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Original Research

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

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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 administrated 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 aantioxidative 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 Disulfate 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 and 2% Phytohemagglutinin (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) (Eurogenetec, Liège, Belgique). 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% Phytohemagglutinin (GIBCO-BRL, France). Cytochalasin B (from *Drechslera dematioidea,* 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 $p \leq 0.05$, ** for $p \leq 0.01$, *** for $p \leq 0.001$, **** for $p \leq 0.0001$. 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 heathy 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

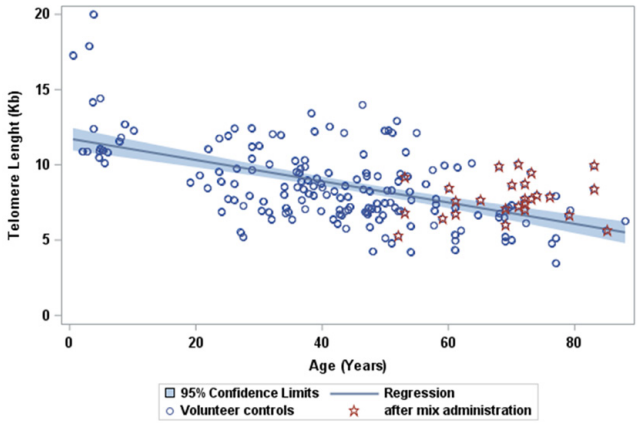

Figure 1. Blood lymphocyte telomere length (kb) as a function of the age of healthy volunteers after taking a mix of SAMe and ASP as a dietary supplement for one year (red stars) compared to that of a large cohort of healthy volunteers (blue circles). A linear regression, as well as the 95% confidence interval, of telomere loss with age are presented.

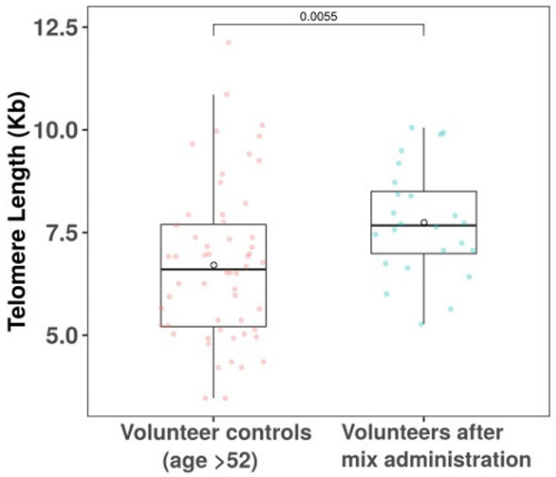

Figure 2. Box plot of mean telomere length of blood lymphocytes of healthy volunteers after administration of a mix of SAMe and ASP as a dietary supplement for one year compared to that of healthy volunteers with similar age. Significant difference was observed.

for one year (Figure1).

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 (Figure2).

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 youger 5-10 years youger based on telomeres medium size) (Figure3).

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 ($p<10^{-3}$).

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, but before genes are affected or chromosomes fuse, cells stop dividing and undergo senescence (4).

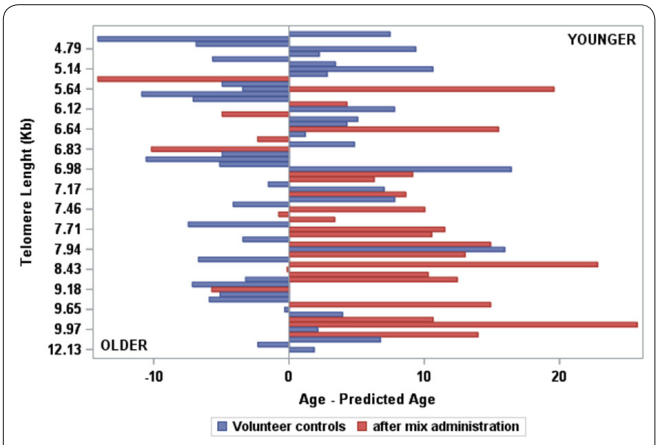

Figure 3. Prediction of age according to telomere length performed on a large cohort of volunteers (blue circles) and the linear regression, corresponding to natural aging. The age predicted by the model for the cohort of donors using the dietary supplement was significantly less than the actual age, by approximately 10 years.

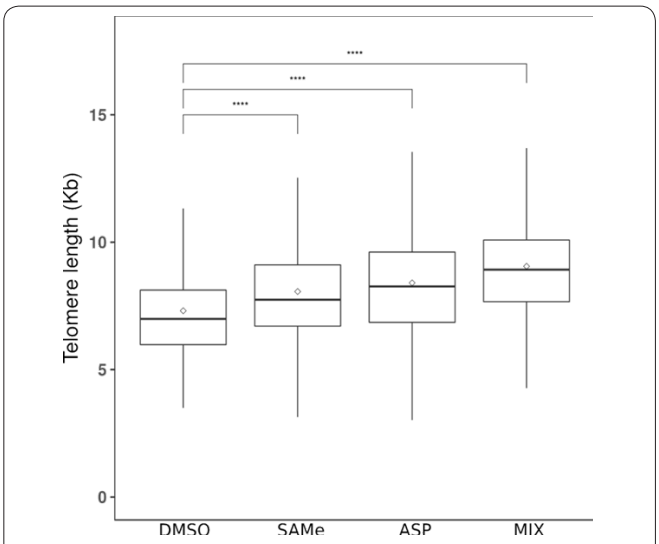

Figure 4. *In vitro* quantification of telomere length in peripheral blood lymphocytes before and after treatment with 3.6 µg/ml SAM, 1.4 µg/ml ASP**,** or a mixture of the two molecules (MIX) . Blood lymphocytes were treated for three days in the presence of S9 fraction. ****p < 0.0001.

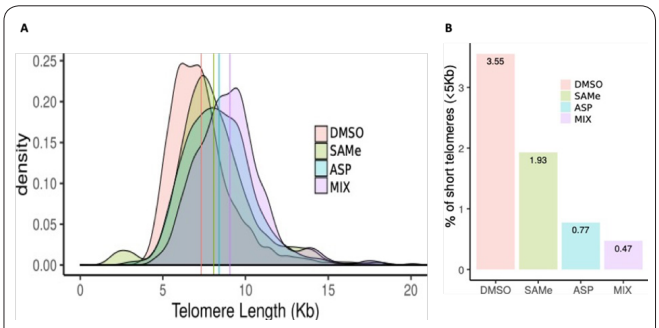

Figure 5. *In vitro* distribution of telomere length of circulating lymphocytes after treatment with DMSO, SAMe, ASP, or a mixture of the two molecules (MIX). (A) Histogram of the distribution of telomere length and the mean telomere is shown. (B) Frequency of cells with short telomeres (< 5 kb), showing a significant decrease in the frequency of such cells after treatment (SAMe, ASP, or MIX) relative to that observed after DMSO exposure.

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.

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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.

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