# Changes in the Count of Pancreatic β- and α-Cells and Blood Glucose Level in Rats with Alloxan-Induced Diabetes

**G. N. Aleeva, A. P. Kiyasov, M. M. Minnebaev, I. M. Burykin, and R. Kh. Khafiz'yanova**

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 133, No. 2, pp. 151-153, February, 2002
Original article submitted November 8, 2001

We estimated the count of pancreatic α- and β-cells and blood glucose level at various stages of alloxan-induced diabetes in rats. Alloxan decreased the count of insulin-producing β-cells, but increased the number of glucagon-secreting α-cells in the pancreas (week 1 of diabetes). These changes were accompanied by hyperglycemia. The decrease in blood glucose level in diabetic rats was associated with an increase in β-cell count against the background of high density of pancreatic α-cells.

**Key Words:** *pancreas; α-cells; β-cells; diabetes; alloxan*

Considerable advances were made in understanding of the pathogenesis, complications, therapy, and prevention of diabetes mellitus (DM). Further studies on animals should elucidate the pathogenetic mechanisms of this disease.

Alloxan is widely used for modeling diabetes mellitus in experimental animals. This agent causes death of β-cells due to activation of lipid peroxidation, which leads to the development of type I DM [1]. However, hyperglycemia can result not only from a decrease in the count of insulin-producing β-cells synthesizing insulin, but also from an increase in the number of glucagon-secreting α-cells. This is consistent with the data on increased blood glucagon concentration and α-cell count in pancreatic islets of patients with type I DM [4]. It can not be excluded that changes in the ratio between α- and β-cells in pancreatic islets play the major role in the pathogenesis of alloxan-induced diabetes.

Here we estimated glucose level and density of β- and α-cells in rat pancreas at different terms after a single alloxan injection.

Department of Normal Human Anatomy, Department of Pathophysiology, Department of Pharmacology, Kazan State Medical University

## MATERIALS AND METHODS

Experiments were performed on 78 male outbred albino rats weighing 150-200 g. The animals had free access to food and water. Diabetes was induced by single subcutaneous injection of 110 mg/kg alloxan. Control animals received an equivalent volume of distilled water. On day 3 the blood was taken from the caudal vein, and glucose concentration was measured. Diabetes was diagnosed, if blood glucose concentration surpassed 8.32 mmol/liter. Glucose level was estimated by the orthotoluidine method using standard kits. The rats were decapitated 12, 24, and 36 h or 3, 5, 8, 12, and 27 days after treatment. The tail of the pancreas was removed and embedded into paraffin by routine techniques. α- and β-cells of PI were visualized using polyclonal antibodies to insulin and glucagon (Dako). Immunohistochemical staining with monoclonal antibodies to proliferating cell nuclear antigen (PCNA, Dako) was performed. Indirect immune peroxidase and streptavidin-biotin methods (peroxidase-conjugated porcine antirabbit antibodies and LSAB kit, Dako) were used for visualization [2]. Morphometry of serial slices stained with

0007-4888/02/1332-0127$27.00 

antibodies to insulin and glucagon was performed on a computer-assisted morphometrical system, which allowed evaluating changes in the ratio between β- and α-cells.

We measured the percent ratio between areas occupied by insulin- and glucagon-containing cells and the total area of PI. The results were analyzed by Student's *t* test.

## RESULTS

Blood glucose concentration in rats was maximum 3 days after alloxan administration 350% above the baseline. Hyperglycemia corresponding to moderate DM was observed by the 12th day. Blood glucose level decreased to 7.1 mmol/liter on day 27 (Table 1).

Immunohistochemical assay showed that in control animals α-cells were localized in the peripheral region of PI (demarcation line). β-Cells were regularly distributed in PI. Morphometry of PI showed that the mean areas occupied by insulin-producing β-cells and glucagon-secreting α-cells were 77.61± 2.08 and 26.53±2.26%, respectively (Fig. 1).

Staining with anti-glucagon antibodies was observed in the peripheral and central PI areas 36 h after alloxan injection. Starting from this term and to day 12 the density of α-cells in PI surpassed the control. Morphometry showed that the density of α-cells in PI increased at all stages after alloxan treatment (Fig. 1).

Changes in β-cell population of PI developed 24 h after alloxan administration and were manifested in a decrease in cell immunoreactivity after staining for insulin. The area occupied by β-cells decreased 36 h after treatment with alloxan. The appearance of solitary empty spaces in PI was probably related to cell death. Morphometry showed that the decrease in the density of β-cells was most pronounced 3 days after alloxan administration (by 80.2%, Fig. 1).

The count of cells with PCNA-positive nuclei increased 24 h after alloxan treatment. The distribution of PCNA-positive cells corresponded to localization of glucagon-producing α-cells. These cells were rarely found in control animals. It should be emphasized that the count of proliferating cells increased 24-36 h after alloxan treatment. After 48 h PCNA-positive cells were found not only in the peripheral, but also in the central region of PI. Insulin- and glucagon-positive cells appeared in the ductal epithelium at various stages after alloxan treatment, which attested to the involvement of ductal epithelium in neogenesis of PI.

Our results suggest that hyperglycemia during alloxan-induced diabetes results from not only a decrease in β-cell population, but also an increase in the number of glucagon-synthesizing α-cells. Normalization of blood glucose level is probably related to β-cell recovery at late stages after alloxan administration. These findings contradict published data on the absence of β-regeneration after alloxan treatment [6].

Studies of proliferative activity of pancreatic PI indicate that islet β-cells are involved in proliferation. The recovery of β-cells at late stages after alloxan treatment confirms the possibility of their regeneration from islet cells. Previous experiments with selective perfusion of the pancreas with alloxan produced similar results [7]. Proliferation potential of ductal cells attests to their incomplete differentiation. *In vitro* culturing of human ductal cells revealed their pluripotency, their ability to differentiate into endo- and exocrine cells [3]. It can not be excluded that ductal cells are stem cells differentiating into exo-, endocrine, and ductal cells under the effect of exogenous stimuli [5].

**TABLE 1**. Blood Glucose Level in Control Animals and Rats with Alloxan-Induced Diabetes (mmol/liter, *M*±*m*)

| Days | Control | Experiment |
|---|---|---|
| 3 | 5.31±0.16 | 22.73±3.28* |
| 5 | 4.99±0.39 | 17.86±1.86* |
| 8 | 4.62±0.40 | 11.63±1.01* |
| 12 | 5.35±0.32 | 9.25±1.11* |
| 27 | 5.22±0.42 | 7.10±1.16* |

**Note.** *$p<0.05$ compared to the control.

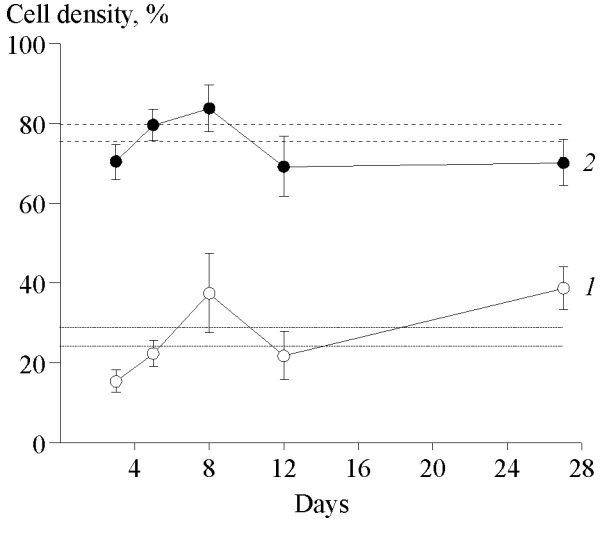


**Fig. 1.** Density of pancreatic β- (*1*) and α-cells (*2*) at different stages of alloxan-induced diabetes. Control densities of β- and α-cells are shown by dashed lines (upper and lower area, respectively). $p<0.05$: significant differences compared to the control.

Thus, hyperglycemia in alloxan-induced diabetes mellitus is characterized by changes in β- to α-cell ratio in PI. Hence, new antidiabetic preparations should prevent the imbalance between these populations of pancreatic cells. This model of diabetes can be used to study regeneration of the endocrine pancreas.

## REFERENCES

1. V. G. Baranov, I. M. Sokoloverova, and A. G. Gasparyan, *Experimental Diabetes Mellitus* [in Russian], Leningrad (1983).
2. A. P. Kiyasov, *Modern Methods for Morphological Assays* [in Russian], Kazan (2001).
3. S. Bonner-Weir, M. Stubbs, P. Reitz, *et al*., *Pancreatic Growth and Regeneration*, Ed. N. Sarvetnick, Basel (1997), pp. 138-153.
4. W. Gepts and P. M. Lecompte, *Am. J. Med*., **7**0, 105-115 (1981).
5. A. Sharma, D. H. Zangen, P. Reitz, *et al*., *Diabetes*, **48**, 507-513 (1999).
6. C. T. Spadella, S. A. Schellini, C. A. Caramori, and C. E. Bacchi, *Transplant. Proc*., **32**, 2820-2823 (2000).
7. M. Waguri, K. Yamamoto. J. L. Miyagava, *et al*., *Diabetes*, **46**, 1281-1290 (1997).