2, IL-4, IL-5, IL-17, IFN-γ, and GM-CSF and behavior of male C57Bl/6 mice were studied on the model of a depression-like state induced by a single intraperitoneal injection of bacterial LPS in a dose of 100 μg/kg. Ladasten was injected 5 times in doses of 30 and 50 mg/kg. LPS was administered 1 h after the last injection of the test agents. Behavioral disturbances and significant increase in the concentration of TNF-α and IL-6 in blood plasma were observed 2 h after LPS treatment. Ladasten was more potent than imipramine in decreasing the content of proinflammatory cytokines TNF-α and IL-6 and preventing the development of behavioral disturbances in mice. Antiasthenic drug ladasten was shown to decrease the elevated level of proinflammatory cytokines TNF-α and IL-6 after LPS treatment. Further studies should be performed to develop new indications for the use of ladasten in adjuvant therapy of depression.

Key Words: *ladasten; inflammation; depression-like syndrome; cytokines; behavior*

Inflammatory changes in the brain serve as one of the major etiopathogenetic factors for depression [6,9]. The severity of inflammatory reactions and content of circulating proinflammatory cytokines were shown to increase in depressive patients [4,7,10]. A positive correlation was found between the concentration of proinflammatory cytokines and risk for the development of depressions, degree of depressive symptoms, cognitive disorders, and low efficiency of standard antidepressants [2,5].

Previous studies showed that psychotropic drugs have the immunomodulatory properties [2]. Administration of some antidepressants is followed by a decrease in the content of proinflammatory cytokines TNF-α, IL-2, and IL-6 and increase in the activity of anti-inflammatory cytokines [8]. Therefore, it is necessary to perform an experimental study of proinflammatory cytokines on the model of depression. Variations in the concentration of these compounds should be evaluated in studying the pharmacological spectrum of new psychotropic drugs [4].

B. Leonard *et al*. [6,7] showed that acute or chronic administration of bacterial LPS to rats and mice causes symptoms similar to those observed in depression. These changes are accompanied by activation of the immune response and increase in the concentration of proinflammatory cytokines IL-1, TNF-α, and IL-6 in the blood [2]. This model is widely used

V. V. Zakusov Institute of Pharmacology, Russian Academy of Medical Sciences, Moscow. Russia. ***Address for correspondence:*** annatall@rambler.ru. A. V. Tallerova

0007-4888/11/1521-0058

TABLE 1. Effect of 2-h Exposure to LPS on the Concentration of Cytokines in Blood Plasma of C57Bl/6 Mice

Group (n=10)		Cytokines, pg/ml		
		TNF-α	IL-6	IL-10
Control		21.2±3.6	57.1±18.0	13.3±5.1
LPS, 100 μg/kg		81.3±18.6*	18 964.7±2096.7*	45.1±27.2
Imipramine, 10 mg/kg		37.7±18.8*x	18 356.8±1757.9*	11.2±6.5
Ladasten	30 mg/kg	25.5±12.5x	11 474.85±3053.7*x+	34.8±11.7
	50 mg/kg	29.7±10.8x	12 359.25±1312.2*x	23.9±9.3

Note. p<0.05: *compared to the control; xcompared to LPS. +$p \leq 0.05$ compared to imipramine.

to study the immunological pattern of a depression-like state [7].

Here we studied the effect of a new antiasthenic drug ladasten on the content of proinflammatory cytokines and behavior of animals with experimental depression-like syndrome induced by LPS.

MATERIALS AND METHODS

Ladasten (N-(2-adamantyl)-N-(*p*-bromophenyl)-amine) was synthesized at the V. V. Zakusov Institute of Pharmacology. This product was shown to possess the psychostimulant, immunostimulatory, and anxiolytic properties [1].

Experiments were performed on male C57Bl/6 mice weighing 18-20 g and obtained from the Stolbovaya nursery. The animals were maintained in a vivarium of the V. V. Zakusov Institute of Pharmacology under standard conditions and had free access to water and food.

The depression-like state was induced by a single intraperitoneal injection of LPS in a dose of 100 μg/kg [7,11]. Ladasten (30 and 50 mg/kg) and reference product imipramine (10 mg/kg; Egis) [8,11] were injected intraperitoneally (5 times) before endotoxin treatment. The doses of preparations were selected from the results of previous experiments [1,11]. LPS was injected intraperitoneally 1 h after administration of study products (day 5 of the experiment). The blood was sampled after decapitation of animals (2 h postinjection). The concentrations of cytokines (TNF-α, IL-6, IL-10, IL-1α, IL-2, IL-4, IL-5, IL-17, IFN-γ, and GM-CSF) in blood plasma were measured on an EPICS XL 4colors flow cytometer with FlowCytomix mouse Th1/Th2 10 plex panel (BenderMedSystem) according to manufacturer's instructions.

Animal behavior was studied 2 h after LPS treatment (100 μg/kg). The spontaneous locomotor activity of mice was recorded on an Ugo Basile actometer. The anxious behavior was studied in the elevated plus maze (EPM). The number of entries to and time spent in open and closed arms of the maze were recorded for 300 sec. An increase in the number of entries to and time spent in open arms of the maze (compared to the control) was considered as a sign of the anxiolytic effect.

The results were analyzed by one-way analysis of variance (ANOVA, post-hoc Fisher's LSD test) and nonparametric Mann—Whitney U test.

RESULTS

Behavioral disturbances and significant increase in the concentration of proinflammatory cytokines TNF-α and IL-6 in blood plasma were observed 2 h after LPS treatment. These results are consistent with published data [3,7,11].

The concentrations of TNF-α and IL-6 in treated mice were higher than in control specimens (by 3.8 and 332.1 times, respectively). The content of other cytokines remained unchanged under these conditions (Table 1).

TABLE 2. Effect of LPS, Ladasten, and Imipramine on Spontaneous Locomotor Activity of C57Bl/6 Mice

Group (n=10)	Spontaneous locomotor activity 2 h after LPS treatment, arb. units
Control	271.5±20.8
LPS, 100 μg/kg	152.2±14.9*
LPS (100 μg/kg)+ imipramine (10 mg/kg)	125.4±17.0*
LPS (100 μg/kg)+ ladasten (30 mg/kg)	193.1±9.9*+

Note. Here and in Table 3: *p<0.01 compared to the control; +p<0.05 compared to LPS.

TABLE 3. Effect of LPS and Study Products on Anxious Behavior of C57Bl/6 Mice in EPM Test 2 h after Endotoxin Treatment

Group (n=10)	Time spent in open arms, %	Time spent in closed arms, %	Number of entries into closed arms	Number of entries into open arms
Control	16.3±5.8	70.3±7.4	6.3±0.8	2.8±1.1
LPS, 100 μg/kg	3.1±1.7*	84.4±3.3	3.7±0.4*	0.5±0.2*
LPS (100 μg/kg)+imipramine (10 mg/kg)	3.3±1.2	84.7±3.4	5.4±1.0	0.8±0.3
LPS (100 μg/kg)+ladasten (30 mg/kg)	9.8±4.2+	67.8±9.4	5.6±1.0	1.6±0.4+

Course treatment with Ladasten in doses of 30 and 50 mg/kg (before LPS injection) was followed by a significant decrease in the concentrations of proinflammatory cytokines TNF-α and IL-6 (by 3.2 and 1.7 times, respectively). No statistically significant differences were found in the effect of Ladasten at various doses. Imipramine significantly decreased the concentration of TNF-α (by 2.2 times; Table 1).

Evaluation of the severity of an LPS-induced depression-like state in mice showed that spontaneous locomotor activity of animals decreased 2 h after LPS injection (by 1.8 times; Table 2). We revealed a significant decrease in the number of entries into closed and open arms and the time spent in open arms of EPM (by 59, 82, and 81%, respectively; Table 3). Ladasten in a dose of 30 mg/kg significantly increased locomotor activity of mice (by 26.9%), time spent in open arms (by 68%), and number of entries into open arms (by 69%). Imipramine had no effect on these parameters of behavior.

We conclude that ladasten (short-term course treatment) is more potent than imipramine in decreasing the content of proinflammatory cytokines TNF-α and IL-6 and preventing the development of behavioral disturbances in mice. Ladasten increases spontaneous locomotor activity and decreases anxiety in the EPM test.

Our results confirm the relationship between a depression-like state and activity of inflammatory mediators [3,5,6].

The ability of antiasthenic drug ladasten to decrease the concentrations of proinflammatory cytokines TNF-α and IL-6 in mice after LPS treatment is associated with the relief of symptoms of a depression-like behavior. Hence, the antidepressive effect of psychotropic drugs is partly related to immunoregulatory activity. Course treatment with ladasten for 5 days was more effective than that of the standard tricyclic antidepressant imipramine. The LPS-induced depression-like state in laboratory animals was abolished only after a 2-week course of treatment with imipramine [8].

Further studies should be performed to develop new indications for the use of ladasten in adjuvant therapy of depression.

REFERENCES

1. Yu. V. Vakhitova, R. S. Yamidanov, V. A. Vakhitov, and S. B. Seredenin, *Mol. Biol*., **39**, No. 2, 276-285 (2005).
2. K. V. Kazantseva, *Farmateka. Spetsvypusk: Psikhiatriya*, 8-14 (2008).
3. R. Dantzer, *Neurol. Clin*., **24**, No. 3, 441-460 (2006).
4. R. Dantzer, *Enciclopedia of Stress (Second Edition)*, 284-287 (2007).
5. Y. K. Kim, K. S. Na, K. H. Shin, *et al*., Prog. *Neuropsychopharmacol. Biol. Psychiatry*, **31**, No. 5, 1044-1053 (2007).
6. B. E. Leonard and A. Myint, *Dialogues Clin. Neurosci*., **8**, No. 2, 163-174 (2006).
7. A. M. Myint, B. E. Leonard, H. W. Steinbusch, and Y. K. Ki, *J. Affect. Disord.*, **88**, No. 2, 167-173 (2005).
8. E. Obuchowicz, J. Kowalsi, K. Labuzek, *et al*., *Int. J. Neuropsychopharmacol*., **9**, No. 1, 27-35 (2006).
9. S. M. O'Mahony, A. M., Myint, H. Steinbusch, and B. E. Leonard, *Neuroimmunomodulation*, **12**, No. 5, 293-298 (2005).
10. O. J. Schiepers, M. C. Wichers, and M. Maes, *Prog. Neuropsychopharmacol. Biol. Psychiatry*, **29**, No. 2, 201-217 (2005).
11. C. Song and B. E. Leonard, *Neurosci. Behav. Rev*., **29**, No. 4-5, 627-647 (2005).