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Protein molecular forms of insulin-like growth factor binding protein-2 change with aging

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ABSTRACT

Aging is considered to be an adaptive mechanism to altered needs of an organism and/or to altered stimuli. Plasma concentrations of insulin-like growth factor binding protein-2 (IGFBP-2) increase with age and it is generally assumed that IGFBP-2 is a negative predictor of healthy aging. The aim of this study was to examine the distribution of IGFBP-2 molecular forms in different age groups and, specifically, the relationship between IGFBP-2 and alpha-2-macroglobulin (α2M). The relative amount of monomer IGFBP-2 was the highest in young persons, making up approximately 2/3 of the total circulating IGFBP-2. This gradually decreased with age down to 1/3 of total IGFBP-2 in elderly individuals. Fragmented IGFBP-2 increased with age and contributed almost 60% to the total immunoreactive IGFBP-2 in the age group 61–80 years. IGFBP-2/α2M complexes represented 10–12% of the total IGFBP-2 in the two younger groups but half this level in the oldest group. The significance of these changes and whether they affect more IGF-dependent or independent interactions are unknown. Due to drastic proteolysis of IGFBP-2, it may be postulated that either over-release of IGFBP-2-bound IGFs causes unwanted events or IGFBP-2 fragments are able to over-stimulate cellular processes.

1. Introduction

Aging is a process that accompanies life itself and the lifespan of every person is limited. Along with aging, the body becomes less able to preserve a homeostatic balance and to accommodate to ever changing external and internal conditions. During this process, the probability of acquiring a disease or dying increases. In the last few decades, development of healthcare and the eradication of widespread and deadly infectious diseases have prolonged the human lifespan. A number of people have been recorded to live more than 120 years (Partridge, 2010).

Many physiological factors play a role in the aging process. One of them, experimentally found to be involved, is the IGF system (Berryman et al., 2008). This system comprises two growth factors (IGF-I and IGF-II), four receptors to which these factors bind [IGF receptor type 1 (IGF-1R), IGF receptor type 2 (IGF-2R), insulin receptor (IR) and the hybrid receptor (IR/IGF-1R)] and a family of six high-affinity binding proteins (IGFBP-1 to -6) (Le Roith, 2003). IGFBPs transport growth factors to their sites of action, preserve them from degradation and potentiate or inhibit IGF activity. IGFBPs are degraded by proteases, thus regulating the bioavailability of IGFs. In general, IGFs stimulate mitosis, cell differentiation and growth, but there are also cell-specific roles (Firth and Baxter, 2002). Certain functions may be beneficial during earlier periods of life, but may become undesirable at older ages (Williams, 1957). It has been discovered that partial inhibition of signaling mediated by IGFs leads to significant increases in the lifespan of invertebrate and vertebrate model organisms (Berryman et al., 2008).

IGFBP-2 is the second most abundant binding protein in the peripheral circulation, after IGFBP-3 (Firth and Baxter, 2002). It is assumed to be a simple protein and there is only one report about the existence of phosphorylated IGFBP-2 as a minor subpopulation (Graham et al., 2007). It exhibits higher affinity for IGF-II than for IGF-I (Oh et al., 1993). IGFBP-2 possesses the special amino acid, so-called RGD sequence (Arg-Gly-Asp), which enables it to bind to integrin receptors and possibly to some other molecules. These interactions may be IGF-independent (Pereira et al., 2004; Srichai and Zent, 2010). Serum IGFBP-2 concentrations rise in acute or chronic non-physiological situations, such as shock, inflammation and various injuries (Wolf et al., 2000). The level also increases in fasting states, especially during protein restriction (Smith et al., 1995).

IGF-I is a stimulator of IGFBP-2 synthesis, while growth hormone is an inhibitor (Hoeflich et al., 2001). The serum concentration of IGFBP-2 decreases between birth and puberty, after which it steadily increases,

Abbreviations: ADU, arbitrary densitometric unit; α2M, alpha-2-macroglobulin; IGF, insulin-like growth factor; IGFBP, IGF-binding protein; RGD, arginine–glycine–aspartate; RGE, arginine–glycine–glutamate.

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especially after the age of 60 years (Mattsson et al., 2008; van den Beld et al., 2003). Data on the influence of IGFBP-2 on health are somewhat controversial. Low serum IGFBP-2 concentration was found to be associated with a higher degree of obesity and insulin resistance (Wheatcroft et al., 2007). On the other hand, it is generally assumed that a low serum concentration of IGFBP-2 indicates overall good physical function in elderly people. High levels of this protein were associated with greater disability, poorer physical performance, lower muscle strength and mineral bone density, together with less lean and fat mass (Hu et al., 2009).

Alpha-2-macroglobulin (α2M) is the only protein in the circulation discovered to bind IGFBP-2. We recently showed that these two molecules form complexes, although the physiological importance of this phenomenon has not been elucidated yet (Šunderić et al., 2013). The glycoprotein, α2M, is a homotetramer composed of 170 kDa subunits, recognized as a general protease inhibitor. Its concentration in peripheral blood ranges from 2 to 4 g/L, or 2.5 to 5.2 μmol/L (Sotrup-Jenssen, 1989) and it acts by trapping and eliminating proteases from the circulation after binding to a specific low density lipoprotein-related protein/α2M receptor (LRP/α2MR). This receptor is expressed on many cells, such as fibroblasts, hepatocytes, adipocytes, astrocytes, monocytes and macrophages (Borth, 1992). Circulating concentrations of α2M are high at young ages (2.8 g/L to 4.0 g/L), declining to a minimum in middle age (2.2 g/L to 2.8 g/L), after which there is a slow increase up to 3 g/L (Tunstall et al., 1975). It was also noted that α2M binds other molecules, playing a protective role by inhibiting their degradation with subsequent delivery to their targets via the LRP/α2MR (James, 1990).

Besides forming complexes with larger biomolecules, α2M binds zinc ions in relatively large amounts and to a lesser extent some other metal ions, such as copper (Moriya et al., 2008) and manganese (Roth, 2006). Approximately 15% of the protein-bound zinc is attached to α2M, whereas the rest is bound to albumin (Craig et al., 1990). There is no evidence to date that zinc directly influences the activity of α2M, but it affects its conformation (Pratt and Pizzo, 1984). Zinc is also a physiological factor which changes with aging. Zinc deficiency occurs in the elderly due to inadequate food intake and loss after interaction with metallothioneins, whose concentrations rise in stress-related conditions and with aging (Mocchegiani et al., 2006).

Besides being a negative predictor of healthy aging, little is known about the mechanism of IGFBP-2 control. Synthesis and clearance are the initial and final control points, but IGFBP-2 distribution among several molecular forms (monomer, fragments, complexes), some of which bind IGFs and the others do not (or trace amounts), represents an intermediate level of control of IGFBP-2 activity (Mark et al., 2005). The aim of this study was to examine the distribution of IGFBP-2 forms at different ages and the relationship between IGFBP-2 and α2M. Besides determining the relative quantity of these complexes in healthy people of different ages, we also studied certain characteristics of this interaction. Thus, we investigated whether zinc ions can influence the formation of complexes and whether the RGD sequence in IGFBP-2 serves as a contact point.

2. Materials and methods

2.1. Serum samples

Serum samples were divided into three groups, according to the age of the donors: 20–40 years, 41–60 years and 61–80 years. Donors were healthy individuals, assessed by determination of common biochemical and hematological parameters (Table 1) and by questionnaire. They were non-smokers and none of them were alcoholics or taking drugs. They were not under any specific nutritional regimes or vegetarians. Their body mass index ranged from 20 to 32 kg/m^2. None of them were professionally involved in sports. They all declared to be healthy and not aware of any specific disease. The total number of samples was 45 (15 donors in each group, 7 males and 8 females). Blood samples were obtained within 1 week in October 2013; the sera were divided into aliquots and stored at −20 °C until analysis. All experiments were performed within a month, using fresh aliquots.

Table 1
Biochemical and hematological parameters in serum and blood samples obtained from healthy persons of different ages (mean ± SD).

Group (n = 15)	20–40 years old	41–60 years old	61–80 years old
Glucose (mmol/L)	5.5 ± 0.41	4.9 ± 0.52	6.1 ± 0.81
Urea (mmol/L)	5.9 ± 2.20	6.1 ± 2.54	7.9 ± 2.52
Creatinine (μmol/L)	74.0 ± 11.50	74.6 ± 14.62	77.0 ± 10.44
Total bilirubin (μmol/L)	16.0 ± 5.05	14.0 ± 4.12	10.8 ± 6.47
Activity of AST (U/L)	26.4 ± 2.72	24.4 ± 3.70	26.3 ± 6.11
Activity of ALT (U/L)	20.2 ± 2.93	27.0 ± 3.63	22.4 ± 7.94
Cholesterol (mmol/L)	4.9 ± 0.86	5.5 ± 0.82	4.5 ± 1.52
Triglycerides (mmol/L)	1.1 ± 0.42	1.2 ± 0.55	1.0 ± 0.34
Iron (μmol/L)	18.0 ± 5.33	19.4 ± 5.42	10.1 ± 2.08[b,c]
Total protein (g/L)	78.9 ± 7.59	72.1 ± 5.00	59.5 ± 11.24[b,c]
White blood cell count ($\times 10^9$/L)	7.2 ± 1.56	6.4 ± 1.14	7.1 ± 1.50
Red blood cell count ($\times 10^{12}$/L)	4.8 ± 0.71	4.8 ± 0.36	4.4 ± 0.33
Hemoglobin (g/L)	141 ± 22.7	142 ± 10.0	135 ± 8.7
Platelet count ($\times 10^9$/L)	287 ± 24.6	250 ± 41.4	223 ± 59.7[c]

Statistically significant differences ($p < 0.05$, Student's t-test) between age groups 20–40 and 41–60 are indicated as [a], between age groups 41–60 and 61–80 as [b], and between age groups 20–40 and 61–80 as [c].

2.2. Determination of IGFBP-2, α2M and zinc concentrations in serum samples

The concentrations of IGFBP-2 and α2M were determined by ELISA (Abcam, Cambridge, UK) and that of zinc by atomic absorption spectrometry.

2.3. Electrophoresis and immunoblotting

Serum samples, diluted to 1:20, were subjected to SDS-PAGE under reducing conditions (according to the Guide to Polyacrylamide Gel Electrophoresis and Detection, BioRad). After electrophoresis (10% gels for IGFBP-2 and 6% for α2M) proteins were transferred to nitrocellulose membranes and analyzed by immunoblotting using goat anti-IGFBP-2 (Santa Cruz Biotechnology, Santa Cruz, USA) or rabbit anti-α2M antibody (AbD Serotec, Kidlington, UK). The secondary antibodies were HRP-conjugated swine anti-goat IgG (Biosource, Camarillo, USA) and sheep anti-rabbit IgG (AbD Serotec, Kidlington, UK), respectively. Immunoreactive proteins were detected by autoradiography using HRP substrate ECL (Pierce, Thermo Scientific, Rockford, USA). The intensity of protein bands was evaluated by densitometry, using Phoretix 1D software (TotalLab Ltd., Newcastle upon Tyne, UK).

2.4. Addition of zinc, RGDS or RGES to serum

To the selected serum samples a solution of zinc (II) chloride in 0.9% sodium chloride was added to achieve a final concentration of 100 μmol/L or 200 μmol/L. These Zn concentrations were approximately ten and twenty times greater than physiological concentrations. In a separate experiment, RGDS (Arg-Gly-Asp-Ser) or RGES (Arg-Gly-Glu-Ser) solutions in 0.9% sodium chloride (Sigma-Aldrich, St. Louis, USA) were added to selected sera to achieve a final concentration of 4 μmol/L. This amount was more than hundred times greater than the physiological concentration of IGFBP-2. A control experiment was carried out with sera diluted in 0.9% sodium chloride solution to obtain the same dilution factor as in the previous experiments. All mixtures