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To Study Various Concentrations of Magnesium and Aluminium on Amylin Hormone Conformation

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Abstract: Type 2 diabetes mellitus can be defined as a conformational disease since a beta cell producing protein called amylin undergoes a change in the tertiary structure followed by self-aggregation and deposition. Amylin deposition causes destruction of pancreatic β-cells. The aim of this study was to investigate whether different concentrations of magnesium and aluminium alter amylin conformation under near-physiological circumstances. Conformational variations were monitored by fluorescent method before and after incubation by shaker incubator in 37°C by LS55 spectrofluorometer instrument. This *in vitro* study showed that magnesium had contradictory effects on amylin folding and these effects were magnesium concentration dependent. Magnesium with concentration of 1 to 1.5 mM had inhibitory effect but in 2.5 to 3.5 mM promoted amylin misfolding significantly ($p<0.05$). The obtained data also demonstrated that aluminium with concentrations of 5, 10 and 20 μM had stimulatory effects on formation of beta-amyloid sheet significantly ($p<0.05$). It may be concluded that islet amyloid misfolding and cytotoxicity to β-cells might be magnesium dose dependent in diabetic patients.

Key words: Elements, human islet amyloid polypeptide, diabetes mellitus, conformational disease, amylin aggregation, protein folding

INTRODUCTION

Protein conformational disease results from misfolding and aggregation of the protein in non-native structure. This is a distinctive of the systemic amyloidosis such as type 2 diabetes mellitus (Carrell and Lomas, 1997; Hayden *et al*., 2005). Prominent feature of diabetes mellitus is hyperglycemia. Hyperglycemia is possible due to lack of insulin and its wrong performance in tissues that may be lead to diabetic complications for instance atherosclerosis (Naqshbandi *et al*., 2008). It is documented that numerous factors for example aging, obesity and oxidative stress may be important in etiology of diabetes mellitus (Houstis *et al*., 2006; Roberts and Sindhu, 2009). Magnesium (Mg) is the most prevalent cation in the cell that has crucial role in basic biological reactions (Saris *et al*., 2000; Delva *et al*., 2002). Diabetes Mellitus (DM), is the most likely related illness to body magnesium diminution (Sales and Pedrosa, 2006; Larsson and Wolk, 2007; Barbagallo and Dominguez, 2007). It has been implicated that Mg has a main regulatory role in insulin function and better glucose metabolism (Lee *et al*., 2009). Aluminium (Al) is a particularly widespread element in surroundings that human are exposed to it easily (Kumar and Gill, 2009). Corn, yellow cheese, tea (Ochmanski and Barabasz, 2000) and pharmaceutical products for example Al based phosphate binders or antacid drugs (Reinke *et al*., 2003) are the major source of aluminium. Al is also used for water purification (Ochmanski and Barabasz, 2000). Lipid peroxidation, reduced antioxidant capacity and pro-oxidative property have been reported for aluminium (Yousef *et al*., 2004; Newairy *et al*., 2009; Exley, 2004). Also, variations in kinetic behaviors of the alkaline phopsphatase isoenzymes of rat has been documented following Al toxicity (Mirhashemi *et al*., 2010).

On the other hand, Amyloid polypeptide is typically established in pancreatic islet of patients suffering diabetes mellitus type 2 (Zheng *et al*., 2010). Amylin is a 37-amino-acid peptide hormone that is normally secreted from the β-cells along with insulin in to blood circulation. Amylin hormone decreases food intake and inhibits pancreatic glucagon hormone discharge and contributes to glycemic control (Cummings and Overduin, 2007; 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

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cell-death (Rhoades *et al*., 2000). For this reason, the main objective of the present study was to investigate the possible roles of different concentration of magnesium and aluminium concerning human amylin hormone conformation and folding, *in vitro*.

MATERIALS AND METHODS

This research project was conducted from March, 2010 to January, 2011 in Kashan University of Medical Sciences, Kashan, I.R. Iran.

Human amylin peptide and other materials were prepared from 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.

Groups designing: In order to assay the effects of different concentrations of Mg and Al on amylin peptide folding and aggregation, control and treated groups were considered. The peptide stock solution was diluted by modified Krebs-Hensleit (KH) buffer (Nacl:123.5 Mm, Glucose: 11.0 Mm, 1.4 mM: $CaCl_2$, 0.05%W/V: NaN_3), pH: 7.4, to the final concentration of 0.8 μM. Different concentrations of Mg (0.5, 1, 1.5, 2.5, 3.5 mM) and Al (0.12, 0.52, 5, 10, 20 μM) were prepared in KH-buffer containing 0.8 μM amylin as treated groups, separately. The samples without Mg and Al were selected as the control group.

Incubating manner: All studied groups were incubated at 37°C for 288 h with shaking by a shaker incubator (GFL 3031, Germany).

Conformational and folding monitoring: To identify the conformational changes and formation of beta-pleated sheets of amyloid, intrinsic and thioflavin T (ThT) fluorescent assay were used.

ThT assay: Thioflavin T assay was performed by adding 40 μL of each incubated solution to 700 μL of 10 μM 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).

If assay: The intrinsic fluorescence of the peptide tyrosine residue was measured for the studied groups after 168 h averaging the fluorescence emission at 304 nm when excited at 270 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

Data indicated that before incubation (at zero time), Th T-fluorescence mean value for each control group (Mg = 0 and Al = 0) was 80.97 a.u. which at the end of incubation (at 288 h) had increased to 101.1 a.u. (Fig. 1, 2). There was statistically significant difference in ThT-fluorescence mean value before and after incubation for each control group (Mg = 0 and Al = 0) ($p<0.05$) (Fig. 1, 2). The data demonstrated that amylin itself readily folded and formed a ThT-Positive material in Mg = 0 and Al = 0 group (Fig. 1, 2). In Mg treated groups (Fig. 1), ThT fluorescence assay showed that compared to Mg = 0 as control group, 0.5 mM of Mg had no significant effect on amylin conformation

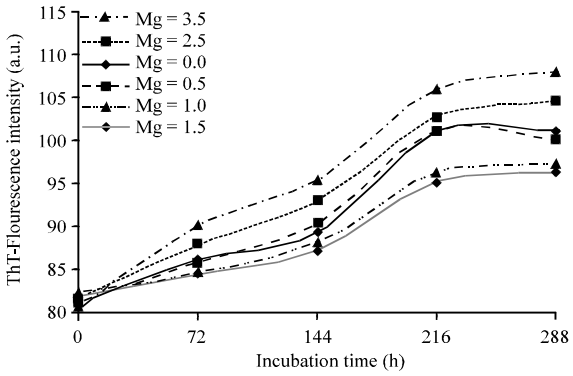

Fig. 1: Effect of different concentrations of magnesium on human amylin hormone conformation. Changes in conformation were monitored by ThT fluorescence in the absence and presence of different concentrations of magnesium for 288 h at 37°C. 1 and 1.5 mM of magnesium inhibited amylin misfolding whereas 2.5 and 3.5 mM stimulated misfolding. Mg = 0 is equal to control group

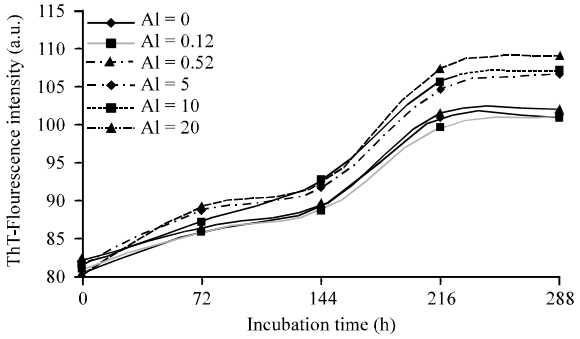

Fig. 2: Effect of different concentrations of aluminium on human amylin hormone conformation. Changes in conformation were monitored by ThT fluorescence in the absence and presence of different concentrations of aluminium for 288 h at 37°C. Aluminium stimulated amylin misfolding and this effect was dose dependent. Al = 0 is equal to control group

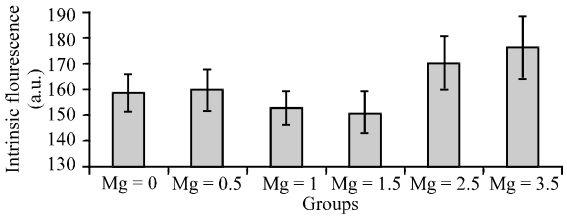

Fig. 3: Intrinsic fluorescence of the control and magnesium treated groups. Tyrosine intrinsic fluorescence of amylin solutions in the absence and presence of the different concentrations of magnesium (0.5, 1, 1.5, 2.5, 3.5 mM) was measured after 168 h incubation in 37°C. Magnesium showed dual effect on amylin hormone conformation and folding. Data have been shown as Mean±SD, n = 6. Mg = 0 is equal to control group

(p>0.05) whereas 1 and 1.5 mM concentrations of Mg significantly (p<0.05) inhibited amylin misfolding by 3.8 and 4.6%, respectively after 288 h incubation at 37°C (Fig. 1). It was very interesting that by increasing of Mg concentration, inhibitory effect of this element was inversed so that 2.5 and 3.5 mM of magnesium elevated the ThT- fluorescence by 4.6 and 7.4%, respectively at the end of incubation time, significantly when compared with Mg = 0 group (p<0.05) (Fig. 1). Figure 2 presents different concentrations effects of Al (0.12, 0.52, 5, 10, 20 μM) on amylin folding. These data indicated that compared to control group (Al = 0), 0.12 and 0.52 μM of Al had no significant effect on ThT-fluorescence of amylin (p>0.05),

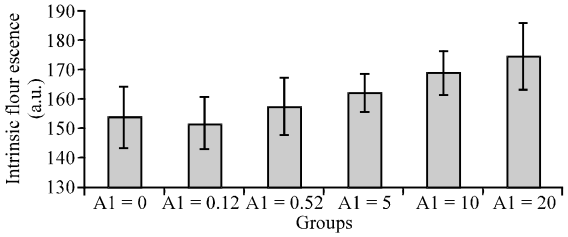

Fig. 4: Intrinsic fluorescence of the control and aluminium treated groups. Tyrosine intrinsic fluorescence of amylin solutions in the absence and presence of the different concentrations of aluminium (0.12, 0.52, 5, 10, 20 μM) was measured after 168 h incubation in 37°C. Aluminum showed one type effect on amylin hormone conformation and folding. Data have been shown as Mean±SD, n = 6. Al = 0 is equal to control group

whereas by increasing of Al concentration to 5, 10 and 20 μM, ThT-fluorescence was increased significantly (p<0.05). 5, 10 and 20 μM of Al induced amylin aggregation and misfolding by 5.8, 6 and 8% related to control group (Al = 0) at 288 h incubation (p<0.05) (Fig. 2). Intrinsic Fluorescence (IF) of human amylin hormone was measured for different groups. Mg had dual effects on IF so that 1.5 mM of this element had the most reducing property (p<0.05) while 3.5 mM concentration of Mg increased IF significantly (p<0.05) (Fig. 3). IF was increased in All treated group proportion to Al concentration. Aluminium with concentration of 20 μM had the most effect on human amylin hormone structure just as measured by IF test (p<0.05) (Fig. 4).

Owing to the increasing prevalence of diabetes, multi disciplinary study with focusing on preventing and treatment of the disease is one of the world-wide research priorities. It is implicated that lack of insulin secreting cells in pancreatic islet of type 2 diabetic patients is related to amyloid depositions resulted from amylin misfolding (Zheng *et al*., 2010). Although, the amyloid formation is well clarified in the diabetic patients, the factors affecting this process remain elusive. Generally, trace elements levels vary in patients suffering diabetes mellitus (Aguilar *et al*., 2007; Viktorinova *et al*., 2009). It has been expressed that a number of elements have impaired metabolism in the diabetic patients and so precise impress in the pathogenesis of diabetes (Meyer and Spencem, 2009; Valko *et al*., 2005; Mirhashemi and Shahabaddin, 2011; Ward *et al*., 2008). The present study was designed because magnesium has a main regulatory effect on insulin function (Lee *et al*., 2009) and so there isn't any data concerning effect of this element on amylin folding.

These findings showed that different concentrations of Mg had dual effects on amylin conformation and aggregation but Al only stimulated misfolding in a dose dependent manner ($p<0.05$), (Fig. 1, 2). As mentioned in method section of this article, the peptide had an intra-molecular disulfide bridge between Cys2 and Cys7. Disulfide bonds are important for proper protein folding and biological activity (Kopito and Ron, 2000; Anelli *et al*., 2002; Fassio and Sitia, 2002). It may be deduced that low level of Mg inhibited amylin misfolding by following possible mechanism: (1) affecting on the intra molecular disulphide bond, (2) increasing the lag-time for fiber formation and (3) declining the amylin addition rate to preformed fibers. The opposite effects might be occurred at higher concentrations of Mg just as described for dual effects of zinc on amylin deposition (Brender *et al*., 2010). Oxidative stress is an important factor in the pathogenesis of diabetes mellitus (Ryu *et al*., 2008; Viktorinova *et al*., 2009). The role of Mg and Al in induction of amyloidogenesis may be result from stimulating of Reactive Oxygen Species (ROS) generation by these elements. ROS may impact the disulfide bond (Cumming *et al*., 2004) and subsequently influence the development of amylin misfolding. Also it is possible that high levels of Mg and Al contribute in amylin dimerization. This process should lead to amylin misfolding and deposition (Wiltzius *et al*., 2009).

CONCLUSION

In conclusion, the present results established that magnesium and aluminium could induce conformational changing in human amylin hormone, *in vitro*. For the first time in literature, this study showed that magnesium had a dual effect on amylin hormone folding. Magnesium had inhibitory and stimulatory roles on the hormone folding and this effect was dose dependent.

REFERENCES

Aguilar, M.V., P. Saavedra, F.J. Arrieta, C.J. Mateos, M.J. Gonzalez, I. Meseguer and M.C. Martinez-Para, 2007. Plasma mineral content in type-2 diabetic patients and their association with the metabolic syndrome. Ann. Nutr. Metab., 51: 402-406.

Anelli, T., M. Alessio, A. Mezghrani, T. Simmen, F. Talamo, A. Bachi and R. Sitia, 2002. ERp44, a novel endoplasmic reticulum folding assistant of the thioredoxin family. EMBO J., 21: 835-844.

Barbagallo, M. and L.J. Dominguez, 2007. Magnesium metabolism in type 2 diabetes mellitus, metabolic syndrome and insulin resistance. Arch. Biochem. Biophys., 458: 40-47.

Brender, J.R., K. Hartman, R.P. Nanga, N. Popovych and R. de la Salud Bea *et al*., 2010. Role of zinc in human islet amyloid polypeptide aggregation. J. Am. Chem. Soci., 139: 8973-8983.

Carrell, R.W. and D.A. Lomas, 1997. Conformational disease. Lancet, 350: 134-138.

Cumming, R.C., N.L. Andon, P.A. Haynes, M. Park, W.H. Fischer and D. Schubert, 2004. Protein disulfide bond formation in the cytoplasm during oxidative stress. J. Biol. Chem., 279: 21749-21758.

Cummings, D.E. and J. Overduin, 2007. Gastrointestinal regulation of food intake. J. Clin. Invest., 117: 13-23.

Delva, P., M. Degan, C. Pastori, G. Faccini and A. Lechi, 2002. Glucose induced alterations of intracellular ionized magnesium in human lymphocytes. Life Sci., 71: 2119-2135.

Exley, C., 2004. The pro-oxidant activity of aluminum. Free Rad. Biol. Med., 36: 380-387.

Fassio, A. and R. Sitia, 2002. Formation, isomerisation and reduction of disulphide bonds during protein quality control in the endoplasmic reticulum. Histochem. Cell. Biol., 117: 151-157.

Hayden, M.R., S.C. Tyagi, M.M. Kerklo and M.R. Nicolls, 2005. Type 2 diabetes mellitus as a conformational disease. J. Pancreas (Online), 6: 287-302.

Houstis, N., E.D. Rosen and E.S. Lander, 2006. Reactive oxygen species have a causal role in multiple forms of insulin resistance. Nat., 440: 944-948.

Kopito, R.R. and D. Ron, 2000. Conformational disease. Nat. Cell. Biol., 2: E207-E209.

Kumar, V. and K.D. Gill, 2009. Aluminium neurotoxicity: Neurobehavioural and oxidative aspects. Arch. Toxicol., 83: 965-978.

Larsson, S.C. and A. Wolk, 2007. Magnesium intake and risk of type 2 diabetes: A meta-analysis. J. Internal Med., 262: 208-214.

Lee, H.J., Y.H. Choe, J.H. Lee, Y.B. Sohn and S.J. Kim *et al*., 2011. Delayed response of amylin levels after an oral glucose challenge in children with Prader-Willi syndrome. Yonsei. Med. J., 52: 257-262.

Lee, S., H.K. Park, S.P. Son, C.W. Lee, I.J. Kim and H.J. Kim, 2009. Effects of oral magnesium supplementation on insulin sensitivity and blood pressure in normo-magnesemic nondiabetic overweight Korean adults. Nutr. Metab. Cardiovascular Dis., 19: 781-788.

Lutz, T.A., 2010. Roles of amylin in satiation, adiposity and brain development. Forum. Nutr., 63: 64-74.

Meyer, J.A. and D.M. Spencem, 2009. A perspective on the role of metals in diabetes: Past findings and possible future directions. Metallomics, 1: 32-41.

Mirhashemi, S.M. and M.E. Shahabaddin, 2011. Evaluation of aluminium, manganese, copper and selenium effects on human islets amyloid polypeptide hormone aggregation. Pak. J. Biol. Sci., 14: 288-292.

Mirhashemi, S.M., A.A. Moshtaghie, M. Ani and M.H. Aarabi, 2010. *In vivo* and *in vitro* studies of kinetic changes of serum, liver and brain high and low molecular weight alkaline phosphatase following aluminium exposure in rat. Asian J. Biochem., 5: 1-11.

Naqshbandi, M., S.B. Harris, J.G. Esler and F. Antwi-Nsiah, 2008. Global complication rates of type 2 diabetes in Indigenous peoples: A comprehensive review. Diabetes Res. Clin. Pract., 82: 1-17.

Newairy, A.S., A.F. Salama, H.M. Hussien and M.I. Yousef, 2009. Propolis alleviates aluminium induced lipid peroxidation and biochemical parameters in male rats. Food. Chem. Toxicol., 47: 1093-1098.

Ochmanski, W. and W. Barabasz, 2000. Aluminium-occurrence and toxicity for organisms. Przegl. Lek., 57: 665-668.

Reinke, C.M., J. Breitkreutz and H. Leuenberger, 2003. Aluminium in over-the-counter drugs: Risks outweigh benefits?. Drug Safety, 26: 1011-1025.

Rhoades, E., J. Agarwal and A. Gafni, 2000. Aggregation of an amyloidogenic fragment of human islet amyloid polypeptide. Biochim. Biophys. Acta, 1476: 230-238.

Roberts, C.K. and K.K. Sindhu, 2009. Oxidative stress and metabolic syndrome. Life Sci., 84: 705-712.

Ryu. S., A. Ornoy, A. Samuni, S. Zangen and R. Kohen, 2008. Oxidative stress in Cohen diabetic rat model by high-sucrose, low-copper diet: Inducing pancreatic damage and diabetes. Metabolism, 57: 1253-1261.

Sales, C.H. and L.F. Pedrosa, 2006. Magnesium and diabetes mellitus: Their relationship. Clin. Nutr., 25: 554-562.

Saris, N.E.L., E. Mervaala, H. Karppanen, J.A. Khawaja and A. Lewenstam, 2000. Magnesium. An uptake on physiological, clinical and analytical aspects. Clin. Chim. Acta, 294: 1-26.

Valko, M., H. Morris and M.T.D. Cronin, 2005. Metals, toxicity and oxidative stress. Curr. Med. Chem., 12: 1161-1208.

Viktorinova, A., E. Toserova, M. Krizko and Z. Durackova, 2009. Altered metabolism of copper, zincand magnesium is associated with increased levels of glycated hemoglobin in patients with diabetes mellitus. Metabolism, 58: 1477-1482.

Ward, B., K. Walker and C. Exley, 2008. Copper(II) inhibits the formation of amylin amyloid *in vitro*. J. Inorg. Biochem., 102: 371-375.

Wiltzius, J.J.W., S.A. Sievers, M.R. Sawaya and D. Eisenberg, 2009. Atomic structures of IAPP (amylin) fusions suggest a mechanism for fibrillation and the role of insulin in the process. Protein. Sci., 18: 1521-1530.

Yousef, M.I., A.M. Esmail and H.H. Baghdadi, 2004. Effect of isoflavones on reproductive performance, testosterone levels, lipid per oxidation and seminal plasma biochemistry of male rabbits. J. Environ. Sci. Health B, 39: 819-833.

Zheng, X., W. Ren, S. Zhang, J. Liu and S. Li *et al*., 2010. Serum levels of proamylin and amylin in normal subjects and patients with impaired glucose regulation and type 2 diabetes mellitus. Acta Diabetol., 47: 265-270.