Benefit of an association of an antioxidative substrate and a traditional chinese medicine on telomere

Radhia M’kacher1, Ludovic Breton2, Bruno Colicchio2, Henry Puget2, William M. Hempel1, Mustafa Al Jawhari1, Eric Jeandidier1, Michel Frey2

1 Cell Environment, DNA Damage R&D, Paris, France
2 IDEC Therapeutics, Paris, France
1 IRIMAS, Institut de Recherche en Informatique, Mathématiques, Automatique et Signal, Université de Haute-Alsace, Mulhouse 68093, France
4 Department of Genetics, Groupe Hospitalier de la Région de Mulhouse Sud-Alsace, Mulhouse 68093, France

*Correspondence to: docfrey@wanadoo.fr, radhia.mkacher@cell-environment.com

Received October 28, 2019; Accepted December 26, 2019; Published December 31, 2019

Doi: http://dx.doi.org/10.14715/cmb/2019.65.8.9

Copyright: © 2019 by the C.M.B. Association. All rights reserved.

Abstract: Telomere shortening is involved in age-related disorders, such as cancer and cardiovascular diseases. Recently, telomerase re-activation strategies have been proposed to counteract telomere shortening and its consequences. Here, we investigated the benefit of dietary supplementation with a mix of S-adenosyl-methionine (SAMe) and a polysaccharide extract of Astragalus (APS) on telomere length of circulating lymphocytes of healthy volunteers. Blood lymphocytes of a cohort of 26 healthy volunteers who were administered the mix of SAMe and APS in a food supplement for one year were collected. In vitro treatment of blood lymphocytes of healthy volunteers with the mix was also performed. A cohort of 150 healthy volunteers was used as a control. Telomere length was measured by Q-FISH. The micronucleus assay was performed to detect genotoxicity of the mix. The telomeres of circulating lymphocytes of the cohort of 26 donors supplemented with the mix were significantly longer than those of matched controls (p < 10^-4). This elongation was essentially observed in the lymphocytes of older donors. Similarly, in vitro treatment of circulating lymphocytes with the mix significantly increased telomere length and decrease the proportion of cells with short telomeres. Here, we observed an increase in telomere length after in vivo and in vitro administration of a mix with SAMe and APS. The benefit of dietary supplementation with this mix opens a new horizon for the battle against aging and could be used in the treatment of chronic age-related disorders.

Key words: Telomere; SAMe; Astragalus membranaceus; Mix; Natural aging.

Introduction

Chronic age-related diseases are currently the most important cause of morbidity and mortality worldwide (1). The goal of healthy aging and the development of anti-aging approaches for maintaining better health in old age is a major target (2).

Telomeres are structures at the end of chromosomes that play a major role in genome stability and integrity, protecting chromosomes from degradation (3, 4). Telomeres are susceptible to DNA damage as they are not as effectively repaired as other DNA and show a high sensitivity to reactive oxygen species (ROS). Critical telomere shortening during cell division signals an irreversible state of growth arrest, known as cellular senescence (5) and globally increases aging and associated chronic diseases (6-8).

Several studies indicate that shorter telomeres are a risk factor for cancer and age-related diseases (9-12). Lifestyle factors and environmental exposure may accelerate telomere shortening and can expose free chromosome ends to the DNA double-strand break (DSB) repair machinery, leading to telomere fusion and chromosomal instability, thus possibly affecting the health and lifespan of the individual.

Moreover, control of oxidative stress is an attractive and safe option to maintain telomere length and control reactive oxygen species (ROS) generated by exogenous (UV, pollutants, chemicals, stress, etc.) and endogenous sources that promote telomere shortening, cellular senescence, and tissue aging (13). S-adenosyl-Methionine (SAMe) is an endogenous substrate of the antioxidative pathway. Low tissue levels of SAMe may provoke molecular changes that result in the dysregulation of cellular homeostasis and DNA methylation disorders, inducing health problems, including increased carcinogenesis (14, 15).

Astragalus membranaceus (Huang Qi) is one of the most well-known and frequently used herbal medicines in traditional Chinese medicine (TCM) and is widely prescribed for the clinical treatment of many indications, including age-related diseases. Astragalus has also been used as a tonic and as food supplements in TCM for many centuries. Astragalus polysaccharides (APS) and Astragalosides are two constituents that are generally claimed to be responsible for the bioactivity and benefits of Astragalus on human health. Recent pharmacological research has shown that components of Astragalus can increase telomerase activity (16), in addition to an antioxidative function (17), both recently linked to the polysaccharides.

In this study, we investigated the benefit of dietary supplementation with SAMe and APS on the telomere length of circulating lymphocytes. This associations had a
significant effect on telomere length without cytotoxicity in vivo and in vitro and confirms the potential benefit of diet between an antioxidative substrate (SAMe) and a mix of traditional Chinese medicine (APS) in telomere stabilization and maintenance. This association could be an interesting approach to stabilize telomeres and to prevent chronic age-related disorders.

Materials and Methods

The formulations of S-Adenosyl-L-Methionine D-sulfate Tosylate (SAMe), Astragalus root HA PE 20% Polysaccharides UV-VIS (ASP), and both (Association) used in this study were produced by Prophar and used by IDEC Therapeutic (France) according to a patented application (WO 2016/092193).

In vivo and in vitro procedure and culture conditions

Peripheral blood lymphocytes from 26 donors (12 men and 14 women), with a mean age of 69 years (range 52-85), were used in this study. These donors took a mix of SAMe and ASP as a dietary supplement for one year. A large cohort of 150 healthy donors (68 males and 82 females) with a mean age of 39 years (range 0.52-79) were used as a control. Informed consent was obtained from all donors included in this study.

For the in vitro study, blood lymphocytes from one male donor 28 years of age were treated with 3.6 µg/ml SAM, 1.4 µg/ml Astragale. DMSO was used as a negative control. Blood lymphocytes were cultured in RPMI 1640 medium supplemented with Glutamax (GIBCO-BRL, France), 10% fetal bovine serum (Invitrogen, France), antibiotics (Invitrogen, France), and 2% Phythojemagglutinin (GIBCO-BRL, France) for 72 h at 37°C in the presence of the liver post-mitochondrial fraction (S9). S9 was prepared from male rats (Sprague–Dawley strain) treated with Aroclor (500 mg kg⁻¹). Aroclor was provided by Molecular Toxicology (Boone, NC, USA) and stored at -80 °C. Blood lymphocytes were exposed to the various substances for 3 h each day in the presence of S9. This treatment was followed by a recovery period of 21 h (without S9 or the product), always in the presence of PHA. Cytogenetic slides were prepared according to conventional procedures and stored at -20°C until used.

Telomere quantification

Telomere quantification was performed using the Q-FISH technique with a Cy-3-labelled PNA probe specific for (TTAGGG) (Eurogentec, Liège, Belgium). The quantification of telomere length was performed in interphase cells, allowing the investigation of intercellular variation in a large number of scored cells. Quantitative image acquisition and analysis were performed using Metacyte software (Metasystem, version 3.9.1, Altlussheim, Germany). The mean fluorescence intensity (FI) of telomeres was automatically quantified in 10,000 nuclei on each slide. Settings for exposure and gain were the same between captures. The experiment was performed in duplicate. Telomere length, measured as mean fluorescence intensity (FI), strongly correlated with telomere length measured by Southern-blot analysis using the telomeric restriction fragment (TRF). The mean telomere length is expressed in kb.

Micronucleus assay

Blood lymphocytes were cultured for 72 h in RPMI 1640 supplemented with 1% penicillin/streptomycin (Invitrogen, France), 1% 200 mM L-glutamine (Invitrogen, France), 10% inactivated foetal serum (Invitrogen, France), and 2% Phythojemagglutinin (GIBCO-BRL, France). Cytochalasin B (from Drechslera dematioida, Sigma) (6 µg/mL) was added 24 h before arrest according to standard procedures. Slides were spread and stored at -20°C until use.

Automatic scoring of MN was performed using MNScore software (version 3.8.101 MetaSystems, Althaussen, Germany) with a Metafer 4 image analyser (MetaSystems, Althaussen, Germany) comprised of a Zeiss Axioplan 2 imager.

Statistical analysis

Data were analyzed using R software. Mean comparisons were computed using the two-sample Wilcoxon test. The convention for symbols indicating statistical significance is: ns for p > 0.05, * for p ≤ 0.05, ** for p ≤ 0.01, *** for p ≤ 0.001, **** for p ≤ 0.0001. The presented regression curve was generating using the mean telomere length of 150 donors and a linear regression model (lm).

Results

Telomere length after treatment with the mix

We assessed telomere length in a large cohort of healthy volunteers (150 healthy volunteers: 2-76 years of age), without any known disease or exposure to genotoxic agents, by the quantitative fluorescence in situ hybridization (Q-FISH) technique using PNA probes. Total fluorescence intensity of telomeres was transformed into kilo-bases based on the previously-published correlation between Q-FISH signals and those of Southern-blot hybridization (11, 12, 18). Telomere length in this cohort was age-dependent, with high inter-individual variation (p and R). The telomere shortening rate per year was approximately 79bp (Figure 1). We also assessed the telomere length of the 26 volunteers who used the dietary supplement containing SAMe and APS.
for one year (Figure 1).

Only five volunteers had telomeres that were short relative to normal age-related telomere shortening. There was a significant difference in the telomere length of volunteers using the mix in the dietary relative to that of the other volunteers of similar age (Figure 2).

Interestingly, we observed this positive effect on telomere length essentially in older volunteers (Figure 3). Telomere length corresponded to a median distribution more usually found in younger people (approximately 10 years younger-5-10 years younger based on telomeres medium size) (Figure 3).

**Telomere length after in vitro exposure to the mix**

Peripheral blood lymphocytes were treated in vitro with 3.6 μg/ml SAMe, 1.4 μg/ml APS, or the mix (SAMs and APS) for three days. Telomere length was then measured. The mean telomere length of blood lymphocytes before treatment was 7.31 kb. The mean telomere length after treatment was 8.07 kb ($p < 10^{-16}$) for lymphocytes treated with SAMe, 8.41 kb ($p < 10^{-16}$) for those treated with APS, and 9.05 kb ($p < 10^{-16}$) for those treated with the mix (Figure 4).

In addition, the frequency of cells with very short telomeres was lower in the presence of the compounds than the control (DMSO) (Figure 5A-B).

**In vivo genotoxicity of mix**

To confirm the potential benefit of the dietary supplement against DNA damages, micronucleus was performed. We scored a total of 76 micronuclei in 11,614 bi-nucleated cells from 26 volunteers who consumed the dietary supplement cohort, 125 micronuclei in 9326 bi-nucleated cells were scored. Significant difference was observed in for one year. In control the frequency of micronuclei in the 26 volunteers using the dietary supplement compared to control cohort ($p<10^{-15}$).

**Discussion**

Over the last few decades, telomere dysfunction has increasingly been considered to be one of the hallmarks of aging (19) and can be used in the prognosis of several age-related chronic diseases. When telomeres become too short, before genes are affected or chromosomes fuse, cells stop dividing and undergo senescence (4).
Preventing the accumulation of short telomeres can ameliorate the symptoms of cardiovascular disease (6, 12), brain aging (20), pulmonary fibrosis (21), hepatic dysfunctions (22) and aging, in general (7).

A better choice of diet and activities has great potential to reduce the rate of telomere shortening, or at least prevent excessive telomere attrition, leading to delayed onset of age-associated diseases and increased lifespan. In addition, women who consumed a diet lacking antioxidants were shown to have shorter telomeres and a moderate risk for developing breast cancer, whereas the consumption of a diet rich in antioxidants was associated with longer telomeres and lower risk of breast cancer (23).

The consumption of Astragalus polysaccharides (APS), which possesses an immunomodulatory function (24), is a promising proposed strategy for adjuvant treatment of cancer (25).

The effect of SAMe, as well as its association with APS, on telomere elongation and stabilization has not been studied yet. Thus, we measured the telomere length of circulating lymphocytes in a small pilot cohort of healthy volunteers using a dietary supplement containing a mix of APS and SAMe. In this study, we observed a significant difference in telomere length between a cohort of 26 volunteers without disease or exposure to genotoxic agents who took a dietary supplement containing a mix of SAMe and ASP for one year and healthy volunteers of similar age who did not. Telomere quantification of circulating lymphocytes of the control cohort of healthy volunteers, who ranged in age from 2 to 76 years, showed telomeres to shorten at a rate of 79 pb/year. This rate of telomere loss is in accordance with that published in other studies (26, 27). We demonstrate that more than 80% of the cohort taking the dietary supplement had longer telomeres than their age-matched controls by linear regression of telomere length with age. This increase in telomere length corresponded to a median gain of approximately 10 years (2 to 20 years). We observed a higher response to the in vivo administration of the mix in the oldest volunteers. In addition, the frequency of cells with drastic telomere shortening (<3kb) was much lower in this cohort. Such cells could be the origin of age-related diseases (28). Moreover, we surprisingly observed that the frequency of micronuclei scored in the circulating lymphocytes of the volunteers taking the supplement was very low relative to data in the literature (29-32) confirming the benefit of healthy diet and supplement.

We also assessed in vitro exposure of blood lymphocytes to the mix to validate our in vivo data. We observed telomere elongation after ASP, confirming the large literature reporting the elongation of telomeres using ASP (33). For the first time, we observed telomeres’ elongation after SAMe addition and even greater elongation after exposure of the cells to the mix. Such telomere elongation was associated with a reduction in the frequency of cells with critical telomere shortening, similarly to that observed after in vivo administration of mix to healthy volunteers.

This study demonstrates that the consuming of a dietary supplement containing ASP and SAMe could be involved in telomere length protection and in the decreases of the proportion of cells with critical telomere shortening, especially in old volunteers. Such telomere elongation was not associated with an increase in the frequency of micronuclei. Our data suggest that dietary supplementation with ASP and SAMe could contribute to the stabilization and maintenance of telomeres. This explorative pilot clinical study opens new horizons for future studies on the mechanistic links between telomere length and nutritional epigenomics as a key factor in chronic age-related disorders.

Authors Contributions
Conceived and designed the experiments: R.M., LL.B., M.F. Performed the experiments: R.M., H.P., M.E. Analyzed the data: R.M., B.C., E.J. Wrote the paper: R.M., L.B., W.H. M.F.

Acknowledgments
We are indebted to Dr Bastien for methodological advice in statistical analysis and Wala Najar for her technical help.

Conflicts of interest
IDEC Therapeutics commercializes a dietary supplement with SAMe and Astragalus ASP. This supplement contains also Vit B6, Vit B12 and oligo elements (Zinc and marine Magnesium).

References
11. M’Kacher R, Bennacerr-Griscelli A, Girinsky T et al. Telomere shortening and associated chromosomal instability in peripheral...
12. Girinsky T, M’Kacher R, Lessard N et al. Prospective coro-
nary heart disease screening in asymptomatic Hodgkin lymphoma
patients using coronary computed tomography angiography: results
and risk factor analysis. International journal of radiation oncology,
Peroxiredoxins: guardians against oxidative stress and modulators
14. Albrecht LV, Bui MH, De Robertis EM. Canonical Wnt is inhi-
bited by targeting one-carbon metabolism through methotrexate or
methionine deprivation. Proceedings of the National Academy of
15. Pascale RM, Foo CF, Calvisi DF, Foo F. Deregulation of methio-
nine metabolism as determinant of progression and prognosis of
hepatocellular carcinoma. Translational gastroenterology and hepa-
tology 2018; 3: 36.
16. Liu BH, Gu YH, Tu Y et al. [Molecular regulative mechanisms
of aging and interventional effects of Chinese herbal medicine].
Zhongguo Zhong yao za zhi = Zhongguo zhongyao zazhi = China
Jul 21 2016; 7(7).
al. Independent Mechanisms Lead to Genomic Instability in Hodg-
kin Lymphoma: Microsatellite or Chromosomal Instability. Cancers
19. Fali T, Papagno L, Bayard C et al. New Insights into Lympho-
cyte Differentiation and Aging from Telomere Length and Telome-
rase Activity Measurements. The Journal of Immunology 2019:
ji1801475.
20. Puhlmann LMC, Valk SL, Engert V et al. Association of Short-
term Change in Leukocyte Telomere Length With Cortical Thic-
ness and Outcomes of Mental Training Among Healthy Adults: A
Randomized Clinical Trial. JAMA network open Sep 4 2019; 2(9):
e199687.
Genetic analysis of telomere-related genes, telomere length measure-
ment—or both? Respirology (Carlton, Vic) 2019; 24(2): 97-98.
22. Ma JJ, Wang XY, Duan M et al. Telomere length variation in
tumor cells and cancer-associated fibroblasts: potential biomarker
for hepatocellular carcinoma. The Journal of pathology Dec 2017;
23. Shen J, Gammon MD, Terry MB et al. Telomere length, oxida-
tive damage, antioxidants and breast cancer risk. International jour-
nal of cancer Journal international du cancer Apr 1 2009; 124(7):
1637-1643.
24. Li Q, Bao JM, Li XL, Zhang T, Shen XH. Inhibiting effect of As-
tragalus polysaccharides on the functions of CD4+CD25 highTreg
cells in the tumor microenvironment of human hepatocellular carci-
25. Li J, Bao Y, Lam W et al. Immunoregulatory and anti-tumor
effects of polysaccharopeptide and Astragalus polysaccharides on
tumor-bearing mice. Immunopharmacology and immunotoxicology
MA. Telomere shortening rate predicts species life span. Proceed-
ings of the National Academy of Sciences of the United States of
27. Vera E, Bernardes de Jesus B, Foronda M, Flores JM, Blasco
MA. The rate of increase of short telomeres predicts longevity in
28. Martinez P, Blasco MA. Telomere-driven diseases and telomere-
29. Fenech M, Kirsch-Volders M, Natarajan AT et al. Molecular
mechanisms of micronucleus, nucleoplasmic bridge and nuclear bud
formation in mammalian and human cells. Mutagenesis Jan 2011;
30. Fenech M, Bonassi S. The effect of age, gender, diet and lifestyle
on DNA damage measured using micronucleus frequency in human
semiautomated FISHbased micronucleuscentromere assay for bio-
monitoring of hospital workers exposed to low doses of ionizing
risk assessment: Reevaluation of the cytokinesis-block micronu-
cleus assay using semi-automated scoring following telomere and
centromere staining. submitted on Mutation Research - Genetic
Toxicology and Environmental Mutagenesis Volumes 850–851,
February–March 2020, 503143.
33. Liu C, Li H, Wang K et al. Identifying the Antiproliferative
Effect of Astragalus Polysaccharides on Breast Cancer: Coupling
Network Pharmacology With Targetable Screening From the Cancer