Inflammation & Cell Signaling 2019; 6: e1539. doi: 10.14800/ics.1539; © 2019 by Takahiro Sakai, *et al*.
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MINIREVIEW

Development of a novel cytoplasmic hydroxyl radical-targeting antioxidant (TA293) that suppresses cellular senescence, inflammation, and apoptosis

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Received: March 15, 2018
Published: May 24, 2019

Hydroxyl radicals (˙OH) exhibit the strongest oxidation potential of any reactive oxygen species (ROS) and react non-specifically with cellular components, such as nucleic acids, lipids and proteins. While mitochondrial ˙OH incites oxidative damage resulting in mitochondrial dysfunction, the actions of cytoplasmic ˙OH remain unknown as no cytoplasmic ˙OH-specific scavenger has been identified to date. To solve this problem, we developed the cytoplasm- and mitochondrion-specific ˙OH-targeted scavengers TA293 and mitoTA293, respectively. As expected, TA293 and mitoTA293 scavenged ˙OH, but not O_2^- or H_2O_2. Notably, TA293 scavenged pyocyanin-induced cytoplasmic ˙OH, but not mitochondrial radicals induced by antimycin A. Conversely, mitoTA293 scavenged ˙OH only in the mitochondria *in vivo* and *in vitro*. Interestingly, we found that cytoplasmic ˙OH plays a central role in cytoplasm ROS-induced oxidative stress, which potentiates cellular senescence, inflammation, and apoptosis in the kidney and lung. Based on these findings, we believe that TA293 could be a novel tool to study the effects of ˙OH damage within the cytoplasm.

Keywords: antioxidant; hydroxyl radicals; oxidative stress; cellular senescence; inflammation

To cite this article: Takahiro Sakai, *et al*. Development of a novel cytoplasmic hydroxyl radical-targeting antioxidant (TA293) that suppresses cellular senescence, inflammation, and apoptosis. Inflamm Cell Signal 2019; 10.14800/ics.1539.

Introduction

Excess reactive oxygen species (ROS) or free radicals oxidize cellular components, resulting in oxidative stress [1, 2]. These molecules react non-specifically with nucleic acids, lipids, and proteins, and are critical mediators of age-related diseases such as cancer, type II diabetes, neurodegenerative disease, cardiovascular disease, macular degeneration, and osteoarthritis [3-8]. NADPH oxidase and xanthine oxidase in the mitochondrial electron transport chain generate most of the superoxide anion radicals (O_2^-), which are subsequently converted to H_2O_2 by either glutathione peroxidase or catalase [9]. Excess H_2O_2 then results in the production of hydroxyl radicals (˙OH) via metal ion catalysis, as in the Fenton reaction [10]. In low concentrations, O_2^- and H_2O_2 act as signaling effectors to play important physiological roles in apoptosis, cell proliferation, and differentiation, amongst others [11]. However, unlike that of O_2^- and H_2O_2, ˙OH

scavenging is a critical antioxidant process, as there is no known detoxification system for these radicals [12].

Recent reports have identified important roles for $^{\bullet}OH$ in the pathophysiology of several diseases [13-15]. Mitochondrial $^{\bullet}OH$ are predicted to oxidize organelle constituents and cause mitochondrial dysfunction [16]; however, the functional significance of cytoplasmic $^{\bullet}OH$ remains to be determined since there is no way to scavenge these radicals specifically. Other $^{\bullet}OH$ scavengers such as, thiourea, H_2, salicylates, and flavonoids are likely distributed throughout the cell because of their low molecular-weight [16-19], and scavenge $^{\bullet}OH$ indiscriminately. In addition, many of these compounds have cytotoxic effects, such as damage of DNA, proteins, lipids, and other macromolecules [20-22]. As such, the function of compartment-specific $^{\bullet}OH$ are still unknown.

We recently developed TA293, a novel antioxidant based on the structure of ascorbic acid (also known as vitamin C). Ascorbic acid is a water-soluble molecule with antioxidant activity derived from its enediol structure and oxidized to dehydroascorbic acid via a radical intermediate [23]. Similarly, TA293 was designed to act as resonance system by adding plural C-C double bonds to the enediol structure; thus, the structure becomes resonance-stabilized after delocalization of its radical, thereby suppressing the oxidation reaction. In addition, TA293 is a more lipophilic compound that can permeate various tissue [24]. We also synthesized mitochondrial-specific mitoTA293 by adding the mitochondrial localization signal triphenylphosphonium to the TA293 molecule [25]. A subsequent analysis of the effects of pyocyanin-induced cytoplasmic $^{\bullet}OH$ in the presence of TA293 demonstrated the radical's central role in inflammation, cellular senescence, and apoptosis.

Effects of TA293 and mitoTA293

A preliminary analysis of TA293 and mitoTA293 function revealed that these compounds exhibit $^{\bullet}OH$ scavenging activity, but have no effect on O_2^- and H_2O_2 in a cell-free system. To study the effect of each compound *in vivo*, cells were treated with pyocyanin and antimycin A to induce ROS formation in the cytoplasm and mitochondria, respectively [26, 27]. As expected, TA293 appears to selectively scavenge $^{\bullet}OH$ in the cytoplasm, whereas mitoTA293 acts solely in the mitochondria. Moreover, conventional $^{\bullet}OH$ scavengers such as thiourea can induce cytotoxicity, but neither TA293 nor mitoTA293 hindered the viability of established cell lines or primary cells in culture. Thus, these data suggest that TA293 and mitoTA293 may help elucidate the physiological significance of $^{\bullet}OH$ localized to the cytoplasmic or mitochondria.

TA293 scavenges pyocyanin-induced cytoplasmic $^{\bullet}OH$.

To further elucidate the effects of TA293 and mitoTA293, we evaluated oxidative stress in primary mouse embryonic fibroblasts (MEF) derived from Keap1-dependent oxidative stress luciferase indicator transgenic (OKD48-Tg) mice that harbor an antioxidant response element (ARE)-regulated luciferase reporter [28]. Notably, TA293 suppressed pyocyanin-induced oxidative stress and apoptosis, but not that induced by antimycin A treatment; while the opposite was true for mitoTA293. Moreover, we evaluated oxidative stress activity in pyocyanin-treated OKD48-Tg mice in the presence or absence of TA293. As expected, TA293 suppressed systemic pyocyanin-induced oxidative stress and biooxidation, but not that resulting from antimycin A. Conversely, mitoTA293 only suppressed antimycin A-induced oxidative stress and biooxidation. Together, these findings suggest that cytoplasmic and mitochondrial $^{\bullet}OH$ play key roles in oxidative stress within their respective compartments *in vitro* and *in vivo*.

We then attempted to identify the organ most impacted by the presence of cytoplasmic $^{\bullet}OH$. This analysis revealed that pyocyanin induced-oxidative stress is markedly increased in the lung and kidney, but not other organs such as the heart, liver, stomach, and spleen. Significantly, TA293 treatment attenuated oxidative stress in these areas, supporting that cytoplasmic $^{\bullet}OH$ specifically induced oxidative stress in lung and kidney *in vivo*. Subsequent histological examination showed pyocyanin-induced oxidative damage in the lung alveoli and kidney tubules, which was mitigated in TA293-treated counterparts. Collectively, these data indicate that the cytoplasmic $^{\bullet}OH$ play a central role in oxidative damage that occurs in organs highly sensitive to radicals in this subcellular compartment.

TA293 suppresses cytoplasmic $^{\bullet}OH$-induced cellular senescence, inflammation, and apoptosis.

Recent studies indicate that pyocyanin elicits oxidative stress and premature cellular senescence [29]. Senescent cells secrete senescence-associated secretory phenotype (SASP) factor, such as IL-6, resulting in chronic inflammation, macrophage infiltration, senescent cell clearance, and tissue remodeling [30, 31]. Notably, we found that TA293 suppressed cellular senescence induced by pyocyanin *in vitro*. The CDK inhibitors p21 (WAF1/ CIP1) and p16 (INK4A) induce cell cycle arrest and cellular senescence [32], and are highly expressed in response to oxidative damaged tissues; however, TA293 dampened the expression of these factors.

IκB phosphorylation-mediated NF-κB signaling has also been shown to promote SASP factor secretion [33].

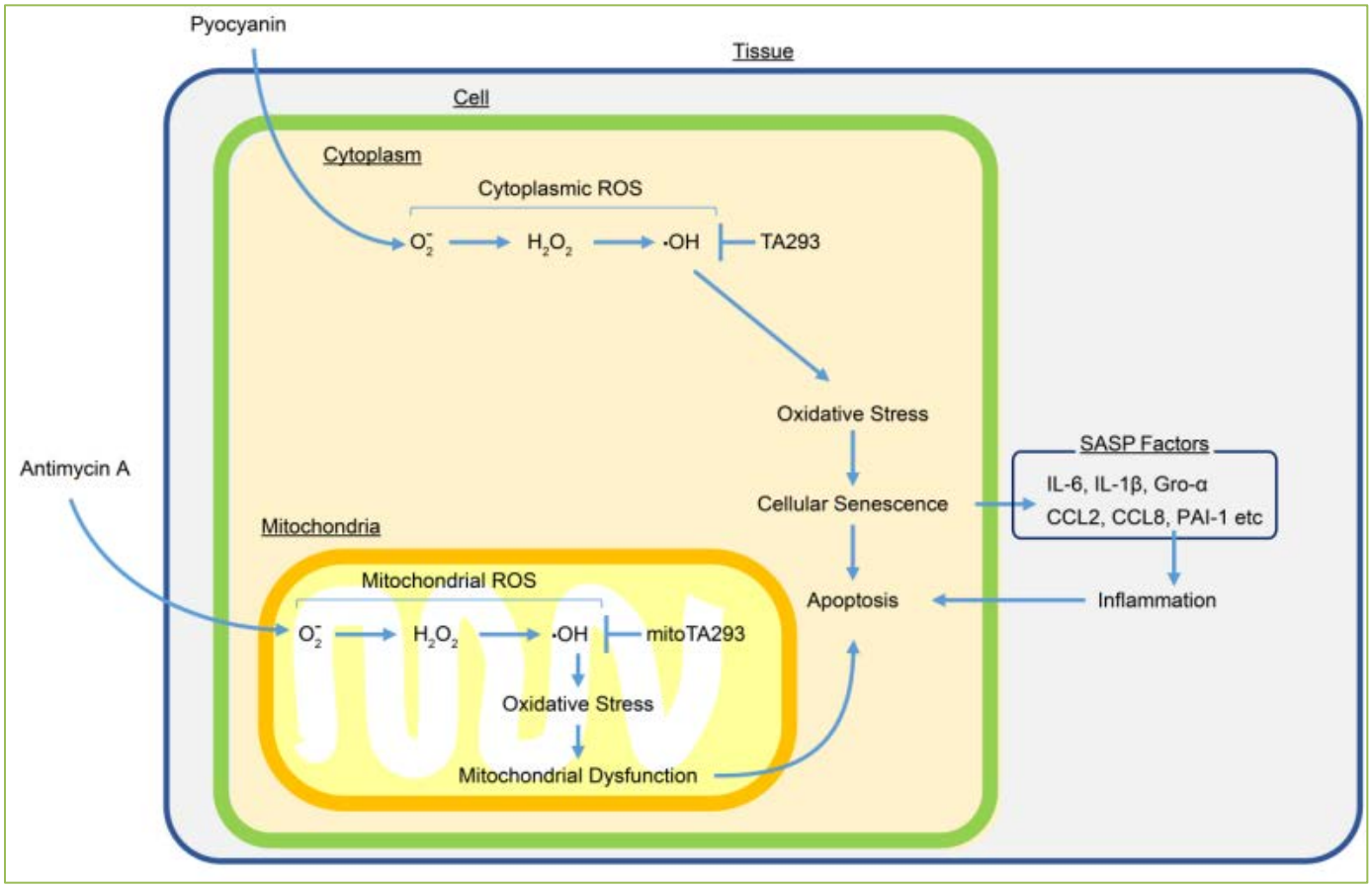

Figure 1. Cytoplasmic ˙OH-induced cellular senescence, inflammation, and apoptosis. TA293 attenuated cytoplasmic ˙OH-induced oxidative stress, cellular senescence, inflammation, and apoptosis; whereas mitoTA293 suppressed mitochondrial ˙OH-induced oxidative stress and apoptosis. These finding suggested that cytoplasmic and mitochondrial ˙OH induce oxidative damage in their respective subcellular compartments.

Consistently, we found elevated IκB phosphorylation in tissues with oxidative damage, but this was attenuated in the presence of TA293. In addition, TA293 was sufficient to block pyocyanin-induced inflammation in IL-1β-based dual-operating luciferase transgenic (IDOL-Tg) mice [34]. Moreover, TA293 suppressed mRNA expression SASP factors (e.g., proinflammatory cytokines, chemokines, and extra cellular matrix remodeling factor) and apoptosis in tissues with oxidative damage in pyocyanin-treated mice. Since cellular senescence, inflammation, and apoptosis are triggered by common stimuli such as DNA damage or oncogenic stress [35], it is not unsurprising that cytoplasmic ˙OH-induced oxidative damage elicited these cellular responses *in vivo* and could be attenuated by TA293 treatment.

Conclusions

In summary, the results of our study provide scientific evidence that TA293 suppressed cytoplasmic ˙OH-induced oxidative stress, cellular senescence, inflammation, and apoptosis; whereas mitoTA293 attenuated mitochondrial ˙OH-induced oxidative stress and apoptosis (Fig. 1). Based on these findings, we propose a novel hypothesis that cytoplasmic and mitochondrial ˙OH respectively induce oxidative damage in cytoplasm and mitochondria, resulting in oxidative stress in each individual subcellular compartment.

Most importantly, this study provided the first experimental evidence that TA293 is a non-toxic compound capable of specifically scavenging cytoplasmic ˙OH. For this reason, we believe that TA293 will be a useful tool to elucidate the mechanisms of ˙OH-induced inflammation, cellular senescence, and apoptosis; and may serve as a prototype to develop therapeutic agents for cytoplasmic ˙OH-induced disease.

Conflicting interests

The authors have declared that no conflict of interests exist.

Acknowledgements

Cactus Communications provided editorial support, in the form of medical writing based on our detailed directions, collating our comments, copyediting, fact checking, and referencing. This work was supported by a JSPS KAKENHI Grant in-Aid for Young Scientists (B) (grant no. 24700434). We also received support from the Science Research Promotion Fund from the Promotion and Mutual Aid Corporation for Private Schools of Japan.

Author contributions

TS made substantial contributions to conception and design, or acquisition of data, or analysis and interpretation of data; has been involved in drafting the manuscript or revising it critically for important intellectual content; and has given final approval of the version to be published. JI, TI, HT, MU, and SH were in charge of the study design and also approved the version to be published.

Abbreviations

$^{\bullet}$OH: hydroxyl radical; ROS: reactive oxygen species; MEF: mouse embryonic fibroblast; Tg: transgenic; SASP: senescence-associated secretory phenotype.

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