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Review

Is antioxidant supplement beneficial? New avenue to explore

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ABSTRACT

Background: In recent years, numerous disappointing clinical outcomes of antioxidant supplement have been reported for various oxidative and nitrosative stress-related diseases. These failures of antioxidant supplement in preventing/treating diseases have become a major hurdle in the therapeutic application.
Scope and approach: This paper described the current status of antioxidant supplement area, and reviewed briefly the important progress in developing probes or sensors for real-time imaging of reactive oxygen species (ROS) and reactive nitrogen species (RNS) in recent years. We then explored the potential of these probes or sensors for real-time imaging of ROS and RNS as new avenues to directly assess whether antioxidants work or not *in vivo*.
Key findings and conclusions: Examining whether or not antioxidant can counteract oxidative and nitrosative stress *in vivo* is crucial to overcome the hurdles in the antioxidant supplement area. We proposed that the recently developed probes or sensors for real-time imaging of ROS and RNS provided a valuable avenue to assess the antioxidant efficacy *in vivo*. Future directions and potential barriers to overcome for clinical translations of these probes or sensors were discussed to direct antioxidant supplement study.

1. Introduction

Oxidative and nitrosative stress have been widely accepted to play an important role in the pathogenesis of numerous diseases, such as cancer, cardiovascular diseases, neurodegenerative diseases, diabetes, and *etc.* (Barnham, Masters, & Bush, 2004; Griendling & FitzGerald, 2003; Reuter, Gupta, Chaturvedi, & Aggarwal, 2010; Sosa et al., 2013; Yang, Jin, Lam, & Yan, 2011). Therefore, antioxidant supplement has attracted tremendous interest in the past decades in preventing or treating these diseases by counteracting the effect of reactive oxygen species (ROS) and reactive nitrogen species (RNS). However, the *in vitro* strong antioxidant ability can not necessarily be translated into *in vivo* effect and numerous disappointing clinical outcomes of antioxidant supplement have been reported for various oxidative and nitrosative stress-related diseases (Table 1) (Grodstein et al., 2013; Lippman et al., 2009; Liu et al., 2006; Sesso et al., 2008; The Alpha-Tocopherol, Beta Carotene Cancer Prevention Study; Group, 1994; Omenn et al., 1996). For instance, a recent systematic review of studies published from 2005 to 2013 concluded that evidence supports benefit from vitamin supplement for cancer or cardiovascular disease prevention is limited (Fortmann, Burda, Senger, Lin, & Whitlock, 2013). Similarly, a recent meta-analysis covering 50 randomized controlled trials with 294,478 participants also found no evidence to support the use of vitamin and antioxidant supplements for prevention of cardiovascular diseases (Myung et al., 2013). Another recent randomized, double-blind, placebo-controlled trial found that long-term use of a daily multivitamin did not exhibit benefits in cognitive decline (Grodstein et al., 2013).

2. Do antioxidants work *in vivo*?

These failures have become a major hurdle in clinical therapeutic application of antioxidant presently, which make it urgent to determine whether or not antioxidant can counteract oxidative and nitrosative stress *in vivo*. New clues challenging our traditional knowledge of the benefits of antioxidant emerge in recent years. For instance, despite the efficient $^1O_2$-quenching activity of β-carotene in homogeneous liquid solutions is invariably invoked when interpreting its biological antioxidant ability, a preliminary study employing unique microscope-based time-resolved spectroscopic methods surprisingly suggested that β-carotene did not

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Table 1
Selected clinical studies of antioxidant supplement in combating oxidative and nitrosative stress-related diseases.

Antioxidant	Disease	Study type	Dose	Outcomes	Ref
Vitamins E and C	cardiovascular disease	randomized, double-blind, placebo-controlled trial	vitamin E (400 IU every other day) and vitamin C (500 mg/d)	Not reduce major cardiovascular events risk	Sesso et al., 2008
Selenium, vitamin E	prostate cancer	randomized, placebo-controlled trial	selenium (200 mg/d) and vitamin E (400 IU/d)	Not prevent prostate cancer	Lippman et al., 2009
Vitamin E, β-carotene	lung cancer	randomized, double-blind, placebo-controlled trial	vitamin E (50 mg/d) and β-carotene (20 mg/d)	Not reduce lung cancer incidence among male smokers	The Alpha-Tocopherol, Beta Carotene Cancer Prevention Study Group, 1994
β-Carotene, vitamin A	lung cancer and cardiovascular disease	multicenter, randomized, double-blind, placebo-controlled trial	β-carotene (30 mg/d) and vitamin A (25,000 IU/d)	No benefit on incidence of lung cancer and on risk of death from lung cancer and cardiovascular disease	Omenn et al., 1996
Multivitamin	cognitive decline	randomized, double-blind, placebo-controlled trial	Not reported	No cognitive benefits	Grodstein et al., 2013
Vitamin E	type 2 diabetes	randomized, double-blind, placebo-controlled trial	600 IU/d	No significant effect in reducing type 2 diabetes in healthy women	Liu et al., 2006

quench $^1O_2$ in mammalian cells (Bosio et al., 2013). Nevertheless, the more direct and reliable antioxidant efficacy assessment in tissues and organs has, unfortunately, encountered technical challenges. Here, we propose that the newly developed real-time sensors or probes for imaging of various ROS and RNS provide good opportunities to determine whether antioxidants work or not *in vivo*.

3. Probes for ROS and RNS imaging

In recent years, much effort has been devoted to develop sensors or probes for imaging of various ROS and RNS *in vivo*, which offers insights into redox dynamics and the ROS/RNS biology (Chen, Tian, Shin, & Yoon, 2011; Woolley, Stanicka, & Cotter, 2013). Multiple ROS and RNS sensors or probes, including $O_2^{\bullet-}$, H_2O_2, •OH, and $ONOO^-$, have been developed and examined in living animal studies.

Several studies have developed probes for *in vivo* detection of $O_2^{\bullet-}$. Zhang et al. developed a novel fluorescent probe (N,N′-di-((2E)-3-(3,4-dihydroxyphenyl)acrylic acyl)-1,3,5-triazine-2,4,6-triamine, Fig. 1a) with one-photon and two-photon fluorescence properties for dynamic imaging of $O_2^{\bullet-}$ fluctuations in both live cells and *in vivo* (Zhang et al., 2013). Very recently, Huang et al. reported the successful application of two near-infrared fluorescent probes (Hcy-Mito, Fig. 1b, and Hcy-Biot, Fig. 1c) for the detection of $O_2^{\bullet-}$ and hydrogen polysulfides (H_2Sn) in organs and tumor issue of mice (Huang, Yu, Wang, & Chen, 2016). Hu et al. recently reported the synthesis of three novel $O_2^{\bullet-}$ fluorescent probes HKSOX-1 (Fig. 1d) and HKSOX-1r (Fig. 1e) for cellular retention, and HKSOX-1m (Fig. 1f) for mitochondria-targeting, and their application in detection of $O_2^{\bullet-}$ in intact live zebrafish embryos (Hu et al., 2015).

For H_2O_2 probes, Shuhendler et al. recently reported the successful use of semiconducting polymer–based nanosensors by combining fluorescence resonance energy transfer and chemiluminescence resonance energy transfer for rapid and real-time imaging of oxidative and nitrosative stress (H_2O_2 and $ONOO^-$) in the liver of live animals for specially testing drug-safety assays for hepatotoxicity (Shuhendler, Pu, Cui, Uetrecht, & Rao, 2014). Van de Bittner et al. synthesized a chemoselective bioluminescent probe (peroxy caged luciferin-1, Fig. 1g) and reported its efficacy for *in vivo* imaging and real-time detection of basal H_2O_2 levels in mice (Van de Bittner, Dubikovskaya, Bertozzi, & Chang, 2010). Bilan et al. demonstrated the successful application of HyPer-3, a genetically encoded fluorescent indicator for intracellular H_2O_2, in imaging of H_2O_2 gradients in zebrafish larvae (Bilan et al., 2013). As to ·OH, Zhang et al. synthesized a novel fluorescent probe (6-triethylene glycol substituted fluorescein hydrazide, Fig. 1h) and examined its applications in real-time imaging of ·OH and HClO in zebrafish (Zhang et al., 2016). Kielland et al. investigated the use of a chemiluminescent probe (8-amino-5-chloro-7-phenylpyrido[3,4-d]pyridazine-1,4(2H,3H)dione) for molecular imaging of ROS and RNS generated from phagocytes during inflammation in living mice (Kielland et al., 2009).

Several studies have investigated the application of fluorescent probe to detect the ROS levels in the eye of mice, which may be useful as novel tools to evaluate oxidative stress in ophthalmic diseases (Prunty et al., 2015). Prunty et al. reported the efficacy of a near-infrared ROS fluorescent probe (Hydrocyanine-800CW, Fig. 1i) in detecting oxidative stress in the eye of mice Similarly, Rayner et al. found that a novel reversible profluorescent probe (methyl ester tetraethylrhodamine nitroxide) can act as a real-time reporter of retinal oxidative status of mice (Rayner, Gole, Bottle, & Barnett, 2014).

Despite these technologies are developed for different research purposes, especially for ROS/RNS detection, we propose that these advancements also open new avenues in antioxidant therapy area by directly investigating the *in vivo* antioxidant efficacy.

4. Opportunity for *in vivo* antioxidant efficacy assessment

Employing the recent technologies aforementioned, we can compare the dynamic imaging of oxidative and nitrosative stress without or with addition of antioxidant (such as the fat-soluble vitamin E, β-carotene and water-soluble vitamin C, polyphenolic compounds, synthetic antioxidant drugs and *etc.*.) supplement as schemed in Fig. 2. If the oxidative and nitrosative stress decrease with antioxidant supplement, it is demonstrated that antioxidant can counteract oxidative and nitrosative stress *in vivo*. We can quantify the antioxidant capacity of the examined antioxidant, and more importantly, then attempt to enhance the antioxidant efficacy by investigating what specific dosage and antioxidant agents combination are most effective to direct further clinical studies.

On the contrary, if the oxidative and nitrosative stress cannot be