Fibroblast growth factors promote pancreatic cell proliferation in normal and STZ – treated hamsters

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Abstract

Introduction: In various organs cell proliferation and differentiation are controlled by growth factors. The knowledge concerning the implication of FGF family members in pancreatic development is still incomplete. The aim of the present study was to examine the structure characteristics, functional and proliferative activity of normal and diabetic hamster pancreatic cells following treatment with FGF 1, 2 and 7 in vitro.

Material and methods: We have studied three groups of healthy hamsters – 5 hamsters at age pnd 1, 10 hamsters at age pnd 5 and 7 hamsters at age pnd 10. We induced experimental diabetes in female hamsters during the first 24 hours after mating of female and male hamsters by intraperitoneal streptozotocin (STZ) administration at a dose of 65 mg/kg. For the study we used only these animals from the litter that had a blood glucose level over 11 mmol/l. Three groups of diabetic hamsters were investigated – 20 hamsters at age pnd 1, 20 hamsters at age pnd 5 and 20 hamsters at age pnd 10. The following methods were used: H.E. staining, P/A.F. staining and histoautoradiography.

Results: Light microscopic studies showed active synthetic processes in pancreatic cells under the influence of investigated FGFs. In our experimental model of diabetes the labelling index of pancreatic cells was higher than in the corresponding groups of healthy hamsters. The most important finding in our study was that FGF2 at a concentration of 10 ng/l has shown the most prominent effect in normal hamsters at age pnd 10 and in the streptozotocin-treated hamsters at age pnd 5.

Conclusions: We conclude that FGF 2 stimulates the initial process of cell aggregation and cluster formation. Our study provides evidence for a positive regulatory effect of FGF1, FGF 2 and FGF 7 on the expansion of the pancreatic cell mass in normal and streptozotocin-treated hamsters.

Key words: fibroblast growth factors 1, 2 and 7, cell proliferation, pancreas, diabetes.

Introduction

Despite the increasing knowledge on the mechanisms that regulate the development of parenchymal organs, many questions remain unsolved regarding the regulation of proliferation and differentiation of pancreatic epithelial cells in the normal growing pancreas and during the pancreatic exocrine and endocrine diseases, including diabetes mellitus. There are two mechanisms by which pancreatic cells develop: differentiation of ductal precursor cells (neogenesis) and proliferation of pre-existing differentiated cells [1-3]. The process of neogenesis mostly takes place during foetal and

neonatal life. In the absence of an external stimulus β-cells have a low mitotic activity after weaning and until adult age [4]. To investigate the mechanisms responsible for abnormal B-cell development that could disrupt glucose homeostasis, many scientists use neonatal experimental models of diabetes. In rats treated with streptozotocin (STZ) a loss of β-cell mass leads to diabetes followed by a reappearance of new B-cells [5-7].

It is generally thought that growth factors regulate cell proliferation, differentiation and survival. Among the growth factors that influence pancreatic development are members of the fibroblast growth factor (FGF) family. Islet-cell regeneration in the diabetic hamster pancreas can be induced by local growth factors [8].

This study was therefore designed to examine the effect of FGF1, FGF2 and FGF7 on pancreatic cell proliferation activity in a normal state and in an experimental model of diabetes in the Golden hamster.

Material and methods

We have studied three groups of healthy hamsters – 5 hamsters at age pnd 1, 10 hamsters at age pnd 5 and 7 hamsters at age pnd 10.

We induced experimental diabetes in female hamsters during the first 24 hours after mating of female and male hamsters by intraperitoneal streptozotocin (STZ) administration at a dose of 65 mg/kg. For the study we used only these animals from the litter that had a blood glucose level over 11 mmol/l. We studied three groups of diabetic hamsters: 20 hamsters at age pnd 1, 20 hamsters at age pnd 5 and 20 hamsters at age pnd 10. Healthy and diabetic pups were decapitated, their pancreases were dissected and cut into small segments. One or two segments from the isolated pancreases were cultivated in each well of 96 – well plates. For the preparation of organ cultures from the pancreases we used Iscove's medium, supplemented with foetal bovine serum (FBS) and 100 U/ml penicillin and 100 µg/ml streptomycin. The incubation medium was additionally supplemented with 4, 10 and 100 ng/l FGF1, FGF2 and FGF7. The pancreatic pieces were incubated at 37°C with 5% CO_2 for 48 hours. After 48 hours, the medium was supplemented with 3H-thymidine in a concentration of 2 µCi/ml for 8 hours. Then the pancreatic segments were fixed in Bouin's solution for 24 hours, embedded in paraffin, sectioned and stained with haematoxylin-eosin (H.E.) and paraldehyde-fuchsin (P.A.F.) according to routine procedures. After 14 days of exposure, the autoradiographs were stained with haematoxylin – Bömer. The proliferative activity of pancreatic cells was determined by the labelling index (LI) according to the formula:

LI (%) = number of labelled nuclei/number of counted nuclei x 100. From each section of the experimental groups we estimated 200 cells. A statistical analysis was made using Student's t-test, Statmost program. The results are expressed as a mean percentage ±S.E.

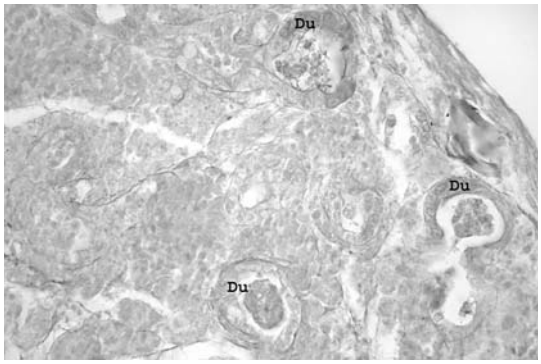

Figure 1. Insulin positive cells in the ductal epithelium in the pancreas of normal hamsters at age pnd 5 after treatment with 4 ng/l FGF 1; Du – duct; P.A.F., x 25

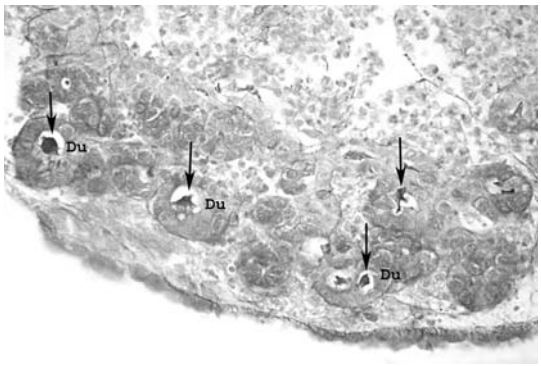

Figure 2. Insulin-positive cells in the ductal epithelium and insulin-positive secretion in the pancreas of diabetic hamsters at age pnd 5 after treatment with 4 ng/l FGF 1; Du – duct, arrows – insulin-positive secretion in the ductal lumen; P.A.F., x 25

Results

Pancreases of normal hamsters (control groups) are distinguished for well-developed mesenchymal tissue, ductal structures mostly in the periphery and differentiated epithelial cells. We observed an increase of the proliferative parts after treatment with FGFs. In the groups of diabetic hamsters we have established more ductal structures in the periphery in comparison with the respective groups of normal hamsters.

In the normal hamsters at age pnd 5 and treated with 10 ng/l FGF 1 we observed mesenchymal tissue. We also observed a process of Langerhans islet formation at the earliest pnd 1 investigated. At ages pnd 5 and pnd 10 we have found a large number of islets of Langerhans throughout the mesenchymal tissue, as well as throughout the exocrine cells in the normal hamster pancreases. In diabetic hamster pancreases we have established more insulin – positive cells in the ductal epithelium than in the

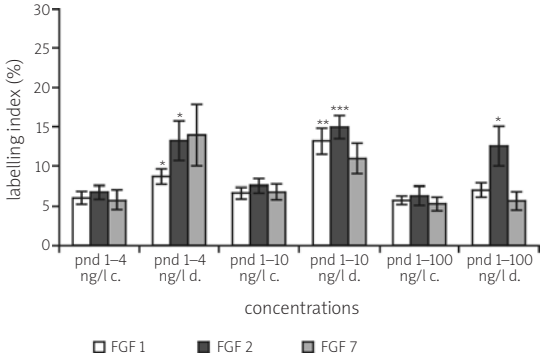

Figure 3. Effect of FGF 1, 2 and 7 on normal and diabetic hamster pancreases pnd 1 (mean ±S.E.); c. – control hamsters; d. – diabetic hamsters; significance of difference to control hamsters *p<0.05, **p<0.01, ***p<0.001

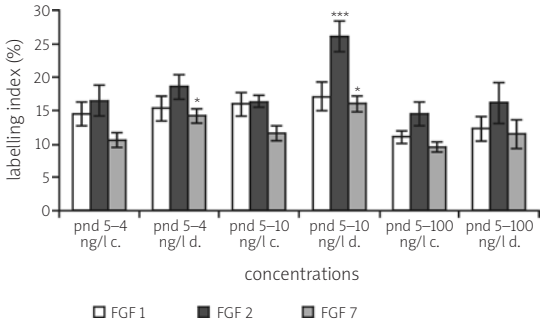

Figure 4. Effect of FGF 1, 2 and 7 on normal and diabetic hamster pancreases pnd 5 (mean ±S.E.); c. – control hamsters; d. – diabetic hamsters; significance of difference to control hamsters *p<0.05, **p<0.01, ***p<0,001

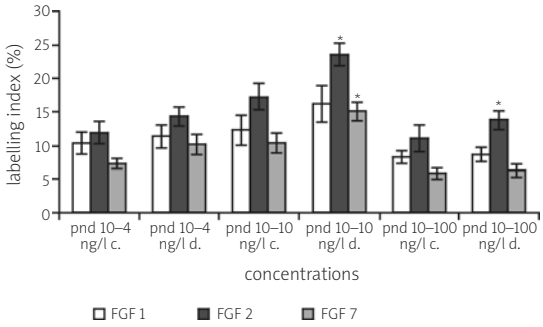

Figure 5. Effect of FGF 1, 2 and 7 on normal and diabetic hamster pancreases pnd 10 (mean ±S.E.); c. – control hamsters; d. – diabetic hamsters; significance of difference to control hamsters *p<0.05, **p<0.01, ***p<0.001

corresponding groups of normal hamsters and insulin – positive secretion in the ductal lumen (Figures 1, 2). We have also established more insulin-synthesising cells close to the ducts, as well as throughout the acinar cells in the diabetic hamster pancreases. In the periphery of some islets of Langerhans we have found vacuolisation, which is due to the effect of STZ.

To evaluate the mitogenic effect of FGFs treatment on the pancreatic epithelial cells we applied the histoautoradiographic method. The histoautoradiographs have shown proliferating ductal cells in the periphery, as well as proliferating exocrine cells in the central part of sections from the control groups of hamsters. In the diabetic hamsters, in comparison with the controls, more marked nuclei mostly at the expense of more proliferating ductal cells have been observed (Figures 3-5).

In the control groups, as well as in the diabetic groups of hamsters, cell proliferation has shown a dose-dependent effect. The most prominent stimulatory effect (26.2±0.6 %, p<0.001 in comparison with the control) was registered at a concentration of 10 ng/l of FGF 2 and at age pnd 5 of the diabetic hamsters and the lowest mitogenic effect – (5.2±0.9%) was registered at a concentration of 100 ng/l FGF7 at age pnd 1 in the control hamsters.

Discussion

In the present work we have investigated the effect of three of FGFs – FGF1, FGF 2 and FGF 7 on the processes of proliferation and differentiation of pancreatic cells from healthy hamsters (control groups) and in STZ – diabetic model hamsters. Our results demonstrate an increased proliferative activity mainly of the pancreatic ductal cells.

Pancreatic development is a classical example of an epithelium – mesenchyme interaction. It has been established that mesenchymal signals control pancreatic cell development by activating the proliferation of these cells and suppressing their differentiation into endocrine cells. A considerable number of studies proved the growth promoting effect of FGF 1, 2, 7 and 10 on pancreatic epithelial cells [3, 9-12]. In our study on the effect of FGF 1, 2 and 7 on early pancreatic postnatal development of healthy hamsters it has been found that these growth factors induce a proliferation of pancreatic epithelial cells. The majority of proliferating cells were ductal cells. We support the concept that the proliferation of pancreatic ductal cells leads to an increase in the number of endocrine precursor cells which could differentiate into beta cells [13-15]. In our experimental model of diabetes the labelling index of pancreatic cells was higher than in the corresponding groups of healthy hamsters. The most important finding in our study was that FGF2 at a concentration of 10 ng/l has shown the most prominent effect in normal hamsters at age pnd 10 and in streptozotocin – treated hamsters at age pnd 5. We have observed a stronger effect of FGF 1 in comparison with FGF 7 both in healthy and in diabetic groups of hamsters and a lesser effect in comparison with FGF 2. These results correlate well with the data that FGF 1 and vascular

endothelial growth factor (VEGF) display an angiogenic effect [16]. We have established that FGF 7 has shown the lowest mitogenic effect both in control and in diabetic experimental groups.

To gain a more precise understanding of the processes of proliferation and differentiation in the postnatal development of the pancreas, we have used PAF staining which is specific for insulin-synthesising cells. We have observed a lot of insulin-synthesising cells in the ductal epithelium. PAF staining has shown an insulin-positive secretion in the ducts as well. There are data in the literature that the epithelia grown in basement membrane – rich gel form multiple cystic structures. These cells stain positively for the pancreatic ductal antigen (cytokeratin 7). There were also insulin-positive cells [17].

Conclusions

In conclusion, our results are in agreement with the statement that FGF 2 stimulates the initial process of cell aggregation and cluster formation, an important step in the development of the pancreas. Our study provides evidence for a positive regulatory effect of FGF1, FGF 2 and FGF 7 on the expansion of the pancreatic cell mass and especially beta cells in normal and STZ-treated hamsters.

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