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Effects Of The Sulfonylurea Glyburide On Catalase Activities in Streptozotocin-Induced Diabetic Rat Muscle

Glibenklamidin Streptozotosin İle Diabet Oluşturulan Rat Kaslarında Katalaz Aktivitesi Üzerine Etkisi

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ABSTRACT: Purpose; Free radicals, those are chemical species possessing an unpaired electron in their molecular or atomic orbit, have been the most attractive subjects in medicine because of their roles in destruction of cell or tissue. Many findings support the view that free radicals and oxidative stress play an important role in etiology of diabetes and its complications. Materials and methods; Hence, in the present investigation, we administrated glyburide to streptozotosin-induced diabetic rats and determined the affect of glyburide on muscle (M. gastrocinemius, M. soleus, M. quadriceps femoris) catalase activities. Rats (Sprague Dawley), weighing 150-200g were used in the present investigation. The experimental group was injected intraperitonally with streptozotosin (STZ, freshly dissolved in citrate buffer, pH 4.5 55 mg\kg) The proceeded data had been provided out from statisticaly (SPSS 10,0) based ware. Results; In the present study, muscle CAT activty was significantly reduced($p<0.001$) in STZ-induced diabetic rats. Effecting glyburide treatment on diabetic rat muscles has been seen to make a measurable improvement creating a slight reduction on the decrease rate; however the above mentioned affect has not been traced on gastrocinemius muscles. Conclusion; This study could not cover an intention to find out the mechanism of restrotation of glyburide‘s decreasing affect level on catalayse. Further studies are needed to provide positive adds both for viewing the levels and mechanism of glyburide restration and expressing the the pathogenesis of type II diabetes.
Key Words: Diabates, Catalase, Rat muscle, Glyburide

ÖZET: Moleküler veya atomik yörüngelerinde çiftleşmemiş e^- bulunduran moleküller olan serbest radikaller; dokularda, hücre hasarı oluşumundaki rolleri ile, son yıllarda, tıbbın en ilgi çekici konularından biri durumuna gelmiştir. Diabet ve komplikasyonlarının oluşumunda, serbest radikaller ve oksidatif stresin rol oynayabileceğine ilişkin birçok bulgu vardır. Bu amaçla, streptozosinle diabet oluşturulan ratlara, glibenklamid (gliburid) uygulanmasından önce ve sonra, kas (M. gastrocnemius, M. soleus, M. quadriceps femoris) dokusunda oksidan savunma sistemi enzimlerinden olan katalazın aktivitesi incelendi. Bu araştırma için 150-200 gr ağırlığındaki ratlar kullanıldı. Ratlarda diabet oluşturmak amacıyla streptozotosin (55mg\kg), pH 4.5 sitrat tamponunda eritilerek intraperitonel olarak uygulandı. Elde edilen veriler bilgisayarda istatistik paket programı (SPSS 10,0) kullanılarak analiz edildi. Kas dokularında ölçülen katalaz aktivitelerinde diabet grubu kontrol grubuna göre her üç kasta da anlamlı derecede azalma ($p<0.001$) saptandı. Diabetik hayvanlara glibenklamid uygulanması ile bu enzimdeki azalmanın düzeldiği gözlendi. Sadece gastrocnemiusta ise glibenklamidin etkisinin olmadiği gözlendi. Glibenklamid’in azalmış katalaz düzeyini restore etmesinin mekanizması bu çalışmada incelenememiştir. Ancak çalışmaların sürdürülmesi, hem glibenklamidin restorasyonunun nedenlerine, hem de diabetin patogenezine katkısı olabileceği için gereklidir.
Anahtar Kelimeler: Diabet, Katalaz, Rat kası, Glibenklamid

INTRODUCTION

Free radicals, those which are chemical species possessing an unpaired electron in their molecular or atomic orbits, have been the most attractive subjects in medicine because of their roles in destruction of cell or tissue[1,2].

Many findings support the view that free radicals and oxidative stress play an important role in etiology of diabetes and its complications [3,4,5]. A growing body of evidence emerging which suggest that reactive oxygen-derived radicals play a crucial role in the diabetogenic effects of alloxan and of streptozotosin (STZ). The activity of antioxidant enzymes in pancreas is low relative to the situation

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in the tissue, making it particulary vulnarable to oxygen radical attack [3,4].

Muscle tissue is unique in it's requirement and ability to undertake very rapid and coordinated changes in energy supply and oxygen flux during contraction. Bearing on several sources,it has been postulared that free radicals play a role in muscle damages induced by various pathological disorders[6,7,8,9].

In-vitro studies have demonstrated that gliclazide, a novel sulfonylurea in routine clinical use, has affects on free radicals scavenging and antiplatelet activities[10]. In addition, glyburide, a member of the second generation sulfonylureas, provides an effective theraphy for patients with type 2 diabetes[11]. Glyburide normalizes blood glucose directly and by increasing insulin secretion, decreasing hepatic glucose production and enhancing pheripheral glucose utilization[12].

Hence, in the present investigation, we administrated glyburide to streptozotosin-induced diabetic rats and determined the affect of glyburide on muscle (M. gastrocinemius, M. soleus, M. quadriceps femoris) catalase activities.

MATERIALS AND METHODS

Induction of experimental type II diabetes

Rats, weighing 150-200g were used in the present investigation. The experimental group was intraperitonally injected with streptozotosin (STZ, freshly dissolved in citrate buffer, pH 4.5 55 mg\kg) whereas the control group was injected with buffer only. All rats were free access to food and water for 5 weeks.

Body weights were obtained before treatment and prior to killing them. Blood samples were collected from the tail vein at the time of killing and blood glucose levels were determined using Ames glucometer.

Glyburide treatment

One week after diabets induction, same of the rats were given glyburide (5 mg\kg orally) for 4 weeks.,

Tissue preparation

M.soleus, m. gastrocinemius, m. quadriceps femoris muscles were rapidly dissected out from rats after ketamine anesthesia. All muscle samples were weighed, frozen in liquid N_2 and stored at -70°C until assayed.

Subsequently muscle samples were thawed, and at the room conditions in a –28 ° C store box homogenated, in 1/9 weight /volume of 50mM potassium phosphate buffer (pH 7.4) containing 10^{-4} M. EDTA Homogenated samples were then centrifuged at 4°C for 15 min at 3400 rpm refrigrated centrifuge. The clear supernatants were removed and kept at -70°C until the subsequent protein[13] and enzyme assays.

Enzyme assay

The catalase mediated decomposition of H_2O_2 was followed directly at 240 nm[14]. A 50 to 100 µ l sample was added to 1 ml of a solution containing 50 mmol\L sodium phosphate pH 7.0, 10 mmol\ L H_2O_2. The blank incubation contained no sample. The results were calculated from the extinction coefficient of H_2O_2 at 240nm.

Statistical analysis

Non-parametric methods were performed in the cross-sectional analysis of biomedical data (Mann-Whitney U test). Two -tailed probability (p) values were calculated throughout, and statistical significance was defined as $p<0.001$. All analyses were performed by statistical software SPSS 10.0.

RESULTS

In the present investigation, after STZ treatment, rats demonstrated polyphagia, polydipsia, polyuria and stable hyperglycemia for 5 weeks.

Body weight measurement before treatment and at the time of killing revealed a significant difference in the body weights of STZ-treated rats relative to controls. Furthermore, blood glucose determinations showed a significant hyperglycemia relative to control animals. Table I (Figure I)summarized the mean changes in body weight and blood glucose levels 5 weeks after STZ treatment. The results shown in table I also indicate that the administration of glyburide to STZ-treated and control rats reversed these changes ($p<0.01$).

Comparing to control group the dıabet group the activity of the muscle CAT has shown a remarkable decrease ($p<0.001$) for the three muscle groups.Threating glyburide to diabetic rats it had been seen that the decrease of the CAT activity has changed into a tendency towards a slight increase.Meanwhile glyburide threatment has not shown any affect on gastrocinemius (Table II, Figure II).

Table I. *Mean changes in body weight and blood glucose levels of control, glyburide - treated control, diabetic and glyburide -treated diabetic rats.*

Group	Body Weight(g)	Blood glucose(mg\dl)
Control	270±35.3	75.2±13.0
Control+GLY	221±34.3	83.4±13.8
Diabetic	164±39.2*	343.5±64.9*
Diabetic+GLY	185±43.8**	230.5±50.4**

*p<0.01 significance relative to control; **p<0.01 significance relative to Diabetic.

Table II. *Muscle CAT activities obtained from control, glyburide-treated control, diabetic and glyburide-treated diabetic rats.*

Group	CAT (mg\U\protein)		
Muscle	**M. Gastrocinemius**	**M. Soleus**	**M.Quadriceps femoris**
Control	0.095±26.6	0.074±12.0	0.047±12.6
Control+GLY	0.053±18.8	0.045±11.1	0.027±8.6
Diabetic	0.019±7.8*	0.019±9.0*	0.016±6.0*
Diabetic+GLY	0.021±5.2**	0.033±13.2**	0.021±6.6**

M.Gastrocinemius *p<0.001
M.Soleus *p<0.001 significance relative to Control
M.Quadriceps femoris *p<0.001

M.Gastrocinemius **p>0.05
M.Soleus **p>0.05 Significance relative to Diabetic
M.Quadriceps femoris **p<0.05

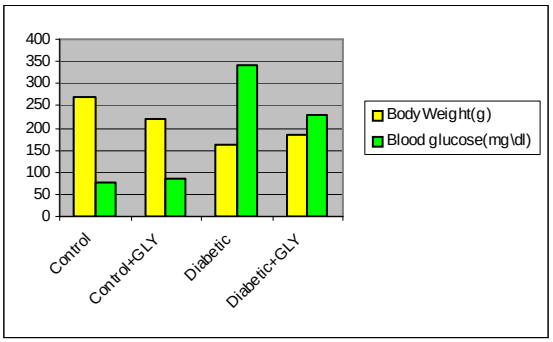

Figure I. Mean changes in body weight and blood glucose levels of control, glyburide - treated control, diabetic and glyburide -treated diabetic rats.

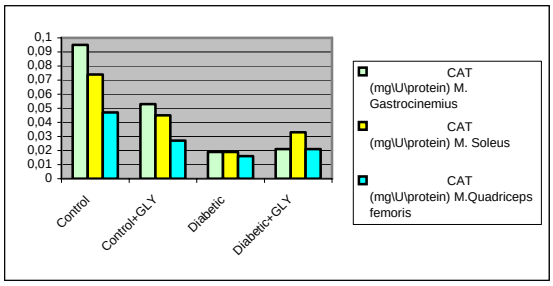

FIGURE II. Muscle CAT activities obtained from control, glyburide-treated control, diabetic and glyburide-treated diabetic rats.

DISCUSSION

In our study, we investigated muscle catalase activities of streptozotocin-induced diabetic rats after 5 week usage of glyburide.

Hypoinsulinemia increases the activity of an enzyme, fatty acyl-CoA oxidase, that initiates β-oxidation of fatty acids resulting in the production of H_2O_2, which is not only toxic, but also permeable through cell membranes. In extracellular environment, H_2O_2 reacts with transition metals such as, iron and copper generating highly reactive hydroxyl radicals, which can react with macromolecules in the vicinity and could cause damage[15,16]. It is possible that increase in oxygen radicals during diabetical period could increase CAT activity, which in turn would protect SOD inactivation by H_2O_2 and hence causes an increase in SOD activity. H_2O_2 is known to inactivate SOD[17]. Increase in SOD activity would protect GSH-Px and CAT against inactivation by superoxide anion, which is known to inactivate catalase[3,18].

The increased CAT activity observed in the heart of diabetic rats agrees with the findings of Asayama et al[18] and Godin et al[3,4]. Insulin deficiency promotes β-oxidation of fatty acids with resulting H_2O_2 formation[19]. The elevation of CAT activity may be due to a compensatory increase in endogenous H_2O_2 production in the muscle. At a

previos study of ours on the heart muscles an increase on the CAT activity had been observed in 24-weeks diabetic rats which probobly due to higher production of H_2O_2.

Glyburide normalizes blood glucose directly and by increasing insulin secretion, decreasing hepatic glucose production and enhancing pheripheral glucose utilization[12].

In comperative researches of normal and diabetic groups, plasma lipid peroxide levels exhibited an increase, compared with healty group[3]. Parallel with this knowledge, we determined a decrease in muscle catalase activity of streptozoticin-induced diabetic rats. Glyburide therapy seems to restore muscle catalase activity.

In one research, after administration of glyburide to diabetic rats, SOD activity of muscle has been investigated and restoration of muscle SOD activity was observed[20]. In another research glyburide seemed to be capable of exerting direct insulin-like and insulin potentiating effects on nonpancreatic tissue in vitro[12] and in vivo[21,22]. Mechanism of alteration in muscle catalase activity in pure diabetic and glyburide administered diabetic rats is still obscure. Effect of diabets on catalase activity differs relatively with tissue type and exposure period.

This study could not cover an intention to find out the mechanism of restrotation of glyburide‘s decreasing affect level on catalayse. Further studies are needed to provide positive adds both for viewing the levels and mechanism of glyburide restration and expressing the the pathogenesis of type II diabetes.

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