

Anti-aging and Anti-oxidation – Salmon Sperm as a Substitute for Nucleotide Sources

Chen-Meng Kuan^{1,*}, Kai-Wen Kan², Jia-Haur Chen², Young-Hao Lin³, Yung-Hsiang Lin¹

¹Research & Design Center, TCI CO., Ltd., Taipei, Taiwan

²Research & Design Center, TCI Gene Inc., Taipei, Taiwan

³Global Business Center, TCI CO., Ltd., Taipei, Taiwan

*Corresponding author: Chenmeng.Kuan@tci-bio.com

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Abstract This research unveils the possibility of a salmon sperm formula, called DNA drink, for anti-aging and anti-oxidation. The applications of salmon sperm have been rarely reported in scientific investigations if compared with salmon roe, but salmon sperm also contains copious nucleotides. Our experiments confirmed that the DNA drink could enhance the anti-oxidant ability and improve the expression levels of aging-related genes (*CCT2*, *CCT6A*, *Atg1*, *Atg8*, and *SIRT1* genes) in peripheral blood mononuclear cells (PBMCs). These results suggest the potential of salmon sperm for boosting cell vitality and protecting cells from oxidative damage.

Keywords: salmon sperm, anti-oxidant, anti-aging, nucleotide supplement, cell viability

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1. Introduction

Human ambition for longevity continues to advance the development of modern technologies, and human life expectancy will extend with technological progress. The American population at ages 65 and older will rise from 16% in 2018 (52 million people) to 23% by 2060 (95 million people) [1]. Aging is the consequence of physiopathological and progressive deterioration with the passage of time and the damages from chronic oxidative stress will hasten the aging process due, in part, to the impact on the regulatory systems (e.g., endocrine and immune systems) [2]. Oxidative stress is associated with the internal and external stressors (e.g., mitochondrial leak, UV radiation, cigarette smoking, etc.) [3]. Oxidative stress results from the imbalance of the formation and neutralization of reactive oxygen and nitrogen species (RONS), which leads to the accumulation of oxidative damage to macromolecules (i.e., lipid, protein, and DNA) [4,5]. Reactive oxygen species (ROS) include superoxide anion ($O_2^{\cdot-}$) and hydroxyl radical (OH \cdot), which is converted from hydrogen peroxide (H_2O_2) through Fenton or Haber–Weiss reaction [2,6]. Superoxide anion may further interact with nitric oxide (NO), and reactive nitrogen species (RNS) resulting [7]. Nevertheless, human body possesses with sophisticated antioxidant systems in mitochondria for eliminating oxidative stress though several detoxifying enzymes, such as glutathione peroxidase [GPx]/glutathione reductase [GRx], superoxide dismutases (SOD), etc. [8]. Exposing

skin to UV radiation, UV-induced ROS may initiate skin-aging-related cascades and elicit metalloproteinase (MMP)-1-mediated aging as well as NF- κ B-TNF- α -mediated and inflammation-induced aging [9]. Moreover, ROS levels are critically connected to oxidative-induced inflammation and the occurrence/progression of various diseases (e.g., cardiovascular diseases, diabetes, neurodegenerative diseases, or cancers) [10,11,12,13]. Nrf2-Keap1 pathway is the main protective system against oxidative stress and electrophiles, but abnormal Nrf2 regulation may facilitate the progression of inflammation and, in turn, cancer formation [14,15]. Accordingly, certain ROS expression is correlated with aging, inflammatory response, and the occurrence of chronic diseases. Dietary nucleotide supplements are indentified as an effective means to alleviate the inflammatory process, oxidative damage, carcinogenic activity, and aging process, along with restoring energy from fatigue [16,17,18]. Nucleotides involve rudimentary biological functions regarding encoding and deciphering genetic information, modulating metabolism and cell signaling, and as cofactors within enzyme reactions [19]. Based on the results of animal studies, dietary nucleotide supplements have been proved to prolong lifespan in tumor-bearing rats by means of long-term nucleotide feeding [20]. Apart from pure nucleotides, fish roe (egg) may be an alternative route to nucleotide sources [21,22]. A study reported that salmon roe could enhance the type I collagen production over 125% and inhibit antioxidant genes (e.g., *OXR1*, *TXNRD1*, and *PRDX* family genes) in human fibroblasts [22]. These evidences imply that dietary nucleotide supplements have the potential to ameliorate

the skin conditions and delay the aging process [23]. To the best of our knowledge, few research groups unveil the salmon sperm as a nucleotide alternative substitution with respect to the utility in anti-aging or anti-inflammation. In this research, we try to investigate such possibilities through using a salmon sperm formula, DNA drink, to evaluate the expression levels of ROS and aging-related genes in PBMCs. We discovered that DNA drink could substantially enhance these gene expression levels and lower ROS levels, which reveals the hope to extend cell lifespan.

2. Material and Methods

2.1. Materials

DNA drink [MelaGene+ Drink, Melaleuca (China); ingredients: salmon sperm extract, yeast powder, brown rice fermented powder, algal-docosahexaenoic acid, snow fungus extract, citric acid monohydrate, pectin, pear juice, fructose, water, plum/apple/vegetable flavors, and potassium sorbate], PBMC (ATCC[®] PCS-800-011[™]), growth media [X-VIVO[™] 10 (Lonza), 10% fetal bovine serum, 1 mM sodium pyruvate, and 1% penicillin/streptomycin], 2',7'-dichlorofluorescein diacetate [DCFH-DA (Sigma-Aldrich)], phosphate buffered saline [PBS (Gibco)], RNA extraction kit (Genaid Biotech), nCounter[®] platform (NanoString Technologies)

2.2. ROS Assay

We dispersed 2×10^5 PBMCs in 2 mL of the growth media into each well in 6-well plates. Following 24 hours incubation, we replaced the media with the refresh media, the media with 0.5 mM H₂O₂, the media with 0.5 mM H₂O₂, or 0.5%/0.25% DNA drink for the corresponding testing cells, and incubated the cells for an hour. Afterwards, we removed the solutions and washed the cells with PBS solutions twice. And we added 10 µg/mL of DCFH-DA (a ROS indicator) to each well and waited for 40 minutes for the staining reaction. Finally, we collected the cells from wells by use of trypsin and loaded the suspending cells into a cytometry (excitation wavelengths: 450-490 nm; emission wavelengths: 510-550 nm).

2.3. Analysis of mRNA Expression

1.5×10^5 PBMCs in 2 mL of the media with 0.25% of DNA drink were dispersed into each well of 6-well plates. Following 24 hour incubation, we collected the PBMCs and extracted their total RNA by the RNA extraction kit. 75 ng/µL RNA extracts were used as templates for analysis of mRNA expression level through the nCounter[®] platform. The operation was following the nCounter protocol.

2.4. Statistical Analysis

The statistical analysis for the experimental results was based on t-test; $p < 0.05$ represented significant difference.

3. Results and Discussion

3.1. Evaluation of ROS Production in PBMC

To evaluate the efficacy of the DNA drink for ant-oxidation, following the treatments of 0.5% and 0.25% of DNA drink, we treated PBMCs with 0.5 mM of hydrogen peroxide (H₂O₂) for an hour to induce the formation and accumulation of ROS (Figure 1). The ROS levels of the cells treated with DNA drink were at least 48% lower than that of the only H₂O₂-induced cells. We used 0.25% of the salmon sperm formula for the following testing considering sample saving and the similar result with 0.5%.

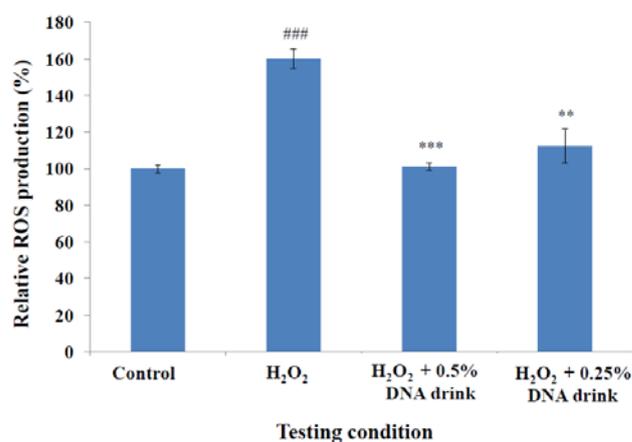


Figure 1. Evaluation of ROS production in PBMCs under the circumstance of different concentrations of DNA drink. ($n = 3$, mean value \pm S.D.) (Comparison with the control group: ###, $p < 0.001$) (Comparison with the H₂O₂ group: ***, $p < 0.001$; **, $p < 0.01$)

3.2. mRNA Expression Level of SOD2 Gene

Figure 2 shows the salmon sperm formula could increase the expression level of SOD2 gene, which encodes superoxide dismutase 2, by ~30%. This result corresponds with the ROS elimination result (Figure 1) and verifies the antioxidant capacity in salmon sperm.

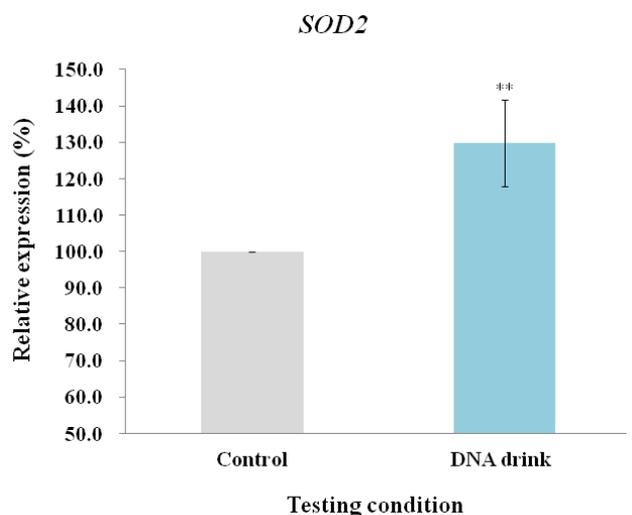


Figure 2. The mRNA expression levels of SOD2 gene in PBMCs after 0.25% DNA drink treatment. ($n = 3$, mean value \pm S.D.; **, $p < 0.01$)

3.3. mRNA Expression Levels of Anti-aging Related Genes

Furthermore, we analyzed the mRNA expression levels for some aging-related genes. Figure 3 shows the increase of the expression levels of *CCT2*, *CCT6A*, *Atg1*, *Atg8*, and *SIRT1* genes after DNA drink treatment; especially, the significant changes in the expression of *CCT2*, *CCT6A*, and *Atg* genes. Chaperonin containing TCP1 (CCP) family chaperonins participate in correct protein folding and their high expression levels represent vigorous cell proliferation [24]. Autophagy-related proteins (Atg) are associated with the regulation of autophagic process to remove damaged and unnecessary molecules or components in cells by lysosomal degradation, which remains the stable cell functions, structures, and viability [25]. Sirtuin 1 (SIRT 1) can deacetylate various cellular proteins and is essential to the control of cell aging, oxidative stress, inflammatory responses, etc. [26]. Therefore, salmon sperm indeed can improve cell vitality and the functionalities of the depletion of cell metabolites as well as prevent cells from oxidative damage.

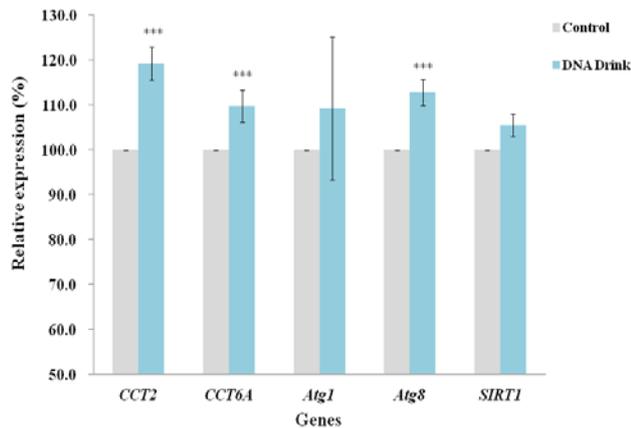


Figure 3. The mRNA expression levels of anti-aging related genes after 0.25% DNA drink treatment. ($n = 3$, mean value \pm S.D.; ***, $p < 0.001$)

4. Conclusion

This work successfully demonstrates the potential of our salmon sperm formula for anti-aging and anti-oxidation. DNA drink can remarkably improve the anti-oxidative capability of ROS elimination and the increment of the expression level of *SOD2* gene. More importantly, DNA drink is able to up-regulate the aging-related genes, which contribute to correct protein folding, the depletion of unnecessary molecules or substances, and the modulation of cell proliferation/aging. In summary, we have proved that salmon sperm can be an alternative route to nucleotide sources apart from salmon roe and improve cell vitality by comprehensive gene regulations.

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