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Medical Sciences

Extra-Islet Intermediate Cells of Pancreas and the Effect of Plaferon in the Alloxan-Induced Diabetes Mellitus

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ABSTRACT. Using the methods of light and electron microscopy, pancreas was studied in the experimental model of alloxan diabetes (AD) at various times of the experiment (1 and 6 months) at different degrees of AD and after the action of plaferon.

Special emphasis was made on the display of compensatory changes in opportunity of occurrence in extra-islet cells special granules, characteristic of insulin producing β-cells.

Data obtained suggest that extra-islet cells possess structural features of both acinar as well as endocrine cells. They represent a transitory structure under transformation into the endocrine cell and rise in number in adaptive-compensatory response to alloxan-induced diabetes mellitus.

Plaferon is shown to have positive influence on the content of sugar and insulin in the blood in alloxan-induced diabetes mellitus. Plaferon is suggested to promote maturation and transformation of extra-islet cells. Presumably plaferon takes part in the neogenesis of β-insulinocytes. *© 2009 Bull. Georg. Natl. Acad. Sci.*

Key words: *pancreas, extra-islet intermediate cells, alloxan diabetes, plaferon.*

Introduction

Prophylaxis and treatment of diabetes mellitus remains a challenge in health and social welfare because of the steady growth of the rate of this ailment all round the world.

Insufficient proliferation of β-cells in the pancreas insulin apparatus and subsequent insulin deficiency is a principal cause of impairment of carbohydrate metabolism.

Investigation of compensatory and adaptation processes in the pancreas during diabetes mellitus is of special theoretical and practical significance [1-13]. Experimental modeling of pancreas morphological reconstructions starting with the onset of clinical manifestation and all along the course of diabetes mellitus is of special import since clinical observation of pancreas reorganization is impossible.

The present study was directed at investigating subcellular and cellular changes in pancreas and the effect of plaferon in alloxan-induced diabetes mellitus.

Material and methods

Wistar laboratory rats (n=20) served as experimental subjects. Subcutaneous administration of Alloxan solution (10%, 300 mg/g) was used to induce diabetes mellitus. Death lethal injection of sodium pentobarbital mixture (1%) was used for cessation of experiment.

Sub-cellular and cellular changes in pancreas have been studied at different periods and severity of diabetes manifestation. Special attention was paid to com-

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pensatory changes in extra-islet cells. Possibility of the formation of special granules, specific to insulin producing β-cells was of principal concern in this respect.

The level of the sugar and insulin in blood was used as a marker for the development of diabetes mellitus. KIT set ("CEA-IRE-SORIN", "INSIK 3, France, Belgium, Italy) was used for radio-immunological detection of insulin.

Pieces of pancreas of 10 experimental subjects were sampled in the first and 6th month after alloxan injection. Plaferon (0.25 mg/g) was administered 7 consequent days in a month over a period of experiment 30 days after the experimental induction of diabetes in 10 subjects.

Plaferon was obtained from the amniotic membrane of human placenta [14]. Plaferon is recognized as an active immune modulator as well as having antioxidant, antiviral, antibacterial and antifungal effect.

To prepare samples for morphological study, pancreas was fixed in Carnua mixture and Buen fluid. Paraffin sections (5 mc) were stained by hematoxylin and picrofuchsin (Van Gison method).

To prepare electron microscopy samples, pieces of pancreas were: 1) treated in fresh 2% solution of osmium tetra-oxide on colloid buffer (PH 7.2 - 7.4) for 2 hr at + 4 ^{0}C; 2) dehydrated in spirits with increasing concentration; 3) poured over araldite mixture and 4) polymerized for 24 hr at +58^{0}C. Ultramicrotom Reichert-42 was used to prepare sections. Reynolds method was used for uranil acetate contrasting of the samples. Sections were covered with silver containing emulsion (1) and observed in microscope ("Tesla-BS 500" ,Czech Republic, magnification of 3000-22000). Negative images were magnified 3-5 times when printed.

Results and Discussion

Positive correlation between the severity of the damage to pancreas islet β-cells and hyperglycemia was revealed. Data obtained point to the principal role of the damage of β-cells in the manifestation of diabetes mellitus.

Pancreas islets were found markedly changed one month after the alloxan administration (content of Sugar in the blood 250-320 mg %): part of the cells atrophied, others – hypertrophied. Destruction – degranulation as well as vacuolization of β-insulinocytes has been revealed. Necrotized cell regions with surrounding connective tissue capsule were present as well. Single fibrous inclusions were detected in the islets. One month after the beginning of the experiment islets as well as adjacent tissue were found markedly infiltrated with macrophages, lymphocytes and fibroblasts. Macrophages were detected at the early stages of the experiment.

Electron microscopy one month after the beginning of the experiment revealed extra-islet cells adjacent to acinar cells and close to islets and pancreatic ducts. These cells are at different levels of differentiation.

The border between acinar and extra-islet cells was well preserved. Presence of granules with dense interior and white aureole and fine mitochondria with light matrix and isolated cristae, as well as small number of organelles, cisterns and vesicles in the rough endoplasmic reticulum in the part of the cell, oriented towards the islet,. is specific to islet β-cells. Large spherical and oval mitochondria with dense matrix and numerous cristae, rough endoplasmic reticulum with cisterns placed in parallel as well as zymogenic granules presented in the opposite part of the extra-islet cell are specific to acinar cells.

Pancreas islet fibrosis manifested in parallel to the progress of pathology 6 months after the beginning of experiment (Sugar level in blood 320 mg%, insulin – 0). Large regions of necrosis surrounded by connective tissue capsule as well as interacinar and intraglobullar fibrosis were detected in the islets. Islet structure was impaired. Highly fuchsinophilic collagen fibers disposed between endocrine cells as well as histio-lymphocytar infiltration in the islets and nearby were detected. The number of macrophages was decreased as compared to earlier stages of experiment.

Numerous extra-islet intermediate cells with signs specific to β-cells were detected 6 months after the beginning of experiment mainly in case of the alloxan-induced diabetes of mild severity (sugar in blood 180-200 mg%, insulin -6.4). No difference in the structure and localization between these cells and those revealed in the earlier period was found. In several extra-islet cells numerous insulin granules were concentrated in one pole or close to the nucleus (Fig. 1).

Sugar content in blood was shown to decrease to 50-80 mg% and the level of insulin made up 2.4 – 5.1. six months after the administration of alloxan against a background of treatment with plaferon (7 consequent days in a month over a period of experiment 30 days after the experimental induction of diabetes),

Extra-islet cells with different degree of maturation were revealed in electronogram of treated animals. Insulin granules with dense interior and light asymmetric aureole were found in most of the cells. Large nucleus with prevailing content of euchromatin was detected (Fig. 2). Dystrophy of acinar part and disorganized cristae in mitochondria gave the impression of disconnection between

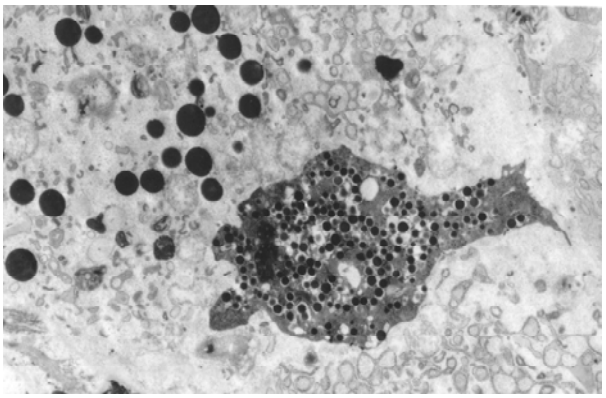

Fig. 1. Extra-islet cell 6 months after the beginning of experiment. SGI - Insulin secretory granules. ZG- zimogenic granules. Magnification- 3 000

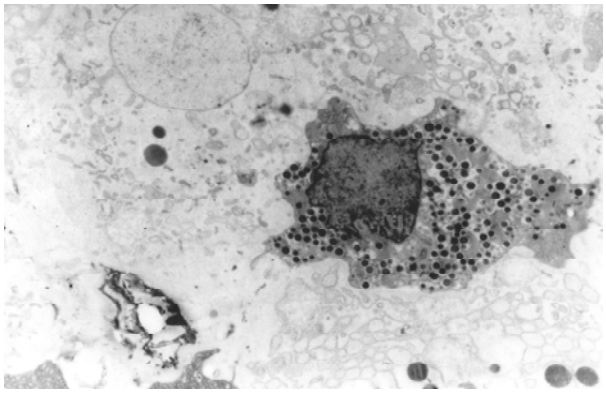

Fig. 2. extra-islet cell under the effect of plaferon 6 months after the beginning of experiment Magnification - 4 000

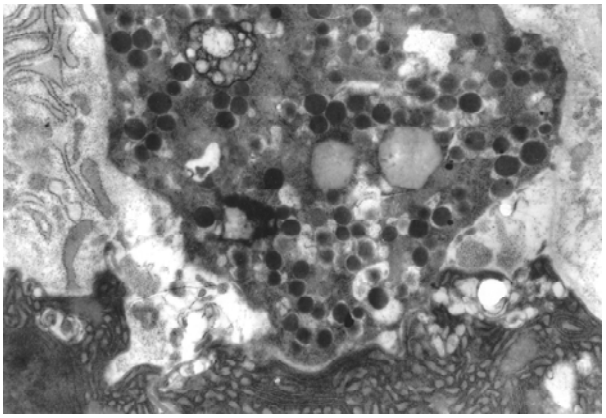

Fig. 3. Extra-islet cell under the effect of plaferon 6 months after the beginning of experiment. Endocrine and exocrine regions are connected with desmosomes. SGI, D - desmosome, C - exocrine region. Magnification - 8 000

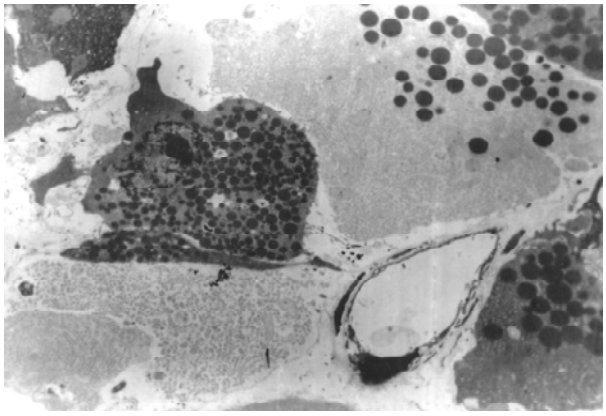

Fig. 4. Capillary of sinusoid type. Magnification 4 000

exocrine and endocrine parts with isolated desmosomes stretched between acinar and endocrine regions (Fig. 3). Numerous less differentiated cells were detected as well.

Endothelial lining of capillaries adjacent to extra-islet cells was refined and fenestrated (Fig. 4). Marked infiltration with macrophages, lymphocytes and plasmocytes was found close to islets and inside the islets as well.

The possibility of transformation of acinar cells into islet cells has been discussed earlier and remains problematic nowadays. Several authors reported on acinar islet transformation in pathological and physiological conditions [1,3,7-9]. These cells are considered transitory structures formed during transformation of acinar cells into endocrine cells [1,3,8,9]. Epithelial cells in the finest pancreatic ducts are suggested to give rise to these structures as well [1,7,8]. The function of these cells, however, remains obscure.

During the development of pathological processes, there were detected intermediate cells including exocrine and endocrine granules. Some of these cells were located near the acini and some of them were in close contact with duct cells.

Conclusion

Data obtained suggest that extra-islet cells possess structural features of both acinar as well as endocrine cells. They represent a transitory structure under transformation into the endocrine cell and rise in number in adaptive-compensatory response to alloxan-induced diabetes mellitus.

Plaferon is shown to have positive influence on the content of sugar and insulin in the blood in alloxan-induced diabetes mellitus. Presumably plaferon promotes maturation and transformation of extra-islet cells and plays a role in the neogenesis of β-insulinocytes.

სამედიცინო მეცნიერებანი

პანკრეასის კუნძულგარეშე შუალედური უჯრედები ალოქსანური დიაბეტის დროს და პრეპარატ პლაფერონის ზემოქმედების შემდეგ

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(წარმოდგენილია აკადემიკოს ნ. ჯავახიშვილის მიერ)

უჯრედულ და სუბუჯრედულ დონეზე შესწავლილია ცვლილებები, განვითარებული პანკრეასში ალოქსანური დიაბეტის (1/6 თვე) სხვადასხვა სიმძიმის პათოლოგიური პროცესის დროს, აგრეთვე პრეპარატ პლაფერონის (ამნიოტური ინტერფერონის) ზემოქმედების შემდეგ.

ჩატარებული გამოკვლევების დროს განსაკუთრებული ყურადღება დაეთმო ე.წ. "კუნძულგარეშე" — შუალედური ტიპის უჯრედებს, რომლებიც იმყოფებიან დიფერენციაციის სხვადასხვა ხარისხში. ეს შუალედური ტიპის უჯრედები შეიცავენ როგორც ეგზოკრინულ, ასევე ენდოკრინულ β ინსულოციტებისათვის დამახასიათებელ გრანულებს. ელექტრონოგრამაზე ამ "კუნძულგარეშე" — შუალედური ტიპის უჯრედებში, პლაფერონის ზემოქმედების შემდეგ მატულობს ინსულინის გრანულების რაოდენობა და სიმკვრივე ელექტროგრამაზე.

როგორც ჩანს, ეს უჯრედები წარმოადგენენ კომპენსაციურ-ადაპტაციური პროცესის გამოვლენას ექსპერიმენტული დიაბეტის დროს. სავარაუდოა, რომ პრეპარატი პლაფერონი დადებითად მოქმედებს შუალედური ტიპის უჯრედების ტრანსფორმაციასა და დიფერენციაციაზე და ხელს უწყობს βინსულოციტების ნეოგენეზს.

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