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Ashraf Seida
Urology and Nephrology, Mansoura University, Mansoura, Egypt

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IMPACT OF PHARMACOLOGICAL AGENTS ON INSULIN SECRETION CELLS TRANSPLANTED IN DIABETIC RATS

ASHRAF M. SEIDA

Urology and Nephrology, Mansoura University, Mansoura, Egypt

ABSTRACT

Cyclosporin A is an immunosuppressive agent that improves survival of transplant. This exciting immunosuppressive agent was first used clinically in renal transplantation **(Ferguson and Fidlus 1982).***The aim of this study was the isolation , purification and transplantation of hamster pancreatic islet as xenograft transplantation for the treatment of diabetic rats. It is also aimed to study the effect of cyclosporin A as an immunosuppressive agent on some biochemical parameters e.g blood glucose and blood insulin levels to induce maximum suppression of mice immune system and consequently realizing maximum survival of transplanted islets. A total of 30 streptozotocin induced rat were randomized to receive islets xenografts from golden syrian hamsters by three different approaches ,the first group of ten rats received islets only in the renal subcapsular space, the 2nd of ten rats received islets in the renal subcapsular space and was given cyclosporin A in a dose of 30 mg/kg/day for three days only.The 3rd group of ten rats were received islets in the renal subcapsular space and were given cyclosporin A orally every day at a dose of 30 mg/kg/day for three days which was gradually decreased to 10 mg/kg/day. Non of rats of group 1 became normoglycemic. All rats of group 2 became normoglycemic for 228± (range 12-36 days) . Only rats of group 3 enjoyed normoglycemic as long as cyclosporin-A was administrated. Consequantly, prolonged survival of islets xenografts may be achieved with administration of cyclosporin-A* **(Springer,*et al.*, 2015).**

INTRODUCTION

Transplantation of normal isolated islets of langerhans for the treatment of diabetes remains an elusive goal in clinical practice. Perhaps the major problem in preventing clinical islet transplantation has been limited by the inability to prevent islet allograft or xenograft rejection. Various approaches and different sites have been suggested to prevent islet graft rejection and maintain long-term islet cell function **.** Immunosuppression by pharmacological agents such as cyclophosphamide, azathioprine and corticosteroids was of minor effectiveness. However, cyclosporin A (CyA) is a an immunosuppressive agent that improves survival of transplant**(Springer,*et al.*, 2014)..**

The structure cyclosporin was established by chemical degradation together with an X-ray crystallographic cyclic peptide composed of eleven amino acids residues, all having the L-configuration of the natural amino acids except for the D-alanine (D-Ala)in position 8 and the non chiral sarcosine **fig (1).**This exciting immunosuppressive agent was first used clinically in renal transplantation in 1978 **(Ferguson and Fidlus 1982).**

Our study was conducted to evaluate the role of cyclosporin-A on the biochemical parameters such as blood glucose, Cylosporin A level in a trial to determine the minimum level of cyclosporin which cause maximum suppression of immune system and minimal nephrotoxicity to attain maximum survival of transplanted islets **(Jain, *et al*.,2007)**

MATERIALS AND METHODS:-

Induction of Diabetes:

Golden Syrian hamsters, weighing 100-120 g were used as islet donors. Spraque dewaly rats were the recipients. Non fasting plasma glucose levels of the recipient rats were determined before the induction of diabetes. Blood sugar level was monitored via orbital sinus blood samples with Aqua trend sensor. The rats were made diabetic by a single intraperitoneal injection of streptozotocin (150 mg/ Kg body weight) and only rats those with serum glucose levels more than 350 mg/ dl were used for transplantation.

Insulin cells Isolation:

Hamster islet was isolated according to the method previously described **Avila *et al.*,2003** by intraduct injection of collagenase solution

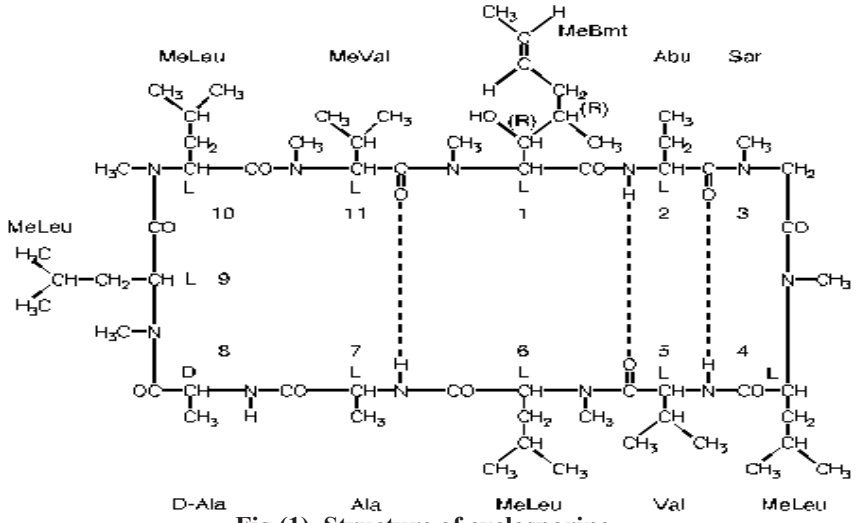

Fig (1). Structure of cyclosporine

followed by digestion and extensive purification on Ficoll gradients. Hand-picked islets were cultured in CMRL-1066 medium containing 10% heat inactivated fetal calf serum (FCS) and antibiotic-antimycotic solution (1 ml/ 100 ml). The islets were then incubated at 37 °C for 3 days in a humidified atmosphere of 5% CO_2. Islets were stained for viability by diphenylthiocarbazone (DTZ). **Kanti, *et al*.,2007**

In Vitro Insulin Secretion:

Insulin secretion in response to glucose was tested according to the method discribed by **Gotoh *et al*, 1987**. Freshly isolated or 24- hour cultured islets were incubated for 30 minutes at 37 °C in a modified Krebs Ringer bicarbonate buffer containing 10 mM HBSS , 0.5 mM NaHCO3, 1mM CaCL2 and 05% bovine serum albumin{KRB-HEPES,OPH 7.4} The basal KRB - HEPES used for incubation also contained low (2.8 mM) glucose or high {16.7 mM} glucose content . After incubation, the supernatant was immediately removed and stored separately at -20°C until assayed for insulin content.

Transplantation of Insulin cells:

At transplantation, Islets were maintained for 3-4 days and checked for viability by diphenyl thiocarbazone (DTZ) staining . Streptozotocin - induced diabetic rats were used as recipients with at least two consuctive blood glucose analysis of 350 mg/dl A total of 30 rats were divided into 3 different treatment groups. A small incision was made in the left lumber region to expose the left kidney. Countered islet were suspended in 0.3 ml CMRL-1066 medium and implanted underneath the renal capsule using 27 gouge needle without making an incision into the capsule. Each diabetic mice received a total of 10 islet per gram of body weight

Cyclosporin-A treatment:-

roup 2,3 were transplanted with islets and treated with Cyclosporin-A whichwas adminsterated orally using stomach canula every day from the day of transplantation. The first dose was 30 mg/kg/day for three days (group 2), and 30 mg/kg/day for three days gradually decreased to 10 mg/kg/day for one month (group3).

Post-transplantion Follow-up:

After transplantation, the rats were transferred to metabolic cages for daily examination. Non-fasting blood glucose levels of the recipients were monitored 3 times weekly for the first 3 weeks, then twice weekly thereafter. The rats were considered cured if exhibited the following criteria: a random plasma glucose less than 200 mg/ dl, a glucosuria with steady weight gain. Rejection was considered when blood sugar concentration exceeded 200 mg /dl on two consecutive bleedings.

Determination of Urine Glucose:

The urine glucose content of rats was measured by glucose stripes (Boehringer Manheim's), when the rat was caught from the tail and transferred from the cage to a clean bench, it urinates at once ; the glucose stripe put up side down on the urine drops for 2 seconds. The stripe

was left for 30 seconds, then was read according to the manufacture instruction. The urine glucose content ranged from +1 to +4.

Determination of Blood Glucose :

The rat was anesthetized with halothan inhalation, blood was collected from the orbital sinus by using fine Pasteur pipette.The blood was then transferred to a centrifuge tube and left for 10 minutes.The tubes was centrifuged for 10 minutes at 4000 r.p.m. and the serum was separated. Blood glucose was measured with Beckman Glucose Analyzer 2.The method employes the enzymatic reaction of B-D-Glucose with oxygen followed by measuring oxygen consumption rather than hydrogen peroxide formation.

B-D-Glucose + O_2 --------à Gluconic acid +H_2O_2

The rate of oxygen depletion was shown to be directly propotional to the concentration of glucose present in the sample.In the reaction Oxygen is cosumed in the same rate as glucose reacts to form gluconic acid . The analyzer readout concentration in mg/dl (Kadish, *et al*.1968)

Determination of Cyclosporin A level:-

Cyclosporin A (CyA) was determined post-transplantation and after 1 week and 14 days according to the method of (**Peso *et al* , 1990**) as follow:

Whole blood sample (150 mL) , 50 mL solublizing reagent and 300 mL precipitating reagent (Supplemented in the CyA kit) were mixed together , vortex for 10 seconds and centrifuged for 5 minutes at 10900 rpm. The supernatant was then analyzed on the TDx analyzer using CyA monoclonal whole blood kit . The Cyclosporin A level was obtained in ng/ml .The constituents of the solublizing reagent are surfactant in water with 0.1% Sodium Azide as preservative . The constituents of the precipitating reagent are zinc sulphate solution in methanol and ethylene glycol.

Radioimmunoassay of Insulin:

The precise measurement of serum insulin by radioimmunoassay (RIA) was an improvement in endocrine chemistry and much knowledge about diabetes has been derived from clinical research. The insulin radioimmunoassay can be performed by the following method :

a-The serum sample, first antibody (guinea pig anti- insulin serum) and tracer (Bovine serum albumin borate buffer) are combined and incubated for 16-20 hours at 2-8°C. the second antibody (normal guinea pig serum, pre-precipitated with goat anti- guinea pig serum and polyethylene glycole (PEG) is then added to separate bound from free insulin.

b-The assay can be centrifuged and decanted after 15-25 minutes incubation at 20-25 C and quality control must be performed using the same method.

c- An optional PEG precipitational step is induced so that endogenous insulin antibodies and other interfering substances may be removed from serum samples perior to measurement of insulin using the RIA kits contain radioactive materials (Iodine 125) and instructions for calculating and quantitative determination of insulin in serum by Gamma system (Kontron)**.**

RESULT:-

The mean number of islets obtained from one pancreas using 0.7 mg/ml collagenase type XI was 414±48 islets**.** The islets were spherical or oval, some of them had exocrine tissue attached to their surface. Morphologically the cultured islets were characterized by golden brown with a distinct outer intact membrane **Fig (2)**.

The insulin release from the islet gradually increased during 24 hours of culture, and the insulin content of cultured islets was relatively lower than that of freshly isolated **fig (3**). This in

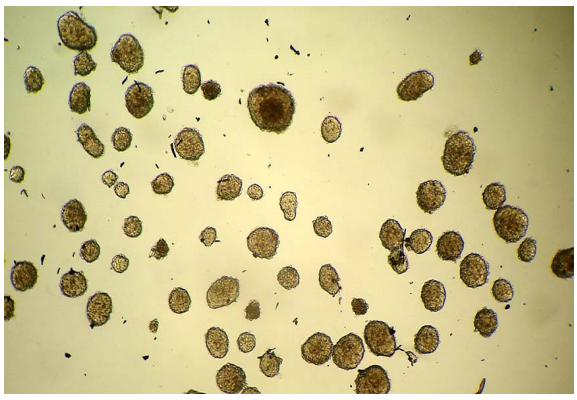

Fig (2) Light photograph showing hamster islet stained with DTZ. Islets appear healthy with viable insulin cells

agreement with some previous reports by other investigators .which showed that fresh islets isolated by conventional agitation with collagenase solution responded to a glucose stimulus immediately after isolation, and that responsiveness of the islets was better than that of the cultured islets **Gotoh, *et al.*, 1987**.

The result of islet transplantation are summarized in table 1. None of rats transplanted with islet only under the kidney capsule (Group 1) without administration of cyclosporin-A became normoglycemic. Rats receiving islets only in the renal subcapsular space and treated with cyclosporin-A 30 mg/kg/day for three days (Group 2) became normoglycemic for a mean of 22 ±8. on the other hand mice receiving islets only in the renal subcapsular space and treated with cyclosporin-A 30 mg/kg/day for three days. gradually decreased to 10 mg/kg/day for one month (group 3) became normoglycemic for a mean of 61 ± 2 and exhibit normoglycemia as long as cyclosporin-A was administrated. **Table1**. The blood insulin started to increase after 2 days of transplantation **Fig (4)**.

Glucose Tolerance Test:

Glucose tolerance tests were carried out in transplanted rats that had been maintained normoglycemic state for more than 60 days**.** Five rats from each group were infused with 1 g /kg glucose after 30 days of transplantation. The rats were able to normalize their blood glucose. In normal rats, Plasma glucose peaked at 30 minutes. And returned to base line levels by 120 minutes. Diabetic rats showed higher glucose levels after 120 minutes. Compared to the normal with transplanted rats there was no significant difference in blood glucose levels at 0,30,60,90 and 120 minutes, The blood glucose decreased to normal level after 2 days of transplantation **Fig (5)**. The body weight increased after 5 days of transplantation **Fig (6)**..

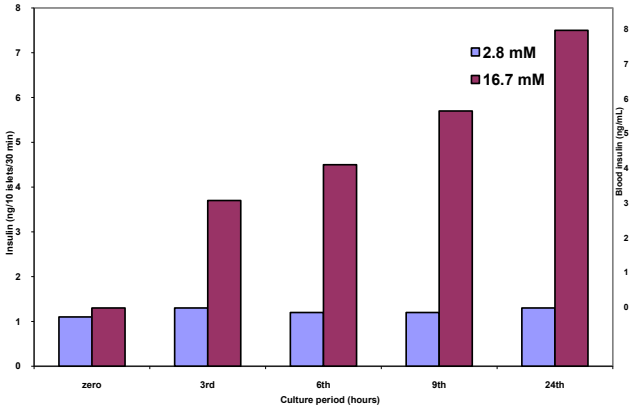

Fig (3) Insulin release from fresh and cultured islets in presence of 2.8 mM (low) and 16.7 mM (high) glucose concentration.

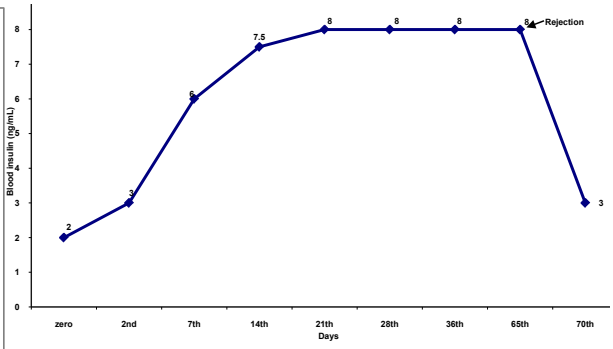

Fig (4) The relation between the mean blood insulin and duration of survival following transplantation of cured rat of group3. the blood insulin started to increase after 2 days of transplantation.

Table1. Results of islet transplanted in the renal subcapsular space with cyclosporine –A administration

No	Treatment	Duration of normoglycemia	Mean graft survival
1	----------	----------	---------
2	Cy-A	12,18,14,22,16,32,18,32,24,36	22± 8
3	Cy-A	52,64,54,,72,72,48,58,64,58,52	61± 2

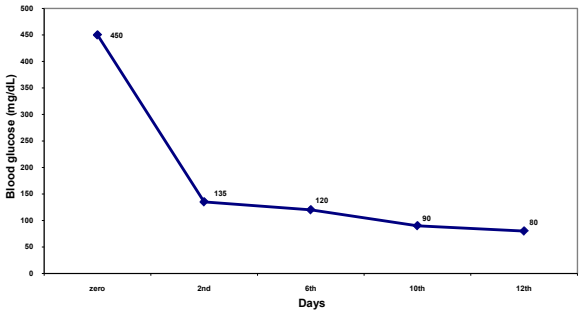

Fig (5) The relation between the mean blood glucose level and duration of survival following transplantation of cured rat of group3. The blood glucose decreased to normal level after 2 days of transplantation

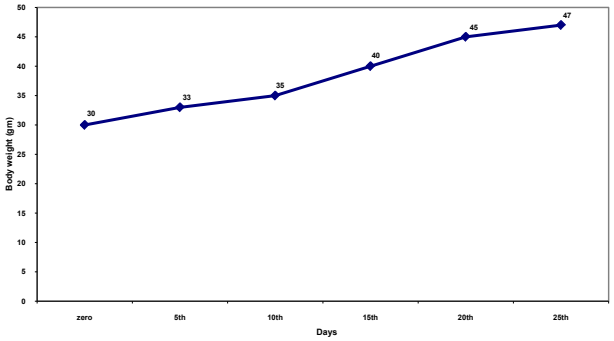

Fig (6) The relation between the mean body weight and duration of survival following transplantation of cured rat of group3. The body weight increased after 5 days of transplantation

DISCUSSION

All streptozotocin injected rats exhibits diabetic criteria including hyperglycemia , polyurea and progressive weight loss. Diabetes induction in this study is shown to agree with the results of **Harlan , *et al*., (1995) and Leow, *et al*., (1995**). The data indicated that rats transplanted with islets under the kidney capsule and treated cyclosporine-A enjoy normoglycemia for a maximum twenty two days , Moreover, a daily administration of cyclosporine-A causes the graft survival to be prolonged and enjoy normoglycemia as long as cyclosporin-A is given.It is clear that the introduction of cyclosporin A (CyA) has resulted in significant improvement in transplant results and has external transplantation a routine procedures.The survival rate of islet transplantation xenografts was significantly improved under cyclosporin A immunosuppression, primarily due to the ability of this agent to prevent rejection of histocompatible grafts (**Opelz, 1992).**

It was suggested that CyA may either directly inhibits the function of unclear protiens critical to the T - lymphocytes activation (**Ream, 1992**) or may prevents the transduction of the mitogenic signal resulting from receptor ligation, to the nucleus at a stage after the rise in intracellular calcium levels **(Angela, 1990 and Riesbeck *et al*., 1994).**CyA may also function as an immune inhibitor by imparing the ability of activated helper T - cells to respond to IL - 2, probably by limiting interleukin -2- receptor (IL-2R) expression in which IL-2 is an essential cofactor in the activation of both cytotoxic T - lymphocytes and B - cells in acute rejection episodes (**Winkelstein, 1994).**

While cyclosporin A has remarkable immunosuppressive properties, it also produces acute nephrotoxicity which may be purely functional or both functional and morphological. This kidney toxicity represents a serious limitation to its use as an immunosuppressive agent, but the benefits of CyA clearly outweight the adverse effects of kidney **(First, 1993 and Cardoso et al., 1996).** It may concluded that , it is possible to create an immunoprivileged site under the kidney capsule in xenograft transplantation of pancreatic islet cells by administration of cyclosporine-A**(Springer,*et al.*, 2015).**

REFERENCES

Angela G.P.(1990). Lymphokine gene expression in vivo is inhibited by cyclosporin A. J.Exp. Med., 171:533-544.

Avilla,J.; Tsujumura,T.; Oberhalzer,J.; Churctell,T.; Salihi,P.; Janes,A.M.; Lakey,J.R.T. (2003): Improvement of pancreatic islet isolation outcomes using Glutamine perfusion during isolation procedures. Cell Transplant., 12: 1230

Cardoso.L.R.,Santos, O.F.P., Biom, M.A., Barros, E.G., Ajzen,H. and Schor.N.(1996). Fructose -1,6diphosphate: potential protection in cyclosporine induced renal impairment. Nephron,72: 67-71.

Ferguson, R. M. and Fidelus, G. R. (1982). Immunosuppressive Action of Cyclosporin in Man. Transplantation Proceeding. 15 : 2485 – 2489

Getoh, M.; Maki, T.; Salomi, S.; Porter, J.; Weir,S.D.; O*Hara,C.j. and Monacco,,A.P. (1987):

Reproducible high yield of rat islets by stationary in vitro digestion following ductal or portal veneous collagenase injection. Transplantation, 43: 725.

First,M.R.(1993).Renal allograft survival after 1 and 10 years: comparison between pre-cyclosporin and cyclosporin data. Nephrol.Dia. Transplant., 9: 90-97.

Harlan, D.M., Barnett,M.A., Abe,K., Pachold,K.,Patterson,N.B.and Gray,G.S.(1995). Very low dose streptozotocin induced diabetes in insulin prometer-mB7-1 transgenic mice Diabetes, 44:816-823.

Jain, K.; Yang, H.; Caj, B.; Haque, B.; Harvitz, A. Diehi, G.; Miyata, T.; Smith, B. H.; Stenzel, K.; Suthanthiran, M. and Rabin, A. L. (2007): Retrievable replaceable macrocapsulated pancreatic islet xenografts. Transplantation, 59 (3): 319.

Kanti,J.; Albert,R.; Barry,S. (2007): Preparation of agarose Coatd, solid agarose collagen beard containing Secretary cells. United state patent RE 39452

Leow,C.K., Gray,D.W.R.and Morris,P.J.(1995). The long term metabolic fun of intrap ortal and renal subcapsular islet isograft and the effect on glomerular basement membrane thickness in rats. Diabetologia.38:1014-1024.

Oples,G.(1992). Collorative study: 10 years report. Transplantation Proceedings, 24: 2342

Pesce, A.J.; Schroeder,T.J. and First, M.R. (1990): Evaluation of cyclosporin monitoring by nonselective fluorescence polarization immunoassay. Transplantation proceedings, 22: 1171-1174.

Ream,G.H.(1992). Molecular mode of actionof cyclosporine and FK-506in human thymocyte.J. Autoimmun.,5: 159.

Riesbeck, K.,Gullberg, M.and Forsgren,A.(1994). Evidence that the antibiotic ciprofloxacin counteracts cyclosporindependent suppression of cytokine production. Transplantation,57(2):267-272.

Springer,M..;kinzer,K.K.;Martellotto,J.;Barbaro,B.. (2014). Five years fellow up patient type 1 diabetes transplanted with allograft islets.Diabetes.

Springer,M..;Naser,A.;Vergani ,J.; Aanch,L.; Pin,E.; Kafaloyiani.(2015). Co-transplantationof autologus MSCsdelays islets llograft rejection and generate alocal immunoprivileged sites.ACTA.

Winkelsein, A.(1994). Immunosuppressive therapy. Cited from: Basic and clinical immunology,ed. by Slites,O.P., Terr,A.J.and Parslow,T.G., Large medical book, Ltd., USA. Pp.765-780