Original Article

Glycation Pattern of Captopril in Diabetics

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ABSTRACT

Objective: Captopril is a chemical which acts as vasodilator and angiotensin- I converting enzyme inhibitor. The present attempt is to study the glycation inhibition pattern of captopril in diabetics and normals.
Study Deign: Experimental Study.
Place and Duration of Study: This study was conducted at the Research Lab., Department of Biochemistry, University of Agriculture, Faisalabad from May 2006 to February 2007.
Materials and Methods: The study was designed on blood plasma from apparently healthy non-diabetic and diabetic persons. Plasma samples with different concentrations of glucose and that of captopril were prepared, followed by incubation for 5 weeks at 37 ºC temperature. Glucose was estimated by glucose oxidase method before and after dialysis. Glycation level was measured by TBA and Periodate assays.
Results: Increased glycation was observed from 1st to 3rd week of incubation while it decreased after 5th week due to the formation of advanced glycation end products. 10 *mM* concentration of captopril showed fairly good response to decrease glycation as compared to its other two concentrations.
Conclusions: Highest concentration of captopril produced overall good enough of inhibition. Periodate borohydride appeared to be more reliable and sensitive glycation assay as compared to TBA.
Key Words: Glycation, Diabetes, Maillard reaction, Captopril, TBA, Periodate

INTRODUCTION

Diabetes is characterized by hyperglycaemia resulting in various short-term metabolic changes in lipid and protein metabolism and long-term irreversible vascular changes[1]. Diabetes and its complications are rapidly becoming the world's most significant disease epidemic[2]. The morbidity caused by diabetes has traditionally been classified into macro vascular complications including, atherosclerosis leading to heart disease, stroke, peripheral vascular disease and micro vascular complications like retinopathy, nephropathy and neuropathy. The same disorders are also two to five times more prevalent in diabetic as compared to normal subjects[1]. Chronic hyperglycemia is a major threat to diabetic microvascular complications. According to Matthew and George,[3] glucose is utilized in a variety of diverse metabolic pathways; hence, chronic hyperglycemia can induce multiple cellular changes leading to various complications.

The adverse effects of high plasma glucose depend upon the type of the cells. Cells which express a high level of the glucose transporter 1 (GLUT1), are unable to regulate intracellular glucose concentrations and are thus very susceptible to hyperglycemia-induced damage[4]. The *in vivo* formation of non-enzymatic glycated compounds were first detected in 1969 from studies on chromotagenic mobilities of fast moving, minor hemoglobin from diabetic patients, in particular HbA1C, now used as a clinical tool in the management of glycaemic control in diabetic patients[5]. Reaction of glucose with proteins is called Maillard reaction. The Maillard reaction has three stages. The early reactions results in the formation of a Schiff base and Amadori products. Then rearrangements of chemical groups take place resulting in the formation of classical Maillard browning products known as AGEs[6]. Non-enzymatic glycation of proteins have now been implicated in the pathogenesis of different diseases like diabetes, renal failure and aging[7].

Living system has devised various defense mechanisms to protect the tissues against deleterious effects of advanced glycation end products. These include glyoxylase system (I and II) having oxaldehyde reductase and aldose reductase that catalyze the deglycation and detoxification of methylglyoxal, the most common reactive intermediates of AGEs to D-lactate[8].

Now it is the need of the day to develop or isolate new compounds either from plants or synthetically to control diabetes and other age accelerating diseases. Aminoguanidine (AG) is the first compound that has been extensively studied *in vitro* and *in vivo*, to be a powerful glycation and AGE inhibitor[9]. AG is a nucleophilic compound that traps reactive carbonyl intermediates partially inhibiting carboxy methyl lysine and carbxy ethyl lysine[10]. Hammes et al.[11] showed that oral benfotiamine supplementation in diabetic rats could effectively block the formation and accumulation

of AGEs and prevent the development of experimental diabetic retinopathy and nephropathy. The major objective of this study was to investigate the glycation inhibition level of captopril in normal and diabetic human plasma and secondly to compare the sensitivities of Thiobarbituric Acid (TBA) with that of Periodate method.

MATERIALS AND METHODS

Experimental concentration and conditions

Four different concentrations of glucose i.e (G_1=500 m*M*, G_2=250 m*M*, G_3=50 m*M and* G_4=5.5 m*M*) were prepared. Blood plasma samples were collected from diabetic (Type II) patients as well as from normal/ healthy male and females. Samples were stored at -20 ºC till use. At the time of use, all plasma were polled together. Thereafter, separately both in normal as well as in diabetics the required sample volume for experiments were obtained. Then all plasma samples were diluted so as to have protein concentration range up to 20mg/mL. Glycation level was assessed by two different methods. Proteins were estimated by Biuret method[12] before and after dialysis of plasma samples. Three different concentrations of captopril, the inhibitor (I_1= 10 m*M*, I_2= 5 m*M* and I_3 = 1 m*M*) were used in this experimental study.

Selection of Combinations

To study the effect of captopril, sixteen combinations with normal (P_N) and diabetic (P_D) plasma were made. All were placed simultaneously for five weeks at 37 ºC (Table No.1).

Table No. 1: Different combinations for Plasma glycation inhibition

S#	Combinations for Normal/ Diabetic Plasma	S#	Combinations for Normal/ Diabetic Plasma
1	$G_1 + P_{N/D}$	9	$G_3 + P_{N/D}$
2	$G_1 + P_{N/D} + I_1$	10	$G_3 + P_{N/D} + I_1$
3	$G_1 + P_{N/D} + I_2$	11	$G_3 + P_{N/D} + I_2$
4	$G_1 + P_{N/D} + I_3$	12	$G_3 + P_{N/D} + I_3$
5	$G_2 + P_{N/D}$	13	$G_4 + P_{N/Ds}$
6	$G_2 + P_{N/D} + I_1$	14	$G_4 + P_{N/D} + I_1$
7	$G_2 + P_{N/D} + I_2$	15	$G_4 + P_{N/D} + I_2$
8	$G_2 + P_{N/D} + I_3$	16	$G_4 + P_{N/D} + I_3$

Glycation of Plasma

All plasma combinations with four different concentrations of glucose were incubated for 1-5 weeks at 37ºC. Plasma samples after incubation were dialyzed to remove free glucose, as free glucose is the major hindrance in ascertaining the glycation level. Glucose was again estimated after dialysis by glucose oxidase method in order to see whether the concentration of glucose is decreased or not.

Glycation inhibition of plasma

Different concentrations of glucose and that of captopril were incubated at 37 ºC for 1-5 weeks.

Thiobarbituric Acid (TBA) Colorimetric Technique

Enzymatic and non-enzymatic glycation was determined by TBA technique. This method is based on the reaction between fructose, amino acids and weak acid that will yield 5-hydroxymethyl furfural (HMF) compound.[13]

Non enzymatic glycation was determined as follows.

NE Glycation = (C Glycation + E Glycation) - E Glycation

NE =Non Enzymatic, E= Enzymatic, C =Collective

Periodate Borohydride Assay

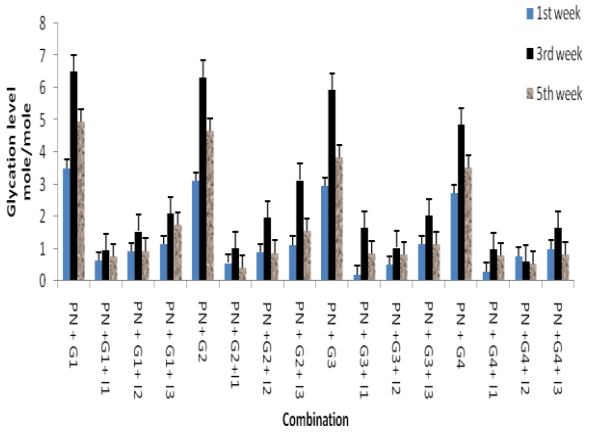
This test is based on the formation of formaldehyde by periodate oxidation of cis-diol, aminol, ketol or ketoamine structures. Two moles of formaldehyde are formed from hexose sugar. The amount of formaldehyde produced was quantified as fluorescent adduct formed by condensation of formaldehyde with acetyl acetone and ammonia.[14,15].

RESULTS

Effect of Captopril on glycation level with normal human plasma

The results obtained from captopril in normal human plasma showed that 500 m*M* (G_1) concentration of glucose had maximum glycation level with value 6.507mole/mole after 3rd week of incubation and it was minimum after 1st week of incubation with G_4 (5.5 m*M*) glucose concentration (Figure No.1). TBA technique showed that 10 m*M* (I_1) concentration of captopril was more active to inhibit glycation while 5 m*M* (I_2) gave variable response and 1 m*M* (I_3) concentration exhibited least glycation inhibition effect.

Figure 1: Effect of Captopril on glycation level with TBA in normal human plasma



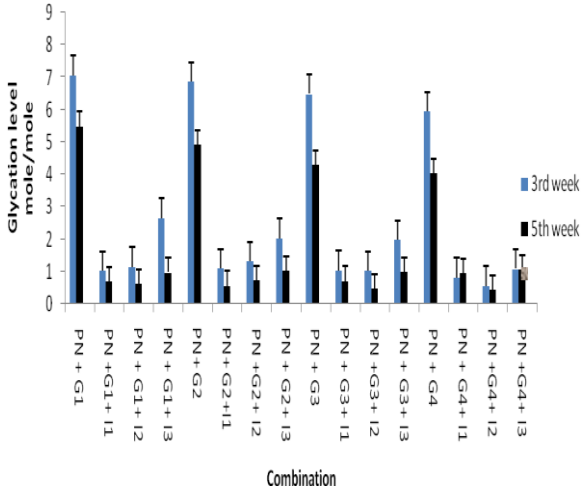
Normal plasma protein ($P_N \approx$ 20 mg/mL) was incubated with all glucose concentrations (G_1= 500 m*M*, G_2= 250 m*M*, G_3= 50 m*M* &

G_4= 5.5 m*M*) and three concentrations of captopril (I_1= 10 m*M*, I_2= 5 m*M* & I_3 =1m*M*) in 0.075 *M* PBS. Reaction mixtures were incubated at 37 °C for 5 weeks at the same time. Samples were analysed after 1st, 3rd and 5th week and glycation level was measured in mole\ mole (glucose\ protein).

***** Values were the average of experiments carried out at n= 3

With Periodate borohydride assay, maximum glycation response i.e. 7.053 mole/mole was observed after 3rd week with G_1 concentration of glucose which dropped to 2.960 mole/mole with G_4 glucose concentration in 1st week of incubation (Figure No.2). Different concentrations of captopril showed comparable inhibitory responses i.e. 10 m*M* (I_1) produced maximum inhibition with G_1 and G_2 concentration of glucose. Likewise, 5 m*M* (I_2) concentration of captopril also exhibited maximum inhibitory response against G_3 and G_4 glucose concentrations.

Figure 2: Effect of Captopril on glycation level with Periodate borohydride assay in normal human plasma

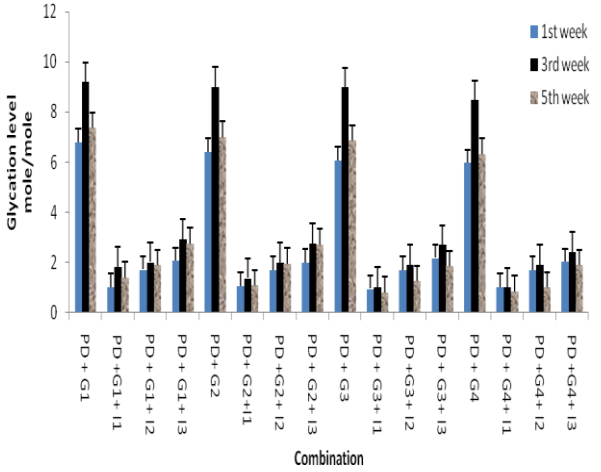


Normal plasma protein (P_N ≈ 20 mg/mL) was incubated with all glucose concentrations (G_1= 500 m*M*, G_2= 250 m*M*, G_3= 50 m*M* & G_4= 5.5 m*M*) and three concentrations of captopril (I_1= 10 m*M*, I_2= 5 m*M* & I_3 =1m*M*) in 0.075 *M* PBS. Reaction mixtures were incubated at 37 °C for 5 weeks at the same time. Samples were analysed after 1st, 3rd and 5th week and glycation level was measured in mole\ mole (glucose\ protein).

***** Values were the average of experiments carried out at n= 3

Results in Figure No. 3, are of diabetic human plasma which showed that G_1 concentration of glucose showed maximum glycation (9.195mole/mole) after 3rd week of incubation with TBA test. Minimum glycation i.e. 5.966 mole/mole was observed after 1st week of incubation with G_4 concentration of glucose. Maximum glycation inhibition response was reflected in 10 m*M* (I_1) concentration of captopril while 5 m*M* (I_2) and 1 m*M* (I_3) had variable response towards inhibition

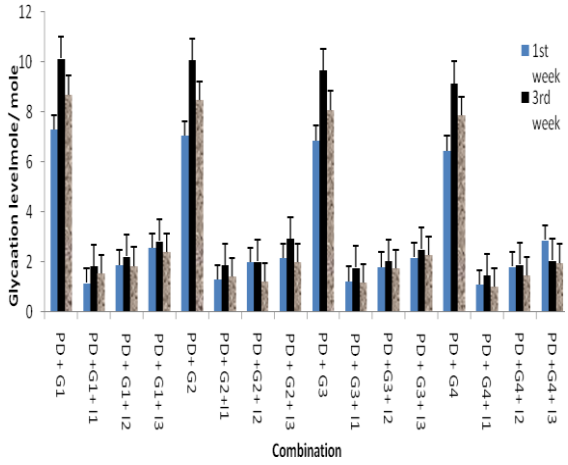
Figure 3: Effect of Captopril on glycation level with TBA in diabetic human plasma



Normal plasma protein (P_D ≈ 20 mg/mL) was incubated with all glucose concentrations (G_1= 500 m*M*, G_2= 250 m*M*, G_3= 50 m*M* & G_4= 5.5 m*M*) and three concentrations of captopril (I_1= 10 m*M*, I_2= 5 m*M* & I_3 =1m*M*) in 0.075 *M* PBS. Reaction mixtures were incubated at 37 °C for 5 weeks at the same time. Samples were analysed after 1st, 3rd and 5th week and glycation level was measured in mole\ mole (glucose\ protein).

***** Values were the average of experiments carried out at n= 3

Figure 4: Effect of Captopril on glycation level with periodate borohydride assay in diabetic human plasma



Normal plasma protein (P_D ≈ 20 mg/mL) was incubated with all glucose concentrations (G_1= 500 m*M*, G_2= 250 m*M*, G_3= 50 m*M* & G_4= 5.5 m*M*) and three concentrations of captopril (I_1= 10 m*M*, I_2= 5 m*M* & I_3 =1m*M*) in 0.075 *M* PBS. Reaction mixtures were incubated at 37 °C for 5 weeks at the same time. Samples were analysed after 1st, 3rd and 5th week and glycation level was measured in mole\ mole (glucose\ protein).

***** Values were the average of experiments carried out at n= 3

With periodate borohydride assay, maximum glycation i.e.10.138 mole/mole was measured after 3rd week with G_1 concentration which dropped to minimum i.e.6.453 mole/mole with G_4 after 1st week of incubation (Figure No.4). Overall maximum glycation inhibitory effect of

captopril was seen in I_1 concentration. One exception was observed where I_2 showed maximum response.

DISCUSSIONS

The results reflected that overall G_1 (500 m*M*) concentration of glucose exhibited maximum glycation impact while G_2 (250mM) has almost similar trend as G_1 at many points. 10 m*M* concentration of captopril showed maximum glycation inhibitory response in both normal and diabetic condition (Figures 1-4). These results are comparable with Mariee et al. studies, [16] who studied comparative inhibitory pattern of captopril and aminoguanidine (AG). Our results also corroborates with report by Jakus et al. [17] as they concluded that captopril decreases AGEs formation. Huang et al. findings are also in conformity with our results as they studied the role of captopril in the pathogenesis of diabetic nephropathy and found dose-dependent inhibition by antisense RAGE oligodeoxynucleotide and captopril. [18]

Glycation assays: TBA and Periodate almost showed same trend of glycation measurement but periodate proved to be more sensitive and affective method to measure glycation level. Our results are also in harmony with Jakus et al[17]. It can be inferred that periodate glycation assay measurement of Amadori product was more convenient.

It can be conclude ed from the present study that glycation increased from 1st to 3rd week of incubation and slight decline in glycation level was seen after 5th week of incubation. This possibly could be due to formation of advanced glycation end products. It was also seen that G_1 and G_2 concentrations of glucose produced maximum glycation as compared to its other concentrations.

CONCLUSIONS

- Glucose concentrations 500mM and 250 mM can produced maximum glycation
- 10 mM concentration of captopril can produce fairly good response to decrease glycation both in normal and diabetics.
- Periodate borohydride technique seems to be more affective and sensitive as compared to TBA test.

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