MODELING ALLOXAN OSTEOPATHY IN RATS WITH CLINICAL, BEHAVIORAL, AND METABOLIC MANIFESTATIONS OF EXPERIMENTAL DIABETES

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Abstract: *The results of an experimental model of alloxan-induced osteopathy in white rats are presented. A comprehensive assessment of body weight dynamics, glycemic levels, clinical status, and behavioral responses was conducted over 28 days after alloxan administration. The development of persistent hyperglycemia, polyuria, weight loss, and signs of general exhaustion, accompanied by decreased motor activity and anxiety, was observed. The data obtained confirm the reproducibility of the alloxan-induced diabetes model and its suitability for subsequent morphological and biochemical studies of bone disorders.*

Keywords: *Alloxan, diabetic osteopathy, hyperglycemia, body weight, behavioral tests, experimental model.*

Relevance of the study. Diabetic osteopathy is considered one of the most common and clinically significant complications of diabetes mellitus, characterized by impaired bone remodeling, decreased mineral density, and an increased risk of fractures [1, 4, 5]. According to modern epidemiological reviews, the incidence of osteopenia and osteoporosis in patients with type 1 diabetes is 2–3 times higher than in the general population [5].

The mechanisms of diabetic osteopathy remain complex and multifactorial. They are underpinned by chronic hyperglycemia, oxidative stress, inflammatory activation, and insulin deficiency, which disrupt the interaction between osteoblasts and osteoclasts [3, 5]. Experimental models allow us to track these changes at early stages and evaluate the effectiveness of therapeutic interventions.

The alloxan model of diabetes mellitus in rats is one of the most widely used in fundamental research due to its ease of reproduction and the stability of its hyperglycemic effect [3]. Alloxan selectively damages pancreatic β-cells, causing severe insulin deficiency and the development of metabolic disorders similar to the clinical picture of type 1 diabetes [2, 3].

Along with metabolic changes, animals exhibit behavioral and somatic disorders—hypoactivity, anxiety, decreased body weight, and physical endurance—reflecting the systemic effects of hyperglycemia and energy deficiency [1, 2]. These manifestations are closely associated with early disruption of bone metabolism and the development of osteopathy [4].

Thus, the creation of a reproducible and controlled model of alloxan osteopathy in rats represents an important step for the subsequent study of the pathogenesis of diabetic bone tissue lesions and the search for effective means of their correction [4, 5] .

The aim of the study was to create and characterize an experimental model of alloxan osteopathy in rats based on an analysis of the clinical, behavioral, and metabolic manifestations of diabetes, enabling further study of the pathogenesis and correction of diabetic bone disorders.

Materials and methods. The study was performed on 48 albino male nonlinear rats weighing 180–220 g, kept in standard vivarium conditions (temperature 22 ± 2 °C, 12-hour light regime, free access to water and food). Alloxan osteopathy was modeled by a single intravenous administration of alloxan monohydrate at a dose of 170 mg/kg in saline after a 12-hour fast. Control animals received an equivalent volume of saline. The glycemia level was determined with a glucometer on days 3, 7, 14, 21, and 28. Animals with blood glucose levels above 11 mmol/l were included in the main group. Body weight was recorded weekly.

Behavioral reactions were assessed using the Open Field test — general motor activity, number of crossed squares; Elevated Plus Maze - Anxiety Measures; Test "Vertical activity"—the number of rearings and exploratory reactions. A detailed breakdown of the study material and a description of the experimental methods are presented in Table 1.

Table 1.

Behavioral response indices of rats in the modeling of alloxan osteopathy (n = 48)

Test / Indicator	**Control group (n = 12)**	**Alloxan group (7th day) (n = 12)**	**Alloxan group (day 28) (n = 12)**	**Significance of differences with control (p)**
Open Field Test				
Number of squares crossed in 3 minutes	82 ± 6	56 ± 5	48 ± 4	< 0.05
Number of washes (grooming)	9 ± 1	6 ± 1	5 ± 1	< 0.05
Freezing time, s	21 ± 3	34 ± 4	46 ± 5	< 0.05
Vertical Activity Test				
Number of stands ("stands")	23 ± 2	17 ± 2	15 ± 2	< 0.05
Exploratory reactions (sniffing, inspection)	18 ± 2	14 ± 2	11 ± 1	< 0.05
Elevated plus maze				
Time in open	34 ± 4	25 ± 3	18 ± 3	< 0.05

sleeves, %				
Number of entrances to open sleeves	6 ± 1	4 ± 1	3 ± 1	< 0.05

Note: values are given as M ± m ; differences are significant at $p < 0.05$ compared to the control group.

Table 1 presents the results of behavioral tests conducted to assess the functional state of rats after modeling alloxan osteopathy. In the Open Field test, a significant decrease in motor activity (a decrease in the number of squares crossed) and an increase in the duration of freezing were observed, reflecting a decrease in overall tone and interest in the external environment. In the Vertical Activity test, a decrease in the number of rearings and exploratory responses was recorded, indicating asthenia and a decrease in motivation to explore. In the elevated plus maze, a decrease in the time spent in the open arms and the number of entries into them was observed, indicating an increase in anxiety.

Thus, the dynamics of behavioral parameters demonstrate the development of neurometabolic disorders and stress-induced hypoactivity in the context of alloxan-induced diabetes, reflecting the systemic effects of hyperglycemia and energy deficiency. Mortality, coat, skin, and mucous membrane conditions were assessed clinically.

Statistical processing of data was carried out using the Statistica 10.0 package ; differences were considered significant at $p < 0.05$.

Results. The experiment yielded data reflecting the dynamics of clinical, behavioral, and metabolic changes in rats modeling alloxan osteopathy. These results allow for a comprehensive assessment of the development of diabetic syndrome and its impact on the overall well-being of the animals, and confirm the reproducibility of the chosen experimental model.

Table 2.

Dynamics of body weight of rats during modeling of alloxan osteopathy (M ± m)

Observation period	**Control group (n = 12)**	**Alloxan group (n = 12)**	**Change, % to original**	**p**
Initial (day 0)	197 ± 5.8 g	198 ± 6.2 g	—	—
Day 7	201 ± 5.6 g	184 ± 5.7 g	-7.1%	< 0.05
Day 14	205 ± 6.0 g	172 ± 5.3 g	-13.1%	< 0.01
21st day	208 ± 5.9 g	166 ± 5.2 g	-16.2%	< 0.01
Day 28	210 ± 6.1 g	162 ± 5.4 g	-18.2%	< 0.01

Note: The control group showed a moderate increase in body weight (+6.3%), while the alloxan group showed a progressive decrease in weight, indicating the development of metabolic disorders.

The table shows the dynamics of rat body weight during a 28-day observation period after the administration of alloxan. Initially, the weight of animals in the control and alloxan groups was comparable (197 ± 5.8 g and 198 ± 6.2 g, respectively). Already on the 7th day, rats with alloxan-induced diabetes showed a significant decrease in body weight by 7.1% ($p < 0.05$) compared to the initial data. By the 14th day, weight loss reached 13.1% ($p < 0.01$), and by the end of the experiment (day 28) - 18.2% ($p < 0.01$). At the same time, in the control group, a moderate increase in body weight of 6.3% was observed, which reflects the normal physiological growth rate of the animals.

Progressive weight loss in animals of the alloxan group indicates pronounced metabolic disorders characteristic of diabetes mellitus and confirms the successful modeling of the state of alloxan osteopathy.

Table 3.

Dynamics of blood glucose levels in rats after administration of alloxan (M ± m)

Observation period	**Control group (n = 12)**	**Alloxan group (n = 12)**	**p**
Initial (day 0)	5.0 ± 0.4 mmol/l	5.1 ± 0.4 mmol/l	—
3rd day	5.2 ± 0.5 mmol/l	11.2 ± 0.8 mmol/l	< 0.001
Day 7	5.1 ± 0.4 mmol/l	14.8 ± 0.9 mmol/l	< 0.001
Day 14	5.3 ± 0.5 mmol/l	15.5 ± 1.0 mmol/l	< 0.001
21st day	5.1 ± 0.4 mmol/l	15.8 ± 1.1 mmol/l	< 0.001
Day 28	5.2 ± 0.4 mmol/l	16.2 ± 1.1 mmol/l	< 0.001

Note: starting from the 3rd day after the administration of alloxan, the glucose level exceeded physiological values by more than three times and remained stably high until the end of the experiment, which confirms the formation of persistent hyperglycemia.

Table 3 reflects the dynamics of changes in the blood glucose level of rats after the administration of alloxan. Initially, glycemia indicators in the control and alloxan groups were almost identical (5.0 ± 0.4 and 5.1 ± 0.4 mmol/l, respectively). Already on the 3rd day after the administration of alloxan, a sharp increase in glucose levels to 11.2 ± 0.8 mmol/l ($p < 0.001$) was observed, indicating the development of acute hyperglycemia. By the 7th day, glycemia reached 14.8 ± 0.9 mmol/l, and by the 28th day it stabilized at 16.2 ± 1.1 mmol/l, while in the control group the values remained within the physiological norm (≈5.1 mmol/l).

Administration of alloxan caused a sustained and statistically significant increase in blood glucose levels, which persisted throughout the experiment. This confirms the development of a stable diabetic state that adequately reproduces the metabolic disturbances characteristic of type 1 diabetes.

Table 4.

Behavioral response indices of rats in modeling alloxan osteopathy (M ± m)

Test / Indicator	Control group (n = 12)	Alloxan group (7th day)	Alloxan group (28th day)	p
Open Field Test				
Number of squares crossed in 3 minutes	82 ± 6	56 ± 5	48 ± 4	< 0.05
Number of washes (grooming)	9 ± 1	6 ± 1	5 ± 1	< 0.05
Freezing time, s	21 ± 3	34 ± 4	46 ± 5	< 0.05
Vertical Activity Test				
Number of stands ("stands")	23 ± 2	17 ± 2	15 ± 2	< 0.05
Exploratory reactions	18 ± 2	14 ± 2	11 ± 1	< 0.05
Elevated plus maze				
Time in open sleeves, %	34 ± 4	25 ± 3	18 ± 3	< 0.05
Number of entrances to open sleeves	6 ± 1	4 ± 1	3 ± 1	< 0.05

Note: A 41% decrease in motor activity, a 36% decrease in vertical rearing, and an almost twofold reduction in the time spent in the open arms of the maze were observed, reflecting asthenia and increased anxiety in animals with alloxan diabetes.

Table 4 presents the results of behavioral tests reflecting the overall physical and emotional state of rats in the alloxan osteopathy model. Already on the 7th day after alloxan administration, a significant decrease in motor activity was observed: the number of crossed squares in the open field test decreased from 82 ± 6 to 56 ± 5, and by the 28th day, to 48 ± 4 ($p < 0.05$). Similar changes were noted in the number of grooming acts and freezing time, indicating increasing asthenia and a decrease in motivation for exploratory behavior.

The Vertical Activity Test also revealed a 36% reduction in rearing and a more than 30% reduction in exploratory responses compared to the control ($p < 0.05$). In the elevated plus maze, the proportion of time spent in the open arms decreased almost by half (from 34 ± 4% to 18 ± 3%, $p < 0.05$), indicating increased anxiety and impaired adaptive behavior.

The observed behavioral changes in rats with alloxan-induced diabetes demonstrate the development of asthenic syndrome, decreased motor and exploratory activity, and increased anxiety. These data confirm the presence of systemic functional impairments accompanying metabolic disorders in diabetic osteopathy and emphasize the validity of the chosen experimental model.

The data demonstrate that alloxan administration induces the development of typical signs of diabetic syndrome—hyperglycemia, weight loss, hypoactivity, and anxiety. These changes are evident as early as the first week of the experiment and persist through the 28-day observation period. Behavioral and metabolic changes confirm the development of a stable model of diabetic osteopathy suitable for further morphological and biochemical studies.

Table 5.

Clinical signs and external condition of animals in the model of alloxan diabetes

Indicator	**Control group**	**Alloxan-induced diabetes (3 days)**	**Alloxan-induced diabetes (7 days)**	**Alloxan-induced diabetes (28 days)**
General condition	Active, mobile, adequate behavior	Moderate lethargy, decreased activity	Severe adynamia, lethargy	Asthenia, decreased tone, long periods of immobility
Caged behavior	Research, regular physical activity	Decreased exploratory responses	Freezing, avoiding contact	Lethargy, rare movements
Coat	Smooth, shiny, well-groomed	Moderately tousled	Disheveled, dull	Distinctly tousled, hair loss
Leather	Elastic, clean	Dryness of individual areas	Dry, flaking	Thinned, dull, possible scratching
Appetite	Saved	Reduced	Significantly reduced	Anorexia or partial refusal to eat
Drinking behavior	Norm	Increased water consumption	Polydipsia	Severe polydipsia (2–3 times higher than normal)
Urination	Norm	Moderate polyuria	Severe polyuria	Stable polyuria, frequent urination
Mortality rate (%)	0	—	10.4	—

Table 5 shows the dynamics of clinical signs and the external condition of rats during a 28-day alloxan-induced diabetes model. In the control group, animals maintained normal behavior, activity, clean fur and skin, adequate appetite, and drinking behavior. As early as the third day after alloxan administration, the animals showed the first signs of diabetic syndrome: moderate lethargy, decreased activity, increased water consumption (polydipsia), and mild polyuria.

By day 7, pronounced metabolic and behavioral disturbances developed: adynamia, dull and ruffled fur, dry skin, and a noticeable decrease in appetite. By day 28, the animals' condition was characterized by asthenia, thinning and flaking of the skin, hair loss, frequent urination, and severe polydipsia. Appetite was sharply reduced, leading to partial refusal to eat. At this stage, a mortality rate of 10.4% was recorded, consistent with known data on the toxicity of alloxan.

The clinical picture of alloxan-induced diabetes in rats is characterized by typical signs of systemic damage: progressive asthenia, polydipsia, polyuria, trophic changes in the skin and coat, as well as decreased motor activity and appetite. These signs, taken together, confirm the successful development of a stable experimental model of diabetic osteopathy, suitable for further morphological and metabolic studies.

Discussion . The alloxan-induced diabetes model in rats reproduces the key clinical and metabolic features of type 1 diabetes: hyperglycemia, weight loss, polydipsia, and hypoactivity. The development of these disorders is due to selective damage to pancreatic β-cells and subsequent absolute insulin deficiency.

The observed behavioral changes reflect energy deficiency and disruption of the animals' neurometabolic status. This is consistent with literature (Bansal et al., 2020; Larionov et al., 2021), which describes the impact of hyperglycemia on activity and stress resistance in rodents.

Weight loss and decreased activity are accompanied by decreased bone mineralization and remodeling, laying the foundation for the development of diabetic osteopathy. Thus, these abnormalities are early indicators of systemic musculoskeletal damage in diabetes.

The developed model is stable, reproducible, and can serve as an experimental platform for subsequent morphological, biochemical, and pharmacological studies aimed at correcting diabetic bone tissue disorders.

Conclusions . A single administration of alloxan at a dose of 170 mg/kg induces persistent hyperglycemia in rats for 28 days. The model is characterized by decreased body weight, decreased motor activity, and increased anxiety, reflecting systemic metabolic stress. Alloxan osteopathy is reproducible, stable, and suitable for further morphological and pathobiochemical studies of bone changes in diabetes.

Literature

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