The Effects of Epidermal Growth Factor on Pancreas in Alloxan-Diabetic Rats: An Ultrastructural Study

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Abstract: To examine the ultrastructural changes of rat pancreas in alloxan induced diabetes mellitus and the follow-up Epidermal Growth Factor treatment. In this study, we used 30 wistar rats. They were divided into three groups; group I: controls, groupII: alloxan induced diabetes mellitus (single intraperitoneal dose 200mg/kg alloxan), group III: diabetes mellitus + EGFtreatment (0.2 ml EGF injected per day for seven days). At the end of the experimental period, animals were sacrificed and their pancreases were removed. The tissue samples were investigated by using electron microscopy techniques. We observed degenerative changes in the β cells, and acinar cells in group II. In contrast, the β cells and acinar cells have revealed positive alterations in the group III. We found that EGF administration on diabetic rats could reserve some of the degenerative changes caused by diabetes.

Keywords: Alloxan, diabetes, EGF, pancreas, ultrastructure.

INTRODUCTION

Type I diabetes is characterized by insufficiency of β cell mass in pancreas (Insulin-dependent diabetes). Type II diabetes is also characterized by deficiency of β cell mass and peripheral insulin resistance (Non-insulin Dependent Diabetes) [1]. Insufficient production of biologically active insulin is a common denominator in almost all forms of diabetes and the degree of insulin deficiency determines both the severity of the disease and the choice of therapy [2, 3]. Is ß cell regeneration possible? Is imbalance between ß cell destruction and ß cell formation result in diabetes. For long years, many invastigaters have tried to find out the answers to these questions [1, 4] Although the previous data are quite restricted there is evidence that of β-cell regeneration can occur early in both types of diabetes [5, 7]. Therefore, it would be of interest to examine the regulation of ß -cell growth, and the factors that prevent or promote the replacement mechanisim of the lost islets. So far, a number of models have been developed related ß cell regeneration and neogenesis [1, 8-10].

Peptide growth factors constitute an important component in the regulation of pancreatic development and regeneration, and are the major external signals that activate the MAPK pathway [10, 11]. Epidermal growth factor (EGF), one of the best-characterized and most comprehensively studied peptide growth factors, has been isolated from most human tissues. EGF produces a cascade of cellular events that are part of the mitogenic responses including initiation of DNA synthesis of extracellular macromolecules. Studies on animals and humans showed that EGF displays have various biological effects including stimulation of cell proliferation, differentiation and maturation [12-14]. The biological effect of EGF is mediated by specific, high-affinity cell membrane receptors, termed epidermal growth factors receptor (EGF-R), which have been found in a wide spectrum of mammalian tissues. EGF-R is a large transmembrane glycoprotein (1,186 amino-acids) containing an extracellular and intracytoplasmic domain separeted by a transmembrane region [14-16]. Therefore, this study have investigated, at ultrastructural level, the changes occured in the pancreatic tissue of rats administered alloxan with EGF and to what extent EGF prevents the pathological changes occured in diabetic pancreas.

MATERIALS AND METHODS

Animals

The study included 30 Wistar Albino male rats with an avarage weight of 220-250 gr. They were maintained under conditions of 20^{o} C temperature and daily light/ dark cycle and were fed with normal diet and water ad libitum for 40 days.

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Research Design and Methods

Some chemical compounds, like alloxan and streptozotosin (having toxicity to Langerhans islet) are used to develop experimental diabetes. In order to develop experimental diabetes, we used alloxan. Thirty rats were used, three groups of ten animals each. Group I animals were used as a control group. Group II and group III rats were fasted overnight and diabetes was induced in rats with a single intraperitoneal injection of a freshly prepared solution of alloxan (200 mg/kg, ALX monohydrate, Sigma A8128) in 0.9% NaCl to constitute a 10%(w/v9) solution only. After a week's time for the development and aggravation of diabetes, the rats with moderate diabetes having persistent glycosuria and hyperglycemia (blood glucose range of above 250 mg/dl) were considered as diabetic rats and used for the experiment. Epidermal Growth Factor (EGF) treatment was started on the 8th day after alloxan injection and this was considered as the 1st day of treatment. Group II rats were not treated and used as a diabetic control group. We measured their blood glucose levels by Dextrotix (Ames, Miles Ltd. U.K.) The animals were gdivided into three groups, comprising of ten animals in each group as follows:

- *Group I*: control rats receiving 0.9% NaCl
- *Group II*: alloxan-diabetic rats;
- *Group III*: diabetic rats were injected per day with 0.2 ml EGF (Sigma E 7755) for seven days

At the end of the experimental period, the animals sacrificed by cervical decaptitation.

Electron Microscopy

The pancreas from experimental animals and controls were cut into pieces of about $1mm^3$, fixed in gluteraldehyde-containing phosphate buffer at pH 7.2 and post-fixed in an 1% osmium tetroxide solution for 1h at 4^0C. After fixation, the specimens were dehydrated and embedded in araldite. Thin sections were stained with uranyl acetate and laed citrate and then examined and photographed under ZEISS 900 transmission electron microscope.

The degenerative changes in endoplasmic reticulum and mitochondria were semiquantitatively evaluated as described below.

Endoplasmic Reticulum

+++: Dilate endoplasmic reticulum and tubules were filled with less dense material

++: Moderate dilatation in endoplasmic reticulum

+: Less dilate endoplasmic reticulum

-: No degenerative changes

Mitochondria

+++: Matrix dilution,vacuolization and swelling

++: Matrix dilution and vacuolization

+: Matrix dilution

-: No degenerative changes

Statistical Analysis

All data were presented as mean± standard deviation (S.D.). A computer program (SPSS 12,0 for Windows, SPSS Inc., Chicago, IL, USA) was used for statiscal analysis. The data were considered to be non parametric, therefore, they were analyzed using Mann-Whitney U- test. $p<0.05$ were considered to be statistically significant.

RESULTS

Group I Animals

Langerhans islets of control animals displayed a normal architecture in which mitochondria with a dense matrix and vissible cristae. Golgi apparatus were evident. β cells contained a number of secretory granules. The granules composed of a central core, usually with moderate homogenous, or slightly heterogenous electron density, and an external single-layered membrane. The granules had a space between the core and the membrane. The granules were diffusely distributed in the cytoplasm.

Similarly, exocrine pancreas ultrastructure from control animals presented a normal structure. The acinar and sentroacinar cells and were observed completely normal. The cytoplasm of acinar cells was filled with zymogen granules.

Group II Animals

In Group II animals, which had been induced with alloxan, the degenerative changes were seen in exocrine and endocrine pancreas. The electron micrographs of β cell in the Langerhans islet of alloxan-induced diabetic rats showed mitochondrial vacuolization and swelling as well as dilatation of the endoplasmic reticulum (Table **1**). It was interesting to

observe that some mitochondria were in normal form whereas the others were consisted of a crystalline appearance and matrix dilution. Just like in acinar cells, mitochondria were in round shape. In addition, there were also spaces in cytoplasm. We noted that ß granules were decreased in number and degranulated in β cells. The degranulated granules were seen in vocuole form and some of them were big and in widening form (Figure **1**).

Table 1: Degenerative Changes in Beta Cells of Alloxan-Diabetic and EGF-Treated Diabetic Rats

	Alloxan- diabetic	EGF-treated
Beta granules	+++	+/-
Mitochondria	+++	+
Granular Endoplasmic reticulum	++	+

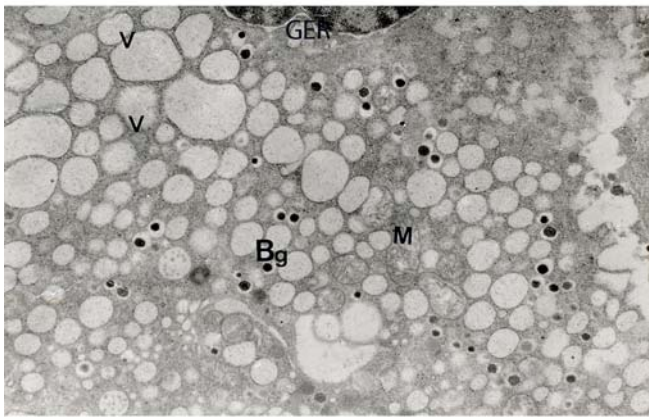

Figure 1: Electron microghraph of ß cell in alloxan- diabetic pancreas. M: mitochondria, Bg: Beta granules, V: vacuole. Lead citrate ×3,200.

Electron microscopic examinations showed that acinar cells of the exocrine pancreas of the diabetic rats were characterized by irregular dilatation and prominent lamellar arrangement of granular endoplasmic reticulum. Mitochondria showed damage consisting of matrix dilution and they were in round shape (Table **2**). Although secretory granules (zymogen) production and secretion were continuing in diabetic acinar cells in number of zymogen granules had decreased. Sentroacinar cells showed a normal appereance but vacuolation was evident in cytoplasm (Figure **2**).

Group III Animals

In particular, exocrine and endocrin pancreas taken from rats treated with EGF prevented ultrastructural

Table 2: Degenerative Changes in Acinar Cells of Alloxan-Diabetic and EGF-Treated Diabetic Rats

	Alloxan- diabetic	EGF-treated
Mitochondria	+++	++
Granular Endoplasmic reticulum	+++	–

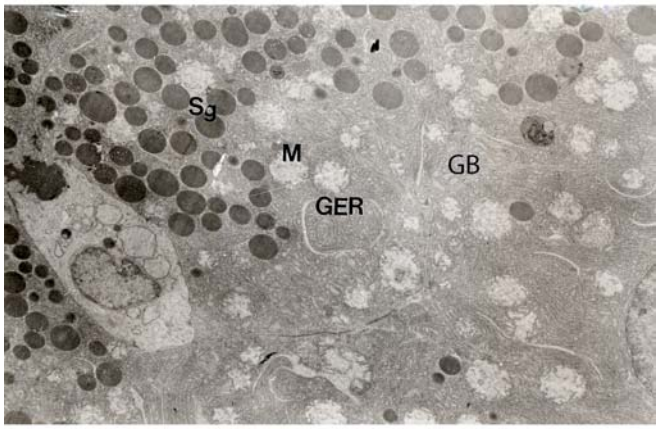

Figure 2: Electron microghraph of acinar cell in alloxan-diabetic pancreas M: mitochondria, GER: Granular endoplasmic reticulum, GB: Golgi Body, Sg: secretory granules. Lead citrate ×3,200.

changes induced alloxan. The cellular structure was better preserved in terms of cells and ß cells as compared with group II animals. Group III animals showed less degranulated sacs and more number of filled secretory granules in comparison to diabetic rats. Round and tubular shaped mitochondria were in normal structure ($p<0.05$). The degenerative changes in granular endoplasmic reticulum had also been reserved (Table **2**). More ß granules and less vesicules were observed than diabetic groups ($p<0.05$) (Figure **3**).

Table 3: Degenerative Changes in Alloxan- Diabetic and EGF Treated Exocrine Pancreas

	Mitochondirion	Gr. Endoplasmic Reticulum
p	0,138	0,002

In group II the most attractive observation in acinar cell was irregular dilatation and prominent lamellar arrangement of granular endoplasmic reticulum (Table **2**). Yet, in this group, rough endoplasmic reticulum lost its dilatated shape and became normal ($p>0.05$). However, mitochondrial degeneration was still present ($p>0.05$). The apex of the cell was filled proenzyme-

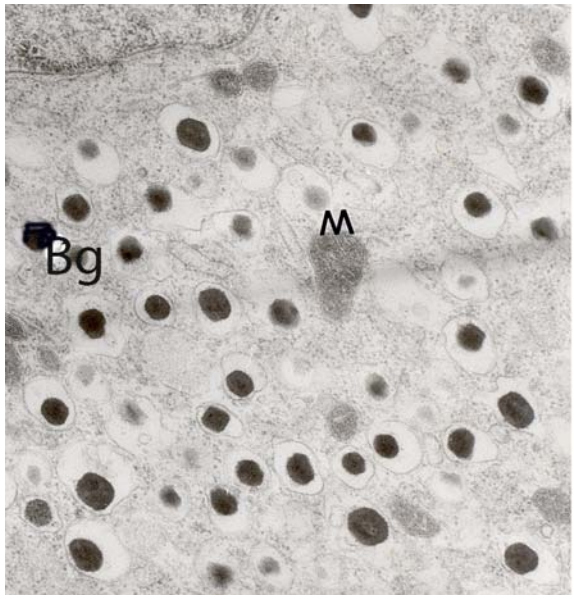

Figure 3: Electron microghraph of ß cell in Langerhans islet of EGF treated-pancreas M: mitochondria, GER: Granular endoplasmic reticulum, Bg: Beta granules. Lead citrate ×3,200.

containing secretory granules similar to those of the controls (Figure **4**).

Table 4: Degenerative Changes in in Alloxan- Diabetic and EGF Treated Beta Cell

	Beta granules	Mitochondrion	Gr. Endoplasmic reiculum
p	0,002	0,001	0,002

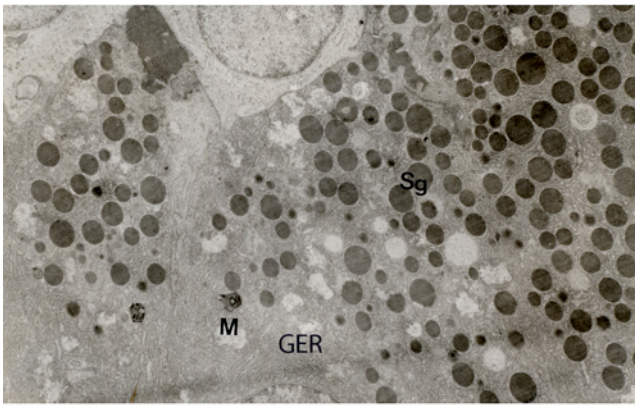

Figure 4: Electron microghraph of acinar cell in alloxan-diabetic pancreas. M: mitochondria, GER: granular endoplasmic reticulum, Sg: secretory granules. Lead citrate ×3,200.

DISCUSSION

Diabetes pathology is due to a combination of ß cells deficiency and peripherial insulin resistance. A

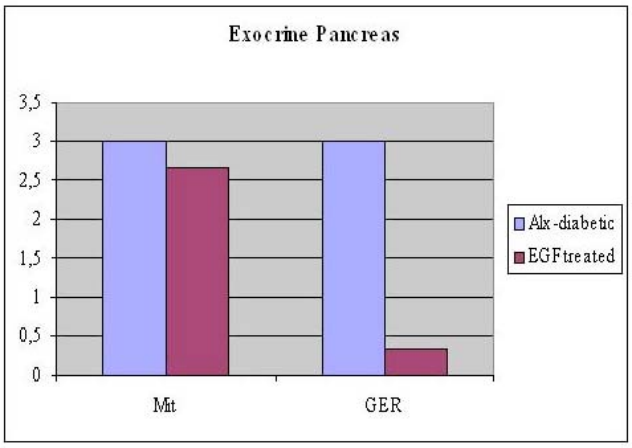

Figure 5: Degenerative changes in alloxan diabetic and EGF treated exocrine pancreas. Mitochondria p>0.05, granular endoplasmic reticulum p<0.05, Golgi body p<0.05.

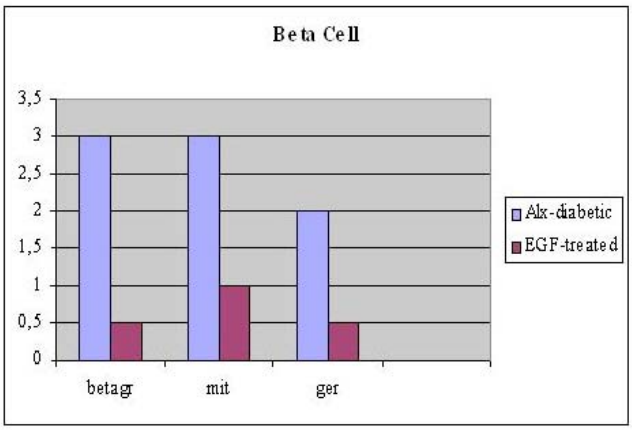

Figure 6: Degenerative changes in alloxan diabetic and EGF treated Beta cell. Beta granules p<0.05 mitochondria p>0.05, granular endoplasmic reticulum p<0.05, Golgi body p<0.05.

major goal of diabetes therapy is to promote the formation of new β-cells, either *in vitro* for transplantation or *in vivo*, i.e., β-cell regeneration [17]. In the present study, we investigated, whether EGF treatment is associated with regeneration of pancreatic tissue following the induction of diabetic pancreas with alloxan by means of electron microscope.

Various models of β regeneration in diabetes have developed in the past years. Pancreaoctomy has been studied as a model of diabetes and substantial regeneration of both endocrine and exocrine pancreas have been noted in many animal models. According to Rosenberg and Vinik, partial duct obstruction of hamster pancreas leads to induction of islet cell regeneration [18, 19]. Regeneration of pancreatic endocrine cells in interferon-gamma transgenic mice has been demonstrated by Sarvetnic *et al*. [20]. In this model, the ductal cells retain the ability to proliferate

and to differentiate into islet cells. Similar results were observed by Papaccio who found neoformed ß cells with low dose injection of streptozotocin (STZ) for 5 days in diabetized 15 mice, and according to their results, they believed that the formation of these newly formed ß cells might be named as " ducto-endocrine proliferation" [21]. This was an attempt to compensate for the loss of cells at the onset of diabetic syndrome.

Peptide growth factors such as epidermal growth factor (EGF), constitute an important component in the regulation of pancreatic development and regeneration, and are the major external signals that activate theMAPK the mitogen-activated protein kinase (MAPK) [12-14, 21, 22]. EGF represent an available agent which has already possessed these effects. The use of EGF for the therapy of diabetic pancreas can be an emerging new treatment modality. To gain insight into the effect of EGF on pancreas, we have used alloxan induced rats.

In our study, the most remarkable degenerative changes have been noted in granular endoplasmic reticulum tubuli in acinar cell and β cells in alloxan group. In addition, another important result was the reduced ßcells in alloxan group. β cells were occupied by large, usually electron-translucent vesicles but β granules were not found in vesicles. It is clear that granular endoplasmic reticulum reversed to normal structure by EGF treatment. However, the number of filled secretory granules was increased in EGF treated animals while they decreased in group II. Also, in other studies, it is possible to observe similar findings to our study such as a decrease in the secretory granules, swelling of mitochondria and endoplasmic reticulum, round-shaped mitochondria, hypertrophic cytoplasmic organelles in the diabetic rat β cells [21, 23-31].

Our study shows that, the alloxan induced pathological changes in exocrine and endocrine pancreas can be prevented by *in vivo* administration of EGF. Paticularly β cells of Langerhans islets were increased in EGF treated groups. These results led us to consider that EGF may increase the synthesis of secretory granules in the beta cells and so may the healing effect in the mitocondrions and in granular endoplazmik retikulum. According to our findings, EGF treatment may be novelity for pancreatic regeneration in diabetes.

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