Ageing in Werner syndrome

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Summary

Oxidative stress markers including pentosidine and homocysteine were examined comparing them with inflammation markers including highly sensitive C-reactive protein (hsCRP) and matrix metalloproteinase-9 (MMP-9) in serum from patients with Werner syndrome (WS) and healthy individuals. Elevation of serum pentosidine correlated significantly with normal aging in healthy individuals (p < 0.0004). Serum pentosidine in WS increased significantly compared with age-matched healthy individuals (p < 0.05). Serum homocysteine levels increased insignificantly with normal aging in healthy individuals and in WS compared with age-matched healthy individuals. As both pentosidine and homocysteine levels did not correlate with hsCRP nor MMP-9, both oxidative stress markers may be differentially regulated by inflammation.

Keywords: Aging, homocysteine, inflammation, oxidative stress, pentosidine, C-reactive protein (CRP), matrix metalloproteinase-9 (MMP-9)

1. Introduction

Human ageing has been believed to be an irreversible and detrimental process counted by the advance of calendar years leading finally to death, though the fundamental mechanism(s) remains unclear. We still do not know whether ageing is a physiological one-way process following development and maturation that may be genetically tuned, or is just the result of a stochastic accumulation of damage resulting from daily metabolic/catabolic activities leading to systemic destruction that may accelerate a chance of death along with the passage of time. Also, we cannot discriminate definitely natural (physiological) ageing and age-related diseases (pathological ageing) (1).

Dyslipidemia (DL) and other risk factors for atherosclerosis include pentosidine and homocysteine (HC). Pentosidines are a family of advanced glycation endproducts (AGEs) generated under oxidative stress and ageing (2-8). AGEs are a group of reactive intermediates resulting from a series of non-enzymatic chemical reactions after an initial glycosylation such as rearrangement, dehydration, oxidation, and fragmentation reactions of glucose or its adducts to protein (9,10). AGEs tend to accumulate with age in long-lived tissue proteins such as collagen, lens crystalline, immunoglobulin chains, amyloid β-peptide and nucleic acids, partly under the influence of oxidative stress (10,11). The chronic, systemic inflammation tightly associated with ageing such as diabetes mellitus (DM) (12,13), atherosclerosis (14), arthritis (15,16) and hemodialysis (17,18) may accelerate AGEs formation through oxidative reactions. Collectively, the term: inflammaging has been proposed to explain pathophysiology of human ageing and ageing-related diseases (1,19).

HC, metabolized into cysteine or recycled into methionine, is a sulfhydryl-containing amino acid exclusively derived from demethylation of dietary methionine in our body (20,21). About 80% of the HC is protein bound and HC promotes oxidative stress of protein via reactive oxygen species (ROS) generation upon disulfide bond formation (22). Elevated serum HC enhanced the expression of acute stress-related genes and pro-inflammatory transcription factor, NF-κB, leading to AGEs formation, vascular inflammation and
accelerated atherosclerosis in mice (23,24). Hyper HC serum is also associated with cardiovascular mortality, osteoporosis, Alzheimers disease, renal failure, and physical dysfunction in elderly people (25-28).

Although plasma HC level has been believed to be a biomarker for atherothrombotic events and folate supplementation has been recommended (29), so-far no convincing preventive data of recurrent vascular events have been reported, even if folate supplementation successfully reduced plasma HC levels (30-32). Even though the magnitude of predictive values of HC has recently decreased after fortification of folate in the United States (33,34) and a variety of Japanese foods has been revealed to contain enough folate (35), the epidemiological, clinical and basic findings addressed an important new link between serum HC level, cardiovascular diseases and oxidative stress (22,36).

Werner syndrome (WS; MIM#27770), the representative progeroid syndrome, has been extensively studied as the natural model of human ageing (37). Patients with WS show a wide variety of ageing-associated clinical manifestations such as gray hair/alopecia, hoarseness, cataracts, skin atrophy, skin hyper-/hypo-pigmentation, skin ulcers (SU), sarcopenia, DM, hypogonadism, DL, atherosclerosis, osteoporosis, and malignancy at a relatively early stage of their life followed by death at around 50 y.o. due to atherosclerosis-related diseases or malignancy. Interestingly, the patients with WS usually manifest a low grade inflammation represented by high sensitivity CRP (hsCRP) and matrix metalloproteinase-9 (MMP-9) despite apparent inflammation such as infection and malignancy (manuscript submitted). Because investigation of oxidative stress monitored by AGEs and HC in WS have never been reported, we conducted a study of serum levels of pentosidine and HC in Japanese patients with WS and healthy Japanese individuals.

2. Materials and Methods
2.1. Subjects
A total of 55 serum samples [35 healthy individuals (M = 14, F = 21) ages from 33 to 83 y.o. and 20 patients (M = 12, F = 8) with mutation-proven WS ages from 35 to 70 y.o. (a part of "Goto collection of Werner syndrome": http://www.brc.riken.jp/lab/cell/gmc/)] was selected for the study as shown in Table 1 (37). Diagnosis of DL was made if one of the following criteria was met: total cholesterol (TC) ≥ 220 mg/dL, low-density lipoprotein-cholesterol (LDL-C) ≥ 140 mg/dL, high-density lipoprotein-cholesterol (HDL-C) < 40 mg/dL, or triglycerides (TG) ≥ 150 mg/dL, as previously described (38). The definition of healthy is based on the following criteria: healthy individuals, enjoying a usual daily life by themselves at home without any specific treatment, had no apparent inflammatory diseases or infection at the time of serum sampling.

2.2. Measurements
Human hsCRP in the sera was measured by using a CircuLex high-sensitivity CRP ELISA kit (CycLex Co., Nagano, Japan) according to the users manual. HC (nmol/mL) was examined by HPLC (39) and pentosidine (μg/mL) was assayed by ELISA as previously described (40). The concentration of MMP-9

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Abbreviations: SU, skin ulcers; DM, diabetes mellitus; DL, dyslipidemia; HC, homocysteine; hsCRP, highly sensitive C-reactive protein; MMP-9, matrix metalloproteinase-9.
in the sera was determined by specific sandwich ELISA using a Human MMP-9 ELISA kit (GE Healthcare, Buckinghamshire, UK) as described previously (41).

2.3. Statistical analysis

Statistical analysis was performed using SAS software version 9.2. Mean values between groups and within each group of patients were compared using Student's $t$ tests after confirming that the data followed a normal distribution, and nonparametric methods (Mann-Whitney $U$ test). Correlations were determined using Pearson's test. $p$ values less than 0.05 were considered significant.

3. Results

The serum level of pentosidine increased significantly with ageing in the healthy population ($r = 0.475; p < 0.0004$), but not in WS patients as shown in Figure 1. The serum pentosidine level (Mean ± S.D.) was significantly elevated in the WS patients ($0.077 ± 0.079 \mu$g/mL, $p < 0.05$) compared with age-matched healthy controls ($0.039 ± 0.015 \mu$g/mL) as indicated in Figure 2. Although pentosidine was usually associated with inflammation, serum levels of pentosidine in the healthy population and WS patients did not correlate significantly with inflammation assessed by hsCRP or MMP-9 in the present study (data not shown). If WS patients were grouped into SU(+)(-), DM(+)(-), and DL(+)(-), respectively, and compared with (+) and (-) in each group, the differences of both serum pentosidine and hsCRP or MMP-9 levels were insignificant between all groups.

The serum level of HC increased insignificantly with ageing in the healthy population ($r = 0.316; p = 0.065$) and in WS patients as shown in Figure 3. Although HC was also usually associated with inflammation, serum levels of HC in healthy individuals and WS patients did not correlate with inflammation assessed by hsCRP or MMP-9 in the present study (data not shown). The serum HC level was comparable between WS patients ($10.7 ± 5.4$ nmol/mL) and age-matched healthy individuals ($9.4 ± 2.8$ nmol/mL) (Figure 4). However, serum HC level in the DL(+) group in WS ($12.7 ± 6.23$ nmol/mL; $p < 0.05$) was significantly elevated compared with the DL(-) group ($7.8 ± 1.84$ nmol/mL).

4. Discussion

The significantly elevated pentosidine level in WS serum may suggest increased AGE production associated with accelerated ageing in WS. However, the other oxidative reaction marker in serum, HC, was not significantly elevated in the same WS serum compared with the age-matched healthy population. Although HC induced oxidative stress may contribute to vascular damage by activating MMP-9 (42), and both HC and pentosidine were in general increased with inflammation monitored by hsCRP, both oxidative markers were neither correlated with each other, nor serum levels of hsCRP and MMP-9 in WS. Serum pentosidine and HC may be differentially regulated by inflammation as was reported (4,6,14,17,23,24,43). Although serum HC in WS did not correlate with TC, HDL-C, LDL-C, and TG (data not shown), the serum HC level in the DL(+) group in WS was significantly elevated irrespective of age compared with the DL(-) group. In animal studies, elevated HC may activate oxidative enzymes leading to podocyte damage resulting in glomerulosclerosis (44). The oxidative damage of HC on the vascular system has been reported in most studies using supraphysiological concentrations of HC or genetic diseases like homocystinuria (45).
In any case, Pagano et al. reviewed that the multiple involvement of oxidative stress in WS and the concomitant association of moderate hyperhomocysteinemia and DL, in addition to elevated AGE production may accelerate oxidative injury in cardiovascular diseases and other age-associated phenotypes in WS.

References

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