Adrenocortical System Response to Induction of Inflammation with Silicon Dioxide in Rats with Alloxan-Induced Diabetes Mellitus

N. V. Kuznetsova, N. A. Palchikova, V. G. Selyatitskaya, and V. A. Shkurupiy

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Alloxan-induced diabetes mellitus in rats was characterized by persistent increase in blood levels of corticosterone, while chronic granulomatous inflammation induced by silicon dioxide and its combination with alloxan-induced diabetes mellitus were associated with transient increase in blood corticosterone level followed by gradual development of hypoadrenocorticism. The content of corticosterone in the adrenal glands of rats with alloxan-induced diabetes mellitus remained unchanged in the dynamics of the disease, but the level of progesterone decreased at the early terms of diabetes and then returned to the initial values. After administration of silicon dioxide to intact rats and to rats with diabetes mellitus, changes in hormone content in the adrenal glands were observed only at the initial stages of inflammation and consisted in elevation of corticosterone concentration against the background of reduced progesterone content.

Key Words: *alloxan-induced diabetes; silicon dioxide; granulomatous inflammation; corticosteroids*

Granulomatosis induced by non-biodegradable minerals silicon dioxide (SiO_2), coal, or asbestos is a variant of chronic inflammation [9]. Hormones of the adrenocortical system (ACS) are modulators of inflammation; they regulate the immune response, including the granulomagenesis process, via modulation of cytokine production by cells [2,14]. We previously demonstrated the relationship between the intensity of chronic candidial granulomatosis inflammation in mice and changes in steroidogenesis in the adrenal glands [10]. In the dynamics of granulomatous inflammation induced by fungi and BCG vaccine, ACS activity was found to undergo phasic changes with periods of hypoadrenocorticism [4,5].

Research Center of Clinical and Experimental Medicine, Siberian Division of the Russian Academy of Medical Sciences, Novosibirsk, Russia. ***Address for correspondence:*** sck@soramn.ru. V. A. Skurupiy

ACS hormones also participate in the pathogenesis of diabetes mellitus (DM) by stimulating gluconeogenesis in the liver. It should be noted that inflammatory processes of different etiology, including granulomatous inflammation, are highly prevalent in DM patients [7,13]. Activation of the pituitary–adrenocortical system in DM patients manifests in increased ACTH and cortisol levels in the blood [1]. Experimental studies also showed that rats with DM are characterized by increased blood content of corticosterone [6,12] and enhanced response of the ACS to stress [11]. Taking into account the physiological role of glucocorticoid hormone, it is important to study the pattern of ACS response under conditions of combination of these two processes.

Here we studied functional state of ACS after administration of non-biodegradable lysosomotropic agent maintaining permanent granulotous inflammation in rats with alloxan-induced diabetes mellitus.

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MATERIALS AND METHODS

The study was performed on 55 male Wistar rats obtained from Nursery of Institute of Cytology and Genetics, Siberian Division of Russian Academy of Sciences (Novosibirsk). The animals were maintained under standard vivarium conditions on standard ration with free access to water and food. The animals were divided into 4 groups.

In group 1 rats, DM was modeled by single intraperitoneal injection of alloxan (17 mg/100 g body weight) dissolved in 0.85% NaCl. In group 2 rats, chronic inflammation was modeled by single injection of SiO_2 (10 mg/100 g body weight) in 0.85% NaCl into the caudal vein. This treatment induced chronic granulomatous inflammation with the formation of macrophage granulomas in the liver followed by their fibrosis [3,8].

In group 3 rats, SiO_2-induced inflammation was modeled 6 days after administration of alloxan as in group 2. Group 4 rats (controls) intravenously received 0.2 ml sterile 0.85% NaCl.

Group 1 animals were sacrificed on days 7, 9, and 20 after alloxan injection; rats of groups 2 and 3 were killed 1, 3, and 14 days after injection of SiO_2, which corresponded to the term of DM development in group 3 rats. Each group consisted of 5 animals.

Glucose content in the serum was measured by the enzymatic method using GLU kits (BioCon); corticosterone concentration was measured by the radioimmune method using [1,2,6,7-^{3}H]-corticosterone (Amersham) and corticosterone antiserum (Sigma-Aldrich). The concentrations of corticosterone and progesterone in homogenates of the adrenal glands were measured by enzyme immunoassay using Steroid IFA-Progesteron kits (Alkor BIO).

The data were processed statistically by dispersion analysis using Kruskal–Wallis test for multiple comparisons and Mann–Whitney test for paired comparisons. The probability of validity of the null-hypothesis was accepted at 5% significance level.

RESULTS

Serum content of corticosterone in group 1 rats surpassed the control value throughout the entire experiment (Fig. 1), which agrees with published data on ACS activation in DM [1,12]. In group 2 rats, changes in corticosterone concentration in blood serum corresponded to those observed during the stress reaction with a peak on day 1 and a 3-fold decrease in hormone concentration on day 14 after SiO_2 injection compared to the corresponding parameter in the control group. Serum content of corticosterone in rats of group 3 corresponded to that in group 2 rats throughout the entire experiment (Fig. 1): the initial rise of blood level of corticosterone was followed by gradual development of hypoadrenocorticism, *i.e.* the effect of SiO_2 treatment predominated.

For evaluation of the relationship of the observed changes in blood corticosterone concentration with possible disturbances in its synthesis we studied the content of this hormone and its precursor progesterone in the adrenal glands in animals of all groups (Fig. 2). In group 1 rats, progesterone content decreased on days 7 and 9 of the disease and returned to control level by days 20. Since corticosterone content remained unchanged in the adrenals and significantly increased in the blood, we can hypothesize that this was due to relatively low activity of the initial stages of steroid hormone biosynthesis at early terms of the disease and their activation in manifest DM. These changes in activity of steroidogenesis processes can be a factor of sustained hypoadrenocorticism in DM.

In rats of groups 2 and 3, corticosterone content in the adrenal glands increased, while progesterone content decreased 1 day after injection of inflammation inductors (Fig. 2); this was paralleled by the increase in corticosterone content in the blood (Fig. 1). These changes in hormone content confirm the assumption on the reaction of ACS on induction of inflammation similar to that in acute stress. On days 3 and 14 after SiO_2 injection, the content of corticosterone and progesterone in the adrenals of group 2 and group 3 rats did not appreciably differ from the corresponding parameters in the control group.

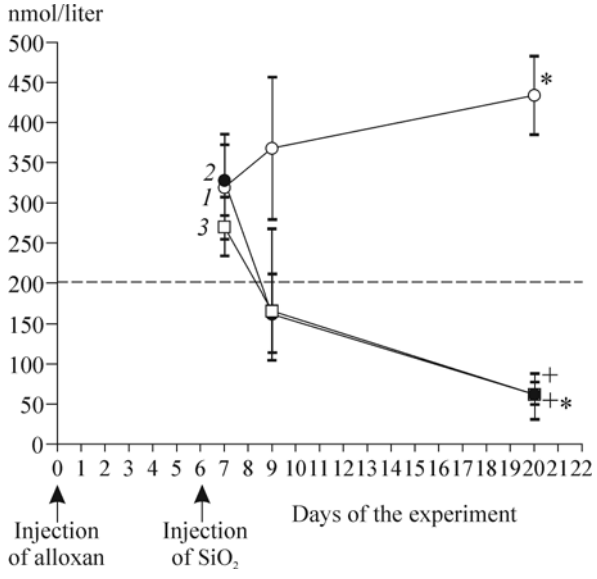

Fig. 1. Corticosterone content in rat serum. Here and on Fig. 3: *1*) group 1; *2*) group 2; *3*) group 3. Solid line: control level. Here and on Fig. 2: $p<0.05$ compared to: *control, $^+$group 1 at the corresponding term.

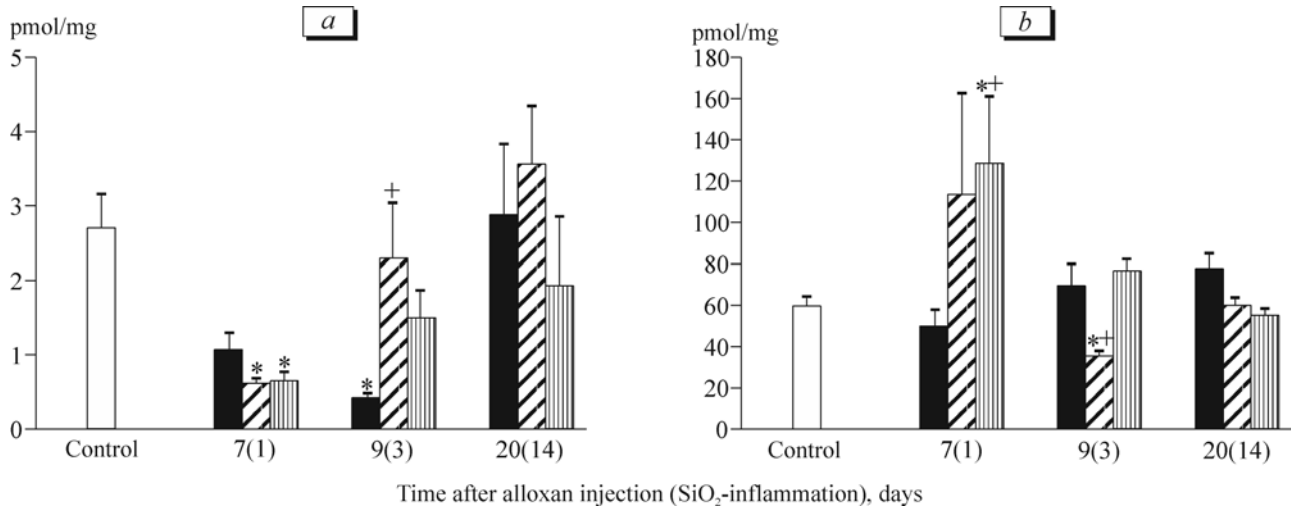

Fig. 2. Content of progesterone (*a*) and corticosterone (*b*) in rat adrenal glands. Dark bars: group 1; horizontal hatching: group 2; vertical hatching: group 3.

These results suggest that the decrease in blood concentration of corticosterone in the dynamics of SiO_2-induced chronic granulomatous inflammation is not related to inhibition of steroidogenesis in the adrenal glands. The decrease in blood corticosterone concentration can be explained by its enhanced metabolism and excretion with the urine due to reduced production of corticosteroid-binding globulin in the liver, because chronic granulomatous inflammation can impair protein-synthesizing functions of the liver. In our previous study, numerous granulomas were detected in rats liver on day 13 after induction of granulomatous inflammation with SiO_2; the number and diameter of these granulomas and the concentration of the fibrotic tissue in the liver increased [3] with time, which attests to pronounced productive component of the inflammatory process.

Hormones of ACS can affect DM development via modulation of gluconeogenesis in the liver [1]. Here we present the results of measurements of glucose content in the blood (Fig. 3). In group 1 rats, glucose concentration in the blood was elevated at all terms of observation. In group 3 rats, a considerable drop of blood glucose concentration was observed 14 days after induction of inflammation. These findings suggest that the decrease in blood corticosterone content in group 3 rats reduces the contribution of this glucocorticoid hormone into *de novo* glucose synthesis in peripheral tissues, which manifested itself in reduced glucose level in the blood.

Thus, injection of SiO_2 to both intact animals and rats with alloxan-induced DM significantly affects the functional state of ACS. It is known that biological effects of SiO_2 are related to stimulation of the immune system [15], but these data do not explain the effects observed in our experiments and, hence, they require further investigation.

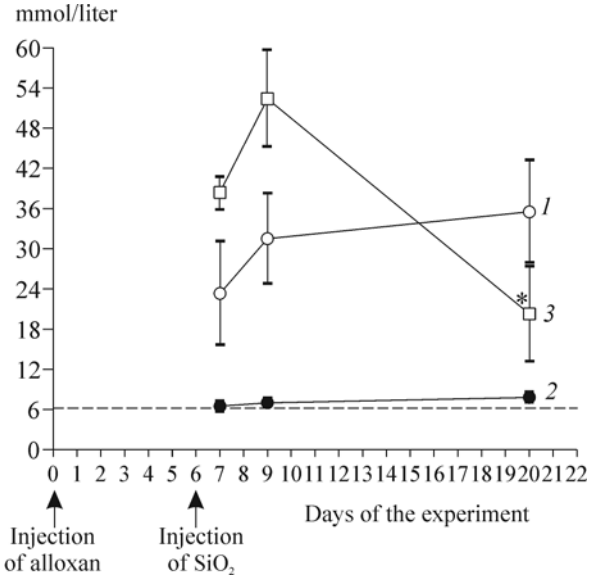

Fig. 3. Glucose content in rat serum. *$p<0.05$ compared to the same parameter at the previous term of observation.

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