Regeneration , Proliferation and Trans-differentiation of Adult Hepatic Oval Stem Cells into Functioning Beta-Cells and Exocrine Acinar Cells of the Pancreas in Diabetic Adult Rats

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Summary:

Back ground: Although adult stem cells possess plasticity that permit differentiation along new lineages, production of exocrine and endocrine pancreatic cells and insulin-secreting beta-cells from adult non pancreatic stem cells has been considered controversial. We present that highly purified adult rat hepatic oval stem cells, which are capable of differentiation to hepatocytes and bile ducts epithelium, can also trans-differentiate into pancreatic exocrine-endocrine tissue, when homogenized hepatic tissue is implanted into subcutaneous tissue .

Methods and Materials: A total of 60 adult Swiss albino rats were divided into two groups . Group I , control group (30 animals) was injected with normal saline into the subcutaneous and pancreatic tissue, group II (30 animals) has been exposed to 90% subtotal pancreatectomy with implantation of hepatic tissue homogenate into marked areas of subcutaneous tissue .

Results: During the first three weeks the animals of group II showed both high blood glucose and serum AFP levels, then gradually started to decline and became normal around the period of six weeks and continued being normal till the end of experiment. The histological study showed regeneration and proliferation of small hepatic oval stem cells in the subcutaneous tissue around the second week and trans-differentiation of these cells into islet like cells and acinar like cells around the sixth week.

Conclusions: Hepatic oval stem cell has multipotent character and can differentiate into new-lineages and formation of different clonal cells, such as islet-like cells and acinar cells of the pancreas.

Key words: Hepatic oval stem cells, Diabetes mellitus, Blood glucose, Alpha-fetoprotein (AFP), Hepatic homogenate, Islet cells of Langerhans.

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Introduction:

A true cure for diabetes mellitus relies on replacement of beta cell mass. Currently, beta cell replacement is accomplished either by ectopancreas transplantation or islet cells implantation. However, these procedures requirelong- term immunosuppression(1,2). .

Over the past few years, several studies have reported the production of endocrine cells, endocrine tissue and / or cluster islet-like cells from embryonic stem cells, adult stem cells isolated from the pancreas, as well as adult stem cells from non pancreatic origins (3,4,5,6) . Although none of these products are considered perfect yet, major advances have been made in understanding the regulation growth and differentiation of beta cells and islet of Langerhans (7,8). This may raise hope for the patients in the near future . However, much attention has focused on the apparent plasticity of adult stem cells, especially the capability of such cells to trans-differentiate into cells of other organs when placed in the environment of a different organ (9,10). Stem cells represent a promising solution to treat degenerative disease (11, 12, 13). Yet, results from a number of laboratories indicate that, there is a lack of some basic understanding to control the process fully (14).In the present study, we have asked whether hepatic oval stem cells originally from transplanted hepatic homogenate can differentiate into endocrine or exocrine cells of pancreas.

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Materials and Methods:

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

Group I , normal control group (30 animals) . The animals of this group were subjected to laparotomy and injected with multiple injections of normal saline into various sites of the pancreas and marked areas in the subcutaneous tissue.

Group II, 90% subtotal pancreatectomy + hepatic tissue homogenate implantation (30 animals) .The animals of this group were subjected to 90% subtotal pancreatectomy. This was followed after that by partial hepatectomy Pieces of hepatic tissue were introduced into 2ml of ringer lactate solution then homogenized. The hepatic tissue homogenate was then implanted into the subcutaneous tissue of the same standard marked areas as group I. All the animals of group I and group II were subjected to the same 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 hematoxylin and eosin stain.

Results:

Biochemical studies.

a. Blood glucose level.

Table-1: Demonstrates the means and standard deviations of the blood glucose in animals in group I and group II.

Periods	Groups	No.	Means Mg/dl	Std.Dv Mg/dl
Days-5	Group-I	30	87.897	8.818
	Group-II	30	287.87	25.225
Days-10	Group-I	30	89.112	9.638
	Group-II	30	291.365	30.808
Days-15	Group-I	30	86.787	9.703
	Group-II	30	301.49	20.407
Days-20	Group-I	30	88.135	9.485
	Group-II	30	291.590	18.951
Days-25	Group-I	30	85.442	9.711
	Group-II	30	240.787	15.152
Days-30	Group-I	30	89.399	7.706
	Group-II	30	184.106	16.080
Days-35	Group-I	30	87.919	9.196
	Group-II	30	170.37	15.895
Days-40	Group-I	30	90.104	9.690
	Group-II	30	112.00	10.439
Days-45	Group-I	30	87.038	11.00
	Group-II	30	102.39	12.991
Days-50	Group-I	30	90.160	8.752
	Group-II	30	88.797	10.421
Days-55	Group-I	30	86.401	8.678
	Group-II	30	87.965	9.333
Days-60	Group-I	30	90.932	8.245
	Group-II	30	86.718	7.911

B. Serum AFP level.

Table-2: Demonstrates the means and standard deviations of serum AFP in animals in group I and group II.

Periods	Groups	No.	Means I.U/ml	Std.Dv I.U/ml
Days-5	Group-I	30	2.221	.493
	Group-II	30	2.370	.417
Days-10	Group-I	30	2.989	.429
	Group-II	30	10.975	2.113
Days-15	Group-I	30	2.744	.547
	Group-II	30	11.544	2.474
Days-20	Group-I	30	2.696	.523
	Group-II	30	11.638	2.321
Days-25	Group-I	30	2.727	.510
	Group-II	30	7.176	.870
Days-30	Group-I	30	2.459	.489
	Group-II	30	6.904	.523
Days-35	Group-I	30	2.181	.411
	Group-II	30	4.987	.566
Days-40	Group-I	30	2.026	.377
	Group-II	30	3.833	.532
Days-45	Group-I	30	2.110	.477
	Group-II	30	2.193	.478
Days-50	Group-I	30	2.324	.457
	Group-II	30	2.297	.495
Days-55	Group-I	30	2.271	.443
	Group-II	30	2.386	.457
Days-60	Group-I	30	2.452.	.441
	Group-II	30	2.324	.468

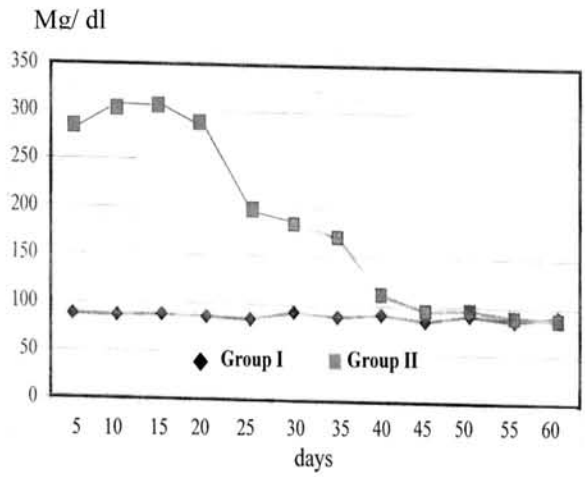

Diagram1: Diagram demonstrating the blood glucose levels in animals of group I and group II through the periods of experiment.

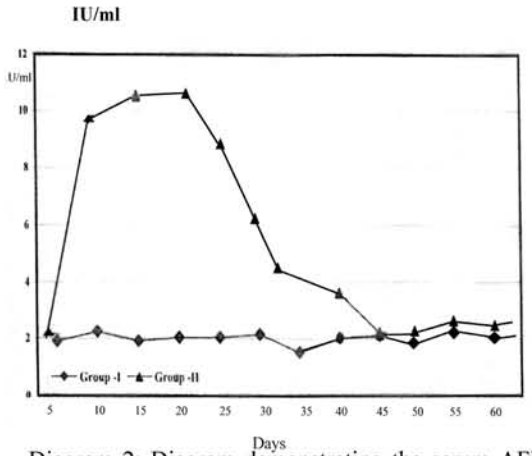

Diagram 2: Diagram demonstrating the serum AFB in animals of group I and group II through the periods of experiment. Histological study.

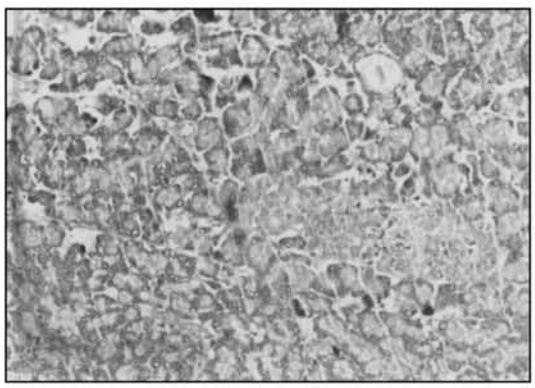

Fig.1: Section demonstrates normal pancreatic tissue, showing serous acini ,intralobbular, interlobular pancreatic ducts and characteristic pancreatic islet. H and E stain, X40.

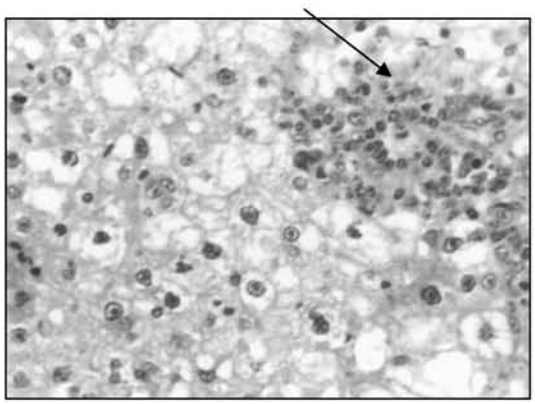

Fig.2: Subcutaneous tissue section 10 days from start of experiment, demonstrating early proliferation of small oval hepatic stem cells (arrow). H and E stain, X100

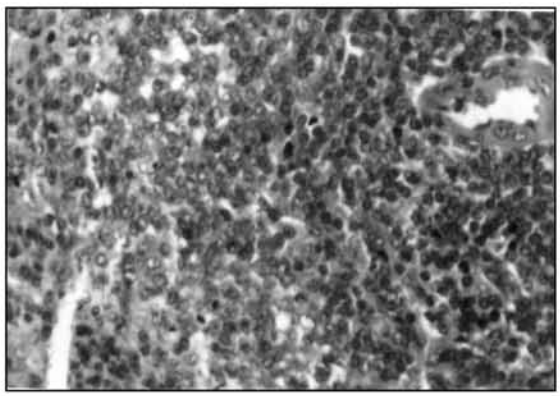

Fig.3: Subcutaneous tissue section around three weeks, demonstrating proliferation of a large number of small oval hepatic stem cells. H and E stain, X40 .

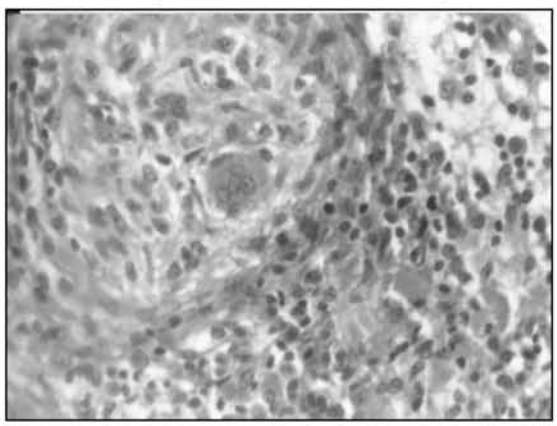

Fig.4: Subcutaneous tissue section around four weeks, demonstrating cluster of hepatic oval stem cells in a round configuration with the formation of ill defined collagenous fibrous capsule. H and E stain, X40..

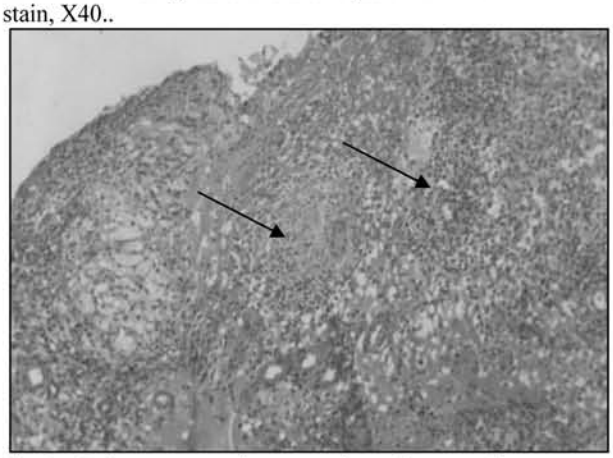

Fig.5: Subcutaneous tissue section five weeks, demonstrating different stages of islets like cells neogenesis. Gomori's stain, X40.

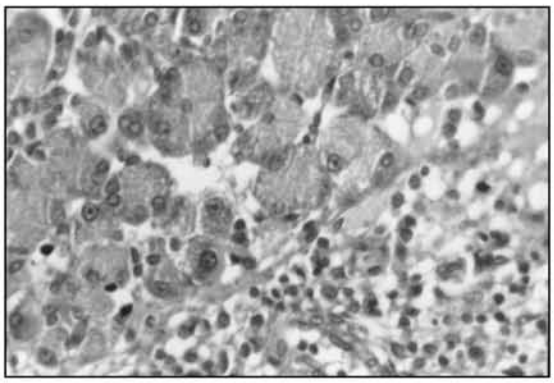

Fig.6: Subcutaneous tissue section six weeks, demonstrating formation of a collection of acinar like cells. H and E stain, X100.

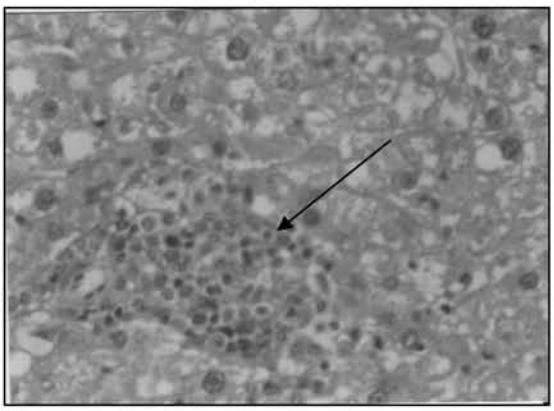

Fig.7: Section in the subcutaneous tissue around seven weeks, demonstrating complete formation of islet like cells. H and E stain, X100.

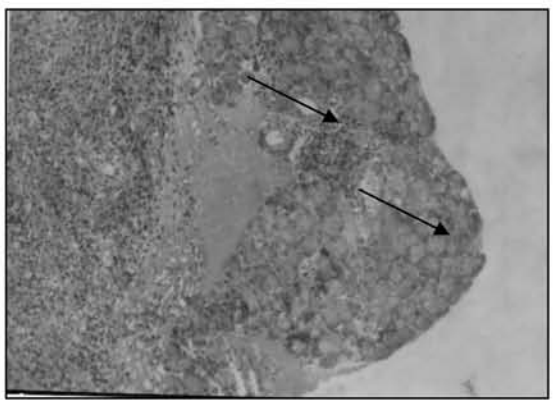

Fig.8: Subcutaneous tissue section eight weeks, demonstrating complete neogenesis of a lobule of acinar like cells with an islet like cells inside lobule. H and E stain, X40.

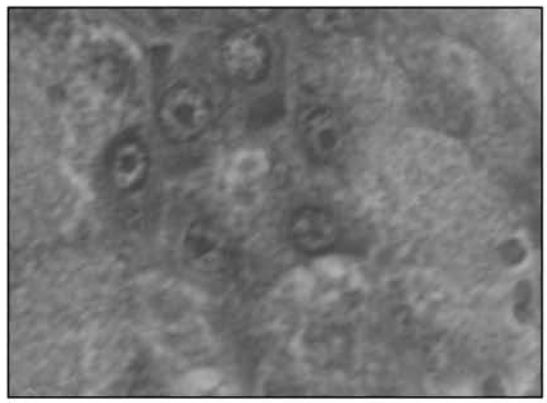

Fig.9: Section through an islet like cells after eight weeks, demonstrating polygonal and round cells that contain small granules of different shapes inside cytoplasm with central or eccentric nuclei. Gomori's stain, X100.

Discussion:

Much evidence has shown the existence of quiescent tissue specific stem cells in the liver that do not normally self-renew or regenerate (15). Stem cells within the liver remain inactive during post-natal life and do not normally respond to injury (16). Many problems emerge in the isolation of hepatic stem cells, that they are rare and difficult to identify their presence in the hepatic tissue, and if they are present only in small number. They may also be hard or even impossible to culture (17, 18).

Total suppression of hepatocytes is mandatory for the proliferation of hepatic oval stem cells (15) .

Tissue environment has an important role in the proliferation of adult stem cells (19). In the liver, there might be suppressive factors that prevent proliferation and differentiation of hepatic oval stem cells. Hepatocytes suppression thus may lead to the inhibition of effects of the suppressive factors on hepatic oval stem cells and the stimulation of quiescent stem cells to start proliferation (20).

An alternative theory stated that stem cells remain undifferentiated when present in their particular niche (remain dormant), stem cells differentiate only when they leave that niche or when there are no longer suppressive signals (21). However, in our current study, we have implanted the hepatic homogenate into subcutaneous tissue to induce total hepatocytes suppression locally. Furthermore, to remove suppressive factors and to provide new environment which contain induction factors helping in proliferation of hepatic oval stem cells.

All types of stem cells (embryonic and adult stem cells) have the characteristic expression of AFP gene and the secretion of this primitive protein (22). AFP is considered as a marker of stem cell presence (23).

The fibroblast cell in mesenchymal and subcutaneous tissue secrets different types of growth

factors (epidermal growth factor , fibroblast growth factor-2 , hepatocyte growth factor) , cytokines and other biomolecules . These biomolecules play an important role in repairing injured tissue,the stimulation of stem cells proliferation, trans-differentiation and tissue organ formation during the embryonic period (24). These biomolecules in the subcutaneous tissue may have mitogenic effect.

It was found that fibroblast present in mesenchymal tissue of the embryo triggers the expression of pancreatoduodenal homeobox gene. This gene may have a role in the formation of pancreas (25,26).

In our experiment hepatic stem cells trans-differentiated into endocrine and exocrine pancreatic tissue. This event may be due to the same phenomena which occur in the formation of pancreatic tissue during embryogenesis. The glucose molecule is one of the biomolecules and may have a role in trans-differentiation of hepatic stem cells into functioning beta cell.

Conclusion :

Hepatic stem cell has multipotent character and can differentiate into new-lineages cells .We are still in the beginning of a long way, and our applications for the study of stem cell depended on animals and not human being .

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