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Plasma biomarkers of oxidative and AGE-mediated damage of proteins and glycosaminoglycans during healthy ageing: A possible association with ECM metabolism

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ABSTRACT

The aim of this study was to examine whether oxidative and AGE-mediated processes correlates with the metabolic changes of proteoglycans (PGs) and proteins during physiological ageing. The age and gender-associated changes of PGs metabolism were evaluated by plasma chondroitin sulfates (CS), dermatan sulfates (DS) and heparan sulfates and heparin (HS/H). We found a linear age-related decline in CS, DS and HS/H, the first one being the predominant plasma GAG during ageing. The possible deleterious effect of oxidative phenomenon on proteins' and proteoglycans' metabolism during ageing process was analyzed by plasma carbonyls (PCO) and thiols (PSH) as well as by total antioxidant capacity (TAS). An age-dependent increase in PCO and decrease in PSH concentrations were found, both strongly correlated with decreasing with age plasma TAS. Intensity of glycation was assessed by circulating N^{ε}-(carboxymethyl)lysine (CML) and endogenous secretory receptor for AGE (esRAGE), both of them founding associated with ageing. Moreover, all markers of oxidative and AGE-mediated damage correlated with CS and DS level and could be contributing factors to age-related changes of these GAG types. Thus, plasma CS and DS could become promising biomarkers of human ageing to date, owning to its close association with oxidative status and glycation processes.

1. Introduction

Physiological ageing is a natural, continuous process associated with progressive structural, functional and metabolic alterations in a variety of tissues and systems. Analysis of the ageing process at the molecular level has shown that integral parts of this process are the structural and functional changes in the extracellular matrix (ECM) components, including glycosaminoglycans (GAGs) (Robert, 2000; Bailey, 2001). GAGs are linear polysaccharide chains that consist of repeated disaccharide units composed of an amino sugar and uronic acid or galactose residue. Depending on the sugar composition of the repeat, they are classified as chondroitin/dermatan sulfate (CS/DS), heparan sulfate/heparin (HS/H), keratan sulfate (KS) and hyaluronan (HA). Among them, only HA is synthesized in a free form. The others are covalently linked to core proteins forming proteoglycans (PGs) (Handel et al., 2005). GAGs are ubiquitously present on the cell surface or in the ECM where their structure facilitates interaction with growth factors, cytokines and proteins, which underlie their numerous biological functions, including cellular communication, cell signaling and regulation of other biochemical pathways (Schönherr and Hauser, 2000). Altered PG/GAG metabolism proceeding as an integral part of physiological ageing process should be reflected by changes in circulating GAGs level. Plasma GAGs represent components of intact PG mainly of hepatic and endothelial origin, secreted into blood as well as products of tissue PG degradation. Plasma CS is derived from both the cell surface and from the extracellular matrix. Similarly, heparan sulfates (HS) are products of matrix turnover, cell surfaces and basement membranes origin. Interestingly, plasma DS comes almost exclusively from ECM localized PGs, mainly from small leucine-rich PGs i.e. decorin and biglycan, widely expressed in the connective tissue (Volpi et al., 1995). The blood GAGs profile remains not fully characterized so far. Few published studies, which focused on the effect of physiological ageing on plasma GAGs, have brought conflicting results (Sames, 1994; Larking, 1989; Volpi et al., 1995; Komosinska-Vassev et al.,

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2008). None of them analyzed the effect of gender on the circulating GAG fractions. Furthermore, blood GAG changes have been investigated in several pathologies or were even used as an activity marker of inflammatory diseases. Thus, the spectrum of serum GAG is believed to be a reflection of connective tissue condition and as such represent a valuable biomarker for the assessment of human health and disease (Ricciardelli et al., 2009; Moseley et al., 2004). Consequently, the knowledge about age-dependent changes of these macromolecules is of great importance.

The mechanism of the ECM components remodeling in human ageing is associated with both the modification of the biosynthesis of these components and with post-synthetic ECM modification. Oxidative damage and glycation represent the main causes of proteins' and PGs' modification of matrix molecules (Yin and Chen, 2005). In the present study, the deleterious effect of oxidative stress on plasma proteins and GAGs was assessed by plasma carbonyls (PCO) and plasma thiols (PSH) measurements. Reactive carbonyl compounds include highly reactive aldehydes and α-dicarbonyls such as 3-deoxyglucosone (3DG), glyoxal (GO) and methylglyoxal (MGO) (Madian and Regnier, 2010). They are formed from several reactions including oxidative modification of proteins, lipid peroxidation, glycation and autooxidation of carbohydrates. Accumulation of reactive carbonyl compounds is known as carbonyl stress. Carbonyl and oxidative stress are both the direct cause of damage to molecules, including proteins and PG core proteins, leading to disturbances in their structure and function (Rahbar and Figarola, 2003).

Protection from adverse effect caused by ROS is achieved with several antioxidants. However, this process seems to be more inefficient with age. Anti-oxidative potential was found to be increased, decreased or remained invariable with ageing (Valko et al., 2007; Pandey and Rizvi, 2010; Dröge, 2005). In order to gain further insight into the status of extracellular antioxidant system during healthy ageing, we analyzed age-related changes in an overall anti-oxidant plasma capacity by TAS measurement.

Glycation is another form of chemical modification of matrix molecules resulting in increased formation of advanced glycation end products (AGEs) such as carboxymethyllysine (CML), carboxyethyllysine (CEL) and glyoxal lysine dimer (GOLD) (Rahbar and Figarola, 2003; Kalousova et al., 2005). Increased accumulation of tissue AGEs has been linked to ageing and age-related disorders (Nagai et al., 2010). AGE formation alters the functional property of matrix protein forming cross-links between proteins (Sell and Monnier, 2012). AGEs contribute to ECM modification and alter cellular function through the interaction with their receptors (RAGE) (Brownlee, 2000). Soluble forms of RAGE, including esRAGE may neutralize AGE-RAGE mediated cellular damage by acting as a decoy receptor. This splice variant form of RAGE lacking transmembrane domain and secreted into ECM space and the circulation could be a powerful predictor or may be involved in the pathogenesis of various AGE-related disorders, including diabetes, cardiovascular disorders or Alzheimer disease (Kalousova et al., 2005; Nagai et al., 2010). However very little information has been obtained about circulating AGE and esRAGE levels during healthy ageing (Crasto et al., 2011; Uribarri et al., 2007; Vlassara et al., 2009). Thus in order to have greater insight into the role of AGEs in relation to ageing, both CML and esRAGE plasma levels were evaluated in our study.

Furthermore, the aim of this study was to investigate the association between oxidative and AGE-mediated processes and plasma GAGs during healthy ageing. To the best of our knowledge, no study has been published so far to confirm direct relationship between these two forms of deleterious reactions and sulfated glycosaminoglycans during physiological ageing.

2. Materials and methods

2.1. Study population

Studies were carried out on 177 healthy individuals of both sex, aged from 1 to 86 years, and divided into seven groups according to the proper decade of life. Subjects were selected after medical history, clinical examination and laboratory screening. Health was defined as an absence of medical or surgical illness in the previous five years, no hospital admission and no current medication. Subjects were also excluded if they had an infection at least 4 weeks prior to investigation. All volunteers enrolled into this study had normal plasma values of fasting glucose, C-reactive protein, complete blood count, lipid profile, creatinine and liver enzymes. None of the subjects included into the study groups were taking vitamin supplements or any other pharmaceutics with antioxidant activity. Exclusion criteria were tobacco smoking, alcohol consumption and high arterial blood pressure (>160/90 mmHg). We selected the people who can maintain a healthful body weight and had body mass index <25 kg/m^2. All of the subjects had similar lifestyle factors (they all were non-smoking, non-drinking and eating at least two portion of fruits and vegetables a day) at least during a few months before blood collection. Blood samples were collected after overnight fasting.

Written informed consent was obtained after explanation of the purpose and nature of this study to the subjects. The experimental protocol was approved by the Ethical Committee of the Medical University of Silesia.

2.2. Biochemical analyses

2.2.1. Isolation and quantitative analysis of sulfated glycosaminoglycans

Sulfated GAGs were isolated by the method of Volpi et al. (1995) and Olczyk et al. (1997). GAGs were released from plasma PG core proteins by alkaline treatment after extensive papain digestion. 25 mg of papain was added to 1 mL of plasma and samples were incubated for 24 h at 60 °C with stirring. After boiling for 10 min, the mixture was brought to pH 9.0 by adding 0.1 mol/L NaOH. After 24 h at 40 °C, the mixture was neutralized with 40% (w/v) trichloroacetic acid (TCA). Then TCA was added to the samples to a final concentration of 5%. The mixture was centrifuged at 5000 × *g* for 15 min, and the pellet was washed twice with 7% TCA. The precipitate was discarded. To the combined supernatants, 3 volumes of 96% ethanol were added and GAGs were allowed to precipitate for 12 h at 4 °C. After centrifugation (20,000 × *g*, 20 min at 4 °C), 1 mL of 0.5 mol/L aqueous CH_3COOK was added to precipitate. The GAGs solution obtained was treated with 3 volumes of 96% ethanol. GAGs were allowed to precipitate for 12 h at 4 °C. After centrifugation (20,000 × *g*, 20 min at 4 °C), precipitate was dissolved in 1 mL of H_2O and GAGs were isolated by precipitation after addition of 0.02 mL of 5% cetylpyridinium chloride (CPC). After incubation (24 h at 4 °C) and centrifugation (11,000 × *g*, 20 min at 30 °C), GAGs precipitated by CPC were finally washed with 3 mL of 96% ethanol containing NaCl (1 h at 4 °C) and once again centrifuged (11,000 × *g*, 20 min at 4 °C). The supernatant was discarded and the final precipitate (isolated and cleared plasma GAGs) was stored at −20 °C until used for biochemical analysis.

Total amount of GAGs was quantified by the hexuronic acid assay according to Blumenkrantz and Asboe-Hansen (1973) as modified by Slim et al. (1994). Isolated GAG samples were submitted to electrophoresis on cellulose acetate as a four fractions (I–IV). The 1st one, containing intact GAGs, was submitted to fractionation without any previous treatment. The other tree fractions were separately treated with enzymes specifically eliminating particular GAG types. The following GAG digesting factors were used: chondroitinase AC for 2nd fraction, chondroitinase ABC (pH 8.0) for 3rd fraction and chondrotinase ABC with heparitinase I and III–for 4th GAG fraction. Chondroitinase AC cleavage was performed in order to remove C-4/6-S and HA and retain DS and HS/H. Digestion was carried out in 0.04 M TrisHCl buffer, pH 7.3, containing 0.04 M sodium acetate and 0.025% (w/v) BSA, for 2 h at 37 °C. Chondroitinase ABC degradation of isolated GAG samples was conducted in 0.05 M TrisHCl buffer, pH 8.0 at 37 °C for 24 h in order to remove C-4/6-S, DS and HA. Heparitinase I and III were used to remove HS. Electrophoresis of four received GAG fractions was performed in 0.017 M aluminum sulfate, at 150 V and 6 mA per acetate plate for 2 h at 20 °C. The cellulose acetate strips were stained with 0.1% (w/v) Alcian blue in 40% ethanol/0.4% glacial acetic acid, and destained in the same solvent mixture but without Alcian blue. The identity of electrophoretic bands was confirmed by comparison of electrophoretic pattern of plasma GAG samples submitted to electrophoresis without any previous treatment (Fig. 1line A) and depolymerized with agents specifically eliminating particular GAG types (Fig. 1lines 2–4).The electrophoregrams obtained were quantitatively analyzed by gel documentation system – G:BOX BioImaging Systems.

2.2.2. Analysis of plasma thiols (PSH)

Plasma protein-bound thiols (sulfhydryl groups) were determined as described by Ellman (1959). This method is based on the thiol-disulphide interchange reaction between 5,5′-dithiobis(2-nitrobenzoic acid) colled Ellman's reagent or DTNB, and thiol groups of proteins under mild alkaline conditions. Released highly chromogenic 5-thio-2-nitrobenzoic acid (TNB) has an intensively yellow color with maximum absorption at 412 nm. Determination procedure started with the addition of 20 μL of plasma sample to 990 μL of distilled water. The 1.0 mL of phosphate buffer (pH 8.0) was added. Three milliliters of this solution was placed