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RESEARCH ARTICLE

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The Adaptive Mechanism of Liver in Response to Hyperglycemic Functional Stress in Alloxan Induced Diabetes

Diana Dzidziguri[1]* , Ekaterine Bakuradze[1], Irina Modebadze[1], Merab Sepashvili[1], Giorgi Aleksanashvili[1], Tornike Gogolauri[1], Nino Kharaishvili[1] and Manana Berulava[2]

[1]Chavchavadze av N1, 0108, Tbilisi, Ivane Javakhishvili Tbilisi State University, Georgia

[2]61 Politkovskaya street, 0186, Sokhumi State University, Georgia

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ABSTRACT

Background: Diabetes is a common disease that poses significant medical and socio-economic challenges due to its widespread prevalence and its impact on disability and life expectancy. Recent studies have demonstrated a reduction in high-ploidy (2c×2) hepatocytes within one month of injecting Alloxan or Streptozotocin in experimental models of diabetes mellitus. This phenomenon is attributed to decreased insulin levels, which regulate cytokinesis and consequently affect the number of high-ploidy cells during cytoskeletal reorganization. However, the dynamics between diploid and high-ploidy cells during the initial phase of the disease (within the first 48 hours), characterized by extreme changes in glucose concentration, have not been thoroughly investigated. This study aims to explore the liver's adaptive responses to hyperglycemic stress during the early stages of diabetes.

Research Objects and Materials: Adult white rats (130-150 g) were used as the experimental subjects. Diabetes mellitus was induced in the animals by administering alloxan (180 mg/kg) intraperitoneally.

Methods: The alloxan-induced diabetes model was validated by measuring changes blood glucose concentration and in insulin-producing pancreatic cells through immunohistochemistry in both intact and experimental groups. The histoarchitecture of the experimental animals was assessed using H&E staining. The proliferative activity of liver tissue was evaluated using the colchicine mitotic index method and liver samples collected 24 and 48 hours later. The smears were stained using Feulgen stain for DNA-specific staining and examined microscopically, with the images analyzed using ImageJ software.

Results: Within 48 hours of Alloxan injection, hepatocyte mitotic activity remained unchanged. Conversely, a significant increase of 4c cells was observed within the liver ($p<0.05$).

Conclusion: At the initial stage of hyperglycemia developed by Alloxan injection (48 hours after injection), the liver's ability to adapt in response to increased functional load is expressed in the quantitative increase of high-ploidy cells through the mechanism of endoreduplication.

Introduction

The liver, multifunctional organ in mammals, owes its capabilities to its unique histoarchitecture and remarkable regenerative capacity. These functions are predominantly mediated by hepatocytes, the parenchymal cells that constitute 80% of the liver's mass. Hepatocytes ensure normal, compensatory, and adaptive organ growth through strictly regulated sequential processes including proliferation, hypertrophy, and polyploidization. Understanding the mechanisms governing these processes remains a major challenge, particularly as disruptions in their sequential progression are observed in various pathologies. For instance, alimentary dyslipidemia has been associated with an initial stage of tissue renewal primarily driven by an increase in liver parenchymal cell ploidy [1]. Similarly, experimental models of bilateral adrenalectomy have demonstrated a significant increase in hepatocyte genome ploidy, while radiation and oxidative stress have also been shown to induce polyploidization [2,3]. Additionally, an increase in cell ploidy has been observed in liver parenchyma under destructive conditions four days after common bile duct ligation [4].

In contrast, experimental diabetes models exhibit a decrease in high-ploidy hepatocytes (2c×2) one month after alloxan or

Contact: Diana Dzidziguri, Chavchavadze av. N1, 0108, Tbilisi, Georgia, Ivane Javakhishvili Tbilisi State University, Georgia.

streptozotocin injection due to insulin deficiency, which normally regulates cytokinesis during cytoskeletal reorganization [5]. However, the dynamics of high-ploidy cell changes at the initial stages of disease development (first 48 hours), characterized by rapid fluctuations in glucose concentration, remain unexplored.

Polyploidy, a fundamental process in development, is facilitated by mechanisms such as incomplete cytokinesis, endoreduplication, and cell fusion. However, current methodologies and approaches are insufficient to definitively attribute which of these mechanisms predominates in the formation of high-ploidy cells during various stages of postnatal development, both in normal and disease states.

Based on the aforementioned considerations, the aim of this study is to investigate the mechanisms underlying the liver adaptation to increased functional load induced by hyperglycemia.

Materials and Methods

Liver tissues from adult male white rats (130-150g) were used as a research material. A diabetic state was induced in animals by intraperitoneal injection of alloxan (180 mg/kg). Glucose concentrations were measured using a blood glucose meter (On Call® Plus) prior to and at 3, 6, 9, 24, and 48 hours following alloxan injection, with results reported in mg/dL units [6].

To identify the mechanisms of liver adaptation, the following objectives were established: 1. Assessment of hyperglycemia severity within 48 hours post-alloxan injection by monitoring blood glucose levels in experimental animals; 2. Evaluation of quantitative changes in insulin-producing cells in the pancreas of intact and experimental group animals using immunohistochemistry; 3. Investigation of changes in mitotic activity and hepatocyte ploidy in the liver of adult rats at 24 and 48 hours post-alloxan injection; 4. Study of proliferative activity changes in rat liver and hepatocyte ploidy during the initial stages of regenerative growth (6 hours post-liver resection).

For these objectives, animals were categorized into three groups: 1. Control group — intact rats; 2. Test group I — animals injected with 180 mg/kg Alloxan (24 hrs.); 3. Test group II — animals injected with 180 mg/kg Alloxan (48 hrs.). The proliferative activity of liver tissue was assessed using colchicine mitotic index determination method (1 mg/kg colchicine injected into rats), with tissue samples collected at 24- and 48-hours post-injection. Liver smears were stained with Feulgen stain for DNA-specific staining, followed by microscopic examination. Images were analyzed using the ImageJ software, with lymphocyte absorption serving as a diploid cell standard.

The Alloxan-induced diabetes model was validated by assessing quantitative changes in insulin-producing cells in the pancreas of both intact and experimental group animals via immunohistochemistry [7,8].

Results: To assess the degree of hyperglycemia, changes in glucose concentration were measured in the blood of each animal over time.

Figure 1: illustrates the change in blood glucose concentration in adult white rats during the first 48 hours after alloxan injection. The figure clearly indicates that three hours post-alloxan injection, the blood glucose levels in the experimental group of rats increased approximately fourfold compared to intact animals. Over the next three hours (3-6 hours), 95% of the experimental animals exhibited a tendency for blood sugar concentration to decrease. At the 20th hour post-injection, the glucose levels equaled of the control animals. However, 24 hours after alloxan injection, blood glucose concentration increased once again. These elevated levels were maintained until the end of the experiment (for 48 hours), indicating that stable hyperglycemia was achieved at this time point after alloxan injection (Figure 1).

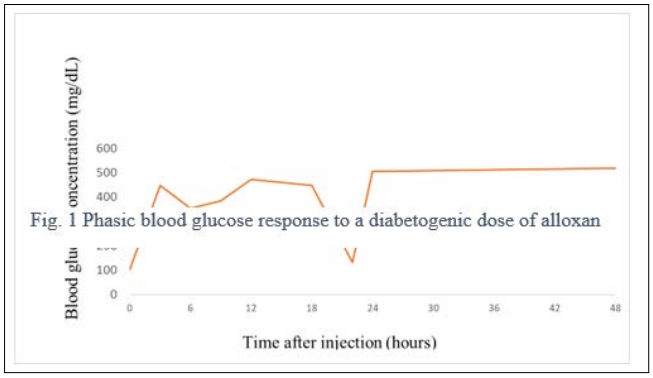

Fig. 1 Phasic blood glucose response to a diabetogenic dose of alloxan

According to the literature, a similar increase in blood glucose concentration has been observed in mice in both alloxan- and streptozotocin-induced experimental diabetes models [9].

In parallel, at the initial stage of the study, we used immunohistochemical methods to assess quantitative changes in insulin-producing cells in the pancreas of both control and experimental groups of animals. Figure 2a and 2b presents microphotographs from the immunohistochemical analysis of pancreatic tissues from these groups. Fig. 2a shows moderate and homogeneous staining of insulin-producing cells in the endocrine part of the pancreas of intact rats. In contrast, 48 hours after alloxan injection, when blood glucose concentrations were stably elevated, the staining intensity of pancreatic β cells using antibodies significantly decreased compared to intact animals (Figure 2b). These findings confirm the occurrence of alloxan-induced hyperglycemia and the onset of diabetes in rats.

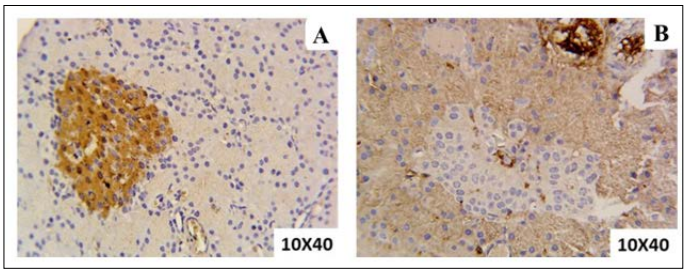

Figure 2: Immunohistochemical Analysis (Using Insulin Antibodies) of the Pancreas of Adult White Rats Under Alloxan-Induced Hyperglycemia.

- Control Group (Intact Animals)
- Animals of the Experimental Group (48 hours)

Figure 3: Depicts changes in liver histoarchitecture at 24 and 48 hours post-alloxan injection. The microphotographs show slight alterations in the liver histoarchitecture of the experimental group of rats at 24 hours post-injection compared to the control group (Figure 3a and 3b). By 48 hours post-alloxan injection (Figure 3c), noticeable structural changes include partial disruption

of the cord-like structures of the liver tissue and cytoplasmic depletion. Additionally, there is a increase in cells with large nuclei, potentially indicative of polyploid cells, with clearly apparent active forms of nucleoli.

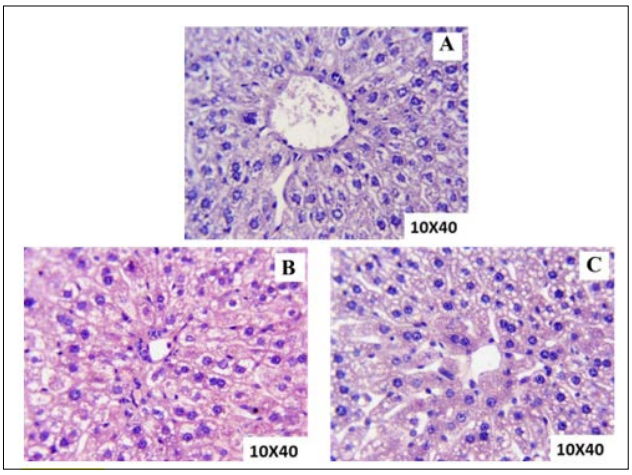

Figure 3: Histoarchitecture of the Liver of Adult White Rats Under Normal Conditions and After Alloxan Injection (24 and 48 hours).

- Intact Anima
- 24 Hours After Alloxan Injection
- 48 Hours After Alloxan Injection

Liver tissue is known for its presence of polyploid cells, reflecting its multifunctional nature. In adult rats, an increase in high-ploidy cells typically occurs in response to liver resection, achieved through incomplete mitosis over several days [10].

Additionally, certain pathologies stimulate early quantitative changes in high-ploidy cells through initiation of polyploidization stages. In rat liver tissue, early mitoses are observed six hours after partial hepatectomy, performed four days post-bilateral adrenalectomy [2].

Notably, this population in adult rat liver forms exclusively through endoreduplication [11].

Hence, it is crucial to determine whether the formation of highly ploidy hepatocytes (4cx2 and 8c) occurs via an alternative polyploidization mechanism, namely endoreduplication.

To investigate this, we examined changes in hepatocyte polyploidization in response to functional load induced by hyperglycemia during the initial stages of alloxan-induced diabetes progression. Specifically, we assessed changes in the ratio of cells with different ploidy in the liver at 24 and 48 hours after alloxan injection. Our findings indicate that at 24 hours post-Alloxan injection, the ratio of cells with different ploidy in the liver of the experimental rat group did not show statistically significant changes (Table 1).

Table 1: Changes In Liver Cell Ploidy After Alloxan Injection ($p<0.05$)

	Control group	24 h.	48 h.
2c	45.5±6.4	31.9±4.2	23.4±7.9*
2c×2	8.4±1.4	8.4±1.4	8.3±1.7
4c	41.4±6.8	55.9±4.8	74.9±4.8*
4c×2	3.1±0.7	2.6±0.5	2.6±0.3
8c	0.9±0.3	1±0.4	1.9±0.5

Simultaneously, a significant change in the ratio of polyploid cells was observed at 48 hours after alloxan injection. Changes in the ploidy of liver parenchymal cells (hepatocytes) in experimental animals at these time points are presented as histograms in Fig. 4. The figure illustrates that 48 hours after alloxan injection, compared to controls, the number of diploid (2c) cells in the livers of experimental animals decreased significantly by 51%. Concurrently, the number of mononuclear tetraploid cells (4c) increased significantly by approximately 55% (Figure 4). These results indicate that during the early stages of alloxan-induced diabetes (48 hours), the liver responds to increased functional demands with a quantitative increase in high-ploidy cells.

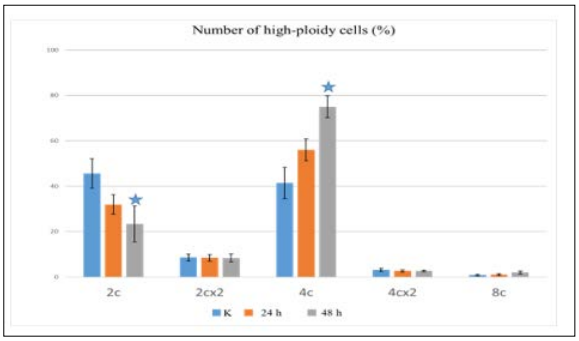

Figure 4: Changes in the Ploidy of Hepatocytes in the Liver of Adult White Rats at Different Times (24 and 48 hours) After Alloxan Injection ($p<0.05$).

Figure 5: displays cell smears isolated from liver tissues of control and experimental rat groups, stained with the Feulgen stain. Notably, at this time point, there is no evidence of increased proliferative activity among hepatocytes in liver tissue. Specifically, compared to control animals, despite observed changes in liver histoarchitecture post-alloxan injection, there are no significant differences in mitotic index values among the three groups (Figure 6).

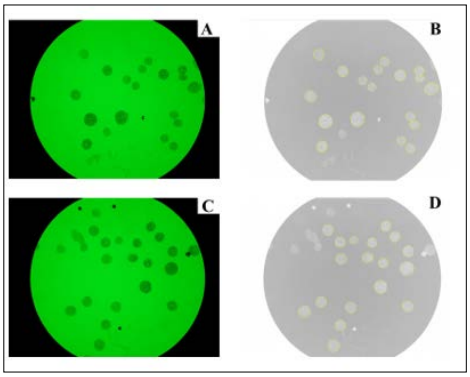

Figure 5: (a, c) Isolated Hepatocytes Stained With the Feulgen Reaction; (b, d) Images Processed Uing Image J Software (10×100).

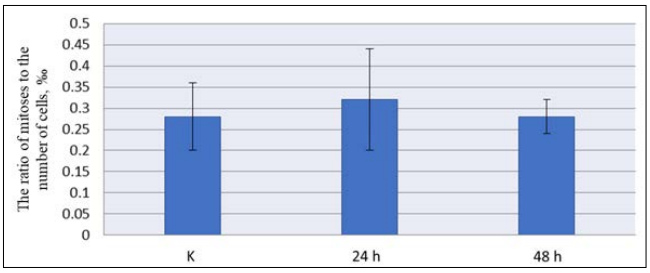

Figure 6: Mitotic Activity of Adult White Rats Under Normal Conditions and at Different Time Points After Alloxan Injection (P<0.05)

According to the literature, early mitotic events occurring six hours post-partial hepatectomy, administered on the fourth day following bilateral adrenalectomy in rat liver tissue, are attributed to the transition of cells into the mitotic phase via the endoreduplication mechanism, coupled with delayed progression through the G2 phase (known as G2-0 cells) [2].

Therefore, in the subsequent stage of the study, rats underwent partial liver resection 48 hours after alloxan injection, and changes in hepatocyte mitotic activity were assessed six hours post-surgery. As illustrated in Figure 7: Partial Hepatectomy Initiated Early Mitotic Events Within Six Hours.

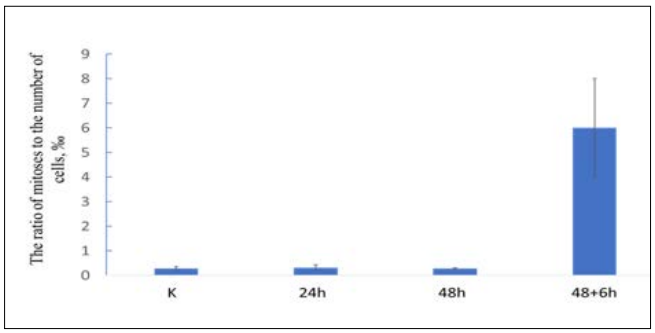

Figure 7: Liver Proliferative Activity Six Hours After Partial Hepatectomy (p<0.05).

Discusion

Based on the obtained results, it can be concluded that hyperglycemia induced by alloxan injection causes increased functional load on the liver and apparent changes in histoarchitecture at the initial stage (48 hours after injection). Significantly, despite these changes, there is no reduction in cellular mass and, consequently, no increase in proliferative activity at this time point. In cases of mass loss, as is known, liver renewal primarily begins with the proliferation of its parenchymal cells.

Notably our data, at the initial stage of alloxan-induced hyperglycemia, there is a quantitative increase in high-ploidy cells in the liver. This suggests that the liver responds to functional load by increasing genome ploidy. The fact that against the fact of a decrease in diploid cells, only the percentage of mononuclear tetraploid cells increases, and there is no change in the mitotic index within 48 hours, suggests that in this case, the increase in genome ploidy is achieved not through classical incomplete cytokinesis, but through the activation of an alternative polyploidization mechanism, namely endoreduplication. Endoreduplication, as is known, ensures the accumulation of cells in the G2 phase and the ability to quickly transition into mitosis itself in response to a signal.wIn our case, the validity of this assumption was confirmed by the emergence of metaphase figures in the remaining tissue six hours after liver resection in the experimental model of alloxan diabetes.

From all of the above, it can be concluded that under conditions of hyperglycemia induced by alloxan injection, the increase of high-ploidy cells through endoreduplication, without changes in mitotic activity in the liver of adult white rats represents an adaptive feature of the liver.

Conclusion

From all of the above, it can be concluded that under conditions of hyperglycemia induced by alloxan injection, the increase of high-ploidy cells through endoreduplication, without changes in mitotic activity in the liver of adult white rats represents an adaptive feature of the liver.

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