

Full Length Research Paper

Effect of two herbal polyphenol compounds on human amylin amyloid formation and destabilization

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Human Islet amyloid polypeptide gathering in the β -cell of the pancreas has been implicated in the pathology of type 2 diabetes. Inhibition of the formation of amylin fibrils could be therapeutic aims for the treatment of type 2 diabetes mellitus. In this study, the fluorimetric assay was used to examine the role of two herbal polyphenol compounds including Rosmarinic acid and Curcumin on amyloid formation and destabilization of human amylin amyloid under near-physiological circumstances. The results showed that after 192 h incubation by shaker incubator at 37°C, Rosmarinic acid at 10 and 40 μ M repressed amylin amyloid formation by 18.6 and 19.3% respectively ($P<0.05$), and the similar values of Curcumin inhibited the formation of β -sheet structure by 17.2 and 29.9%, respectively ($P<0.05$). The obtained data also confirmed that amyloidal sheet opening was induced by Rosmarinic acid and Curcumin significantly ($P<0.05$). It was concluded that islet amyloid cytotoxicity to β -cells may be reduced by these two herbal extracts and these compounds should be key molecules for the development of the therapeutics for diabetes mellitus.

Key words: Amylin, diabetes, rosmarinic acid, curcumin, amyloid.

INTRODUCTION

Human islet amyloid polypeptide also called amylin or diabetes-associated peptide is normally formed by pancreatic beta cell and co-secreted with insulin in to blood circulation (Sasahara, 2010). Amylin is a 37-amino-acid peptide hormone (MW: 3.9 kDa) (Figure 1) and contributes to glycemic control (Lee et al., 2011; Lutz, 2010). The relationship between amylin deposition and the development of type 2 diabetes has been known. Recent data indicated that amyloid deposition can be toxic to β -cells and induce the cell-death (Brender et al., 2008). Therefore preventing amylin amyloidogenesis should be therapeutic aims for the treatment of type 2 diabetes mellitus. Rosmarinic acid (Figure 2) is a phenolic derivative of caffeic acid, found in many *Lamiaceae* herbs used commonly as culinary herbs such as lemon balm, rosemary, oregano, *Salvia officinalis*, thyme and peppermint (Maroo et al., 2002). Rosmarinic acid has a number of biological characteristics such as

antiviral, antibacterial, anti-inflammatory and antioxidant. It is also a potential anxiolytic as it acts as a GABA transaminase inhibitor. The presence of rosmarinic acid in medicinal plants, herbs and spices has beneficial and health promoting effects. In plants, rosmarinic acid is supposed to act as a preformed constitutively accumulated defense compound (Petersen, 2003). Curcumin (Figure 2) is the active component of the nutritional spice turmeric and has been used for medical purposes for thousands of years (Gupta et al., 2011). In vitro and animal studies have demonstrated that Curcumin has antitumor (Strofer, 2011), anti-ischemic and anti-inflammatory properties (Shukla, 2008; Aggarwal, 2006). Curcumin acts as a free radical scavenger and antioxidant, inhibiting lipid peroxidation and oxidative DNA damage (Shukla, 2003). The main objective of the present study was to investigate the possible roles of different concentrations of two herbal polyphenol compounds named Rosmarinic acid and Curcumin on human amylin amyloid formation and destabilization, *in vitro*.

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MATERIALS AND METHODS

Human amylin peptide and other materials were prepared from

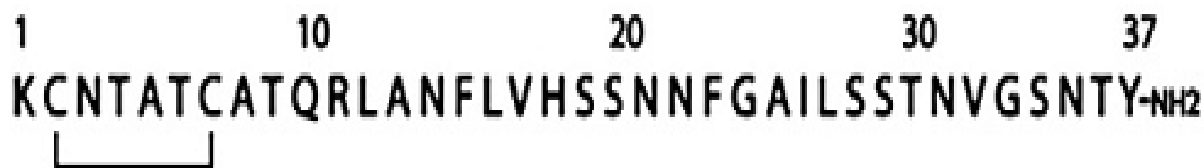
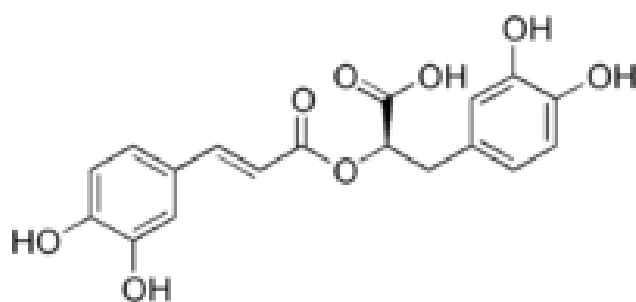
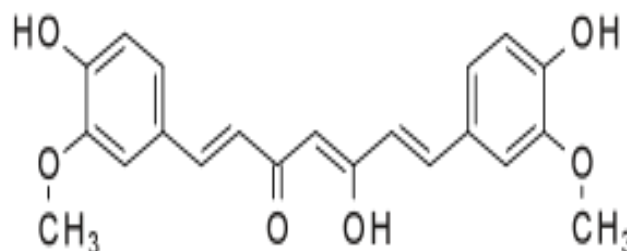


Figure 1. Primary sequence of human-IAPP including the Disulfide Bridge between Cys2–Cys7 and amidated C-terminus.



Rosmarinic acid



Curcumin

Figure 2. Structures of Rosmarinic acid and Curcumin.

Sigma-Aldrich Company.

Amylin stock solution

Human amylin used in this project had the following characteristics: (1-37)(Lys-Cys-Asn-Thr-Ala-Thr-Cys-Ala-Thr-Gln-Arg-Leu-Ala-Asn-Phe-Leu-Val-His-Ser-Ser-Asn-Asn-Phe-Gly-Ala-Ile-Leu-Ser-Ser-Thr-Asn-Val-Gly-Ser-Asn-Thr-Tyr-NH₂, intra-molecular disulfide bridge: between Cys2 and Cys7). Its purity was 97% and the lyophilized salt included 70% peptide by weight. Amylin stock solution was prepared by adding 1.0 ml dimethylsulfoxide (DMSO) to dry purified peptide, sonicating at room temperature for 15 min. Experiments were performed in the two different phases as follows.

The first series of experiment

The peptide stock solution was diluted by PBS 50 mM, pH: 7.5, to the final concentration of 10 μ M. In order to assay for the effects of different concentrations of Rosmarinic acid and Curcumin on amylin aggregation, control and treated groups were designed as follows. PBS buffer containing only 10 μ M amylin, without any Rosmarinic acid and Curcumin was selected as the control group. 10 and 40 μ M concentrations of each of the two herbal compounds were prepared in PBS buffer containing 10 μ M amylin, separately and considered as treated groups. All studied groups were incubated at 37°C for 192 h with shaking by a shaker incubator (GFL 3031, Germany).

The second series of experiments

The second series of experiments were carried out to elucidate the destabilizing effect of Rosmarinic acid and Curcumin on preformed amyloid sheet of amylin. For this purpose, prepared amyloid from

the previous step was used. Amyloid was incubated with different concentrations of each of the agents for 6 h at 37°C.

Amyloid formation and destabilization assay

To determining the level of amyloid beta-pleated sheets at the end of the two series of experiments, Thioflavin T (ThT) fluorescent assay was used. Thioflavin T assay was performed by adding 40 μ l of each incubated solution to 700 μ l of 10 μ M ThT solution. Fluorescence measurements were recorded in a Perkin-Elmer LS55 fluorescence spectrometer (Perkin-Elmer LS55, USA) at room temperature using a 1 cm path length quartz cell. The ThT signal was quantified by averaging the fluorescence emission at 485 nm (slit width = 10 nm) when excited at 440 nm (slit width = 5 nm).

Statistical analysis

Descriptive statistics was accomplished to obtain means and standard deviations. Statistic significance level was established at $P < 0.05$. Analysis of data was performed using SPSS statistical software package.

RESULTS AND DISCUSSION

The first series of experiments showed that amylin itself readily aggregated and formed a ThT-positive material in control group. Data indicated that at zero time, ThT-fluorescence mean value for control group was 34.02 which at 192 h had increased to mean value of 51.07 ($P < 0.05$). In Rosmarinic acid treated groups, ThT fluorescence assay indicated that, 10 and 40 μ M of

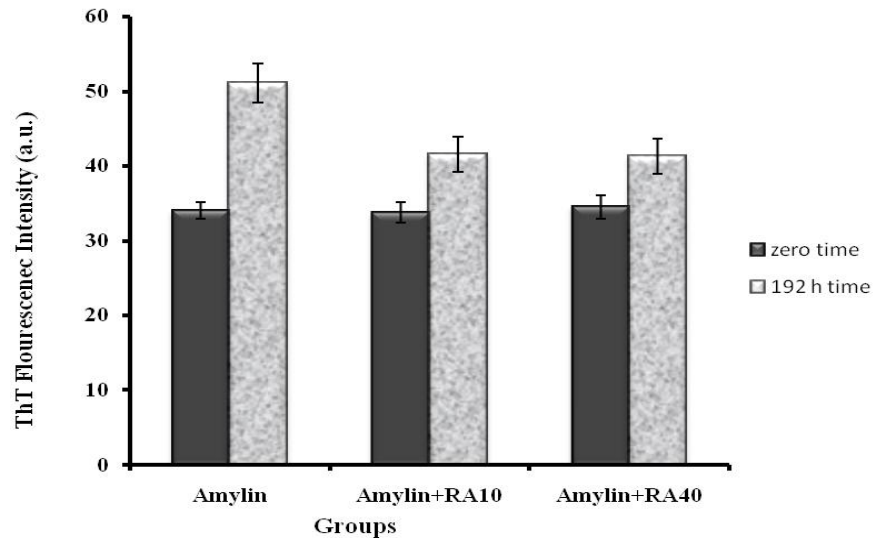


Figure 3. Thioflavin T fluorescence assay of protective effects of Rosmarinic acid on amylin deposition. All groups were incubated at 37°C for 192 h with shaking by a shaker incubator. At zero time (before incubation) there were no significant differences between three groups: amylin, amylin+RA 10 and amylin+RA40 ($P>0.05$). Rosmarinic acid (RA) inhibited amylin aggregation. Data have been shown as Mean \pm SEM, n=5.

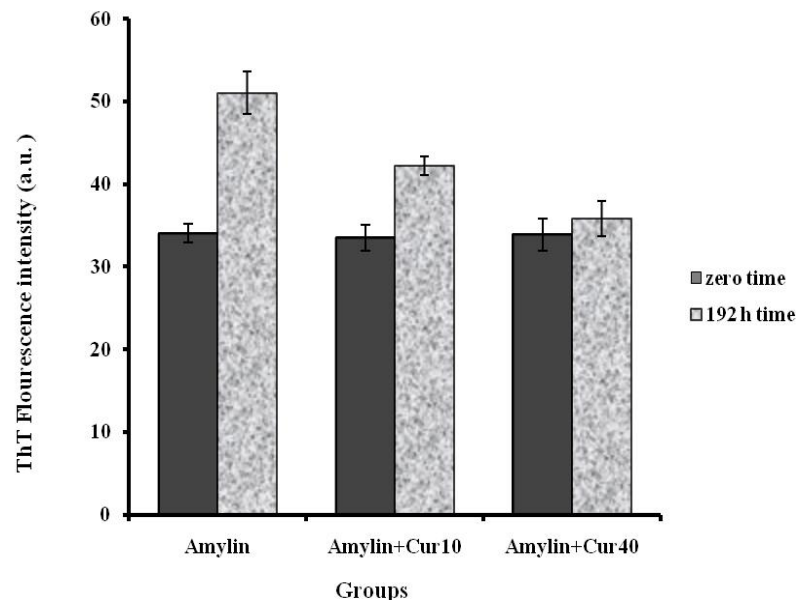


Figure 4. Thioflavin T fluorescence assay of protective effects of Curcumin on amylin aggregation. All groups were incubated at 37°C for 192 h with shaking by a shaker incubator. At zero time (before incubation), there were no significant differences between three groups: amylin, amylin+Cur 10 and amylin+Cur40 ($P>0.05$). Curcumin (Cur) inhibited amylin aggregation in a dose-dependent manner. Data have been shown as Mean \pm SEM, n=5.

Rosmarinic acid inhibited amyloid formation by 18.6 and 19.2% respectively after 192 h incubation at 37°C ($P<0.05$) (Figure 3). Different concentrations effects of

Curcumin on amylin aggregation were demonstrated in Figure 4. These data compared to control group indicated that, ThT-fluorescence was increased significantly in the

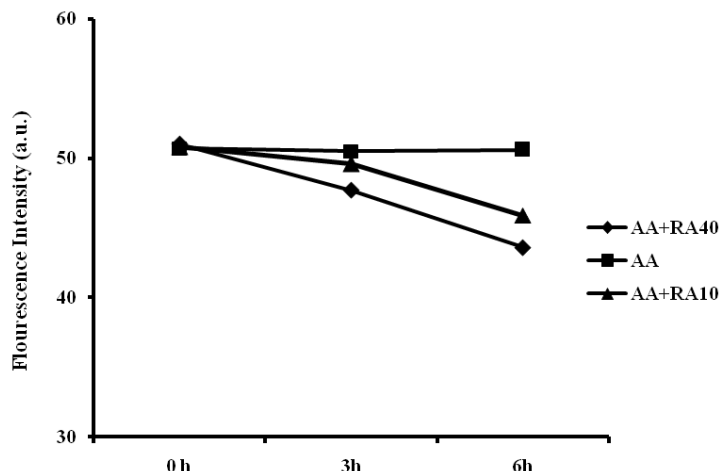


Figure 5. Rosmarinic acid effect on amylin fibril destabilization. Rosmarinic acid with two different concentrations destabilized amylin fibril significantly after 6 h incubation.

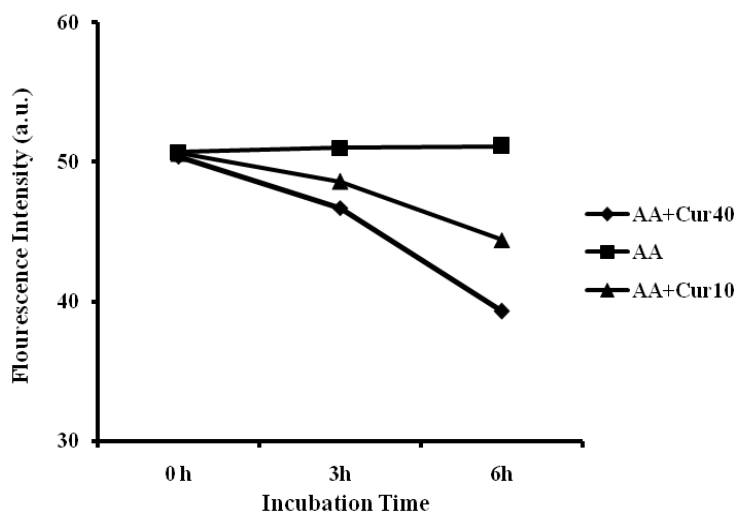


Figure 6. Curcumin effect on amylin fibril destabilization. Curcumin with two different concentrations destabilized amylin fibril significantly after 6 h incubation in a dose dependent manner.

presence of 10 and 40 μM of Curcumin by 17.3 and 29.9%, respectively ($P < 0.05$). It was very interesting that by increasing of Curcumin concentration, inhibitory effect of this herbal component was elevated significantly ($P < 0.05$) but Rosmarinic acid did not show dose dependent effect ($P > 0.05$). Amyloid destabilizing effects of these components were shown in Figures 5 and 6. The obtained data from the 2nd run of experiments confirmed that both compounds were able to open the amyloid sheet significantly ($P < 0.05$). Diabetes mellitus persists to generate a significant burden on healthcare services worldwide because of its high prevalence, so multidisciplinary study aimed at preventing and treating is

one of the world-wide research priorities. It is implicated that human amylin is a small fibrillogenic protein, that is, the major constituent of pancreatic islet amyloid, which occurs in most subjects with type 2 diabetes (Konarkowska et al., 2006 Zheng et al., 2010 and Wang et al., 2011). Although, the amyloid contribution to islet β -cell dysfunction is well clarified in the diabetic patients, the factors affecting this process remain elusive. Amylin is degraded by insulin-degrading enzyme which also takes part in the elimination of insulin (Shen et al., 2007). There are evidences that some essential and toxic elements influence the aggregation of amylin (Mirhashemi et al., 2011a, b, c; Ward et al., 2008) *in vitro*.

Since there is no data in the literature concerning the effect of Rosmarinic acid on amylin amyloidogenesis, and documents regarding correlation between Curcumin and amylin depositions are very little (Daval et al., 2010), thus, the present study was designed. This project showed significant inhibitory role for Rosmarinic acid and Curcumin concerning amylin amyloidogenesis and so demonstrated the β -sheet opening ability for these compounds. It was indicated that Curcumin induced its effect in a concentration-dependent manner but Rosmarinic acid effect was not dose dependent.

The formation of amyloid fibrils, via self-assembly of peptide, is assumed to be a crucial step in the pathogenesis of many amyloid diseases, including type-2 diabetes mellitus (Tabner, 2001). Previous investigations have shown that fibrillization of several polypeptides such as amylin is accompanied by formation of free radicals. In turn, reactive oxygen species (ROS), mainly free radicals, accelerate fibril formation, possibly via oxidation reactions; so that, the free radicals formed during amyloid fibrillization enhance fibrillization (Schoneich, 2005; Shoval et al., 2007).

ROS may impact disulfide bond formation (Cumming et al., 2004) and subsequently influence the development of IAPP misfolding. Disulfide bonds are important for proper protein structure, biological activity, and stability of many secreted and membrane proteins (Kopito and Ron, 2000; Anelli et al., 2000; Fassio and Sitia, 2002). Although, the exact mechanism by which Rosmarinic acid and Curcumin inhibit amyloid formation and destabilize preformed amyloid in vitro remains unclear, it may be suggested that the inhibitory power of these compounds in amyloid fiber formation may be due to their antioxidant properties. Further study is required to elucidate the exact mechanism.

Conclusion

Using fluorescence spectroscopic analysis with thioflavin T, we examined the effects of Rosmarinic acid and Curcumin on the formation, and destabilization of amylin amyloid fibril at pH 7.5 at 37°C in vitro. We expressed that these two compounds inhibited amylin amyloid formation significantly. In addition, they destabilized preformed amylin aggregates. These compounds should be key molecules for the development of the therapeutics for diabetic patients.

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