Diabetes Mellitus Correlation with Free Radicals and Creatine Kinase Isoenzymes Activity

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Abstract

To explain the relationship between free radicals production in experimentally diabetic animals and expected glutathione (GSH) reducing levels with its enhancement due to feeding of cysteine, N-acetyl cysteine and to evaluate the action of antioxidant to relief the damage to the active site of creatine kinase (CK) caused by free radicals, three doses during 48 hours of 150 mg alloxan / kg body weight was injected to forty eight males albino mice to produce the diabetes mellitus.

Blood glucose and serum glutathione were determined using colorimetric methods, whereas, fractionation of CK isoenzymes in sera of diabetic mice was conducted by mini column ion exchange chromatography using Sephadex A-50. Agarose gel electrophoresis was used to quantitize the CK isoenzymes.

Results of present study show that after long period of induced diabets in males albino mice cause a change in CK isoenzyme distribution pattern The total serum CK activity decreased in 75% of animals and reached to 63.5% of the control value whereas CK - MM isoenzyme was also decreased to 54.1% of its initial activity. Other isoenzymes showed variation depending upon the period of diabetes induced which caused elevated levels after 30 days of CK -BB isoenzyme in 62.5% of the animal studied and reached to 68% more than its initial activity which in < 3%. CK - MB which is found in 25% of albino mice was elevated to 17.1% more than that found in control sera which is < 3%. Insulin affects total CK activity and its isoenzymes distribution pattern as a therapeutic agent, and CK activity found to be elevated

Thiol containing compounds including cysteine, N-acetyl cysteine dithiothreitol and glutathione have been suggested to reactivate the CK activity.

الخلاصة

صممت هذة الدراسة لتفسير العلاقة بين الجذور الحرة الناتجة من إصابة فئران مختبرية بداء السكري والنقصان المتوقع بمستويات الكلوتاثايون ثم زيادتة نتيجة لتغذية الفئران بالسستين و N - استايل سستين ولتقدير فعل مضادات الأكسدة في تفادي الضرر الحاصل في الموقع الفعال لإنزيم كرياتين كاينيز(CK) ، تم حقن ثمانية وأربعون فأراً مختبرياً بـــ 150 ملغم/لكل كغم من وزن الجسم من مادة الالوكسان وعلى شكل ثلاث جرعات لمدة 48 ساعة لإحداث داء السكري فيها.

تم تقدير سكر الدم وكلوتاثايون مصل الدم باستخدام طريقة طيفية لونية، وتم فصل ايزوانزيمات الـــ CK في أمصال الفئران المحـــدث داء السكري بها بواسطة تقنية كروماتوغرافيا التبادل ألايوني باستخدام السيفادكس A-50 ، واستخدمت تقنية الترحيل الكهربائي على هلام الاكار لتقدير كمية ايزوانزيمات الـــ CK .

أظهرت النتائج تغيرا كبيرا في توزيع ايزوانزيمات الـــ CK إذ قلت الفعالية الكلية لانزيم CK وقلت كذلك فعالية الـــ CK – MMبينما ازدادت فعالية الـــ CK–BB و CK – MB مقارنة بمجاميع السيطرة. ورفع الانسولين فعالية انزيم CK كمادة علاجية واثر كذلك على توزيع ايزوانزيمات الـــ CK .

نستنتج من نتائج البحث إن تناول المركبات الحاوية على مجاميع الثايول ينشط فعالية إنزيم CK.

Introduction

Free radicals and other reactive oxygen species are derived either from normal essential metabolic processes in human body or from external sources such as exposure to X- rays and ozone [1]. Types of free radicals include the hydroxyl radicals ($OH^{\cdot}$), the superoxide radical ($O_2^{\cdot}$), the nitric oxide radical ($NO^{\cdot}$) and the lipid peroxyl radical ($LOO^{\cdot}$) [2]. However free radical formation occurs continuously in the cells as a consequence of both enzymatic and non-enzymatic reaction. Enzymatic reactions which serve as a source of free radicals include those involved in the respiratory chain and in prostaglandin syntheses, while the non-enzymatic arise from reaction of oxygen with organic compounds [3]. Free radical reactions are expected to produce progressive adverse changes that accumulate with age throughout the body. Such "normal" changes with age relatively common to all. However, superimposed on this common pattern are patterns influenced by genetics and enviromental differences that modulate free radical damage. These are manifested as diseases of certain ages determined by genetic and enviromental factors. Diabetes mellitus or what is called alteration in carbohydrates metabolism at different cellular levels found to be associated with excess production of free radicals.[4]. Also the amounts of partially oxidized LDL in plasma are correlated with insulin resistance and glucose in diabetic diseases [5]. These information lead to thinking that some enzymes and its isoenzymes will have a change in its activity and its electrophoretic pattern since the free radicals overproduction can cause an increase in radical generating enzymes such as xanthine oxidase and / or their substrates, hypoxanthine, creatine kinase and its isoenzymes could be affected by the over production of free radicals due to diabetes mellitus and could also affect the defense system such as catalase, glutathione and other materials which reduce the effect of free radicals to prevent tissue damage since glutathione depletion and / or low serum concentration is reported in the following pathological conditions; Diabetes mellitus, coronary artery disease and liver diseases [6].

The present study tries to explain the relationship between free radicals production in experimentally diabetic animals and the glutathione reducing level with its enhancement due to addition of cysteine, N- acetyl cysteine to evaluate the action of antioxidant to relief the damage to the active site of creatine kinase enzyme caused by free radicals .

Materials and Methods

Forty - eight males albino mice were included in this study which was fasted overnight 18 hours. Experimental diabetes was induced by injecting 150 mg alloxam / kg body weight in normal saline in three doses during 48 hrs [7]. Then diabetes was diagnosed after 7 days when the amount of blood glucose exceeds 300 mg/100 ml and polyuria was also observed. The diabetic mice was classified into five groups and treated for 2 months as a whole period of experiment as indicated below:

Group 1 (n = 8) represents diabetic animals that were fed with normal diet for whole period of experiment. Group 2 (n = 10) represents diabetic animals which were fed with normal diet and treated with insulin 1.0 u /day / Animal for the period of the experiment. Group 3 (n = 10) represents diabetic animals which were fed with normal diet plus 2.0 ml of 30 mM /L cysteine / day (calculated as 15 mM/L cystine) for whole period of experiment. Group 4 (n = 10) represents diabetic animals fed with normal diet plus 2.0 ml of 30 mM/L N - acetyl cysteine / day for the period of experiment. Group 5 (n = 10) represent diabetic animals which were fed with normal diet plus 2.0 ml of 30 mM/L dithiothreitol / day for whole period of experiment. The other group which in Group 6 (n = 16) was treated with normal saline as a control animals.

The amount of blood glucose was measured calorimetrically each week by modefied Folin method [8]. The effect of free radicals in diabetic animals was studied by measuring the amount of serum glutathione produced by using method [9] after giving 30 mM/L N- acetyl cysteine, cysteire and dithiothreitol in diet per each day to the diabetic albino mice.

Fractionation and quantitization of CK isoenzymes in sera of induced diabetic mice was performed by mini column ion exchange chromatography by using DEAE - Sephadex A - 50 as anionic exchanger at pH 7.0 [10], and then quantitated by agarose electrophoresis [11]. The activity of CK and its isoenzymes in serum and their fractions were measured by Oliver method [12].

The effects of cysteine, N - acetyl cysteine and dithiothreitol were compared for their effectiveness in vitro in reactivation of total CK and its isoenzyme, isolated from albino diabetic mice.

Results

The effect of diabetes mellitus induced by alloxan on serum CK; and its isoenzymes activity levels was presented in Table 1, which shows that after long period of induced diabetes in male albino mice (60 days) causes a change in CK isoenzyme distribution pattern found in control sera which was fractionated by ion - exchange chromatography and detected by agarose electrophoresis as shown in Figure 1.

Table 1 Ilustrate the CK activity levels and its isoenzymes fractionated from sera after long term induced diabetic male albino mice by alloxan as compared with the control animals.

Group	No. of Animals	Total CK U/L ± S.D	CK isoenzymes fractionated U/L ± S.D.		
			MM	MB	BB
Control	16	79.2±18.3	74.8 ± 17.1	1.75±0.5	2.5±0.9
Diabetic mice	8	50.6±13.7	40.5±10.3	2.05±0.7	4.2±1.2
Diabetic mice (treated with insulin)	10	70.2±15.2	63.8±12.7	2.0±0.6	3.5±1.1

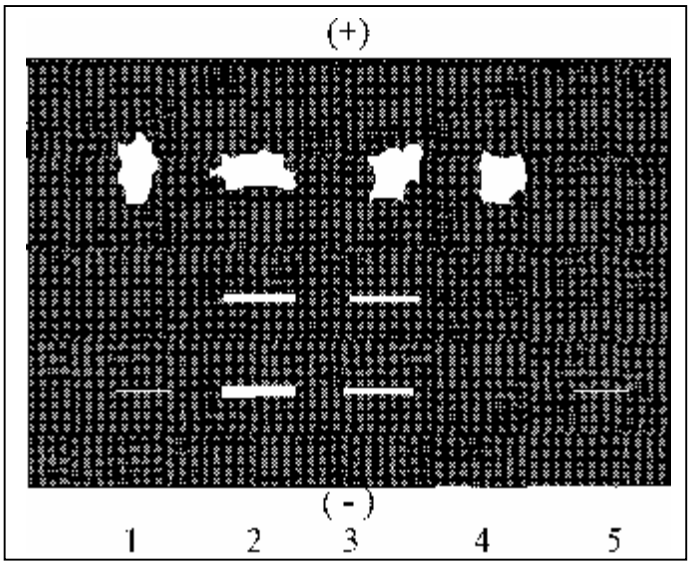

Figure 1 CK electrophoretogram. Agarose electrophoresis 50 mM barbital buffer, pH 8.0 , 90 V for 60 min by using control male albinon mice (1) ; induced diabetic by alloxan (2); Insulin treated of induced diabetic (3) ; CK - MM isoenzyme fractionated by ion - exchange chromatography of diabetic albino mice (4); CK - BB isoenzyme fractionated (5).

The total serum CK activity decreased in 75% of animals and reached to 63.5% of the control value whereas CK - MM isoenzyme which predominantely found in normal sera in more than 94% [13] was also decreased to 54.1% of its initial activity. The other isoenzynes was also varies and depending upon the period of diabetes induced which caused and elevated levels after 30 days of CK -BB isoenzyme in 62.5% of the animal studied and reached to 68% more than its initial activity which in < 3%. The other CK isoenzyme fractionated is CK - MB which is found in 25% of albino mice and elevated to 17.1% more than that found in control sera which is < 3%. [13]

Table 1 shows the effect of insulin as a treatment on total CK activity and its isoenzyme distribution pattern. After 30 days of insulin treatment, 28.5% of CK activity was elevated as compared with its activity in diabetic mice, due to elevation in CK -MM isoenzyme which increased and reached to 33.5% more than that found in diabetic mice. The other CK isoenzyme was also detected in variable degrees include CK - BB which was slightly elevated and myocardium CK - BB isoenzyme as shown in table 1 which shows that CK isoenzyme activity levels in diabetic mice is insulin dependent. The concentration of blood glucose in group 3 is decreased to 145 mg/100 mg after 30 days of insulin treatment.

Table 2 shows the effects of several thiol compounds on CK isonzymes in sera of diabetic mice as compared with control group in which they reactivate the active site of CK isoenzymes due to increased glutathione concentration as shown in table 3 which reveal that the amount of glutathione concentration increased to 44.3%, 33.5%, 37.6% when the animal treated with 30 mM/L of cysteine, N-autqluysteine & dithiothreitol respectively as compared with long period induced diabetic albino mice.

Table 2 Effect of thoil activators on serum total CK and its isoenzymes activity levels in long term induced diabetic male albino mice as compared with control and diabetic groups (treated with insulin & without treatment)

Group	Type of treatment	Total CK U/L ± S.D.	CK isoenzymes fractionated U/L± S.D.		
			MM	MB	BB
Control		79.2± 18.3	74.8± 17.1	1.75± 0.5	2.9± 0.9
1	Diabetic	50,6± 13.7	40.5 ± 10.3	2.05± 0.7	5.8± 1.2
2	Diabetic treated with insulin	70.2± 15.2	63.8 ± 12.7	2.0± 0.6	3.5± 1.1
3	Diabetic treated with 30 mM/1 cysteine	67.4± 16.2	57.7± 10.9	1.7± 0.5	3.7 ± 1.2
4	Diabetic treated with 30 mM/1 N - acetyl cysteine	63.5± 12.9	54.2 ± 10.1	1.9± 0.7	3.4± 1.3
5	Diabetic treated with 30mM/1 Diathiothreitol	66.5± 14.9	59.7 ± 11.4	1.2± 0.6	3.6 ± 1.7

Table 3 Glutathione concentration in sera of normal male albino mice as compared with long term diabetic treated with insulin and without treatment. The effect of thiol activators on its concentration was also presented in diabetic male albino mice.

Group	Type of Treatment	Serum GSH mg/100 ml ± S.D.
Control		7.26± 0.85
1	Diabetic	3.96± 0.52
2	Diabetic treated with insulin	6.37± 0.56
3	Diabetic treated with cysteine	5.72± 0.47
4	Diabetic treated with N - acetyl cysteine	5.16± 0.38
5	Diabetic treated with dithiothreitol	5.45 ± 0.42

To prove that CK isoenzymes in serum are rapidly inactivated, urate in concentration of 5 mM/L added to experimental design and Figure 2 shows the inactivation of CK isoenzymes and that it obeys the first order law behavior. However thiol compounds prevent or completely reverse urate inactivation, as shown in Figure 3 which presents that urate retarded the irreversible inactivation of CK activity. These results may suggest that CK isoenzymes in serum are rapidly inactivated, but they can reactivated by adding sulfhydryl reagents [15 , 16].

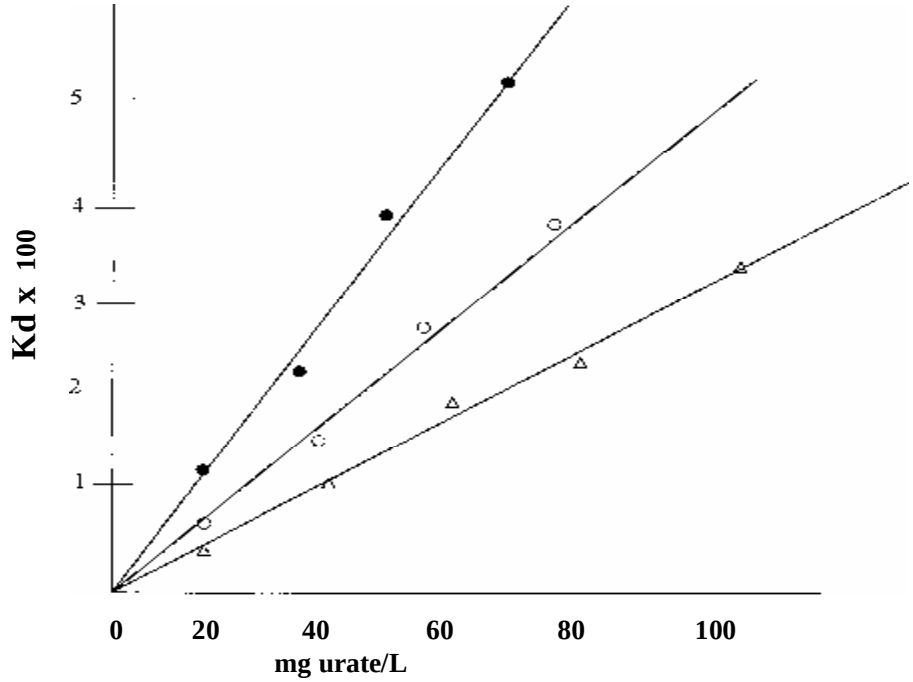

Figure 2 Activity decay constant for serum CK isoenzymes with urate at 25°C.
• CK-MM o CK-MB Δ CK-BB

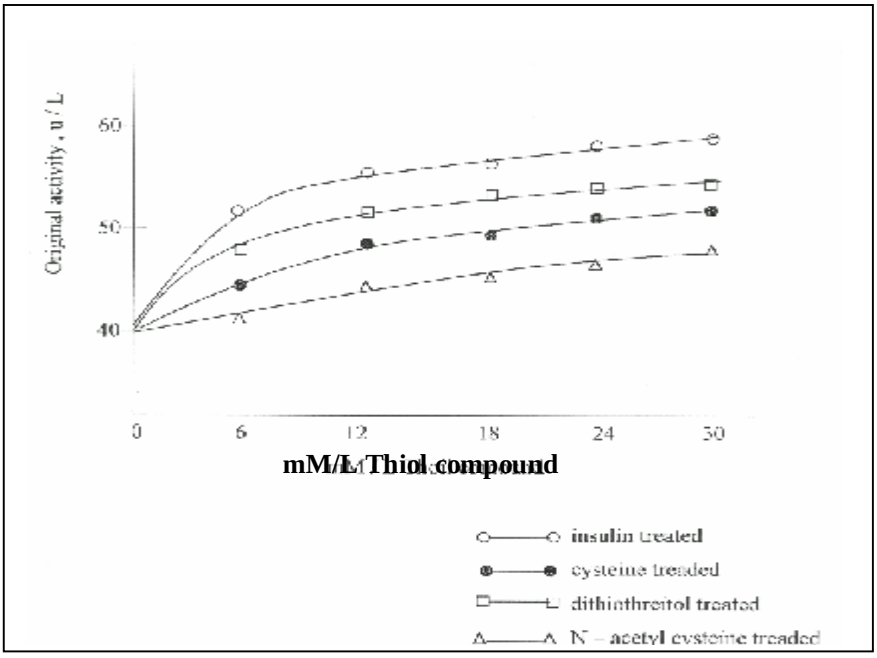

Figure 3 Reactivation of CK - MM fractionated from induced diabetic sera of male albino mice with cysteine, N - acetyl cysteine and dithiothreitolas compared with its activity after 30 days of insulin treatment

Discussion

Elevated plasma free radicals concentration is related to different patho physiological conditions such as aging, cancer and diabetes. Nevertheless even in healthy subjects a rise in plasma free radicals is due to hyperglycemia, elevated free fatty acids and hyperinsulinaemia

Therefore alloxan injection induced hyperglycemia after one week in male albino mice, then blood glucose concentration exceeds 300 mg/l00ml. In this case the persistence of hyperglycemia has been reported to cause an increase in free radical production through glucose auto oxidation and non enzymatic glycation.[18] This was shown by the amount of reduced glutathione which was decreased in diabetic mice as shown in table 3.

Glutathione plays a key role in detoxification by reacting with hydrogen peroxide and organic peroxides, which are the harmful byproducts of aerobic life.[14], therefore the decrease in glutathione concentration caused an increased in hydrogen peroxide produced as a byproduct of metabolic processes which affects every metabolism in muscle by decreasing CK activity levels due to its effect on the sulfhydryl group of the enzyme at the active site. The other data obtained table 2 show that the addition of thiol compounds like cysteine, N - acetylcysteine and dithiothreitol showed a significant reversal effects because some of these sulfhydryl compounds play an important role in glutathione biosynthesis.[14]

According thiol compounds have been suggested for the reactivation of CK including cysteine, N - acetyl cysteine, dithiothreitol, glutathione mercaptoethonol and 2 - amino ethyl isothiouronium [19,20].

Acknowledgment

The authors would like to express a great thankful to Dr. Samr T. Mkhlof from Marbury University, GERMANY for his assistant in running the electrophoresis samples .

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