Endogenous Pancreaatic Regeneration from Pancreatic Duct Epithelium in Diabetic Adult Rats

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Summary:

Background: Deficiency in beta cell mass is the hallmark of most forms of diabetes, it is worthwhile understanding pancreatic regeneration in the context of this disease.Of crucial importance in the development of diabetes, both type I and type II, is the insufficient beta cell replication after the onset of disease.This is why we are always in search of new sources of beta cells to be generated by neogenesis to induce beta cells. In this regard, pancreatic stem or progenitor cells may offer a promising therapeutic approach for diabetes.

Methods and Materials: A total of 60 adults swiss albino rats were divided into two groups. Group I, control group (30 animals) were injected with normal saline into the subcutaneous and pancreatic tissue, and group II (30 animals) were exposed to 90% subtotal pancreatectomy for the study of the possible regeneration, and trans-differentiation of pancreatic stem cells from pancreatic duct epithelium into functioning islet cells.

Results: The animals of group II showed high blood glucose and normal serum AFP during the period of experiment. The histological study revealed regeneration and proliferation of small cells from epithelial cells of the pancreatic duct during the first three weeks of the experimental period and then formation of islet like cells attached to epithelium of pancreatic duct around the period of six weeks. The animals of group I (control group) showed no changes in the pancreas.

Conclusion: Pancreatic islet cells may originate from pancreatic duct epithelium.

Keywords: diabetes, pancreatic regeneration, pancreatic stem cells , replication , alpha-feto protein (AFP) , Blood glucose

Introduction:

Insufficient beta-cell mass is fundamental to the pathogenesis of both types 1 and 2 diabetes and constitutes the basis for the goal of beta-cell replacement therapy (1). Currently being accomplished through ecto-pancreatic transplantation and islet implantation. The number of donars human pancreas organs that can be transplanted directly or used for islet of Langerhans isolation is limited(2). Recently, the potential of adult stem cells for islet regeneration has been explored(3). During pancreatic development, stem cells have a central role in generating endocrine, acinar and duct cells(4,5). Panceatic stem cells are powerful tools for future cell therapy for diabetes, pancreatic carcinoma and chronic pancreatitis(6). However, it is unclear whether pancreatic stem cells exist in adult pancreas(7). It has been shown that pancreatic tissue is able to regenerate in several species of mammals after surgical insult(3,8), and is also known to have the potential to maintain or increase its beta-cell mass in response to metabolic demand during pregnancy and obesity(9). In this regard pancreatic stem or progenitor cells may offer a promising therapeutic approach for diabetes (10). A substantial amount of evidence has accumulated over the last few years supporting the hypothesis that islet neogenesis in the mature pancreas occur via cells which are located in, or which are associated with the ductal epithelium (11). Our own study has demonstrated that the induction diabetes in adult rats through subtotal pancreatectomy is able to trigger regenerative process of pancreatic islet-like structure from stem cells originating from pancreatic duct epithelium.

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Materials and Methods:

A total of sixty normal, healthy, adult swiss albino rats of both sexes, have been kept in good environmental conditions and fed good diet. These animals were divided into two groups:

Group I , normal control group(30 animals) : The animals of this group were subjected to laparatomy and injected with multiple injections of normal saline into varies sites of the pancreas .

Group II, 90% subtotal pancreatectomy(30 animals): The animals of this group were subjected to laparatomy and 90% subtotal pancreatectomy to study the possible regeneration of pancreatic stem cells from epithelial cells of pancreatic duct .

All the animals in group I and group II were subjected to biochemical and histological studies.

The biochemical studies included measuring blood glucose and serum AFP levels at regular intervals (every five days). The histological studies were done also at regular intervals (every 1-2 weeks), using hematoxlyin and eosin stain, Gomori's stain. The period of experiment extended for two months.

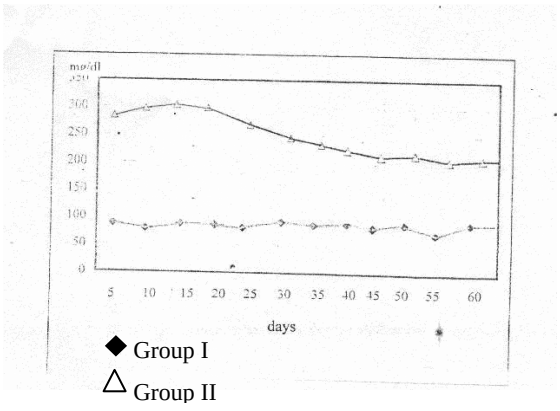
Results:

Biochemical studies.

a . Blood glucose levels in animals of group I and group II at regular intervals through the period of experiment (two months) are presented in table-1 and figure-1 .

Table-1: Demonstrates blood glucose levels in I animals of group I and group II through the periods of experiment.

Periods	Groups	No.	Means Mg/dl	Std.Dv Mg/dl
days-5	group-I group-II	30 30	88.611 285.40	8.818 32.385
days-10	group-I group-II	30 30	87.235 290.791	11.728 19.873
days-15	group-I group-II	30 30	88.361 293.44	7.672 19.o54
days-20	group-I group-II	30 30	86.697 290.95	7.045 13.268
days-25	group-I group-II	30 30	84.442 260.29	9.782 18.634
days-30	group-I group-II	30 30	92.399 235.58	7.701 16.267
days-35	group-I group-II	30 30	87.979 230.44	10.196 15.732
days-40	group-I group-II	30 30	91.000 220.653	8.1o6 11.701
days-45	group-I group-II	30 30	85.380 230.17	11.000 18.388
days-50	group-I group-II	30 30	91.160 216.60	8.443 13.226
days-55	group-1 group-II	30 30	86.468 205	8.443 14.522
days-60	group-I group-II	30 30	90.987 202	7.589 17.999

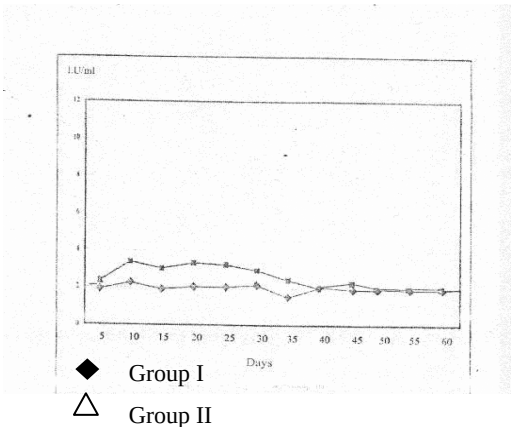


Diag1: Diagram demonstrating the blood glucose levels through the periods of experiment in animals of group I and Group II.

b. The serum AFP levels in animals of group I and group II at regular intervals through the period of experiment (two months) are presented in table-2 and figure-2 .

Table-2: Demonstrates blood glucose levels in I animals of group I and group II through the periods of experiment.

Periods	Groups	No.	Means I.U/ml	Std.Dv I.U/ml
days-5	group-I group-II	30 30	2.221 2.312	.493 .476
days-10	group-I group-II	30 30	2.997 3.391	.486 .567
days-15	group-I group-II	30 30	2.714 3.033	.547 .697
days-20	group-I group-II	30 30	2.696 2.894	.488 .597
days-25	group-I group-II	30 30	2.726 3.012	.511 .767
days-30	group-I group-II	30 30	2.459 2.945	.661 .595
days-35	group-I group-II	30 30	2.386 2.712	.441 424
days-40	group-I group-II	30 30	2.563 2.601	.457 501
days-45	group-I group-II	30 30	2.528 2.423	.444 .353
days-50	group-I group-II	30 30	2.623 2.457	.511 .363
days-55	group-1 group-II	30 30	2.420 2.399	.413 .367
days-60	group-I group-II	30 30	2.012 1.981	.522 .432



Diag.2: Diagram demonstrating serum APF levels through the periods of experiment in animals of group I and Group II.

- **Histological study**

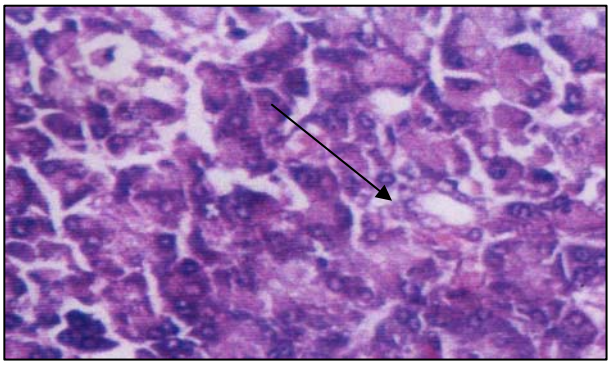


Fig.-1: Section demonstrates normal pancreatic tissue, showing serous acini, intralobular, interlobular pancreatic ducts. H and E stain, X40.

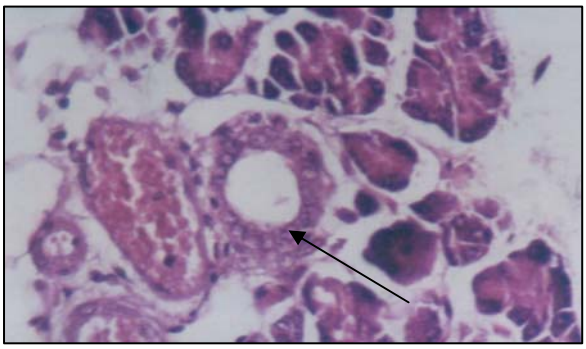


Fig..2: Section in pancreatic tissue after two weeks, demonstrating cluster of few small cells attached to pancreatic duct H and E stain, X40.

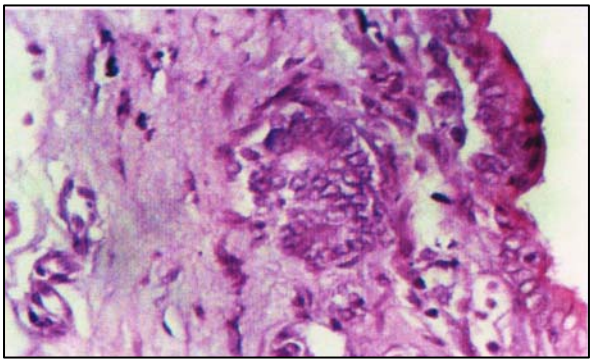


Fig..3: Section in pancreatic tissue after three weeks, cluster of many small cells (pancreatic stem cells) attached to epithelial cells of pancreatic ducts. H and E stain, X40..

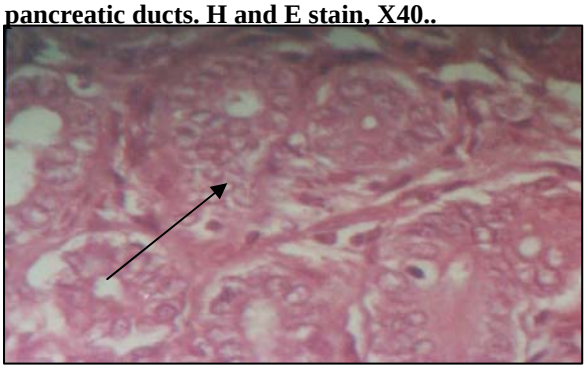


Fig.4: Section in pancreatic tissue after four weeks, cluster of many small cells (pancreatic stem cells) attached to epithelial cells of pancreatic ducts. H and stain, X40..

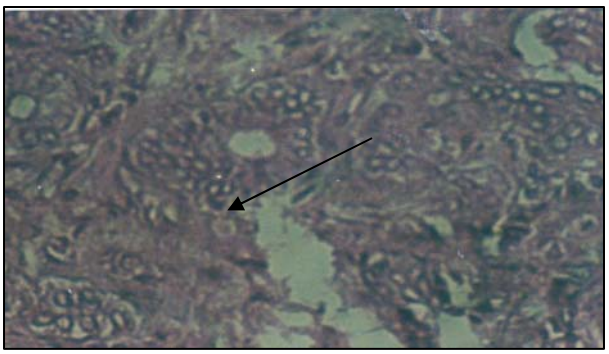


Fig.5: Section in pancreatic tissue after five weeks, demonstrating collection of pancreatic stem cells origenating from epithelium of pancreatic duct. H and E stain, X40.

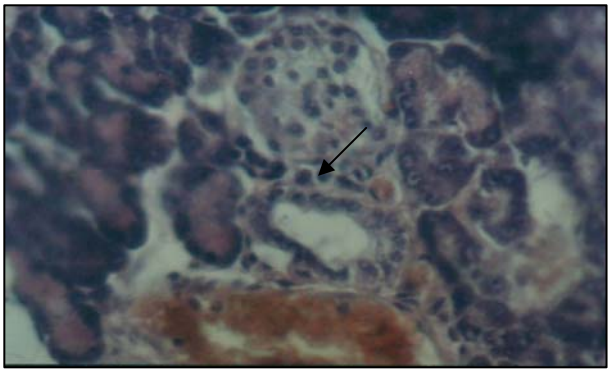


Fig.6: Section in pancreatic tissue after eight weeks, demonstrating complete neogenesis of islet like cells attached to pancreatic duct. H and E stain, X100.

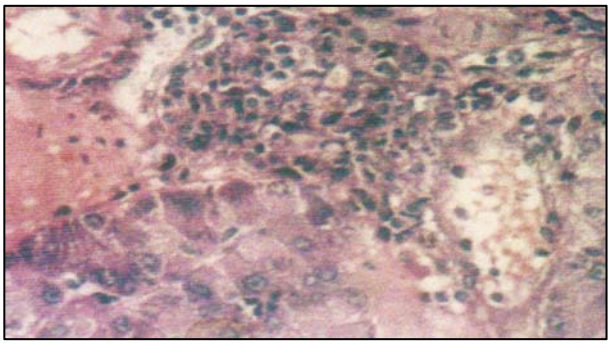


Fig.7: Section in pancreatic tissue after eight weeks, demonstrating complete neogenesis of islet like cells between two pancreatic ducts. H & E stain. X100.

Discussion:

Many recent studies, not proved the presence of pancreatic stem cells, and stated that the pancreas has no power of regeneration in adult during the progression to diabetes or injury(12) . However, a substantial amount of evidence has accumulated over the last few years supporting the hypothesis that islet neogenesis in mature pancreas may occur via cells which are located in or associated with the pancreatic epithelium(13,14) . However, in our current experiment , we observed collection of small round cells (stem cells) near the pancreatic ducts and these may originated from epithelium of pancreatic ducts and later on formation of islet like cells attached to pancreatic duct and was seen at the end of experiment .There was mild decline in the level of blood glucose in the animals of group II after the period 25 days but have not returned to the normoglycemic status and remained in the hyperglycemic status, this may be due to the fact that remained pancreatic tissue is very small and pancreatic islet neogenesis is not sufficient to control hyperglycemia . Furthermore, subtotal pancreatectomy (90%) may lead to sever damage (fibrosis) of the pancreatic remnant which in turn limit neogenesis . The effect of the above events on the serum AFP level is statistically not significant. However, there was a small visual rise in the AFP

level which may indicate stem cell neogenesis . Our experiment is supporting the hypothesis that pancreatic stem cells may originate from epithelial cells of pancreatic duct.

Conclusion:

Pancreas has the potential of regeneration of pancreatic islet cells when exposes to disease or injury through pancreatic stem cells which originating from epithelium of pancreatic duct .In near future we may manage diabetes mellitus especially type 1 by substitution the loss beta-cell mass.

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