Effects of Thermoextracts of Brucella S and L Forms on Lipid Peroxidation and Antioxidant Defense in Organs of Laboratory Animals


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The dynamics of LPO marker malondialdehyde formation and peroxidase-destroying activity was studied in homogenized organs of guinea pigs, immunized with thermoextracts from S and L forms Brucella abortus I-206. The L form brucella thermoextract exhibited a lower reactogenicity and adequately activated the antioxidant system, due to which the destructive effects of ROS could be partially neutralized during the vaccinal process.

Key Words: immunization; oxidative stress; lipid peroxidation; antioxidant status

Creation of new preparations for prevention of brucellosis remains a pressing problem, because of residual virulence and reactogenic activity of live vaccines. Study of immunogenic and reactogenic characteristics of L form low virulent brucellas is particularly important for creation of vaccine preparations [2,8].

Postvaccinal reactions, such as allergization and intoxication, are very important characteristics of new immunobiological preparations. One of the biochemical processes associated with these reactions is oxidative stress, developing as a result of enhanced generation of ROS by the cells. Hyperproduction of ROS by the cells during a complicated vaccinal process can lead to LPO-mediated inflammatory and allergic reactions. Peroxide derivatives of unsaturated fatty acids in the cell membrane are involved in the biosynthesis of prostaglandins and leukotrienes (mediators of inflammation and allergic reactions). Accumulation of ROS in the body leads to mobilization of numerous mechanisms, including H₂O₂-utilizing enzymes. It is obvious that during normal vaccinal process, the pathogenic effects of ROS are neutralized by activated antioxidant system, including the peroxide-utilizing enzymes. If the antioxidant compensatory resources are insufficient, LPO can lead to cell membrane destabilization and subsequent pathological processes [7,9]. Activation of LPO in severe intoxication is realized under conditions of suppressed antioxidant defense of cells, and hence, improvement of the status of immunized organism is an important indicator of the reactivity of antigenic preparations [1].

We study the effects of experimental preparations of S and L forms brucellas on LPO activity and on peroxide-destroying activity as an indicator of the antioxidant status in the organs of laboratory animals.

MATERIALS AND METHODS

The study was carried out on outbred certified guinea pigs of both genders (250-300 g; Vector) in accordance with Regulations for Studies with the Use of Experimental Animals, Order No. 755 of the Ministry of Health of the USSR (August 12, 1977), and Supplement to Order No. 708n of the Ministry of Health and Social Development of the Russian Federation (June 23, 2010).

The animals were immunized with Brucella abortus I-206 antigen prepared by thermoextraction from
S and L forms *B. abortus* I-206 strain [4], in a dose of 2 µg (in conversion to protein). The preparations were dialyzed against distilled water, frozen in liquid nitrogen, and lyophilized.

Controls were immunized with live *B. abortus* vaccine strain 19 BA, characterized by manifest reactivity (positive control) [6]. The liver, spleen, and kidneys were collected on days 1, 3, 7, and 14 after immunization. The content of LPO marker malondialdehyde (MDA) and the peroxide-destroying activity (indicator of antioxidant status) were measured in homogenized organs.

The content of MDA was measured by the method based on MDA capacity to react at high temperature in acid medium with TBA with the formation of a colored trimethyl complex with the maximum absorption at λ=532 nm [5].

The peroxide-destroying activity was evaluated by the method [3] based on ammonium molybdate capacity to form with H2O2 a stable colored complex (maximum absorption at λ=410 nm).

The results were statistically processed using Statistica 6.1 software with Mann—Whitney nonparametric test for independent variables and Wilcoxon’s test for dependent variables. The differences were assumed to be significant at *p*<0.05.

**RESULTS**

The dynamics of LPO marker MDA production and peroxide-destroying activity was studied in homogenized livers and spleens of guinea pigs immunized with thermoextracts (TE) from S and L forms *B. abortus* I-206. The highest level of MDA was found in the organs of animals immunized with *B. abortus* 19 BA vaccine. The levels of peroxidase-destroying activity in animals immunized with TE from S and L forms *B. abortus* I-206 were the same as in the control (immunization with *B. abortus* 19 BA). Obviously, a significant increase of MDA concentration in the presence of low peroxidase-destroying activity indicated a high cytopathogenic effect of *B. abortus* 19 BA.

The S form brucella TE caused a rather significant, though much weaker reaction (Fig. 1, *a*). In addition, in contrast to the live vaccine group, the content of MDA in the group of animals immunized with S form brucella TE decreased by day 14, while the level of peroxide-destroying activity was more than 2-fold higher than in the control (Fig. 1, *b*). Importantly that the S form brucella TE exhibited peroxide-destroying activity comparable to that in the control group but did not suppress the antioxidant system, as was the case with live vaccine.

The effect of L form brucella TE on MDA accumulation was less expressed than that of S form brucella TE. The peroxide-destroying activity in this group clearly increased after immunization only in the liver, where MDA concentration also increased. By day 14, the concentration of MDA and peroxidase-destroying activity (Fig. 2) in the liver of animals immunized by L form brucella preparations decreased almost to control level. This fact indicated the lowest reactogenic activity of L form brucella TE. High reactivity of the S form brucella preparation was obviously due to LPS in its composition.

Comparative evaluation of MDA content and peroxidase-destroying activity showed that LPO activity was maximum in the kidneys (Fig. 3). This fact can be explained by the filtering and excretory function

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**Fig. 1.** Dynamics of MDA content (*a*) and peroxide-destroying activity (*b*) in the spleen of guinea pigs immunized with TE from S and L forms *B. abortus* I-206.