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Vitamin C Improves the Health Span of Animal Models of Werner Syndrome

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ABSTRACT

Werner syndrome (WS) is a rare autosomal recessive disorder characterized by a pro-oxidant/pro-inflammatory status, genomic instability, and by the premature onset of several age-associated diseases. The protein defective in WS patients (WRN) is a helicase/exonuclease involved in DNA replication, repair, and transcription. This review focuses on the beneficial impact of vitamin C treatment in mutant mouse and worm models of WS with an emphasis on serum metabolomic and cytokinome profiles in Wrn mutant mice. Vitamin C normalizes the health and life span of these mutant animals. More importantly, our recent results indicate that it will be possible to follow the beneficial impact of vitamin C at a systemic level by monitoring specific serum metabolites and inflammatory cytokines in a longitudinal study involving WS patients.

Main text

Werner Syndrome (WS; MIM# 277700) is an autosomal recessive disorder characterized by genomic instability and the premature onset of a number of age-related diseases including ocular cataracts, dyslipidemia, diabetes mellitus, osteoporosis, atherosclerosis, and cancer[1-3]. The gene responsible for WS (*WRN*) was identified by positional cloning[4] and its product contains an evolutionary conserved RecQ DNA helicase consensus domain[4-6]. The protein also has a 3'-5' exonuclease activity[7-9]. WS is a rare disorder worldwide. However, the frequency of WS can be highly prevalent in certain regional populations showing high degrees of consanguinity. For example, the frequency of carriers has been estimated to be as high as 1/150 to 1/200 in several prefectures of Japan with a prevalence of homozygotes of ~three in one million newborns (see http://atlasgeneticsoncology.org/Kprones/WernerID10017.htm). More than eighty distinct mutations potentially inactivating the WRN protein have been described in WS patients to date based on The International Registry of Werner Syndrome; Department of Pathology, University of Washington, Seattle, WA, USA (www.wernersyndrome.org). These mutations include missense and nonsense substitutions, frame shifts and premature translation termination mutations, deletions and insertions. All these mutations are believed to disrupt the normal function of the protein or to cause a truncation of the protein such that it cannot localize to the nucleus, the normal site of WRN protein action. Accumulating evidences indicate that WRN is involved in DNA replication, DNA repair, and telomere maintenance[10-18]. WRN interacts with several proteins

important for homologous recombination, nonhomologous recombination, and long-patch base excision repair pathways[12,14,17,19-24]. In addition to defects in DNA replication and repair, alterations of gene expression at the RNA level have also been observed in WS cells implicating WRN in some aspects of transcription as well[25-27]. Accordingly, mass spectrometry analysis of an immunoprecipitated tagged WRN protein revealed the presence of several transcription factors (such as SAFB1, Scaffold Attachment Factor B1)[28] and two subunits of the RNA polymerase II machinery (POLR2A and POLR2B)[19]. However, such interactions with WRN were lost upon nuclease treatments of the lysates prior to the immunoprecipitation step. These results suggest that the interaction between WRN and the RNA polymerase II machinery requires nucleic acids. In addition, the WRN protein modulates the expression of genes containing G-quadruplex DNA structures, a family of non-canonical nucleic acid structures formed by certain G-rich sequences at several chromosomal sites in WS fibroblasts[26,27]. However, WS cells contain several chromosomal rearrangements that may affect gene expression and the interpretation of the data. To avoid using WS fibroblasts from patients that accumulated DNA rearrangements with time, we depleted WRN protein levels in normal human fibroblasts for a short period of time (48 hours) with small interfering RNAs specific for the WRN mRNA to perform expression-profiling studies[29]. We determined that such WRN-depleted cells did not have time to accumulate mutations within 48 hours but exhibited increased oxidative stress. Oxidative stress is believed to exacerbate several age-related diseases[30]. More importantly, our microarray analyses determined that a short-term knock down of WRN was sufficient to induce an expression profile resembling the one obtained with fibroblasts derived from old individuals[29]. Thus, besides the already known impact of WRN on DNA replication, DNA repair, the p21/p53 pathway and cell cycle, gene set enrichment analyses of our microarray data uncovered significant impact of WRN levels on the expression of genes involved in adipocyte differentiation, oxidative stress, and inflammatory responses[29]. Remarkably, several defects observed in WS patients are reminiscent of a chronic inflammatory metabolic syndrome, which can also be observed in the general aging population[31]. Metabolic syndrome afflicts up to half the population of western countries and is considered an age-related pro-inflammatory lipid disorder[32]. Accordingly, increased oxidative stress has been described for WS subjects in addition to the abnormal metabolic phenotypes such as enhanced intra-abdominal visceral fat accumulation or non-alcoholic hepatic steatosis[33-36]. Finally, cytokine analyses of the serum of WS patients indicated abnormal elevation of several inflammatory cytokines or interleukins like IL-4, IL-6, or IL-10 in addition to an abnormal increase of the cardiovascular risk factor plasminogen activator inhibitor 1 (PAI-1)[37,38]. Several of these cytokines are collectively referred to as the senescence-associated secretory phenotype and are important hallmarks of aging. Interestingly, this senescence-associated secretory phenotype is suppressed by reprogramming WS fibroblasts to generate WS induced-pluripotent stem cells upon transduction of the Yamanaka factors (OCT3/4, SOX2, KLF-4, and c-myc)[39].

A mouse model containing a deletion of part of the helicase domain was generated to understand the molecular basis of the premature aging phenotype in WS[40]. This mutant mouse (referred as $Wrn^{\Delta hel/\Delta hel}$) synthesizes a stable mutant protein that has no helicase and exonuclease activities[41]. Interestingly, the metabolic and cytokine profiles of $Wrn^{\Delta hel/\Delta hel}$ mice is different from age-matched wild type mice or the mutant *Wrn* null mice that do not synthesize a Wrn protein[41]. Overall, $Wrn^{\Delta hel/\Delta hel}$ mice exhibit a marked dyslipidemia, show evidence of a low but chronic systemic inflammation, and a 17-22% reduction in mean life span compared to wild type and *Wrn* null mice. The $Wrn^{\Delta hel/\Delta hel}$ mouse model phenocopies several other aspects of the human WS such as hyperglycemia and insulin resistance, elevated blood hyaluronic acid, increased serum IL-10 and PAI-1, hepatic steatosis, aortic stenosis, cardiac fibrosis, and several types of cancer[42-45]. Importantly, we found that the Wrn helicase mutant protein is mislocalized to the cytoplasm in tissues of $Wrn^{\Delta hel/\Delta hel}$ mice[41]. In addition to the loss of Wrn activites in the nucleus, this cytoplasmic mislocalization affects the normal function of several organelles, including the peroxisomes, the endoplasmic reticulum, and the autophagosomes, leading potentially to the alteration of serum metabolites seen in $Wrn^{\Delta hel/\Delta hel}$ mice. Such alterations may be the precursor of molecular events responsible for the age-related changes observed in older $Wrn^{\Delta hel/\Delta hel}$ mice. Of relevance to this mouse work, a recent report indicated that WS patients with a nonsense mutation at position 1256 of the human WRN protein synthesized a stable truncated protein localized in the cytoplasm of their cells[46]. These patients exhibited type 2 diabetes, cataracts, hypercholesterolemia, short stature, bird-like facies, skin ulcers on the lower limbs, osteoporosis, and arterial atherosclerosis. A survey of different WS derived cells with different mutations will be required with the appropriate antibodies to assess the impact of abnormal WRN protein in such cells. It will be important to determine whether the phenotype of human WS cells expressing a detectable mislocalized truncated WRN protein is more severe than WS cells with no measurable level of WRN protein.

One advantage of using animal models is the possibility of modifying their diet in a controlled laboratory setting. Interestingly, $Wrn^{\Delta hel/\Delta hel}$ mice show a decrease in serum glutathione (GSH) level and an increase in serum vitamin

C level[47]. Notably, WS patients also exhibit an imbalance in plasma GSH level and an increase in serum ascorbic acid (vitamin C), suggesting a pro-oxidant status in such individuals[33]. The increase in vitamin C levels in *Wrn*$^{\Delta hel/\Delta hel}$ mice may be due to a response to the abnormal redox status in such animals inferred by the elevated oxidative DNA damage, the augmented lipid peroxidation and reactive oxygen species levels in several tissues of these mutant mice[43, 47]. Importantly, supplementation of *Wrn*$^{\Delta hel/\Delta hel}$ mice with 0.4% vitamin C (weight/volume) in drinking water reversed all the phenotypes observed in *Wrn*$^{\Delta hel/\Delta hel}$ mice and increased the mean life span of these animals to a normal wild type life span[47]. Such a complete reversal of the phenotypes observed in *Wrn*$^{\Delta hel/\Delta hel}$ mice was not obtained with other known antioxidants like resveratrol or catechin[43,48]. It is important to mention that vitamin C is not only a soluble antioxidant but also an important co-factor for hydrolase and monooxygenase enzymes involved in the synthesis of collagen, carnitine, and neurotransmitters[49]. Carnitine is required for the transport and transfer of fatty acids into mitochondria where it can be used for energy production. Vitamin C is also necessary for the transformation of cholesterol to bile acids as it modulates microsomal hydroxylation reactions in the liver[49]. In addition, vitamin C regulates the active levels of several transcription factors like HIF1α (Hypoxia induced factor 1α) or NF-κB (a factor that can modulate inflammatory responses)[50,51]. Accordingly, we observed that vitamin C treatment reversed the abnormal high levels of active HIF1α and NF-κB found in the liver of *Wrn*$^{\Delta hel/\Delta hel}$ mice[47]. Vitamin C is also important for the activities of enzymes involved in the oxidative protein folding reactions in the endoplasmic reticulum[52]. Noticeably, we found that the mislocalization of the Wrn helicase mutant protein in the endoplasmic reticulum fraction from liver tissues increased oxidative stress in that cellular compartment even in younger four-month old mice before they exhibit hepatic steatosis, cardiac fibrosis, or cancer. Thus, although younger *Wrn*$^{\Delta hel/\Delta hel}$ mice did not exhibit a pro-oxidant status at the systemic level, they did show oxidative stress at the sub-cellular level in tissues. Vitamin C treatment reversed this stress in the endoplasmic reticulum of *Wrn*$^{\Delta hel/\Delta hel}$ mice[45].

We have evidence that vitamin C is not only efficient in reversing the age-related phenotypes observed in *Wrn*$^{\Delta hel/\Delta hel}$ mice, but it is also efficient in reversing aging in the worm *Caenorhabditis elegans* bearing a nonfunctional *wrn-1* DNA helicase ortholog[53]. The longevity of *wrn-1* mutant worms is reduced compared to wild type worms when grown at 25˚C. The median life span of vitamin C treated *wrn-1* mutant worms was significantly increased by 26% compared to untreated *wrn-1* mutant animals and was comparable to the normal median life span of wild type worms.

Altogether, the results obtained with both worms and mice support the hypothesis that vitamin C treatment may improve the health and/or life span of very different species bearing a debilitating mutation in the *WRN* gene ortholog. Thus, a long-term vitamin C supplementation could have beneficial effects for human patients with WS. A major advantage of using vitamin C as a potential treatment for WS is the wide range of concentrations that can be used without toxic effect in humans. Another advantage is the possibility of following the impact of vitamin C treatment in patients with WS by simply monitoring several metabolites and secreted factors in their serum such as specific lipids, glucose, PAI-1, IL-6, or IL-10[37,38]. Indeed, vitamin C treatment normalized the serum cardiometabolic and inflammatory profiles of *Wrn*$^{\Delta hel/\Delta hel}$ mice[45]. We hypothesize that similar results could be obtained with WS patients. Of interest, a recent case study indicated that the lipid-soluble antioxidant molecule astaxanthin improved the nonalcoholic fatty liver disease of WS patients with diabetes mellitus[54]. In addition, the reduction of oxidative stress observed in WS fibroblasts alleviates their abnormal in vitro morphological phenotype[55]. Importantly, vitamin C not only regenerates GSH (a natural anti-oxidant) in the body, but it is also capable of recycling the lipid-soluble vitamin E[56] providing potentially additional beneficial effect. Thus, it should be possible to perform a longitudinal study by simply examining the systemic inflammatory and oxidant status in vitamin C treated WS patients by means of a straightforward clinical time-course noninvasive blood sampling protocol.

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