

Redox Status Impairment in Liver and Kidney of Prematurely Senescent Mice

Effectiveness of DTS Phytotherapeutic Compound

F. MAROTTA,^{a,d} F. LORENZETTI,^d M. HARADA,^b S. K. ONO-NITA,^c
E. MINELLI,^a AND P. MARANDOLA^d

^aWHO-Center for Biotechnology and Traditional Medicine,
University of Milano, Milan, Italy

^bMCH Hospital, Tokyo, Japan

^cHepato-Gastroenterology Department, Sao Paulo University, Sao Paulo, Brazil

^dA. Scarpa-G.A.I.A. Age-Management Foundation, Pavia, Italy

ABSTRACT: T-maze test–selected prematurely senescent mice (PSM) were allocated into two groups: (A) those given DTS (150 mg/kg) orally for 30 days and (B) untreated PSM with age-matched fast T-maze performers as control. After sacrifice, the liver and kidney were analyzed for catalase (CAT) activity, glutathione peroxidase (GPx), superoxide dismutase (SOD), malondyaldehyde (MDA), and plasma thiols. Untreated PSM showed decreased plasma thiols and tissue level of CAT, SOD, GPx, with higher MDA ($P < 0.01$ vs. fast performers), while DTS (Denshichi–Tochiu–Sen) significantly improved glutathione and cysteine ($P < 0.05$) and tissue concentration of the above parameters ($P < 0.05$). Such preliminary data suggest that DTS mitigated oxidative damage in PSM, with likely action on the cytoplasm and mitochondrial matrix.

KEYWORDS: prematurely senescent mice; oxidative stress; DTS

INTRODUCTION

The aging process is associated with a decrease in protein-bound thiol levels and their related antioxidant capacity, with oxidative modification of DNA, proteins, lipids, and small cellular molecules by reactive oxygen species (ROS). In particular, thiol compounds (cysteine 80%, glutathione 17%, and homocysteine 2–3%) have a relevant role as key factors in regulating the intracellular

Address for correspondence: Prof. F. Marotta, M.D., Ph.D., via Pisanello, 4, 20146 Milano, Italy.
Voice and fax: +39-0240 77243.
e-mail: fmarchimede@libero.it

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and extracellular redox buffer capacity. Decreased tissue GSH levels are also associated with depressed immunity and the progression of aging, and it may also increase the risk of cancer development. Recently, De la Fuente *et al.*¹ using the T-maze test as a clear-cut parameter, have shown that some mice express overt features of premature aging with immunologic impairment and a shorter life span when compared to their age-matched fast-performing counterparts. The aim of this study was to apply the same methodology in Balb-c mice to test a novel nutraceutical that was shown in preliminary in-house experiments to be endowed with significant antioxidative/anti-inflammatory effects in agreement with the literature.²⁻⁶

MATERIALS AND METHODS

Balb-c mice (25–30 g) were bred under conventional conditions, housed in a pathogen-free environment at $23 \pm 1^\circ\text{C}$ with an alternating 12-h light/dark cycle and supplied food and water *ad libitum*. At 70 weeks of age, the T-maze test was performed once a week for 4 weeks and prematurely senescent mice (PSM) were regarded as those animals that failed at all times to complete the test within the maximum allotted time (60 s). Animals with intermediate performances were excluded so as to obtain a “fast” and a “slow” group, containing 100% and 0%, respectively. Altogether, 28 PSM and 26 non-prematurely senescent mice (NPSM) were chosen. PSM were allocated into two groups: Group A: fed standard food for 4 weeks; Group B: fed standard food added with DTS (*Panax pseudoginseng*, *Eucommia ulmoides*, ginseng radix, kindly donated by the Institute of Health Care Oriental Herbs and Medicine, Tokyo, Japan) 150 mg/kg of body weight daily. After a 4-week supplementation study, the liver and kidney were quickly removed and tissues were kept at -80°C until analysis. A part of the tissues was used to separate the cytosolic and mitochondrial fraction by means of a standard methodology used to measure SOD.

Preparation of Phytotherapeutic Compound

DTS, which is produced under quality-controlled procedures and ISO 9001 and 140001 regulation, was kindly donated by the Institute of Health Care with Oriental Herbs and Medicine, Tokyo, Japan. This compound presents in the form of tiny grains of medium consistency and is palatable and can be easily mixed with food.

Plasma Analysis

The total thiol levels as well as their free and protein-bound fractions were measured by high-performance liquid chromatography (HPLC) after

pre-column derivatization with 2-chloro-1-methylquinolinium tetrafluoroborate and the samples were reduced with sodium borohydride.

Liver and Kidney Tissue Analysis

Catalase activity was determined at 240 nm by measuring the rate of H₂O₂ utilization with the molar extinction coefficient for H₂O₂ being 43.6 M/cm. The amount of the enzyme utilizing 1 μmol H₂O₂/min was taken as one activity unit.

Glutathione peroxidase (GPx) activity was determined at 340 nm by spectrophotometry and the amount of the enzyme converting 1 μmol GSH/min was taken as one activity unit.

Glutathione reductase activity was measured at 340 nm by spectrophotometry and the amount of the enzyme reducing 1 μmol GSSG/min was taken as one activity unit.

Superoxide dismutase activity (SOD) was measured at 560 nm as the rate of reduction of nitrotetrazolium blue and for one unit of activity, the amount of protein was taken, which provided a 50% inhibition of nitrotetrazolium blue reduction under standard conditions.

Malondyaldehyde Determination

Malondyaldehyde (MDA) in liver and kidney tissues was assayed by spectrophotometric measurement and the concentration of thiobarbituric acid was calculated by the absorbance coefficient of MDA-TBA complex and expressed as nmol/mL.

Statistical Evaluation

For statistical analyses, normality was investigated first, and it was shown that some values of the parameters did not fit the normal distribution. Therefore, the nonparametric Kruskal-Wallis test and Mann-Whitney *U* test were used to compare groups.

RESULTS

Plasma Thiol Analysis

As compared to age-matched mice, NPSM mice showed a statistically significant decreased level of total plasma level of thiols (a decrease of 30–33%, $P < 0.01$), which affected all the separate components of thiol (data not shown). DTS administration yielded a partial (17–23% increase) but significant improvement of this parameter ($P < 0.05$ vs NPSM) and further analysis

identified glutathione and cysteine as the thiol components, which significantly improved (data not shown). The free/bound thiol ratio showed that PSM mice had a statistically higher ratio ($P < 0.05$ vs. NPSM), and this parameter was not influenced by DTS treatment.

Liver and Kidney Tissue Analysis

The activity of antioxidant enzymes did not show any difference when testing the cytosolic fraction of both tissues. As compared to NPSM, PSM showed a number of significant modifications ($P < 0.01$), that is, a decrease of GSH ($\mu\text{mol/g}$ tissue: 4.7 ± 0.02 vs. 5.4 ± 0.02 in liver; 2.9 ± 0.09 vs. 4.1 ± 0.04 in kidney), of GSH-Px (U/mg protein: 148 ± 7.3 vs. 163 ± 8.3 in liver; 135 ± 3.6 vs. 168 ± 5.7 in kidney) and of GSH/GSSG ratio (25–40% in both tissues). DTS administration yielded a partial but significant ($P < 0.05$) improvement of these parameters and a normalization of GSH redox, expressed as GSH redox $\text{GSSG}/(\text{GSH} + \text{GSSG}) \times 100$ ($P < 0.05$). As shown in TABLE 1, besides kidney SOD, the tested oxidative/antioxidative parameters were improved by DTS administration ($P < 0.05$). Subcellular analysis revealed that while cytosol SOD was unaltered in PSM, the mitochondrial compartment was significantly ($P < 0.01$) depleted in both tested tissues (units/mg protein: 7.4 ± 2.7 vs. 12.6 ± 1.4 in liver; 6.5 ± 1.0 vs. 10.6 ± 0.9 in kidney). Both parameters were partially improved by DTS (units/mg protein: 11.2 ± 1.1 and 9.8 ± 0.7 , respectively, $P < 0.05$).

CONCLUSION

Aging is associated with a decrease in the level of the most relevant antioxidant, glutathione, and of cysteine, which can be a result of both an elevated

TABLE 1. Effect of DTS on catalase, SOD, and MDA in control and PSM mice

Group	Catalase U/mg protein	SOD U/mg protein	MDA nmol/mg protein
<i>Liver</i>			
NPSM/Balb-c	276 ± 6.4	25.6 ± 3.32	55 ± 55
PSM			
Untreated	$255 \pm 5.8^*$	24.7 ± 2.82	$0.83 \pm 0.06^*$
+ DTS	$271 \pm 5.8^{**}$	24.9 ± 2.01	$0.62 \pm 0.02^{**}$
<i>Kidney</i>			
NPSM/Balb-c	187 ± 6.9	18.9 ± 2.66	0.21 ± 0.01
PSM			
Untreated	$164 \pm 4.1^*$	$16.4 \pm 3.21^*$	$0.32 \pm 0.03^*$
+ DTS	$171 \pm 5.1^*$	$18.0 \pm 3.42^{**}$	$0.26 \pm 0.02^{**}$

* $P < 0.01$ vs. NPSM; ** $P < 0.05$ vs. untreated PSM.

demand and inhibited GSH biosynthesis. In particular, glutathione is an important defense mechanism in living cells and, as a substrate for the antioxidant enzyme glutathione peroxidase, GSH protects cellular constituents from the damaging effects of peroxides formed by metabolism and through other ROS reactions. Thus, a change in the thiol:disulfide ratio (i.e., an alteration of the thiols redox status), significantly affects the morphology and function of cellular and extracellular proteins. By applying previously described selection criteria to detect mice with prematurely aging features,¹ we found that such animals have a defective redox status due to an unbalanced enzymatic antioxidant apparatus. Although specific immunologic deficits have been shown in these animals,¹ the damaging effects on cellular macromolecules and functions by such sustained oxidative stress are likely to argue for a further mechanism for explaining their defective behavioral responses and life span. On the other hand, the same group⁷ has shown that an antioxidant intervention with thioproline or *N*-acetylcysteine would significantly improve the immunologic function in such animals. Nonetheless, the observed increased level of endogenous GSH together with the decrease of GSH redox ratio, which represents the degree of H₂O₂ generation, when PSM were given DTS suggest that this nutraceutical might act directly on the regulation of GSH/GSSG redox status while also increasing glutathione reductase activity. In particular, the mitochondrial SOD fraction was significantly improved. Interestingly, although outside the aim of this study, it has been shown that some saponins contained in DTS might exert beneficial immunologic effects and potent anti-inflammatory properties.^{8,9} While senescence-accelerated mice represent a valuable source of investigations in aging research, the present model in normal strain animals may provide useful insights into the “physiological” aging process that is potentially amenable to clinically oriented therapeutic interventions. While more detailed studies on the mechanisms of action of DTS are in progress, given its inner components’ variety as well,¹⁰ DTS seems a promising nutraceutical in old age.

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