

Impress of Betatrophin in The Treatment of Mice with Diabetes type 1

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ABSTRACT

Betatrophin is a secreted protein of 198 aa that promotes β cell proliferation. It is expressed in the, white and brown adipose tissue of mice. In humans, betatrophin is expressed in the liver. Evaluation the potency of betatrophin therapy as alternative treatment instead of insulin injection in mice with type1 diabetes. This experimental study was based on 30 mice, the study was carried out at animal house, college of medicine-University of Baghdad. These rats were provided from the animal house of medicine, pharmacology Colleges -University of Baghdad. These mice divided into three group's first group are mice treated with insulin (10 mice), second group are mice treated with betatrophin (10 mice) and third group consider as a control group non-diabetic mice (10 mice). Plasma mice C-peptide concentration were measured using an enzyme-linked immunosorbent assay. Fasting blood glucose was measured by using colorimetric methods, these measurements was done in the Teaching laboratories of Baghdad Medicine Hospital. Level of fasting blood glucose show no significant differences among inducible type1 diabetic(T1DM) mice treated with insulin, inducible type1 diabetic(T1DM) mice treated with betatrophin and their control group (90.0 ± 10.8 , 75 ± 12.9 , 85 ± 17) mg/dl with $p=0.016$. That inducible type1 diabetic(T1DM) mice with betatrophin inj. treatment had higher significant mean of c-peptide (3.3 ± 2.2 ng/ml) than inducible type1 diabetic(T1DM) mice with insulin inj. Treatment (2.9 ± 0.8 ng/ml) but both groups had significant lower mean than control non-diabetic mice (5.3 ± 1.2 ng/ml). Data suggested that betatrophin is lower blood sugar and might be lower the dependence of diabetic patients on insulin injection treatment, but this study need for further work to say that betatrophin can replace the insulin treatment.

Keywords: Betatrophin, Mice, Diabetes type 1.

INTRODUCTION

Betatrophin (also called RIFL, Lipasin or Angiopoietinlike protein 8 (ANGPTL8)) is a new secreted protein of 198 aa that promotes β cell proliferation and improves glucose hemostats in mice and may also inhibit lipase activity and on serum triglyceride regulation^{1, 2}. Betatrophin gene is expressed in the liver and in white and brown adipose tissue of mice. In humans, it is expressed to be predominantly in the liver. Betatrophin levels are reduced by fasting and are elevated upon insulin resistance and during pregnancy. The receptor and

the mechanism of action of betatrophin is unknown, identification of this new protein as a hormone renewal β cell replication produce a new potential therapy for diabetes^{3, 4}.

The researchers^{5,6,7} here have taken an interesting step in identifying betatrophin, and it is exciting to think what might come of this over the next few years and how exactly betatrophin is working, and how influence its activity and the activity of the other proteins it effects to increase beta cell replication in humans who have a shrinking supply of beta cells.

MATERIAL AND METHODS

This prospective experimental study was based on 46 mice with 1.5-2.0 months-old, the study was carried out at animal house, College of Medicine-University of Baghdad. Medicine, Pharmacology Colleges -University of Baghdad were provide these mice. The animal protocols were approved by the Use and Care of Experimental Animals Committee of the Jichi Medical University Guide for Laboratory Animals⁸.

Study Design

Thirty six mice were induced type1 diabetes by subcutaneously injection of a single dose of alloxan 0.1mg/g, then the whole mice forty six mice were divide into three groups:

Group 1: Involve 12 DMI mice, after a day from diabetes inducible, they were treated with 0.75 IU insulin/g body weight. (prepare 0.25 IU insulin solution by diluting insulin in sterile saline (and sterile tubes); mix by overtaking.

Dispense the required volume of insulin solution for each mouse into separate 1.5 ml tubes (Volume is calculated as follows: 0.75 IU insulin/g BW). Vol (il) = 3 x BW). Two of them were dead.

Group 2: Involve 15 DMI mice, after a day from diabetes inducible, they were treated with 0.2 mg betatrophin/g body weight. Six of them were dead.

Group 3: Involve 9 DMI mice, consider as a pathological control for the above group.

Group 4: Involve 10 mice represented control non-diabetic mice.

All mice were no significant difference in their body weigh

METHODS

Serum glucose was measured by colorimetric methods, Plasma mice C-peptide concentration were measured using an enzyme-linked immunosorbent assay. Fasting blood glucose

Table1: Descriptive characteristics of mice sample (mean ± SD) before treatment

Parameters	Mice groups	No.	Mean	SD	Comparison of significant ANOVA, LSD (f-test)
F.S.G(mg/dl)	Inducible type1 diabetic(T1DM) mice before insulin treatment (A)	12	250	25.8	0.001HS A VS
BNS	Inducible type1 diabetic(T1DM) mice before betatrophin treatment (B)	15	253	26.1	A VS
	pathological control- Inducible type1 diabetic (C)		9		248
	Control non-diabetic mice group (D)	10	25.5	85	CNS
			11.5		VS D0.001 HS
S.c-peptide (ng/ml)	Inducible type1 diabetic(T1DM) mice before insulin treatment (A)	12	2.1	0.9	0.001HS A VS
	Inducible type1 diabetic(T1DM) mice before betatrophin treatment (B)		15		
	pathological control- Inducible type1 diabetic (C)	9	0.5	0.7	CNS
			2.2		B VS
	Control non-diabetic mice group (D)	10	0.7	5.3	CNS
			1.2		A,B,C VS D0.001 HS

was measured by using colorimetric methods, these measurements was done in the Teaching laboratories of Baghdad Medicine Hospital.

Subcutaneous injection of prepared Protamine zinc insulin (PZI; a product comprising 90% beef insulin and 10% pork insulin) and lyophilized recombinant betatrophin obtained from phoenix pharmaceuticals.inc

RESULTS

Table 1 shed light on the difference of serum fasting glucose and serum C-peptide among the four mice groups before treatment, which were selected at the age 1.5 -2 months -old.

The mean values of F.B.G. of the three inducible type1 diabetic(T1DM) mice groups didn't significantly differ (250 ± 25.8 mg/dl, 253 ± 26.1 mg/dl, 248 ± 25.5 mg/dl) but they were significantly higher than control –nondiabetic mice group group (85 ± 11.5 mg/dl).C-peptide of the two inducible type1 diabetic(T1DM) mice groups also didn't significantly

differ(2.1 ± 0.9 ng/ml, 1.1 ± 0.5 ng/ml),and had significantly lower level when compared with control mice group (5.3 ± 1.2 ng/ml).

In addition, a negative correlation was found between serum betatrophin levels and F.S.G in mice before treatment ($r = - 0.45$; $P < 0.01$).

In the whole mice before treatment, the circulating levels of serum betatrophin positively correlated with c-peptide ($r = 0.41$; $P < 0.01$).

Management of glucose level by insulin and betatrophin injection clarify by table2

The data in table 2 revealed that inducible type1 diabetic (T1DM) mice with betatrophin inj. treatment had highest significant mean of c-peptide (3.3 ± 2.2 ng/ml) among other mice group, inducible type1 diabetic(T1DM) mice with insulin inj. Treatment (2.0 ± 0.85 ng/ml), pathological control- Inducible type1 diabetic and Control non-diabetic mice group .but both groups had significant lower mean than control non-diabetic mice (5.3 ± 1.2 ng/ml).

Table 2: Mean distribution of F.S.G and c-peptide in mice groups that treated with insulin injection, betatrophin and their control

Parameters	Mice groups	No.	Mean SD	Comparison of significant ANOVA, LSD (f-test)
F.S.G(mg/dl)	Inducible type1 diabetic(T1DM) mice with insulin inj. treatment (A)	12	90.0 10.8	0.01 HS
	Inducible type1 diabetic(T1DM) mice with betatrophin inj. treatment (B)	15	75.0 12.9	
	pathological control- Inducible type1 diabetic (c)	9	250 25.9	A,B,D VS CHS
	Control non-diabetic mice group (D)	10	85.0 17.0	
S.c-peptide (ng/ml)	Inducible type1 diabetic(T1DM) mice with insulin inj. treatment (A)	12	2.3 0.85	0.001 HS
	Inducible type1 diabetic(T1DM) mice with betatrophin inj. treatment (B)	15	3.3 2.2	
	pathological control- Inducible type1 diabetic (c)	9	1.9 0.9	A VS B 0.01 S A VS C,D 0.001 HS B VS C,D 0.001 HS C VS D 0.001HS
	Control non-diabetic mice group (D)	10	5.3 1.2	

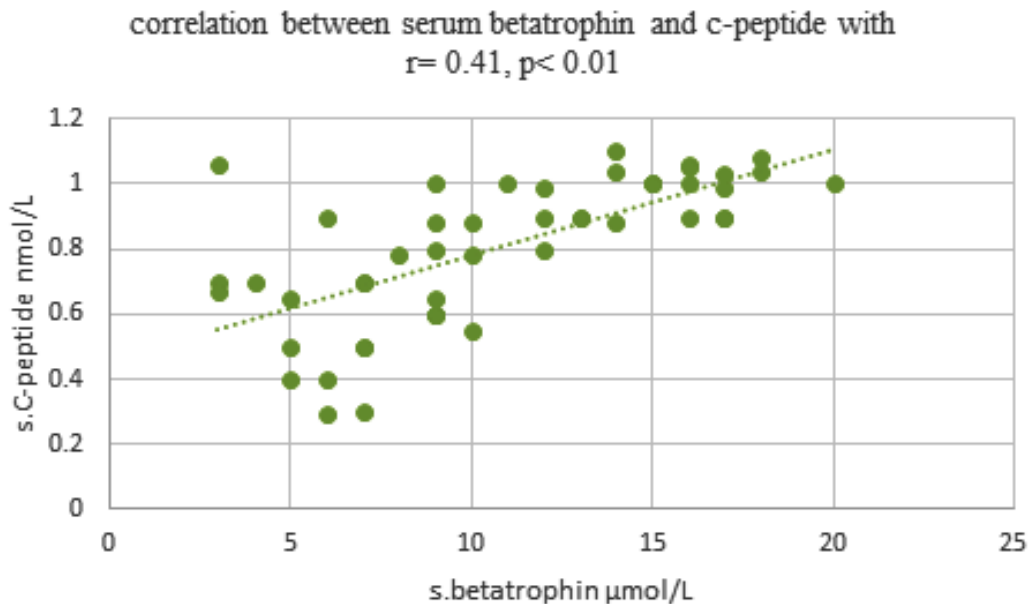
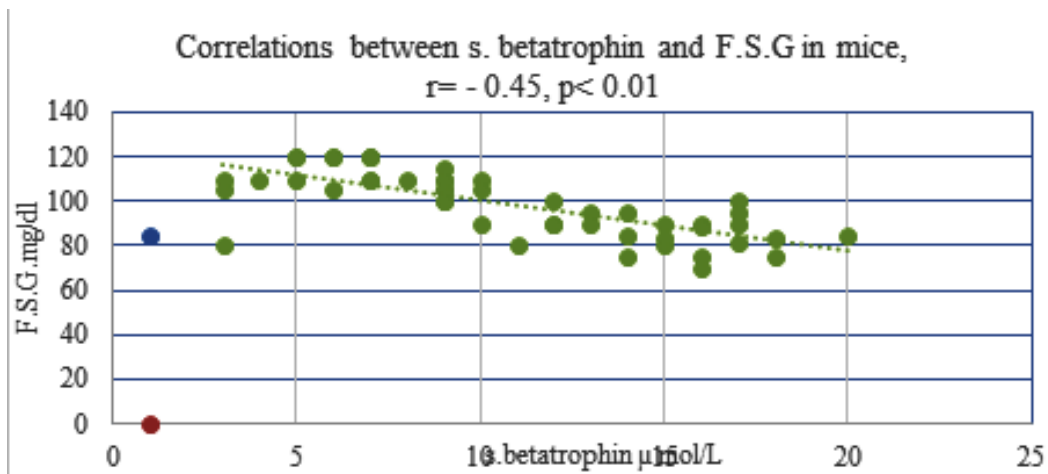
Fasting glucose level showed the highest significant in level pathological control- Inducible type1 diabetic among groups $p > 0.001$.

DISCUSSION

To develop an insulin-dependent diabetes mellitus mice (table 1, the mice became hyperglycemic) and because the selectivity of alloxan in killing the insulin-producing beta-cells, it is used to develop diabetes type 1 in laboratory animals (9,10) the possible mechanism is the uptake of the alloxan due to its structural similarity to glucose as well as

the beta-cell's highly efficient uptake mechanism (GLUT2). Over and above, alloxan has a high affinity to SH-containing cellular compounds which essential for insulin secretion and, as a result, decrease glutathione content¹¹. When a type 1 or type 2 diabetic starts to lose beta cells, the body cannot adequately refill them, and thus diabetics become hyperglycemic as their shrink stores of beta cells which become unable to produce enough insulin^{12,13,14}.

The results of F.B.G. in table 2 revealed to the significant improve of glucose level in inducible



type1 diabetic(T1DM) mice with betatrophin therapy at the same time it was found a notable increment in c-peptide of this group, the decline in the level of glucose in group that injected with betatrophin reflect the good management of the diabetes status. The possible explanation that the ability of Betatrophin in glucose hemostats management, related to its role in controlling the proliferation of the Pancreatic β Cells which are responsible for the secretion of Insulin, thereby making Betatrophin a bright hope for diabetes control^{14,15,16}. Study's findings support previous suggestions that a notable increment in c-peptide level of mice group treated with betatrophin while mice group treated with insulin keep the same level of c-peptide (table2).

Several studies^{17,18} establish there are limited supply of beta cells, there is much to be gained from having a rebuilding source of new beta cells. A number of advances have been made towards transplanting donor beta cells or generating beta cells from stem cells, but now scientists have found a hoping of new way to stimulate the body's own beta cells to replicate.

The researchers^{14,19,20,21} first saw whether the drug (betatrophin) was causing beta cells to expand directly, but they found the drug alone had no effect on beta cells. The researchers next looked at the levels of genes in the liver, fat tissue, and skeletal muscle of the mice. They found specific gene in livers and fat in very high rat in mice treated with drug but not in the untreated. This gene had previously been predicted and identified as Gm6484.

Further experiments showed that 8-week-old mice injected with betatrophin showed an average 17-fold rise in the replication of their β cells²².

All of mentioned studies were discussed effect of betatrophin in type 2 subjects but the current study create a hope treatment for diabetes type1.

CONCLUSION

Data suggested that betatrophin is lower blood sugar and might be hold a new treatment for the dependence of diabetic patients on insulin injection treatment, but this study need for further work to say that betatrophin can replace the insulin treatment.

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