otExendin-4 inhibits mitochondrial apoptotic pathway via clusterin in the pancreatic islets in neonatal STZ treated rats

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Clusterin is a disulfide-linked heterodimeric glycoprotein expressed ubiquitously in a wide variety of tissues and play an important roles in tissue remodeling/regeneration, differentiation and apoptotic cell death in damaged tissues. Clusterin is specifically expressed in the early process of pancreas regeneration in both developing exocrine and endocrine cells [1,2]. The streptozotocin (STZ) induced diabetic neonatal rats are one of the useful experimental models to study the regeneration of β cells [3]. The glucagon like peptide-1 receptor selective agonist exendin-4 sustains pancreatic β cell proliferation and prevents cell death by apoptosis [4]. In this study we investigated that the effect of exendin-4 (Ex4) on apoptosis regulating genes and clusterin expressions in pancreatic islet cells after STZ administration in newborn rats.

Three groups were performed. (1) The n2-STZ group; on the second day after birth 100mg/kg STZ was given i.p. to two groups of newborn rats. (2) n2-STZ+Ex4 group; 3μg/kg/day Ex4 was given for 5 days by starting from third day. (3) Control group. The pancreatic tissue sections were immunostained with insulin, glucagon, somatostatin, clusterin β, clusterin α/β, Apaf-1, caspase-3 and caspase-8 antibodies. In addition, pancreas sections were stained double immunohistochemistry for insulin and clusterin-β. For detection of apoptotic cells TUNEL assay was used. In situ hybridization carried out with clusterin probe. Blood glucose levels of the animals in all groups were measured. All values were analyzed with statistical methods.

In n2-STZ, blood glucose levels significantly increased ($p<0,001$) when compared with the control and n2-STZ+Ex4 groups**.** The islets sizes of STZ treated groups were smaller than the control group. Insulin immunopositive cells in the islets were higher at the n2-STZ+Ex4 groups ($p<0,001$) compare to n2-STZ. The number of glucagon and somatostatin immunopositive cells increased in the Ex4 treated and the untreated n2-STZ groups. In the n2-STZ group the number of Apaf-1 and caspase-3, clusterin β positive cells and apoptotic cells were higher than the other groups(Figure 1 and 3). Clusterin immunoreactive cells were seen in the periphery of the islets similar localization to glucagon cells. When compared with the control group, the number of clusterin mRNA signal positive cells were higher than in the islets of the n2-STZ+Ex4 and the n2-STZ groups (Figure 2). In the Ex4 treated STZ group insulin/clusterin double-positive cells were significantly increased ($p<0.001$) compared to the n2-STZ group (Figure 4).

Our results show that Ex4 treatment increases the expression of the clusterin which stimulates β-cell regeneration and prevents mitochondrial pathway of apoptosis in the pancreatic islets cells in n2-STZ rats.

References

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Figure 1; Apaf-1

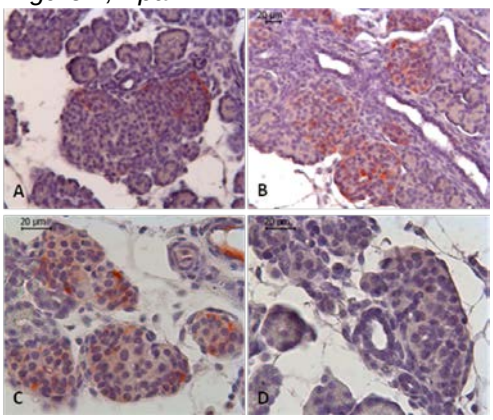

Figure 2; Clusterin

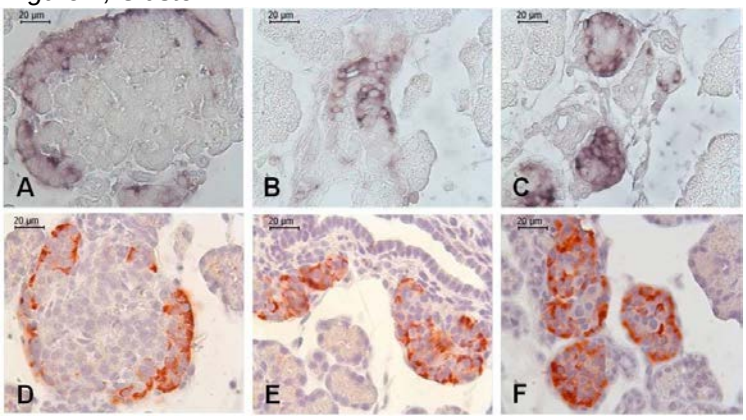

Figure 1. *Apaf-1 immunopositive cells distribution in islets. A:Control group, B:n2-STZ group, C:* n2-STZ+Ex4 group. (A,B X20; C,D X40),

Figure 2. *Clusterin expression in all groups. A-C; In situ hybridization. Clusterin mRNA positive cells are localized in periphery of islets. D-E: Immunohistochemistry. Clusterin-beta immunopositive cells are seen similar localization with the mRNA signal positive cells. (A,D: Control group, B,E:n2-STZ group, C,F:* n2-STZ+Ex4 group).

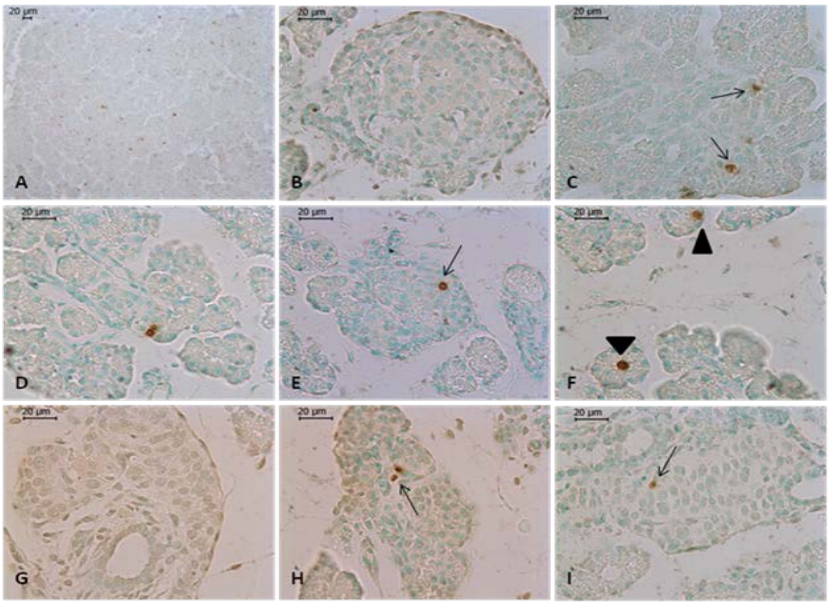

Figure 3. *Apoptosis positive cells both within the islets(↑) and in the exocrine pancreas (▲). A: Positive control for TUNEL, B:Control group, C-F:n2-STZ group, G-I:* n2-STZ+Ex4 group.

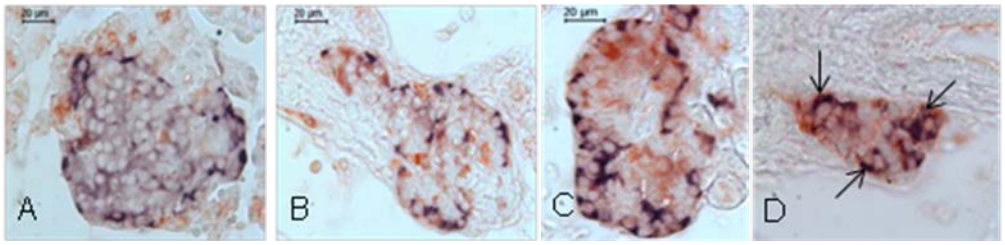

Figure 4. *Insulin/clusterin double immunostained positive cells(↑). A: Control group, B:, n2-STZ group, C,D:* n2-STZ+Ex4 group. *(dark purple;insulin, red;clusterin)*