Naloxone inhibits human serum albumin Glycation

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ABSTRACT

Advanced glycation end-products (AGEs) are formed by non-enzymatic reaction between reducing sugar and protein. AGEs play important roles in pathogenesis of diabetic, aging complications, endothelial dysfunction and neurological diseases such as the Alzheimer's disease. Therefore compounds that prevent the glycation reaction are purported to have therapeutic effect on patients with diabetes and age-related complication. In this study, the Human serum albumin at concentration of 10 mg/ml was incubated in PBS with 40 mM Glucose, and in different concentrations of Naloxone (25,100,250μM) for 42 days at 37˚C. HSA with no additives and with Glucose 40mM were incubated as control and as glycated sample, respectively. Following the incubation, the samples were prepared for Circular Dichroism, Fluorescence and absorbance techniques. It was shown different Naloxone concentration can prevent Human serum albumin glycation.

Keywords: Glycation, Human serum albumin, Naloxone, Glucose

INTRODUCTION

Diabetes mellitus is known as metabolic disorders and is manifested by hyperglycemia[1].The none enzymatic glycation of protein is a process which is happened in patient with hyperglycemia ,it causes a series of pathophysiological changes and different disease[2]. None enzymatic glycation of protein subdivided into two steps: early and late. In the early phase, reducing sugar react with the free amino group of protein, forming Schiff base and Amadori products. In the late phase in order to formation advanced glycation end products (AGE), Amadori more rearrangements to produce AGE products [3]. AGEs have an important effect on the development of atherosclerosis, neurological disorder; also AGE causes damage to eyes, kidneys joint and they are responsible for aging and tissue damage [4-6]. Non enzymatic glycation of serum albumin occurs at multiple sites, in vivo. Glucose gets attached to lys-199, lys-281, lys-439, and lys-525 as well as to some other lysine residues [7]. Naloxone is a drug that is used as an opioid antagonist; it uses specifically to block the side effects of opiate overdose and counteract depression of the CNS and respiratory system [8]. Naloxone is made up of thebaine that is obtained of plant *papaver somniferous* [9]. Compounds that inhibit the formation of AGEs are purported to have therapeutic potentials in patients with diabetes and age-related diseases. Researches show that many agents can prevent AGE formation; it has been shown Aminoguanidine, Zea mays, garlic, pepper , ginger and some other plant exteract have good antioxidant as well as antiglycation potential [10-12].in present study we have decided to examine the effect of Naloxone on human serum albumin glycation.

MATERIALS AND METHODS

Materials

Human serum albumins, sodium bicinchoninic acid were purchased from Sigma .The membrane filters (0.2 μm pore size, 25mm in diameter) and dialysis tubing (cut-off 10,000MW) were obtained from Whatman (UK). 2, 4, 6-trinitrobenzene sulfonic acid (TNBSA) were acquired from Fluka. Papaverine was received from pharmacy. All others materials were

purchased from Merck (Germany). All solutions were prepared with deionized water.

In vitro glycation of HSA

HSA 10mg/ml were incubated in PBS 50 mM (pH7.4) containing glucose 40 mM and different concentration of Naloxone 25,100,250µM for 42 days at 37˚C. In addition, HSA were incubated with and without glucose as glycated (H+G sample) and as control (H sample), respectively After the 42 days, the samples were dialyzed extensively against PBS at 4˚C and stored at -20˚C [13].

AGE-specific fluorescence

The AGE-fluorescence of all samples (1mg/ml) were obtained on a Cary Eclipse fluorescence spectrophotometer at Ex/Em (380/390-540 nm). Each point represents the mean of three independent experiments [14].

Statistical analysis

The results were expressed as Mean ± SEM. The significance of the positive effect of Papaverine was determind by a chi-square test and the results were regarded as significant at $P \leq 0/05$.

RESULTS

The far-UV CD spectrum of HSA is recognized by the presence of two strong negative bands at 208 and 222 nm, which represent the helical characteristics of HAS(see H sample in Fig,1). As seen in Fig. 1, there is a loss of helical structure as shown by a decrease in the negative ellipticity at 208 and 222 nm due to the 42-day incubation of glucose with HSA (H+G) compared to the control (H). HSA in the presence of glucose and different Naloxone concentration show significantly more negative ellipticity values at 208 and 222 nm on the 42 day of incubation in comparison with glycated sample (H+G). Fig. 3 and 4 show the enhancement in fluorescence intensity in glycated HSA (H+G) compared to the control HSA (H). The results indicate that HSA incubated with glucose and different Naloxone concentration show a decrease in fluorescence intensity compared with glycated HSA (H+G).

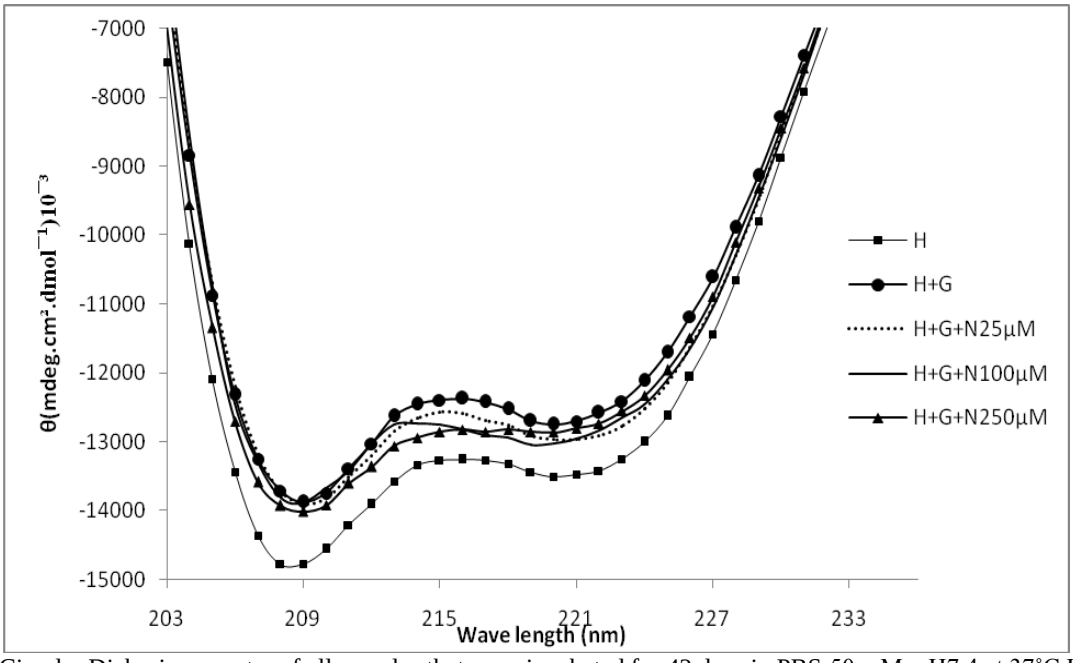

Figure 1. Circular Dichroism spectra of all samples that were incubated for 42 days in PBS 50 mM, pH7.4 at 37˚C.H, G and N indicate HSA, Glucose and Naloxone respectively.

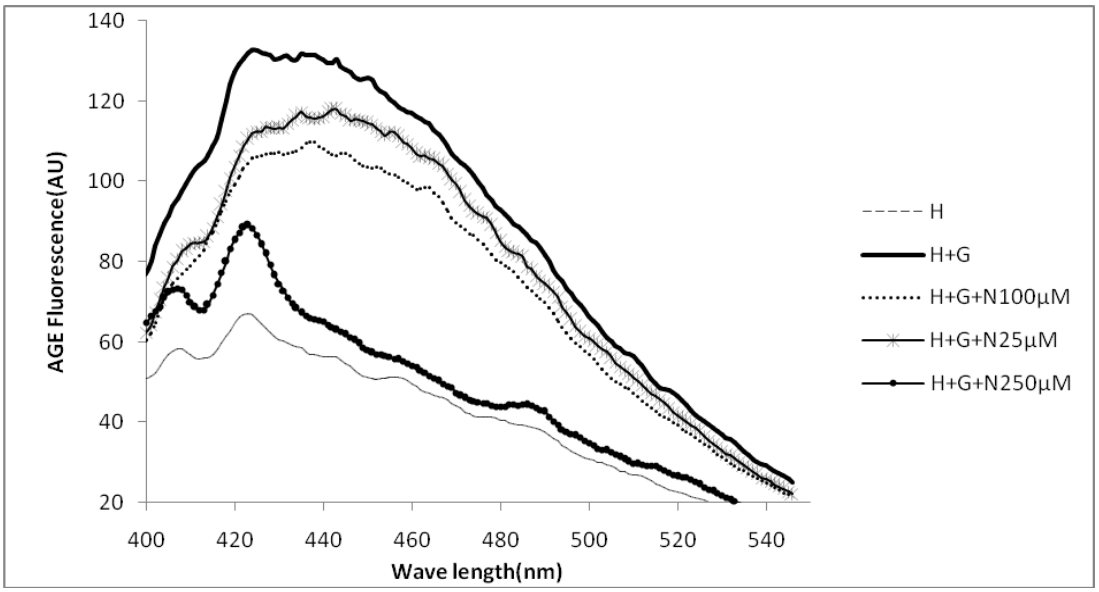

Figure 2. AGE fluorescence spectra of all samples that were incubated for 42 days in PBS50 mM, pH7.4 at 37˚C Ex/Em (380/390-550nm) .H, G and N are the same as Figure1.

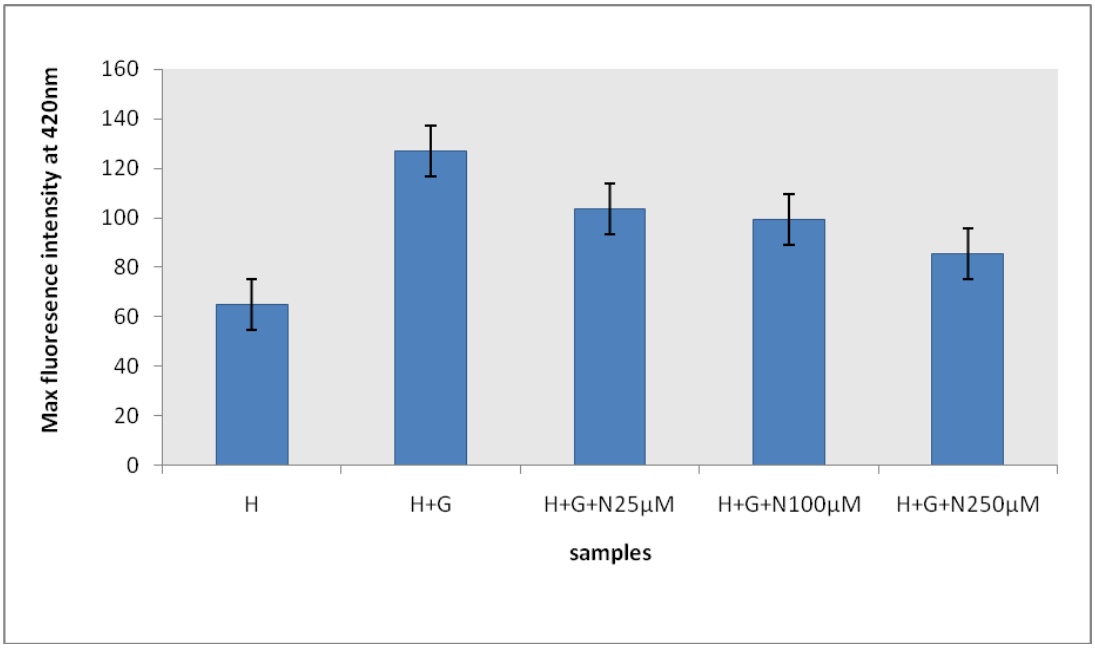

Figure 3. Maximum AGE fluorescence spectra of all samples that were at 420 nm. H, G and N are the same as Figure1.

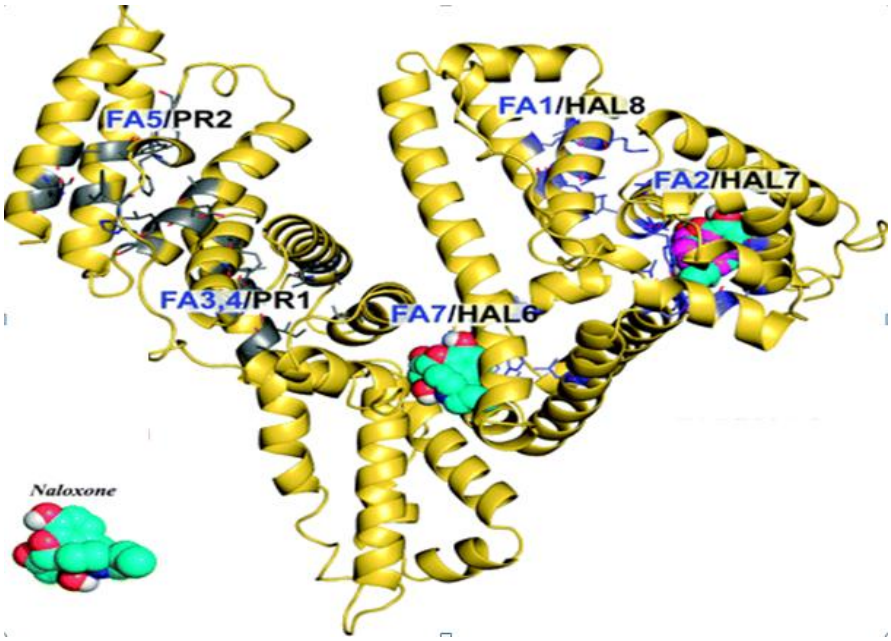

Figure 4. Naloxone can get attach to halothane site in human serum albumin [15]

As it is shown in figure 3, previous study has shown that Naloxone has ability to interact with human serum albumin. In accordance with earlier study Naloxone is connected to the halothane site in HSA [15].

DISCUSSION

The AGEs are formed as the result of the Reducing sugars reaction with proteins [16]. Because of long life period and high concentration HSA in plasma; it is one of the most important targets for glycation reaction. Naloxone is made up of thebaine that is obtained of plant *papaver somniferous.* Naloxone prevents or reverses the effects of opioid including respiratory depression, sedation and hypotension.
Also, Naloxone can reverse the psychotomimetic and dysphoric effects of agonist-antagonists, such as pentazocine [9]. An extensive change in the secondary structure of proteins takes place after the glycation process; it means glycation induces α-helix to β-sheet transition that is detectable with Circular Dichroism (CD)[18]. CD spectrum of HSA is characterized by present two strong negative bands at 208 and 222 nm in control(H sample) but in glycated sample (H+G) these two negative band have decreased as a result of glycation [3]. In comparison with glycated sample (H+G), samples which contain HSA with glucose and Naloxone show enhance in negative band.
The fluorescence, as an index of advanced glycation, increased linearly over time for HSA incubated with reducing sugar; it means glycated protein has fluorescence property [17, 19,20,21]. On the other hand at those Figures, samples with HSA plus glucose and Naloxone show decrease in fluorescence feature compared with glycated sample (H+G).we think HSA in present Naloxone can be protected against Glycation reaction because it prevent attachment between HSA and sugar [15].

CONCLUSION

The main goal of this study was to examine antiglycation activity of Naloxone on pathway of AGEs formation and this data were acquired by Circular Dichroism, fluorescence. Circular Dichroism and fluorescence have confirmed in samples which have HSA plus Naloxone and glucose, Naloxone inhibit reaction between HSA and glucose. So it seems that it has antiglycation activity. As previous study has shown Naloxone get attach to halothane site in HSA [15].
It seems Naloxone through attachment to HSA prevent glycation reaction between HSA and glucose. Overally it can be concluded that

Naloxone can inhibit glycation reaction so it is an AGE inhibitor. Finally it can be summarized that Naloxone inhibits glycation of HSA and leads to decrease AGE formation.

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