

## Disruption of Vitamin E and Butylated Hydroxytoluene Antioxidant Function in Response to Paraquat-Induced Chromosomal Damage in Cultured Anuran Leukocytes

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Received date: July 22, 2014, Accepted date: December 22, 2014, Published date: December 29, 2014

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### Abstract

Researchers have reported that the phenolic antioxidants vitamin E and butylated hydroxytoluene (BHT) effect on cultured cells and human low-density lipoprotein is disrupted by various synthetic chemical compounds and  $\text{Cu}^{2+}$  involved in reactive oxygen species (ROS) generation by redox reaction. In this paper, information involved in oxidative action of vitamin E and BHT in cultured anuran leukocytes is provided in attempt to clarify such disruption mechanisms of antioxidant function.

**Keywords:** Vitamin E; Butylated hydroxy toluene; Antioxidants

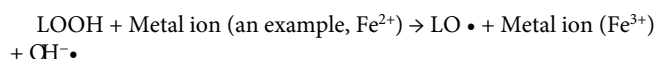
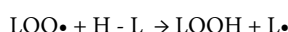
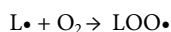
### Introduction

Researchers have reported that the phenolic antioxidants vitamin E effects on cultured cells and human low-density lipoprotein is disrupted by various synthetic chemical compounds and  $\text{Cu}^{2+}$  involved in reactive oxygen species (ROS) generation by redox reaction [1-8]. Phenolic antioxidants vitamin E effect on cultured cells and human low-density lipoprotein is disrupted by various synthetic chemical compounds and  $\text{Cu}^{2+}$  involved in reactive oxygen species (ROS) generation by redox reaction [1-8]. Phenolic antioxidants vitamin E and BHT have capability to inhibit autoxidation of unsaturated fatty acids (USFAs) followed by a chain reaction of free radical [9-11]. Preventive effect of phenolic antioxidants on autoxidation of USFAs is thought to be induced by two chemical reactions, hydrogen transfer reaction and termination reaction [10-15], shown below.

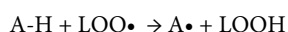
### Antioxidant Action by Phenolic Antioxidants

#### USFA autoxidation chain reaction

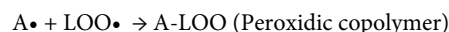
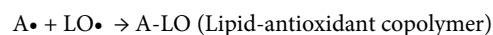
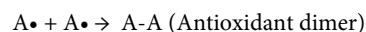
L-H + various stimulations (irradiation, heat treatment, reaction with metal ion and reaction with another free radicals) ( $- \text{H} \cdot$ )  $\text{L} \cdot$



#### Hydrogen transfer reaction



#### Termination reaction



H, phenolic antioxidant;  $\text{A} \cdot$ , free antioxidant radical;  $\text{H}^-$ , hydrogen transfer reaction; L-H, unsaturated fatty acids;  $\text{L} \cdot$ , lipid radical;  $\text{LO} \cdot$ , alkoxy radical;  $\text{LOO} \cdot$ , peroxy radical; LOH, hydroxy acid; LOOH, lipid hydroperoxides;  $\text{OH}^-$ , hydroxyl radical)

Phenolic antioxidants-inhibited mechanism of lipidic free radical production is as follows; hydrogen transfer reaction producing hydroxy acid and lipid hydroperoxides; termination reaction forming lipid-antioxidant copolymers and peroxidic copolymers. Free antioxidant radical generated at the USFA autoxidation also reacts with another free antioxidant radical, and thereby inactivating free antioxidant radical by forming antioxidant dimer. Particularly, representative phenolic antioxidant vitamin E is seen to function as protective compound against lipidic free radicals generated in human and wild life body by such chemical reactions [9, 16-19]. It is a vitamin E synthetic analog, and also has vitamin E-like antioxidant activity, mentioned above [10,13]. Disruption of antioxidant function is therefore thought to promote unexpected accumulation of reactive oxygen species (ROS), resulting in adverse influence [6-8].

This paper focuses on the mechanism involved in antioxidant function-disruption of phenolic antioxidants (vitamin E and BHT).

### Vitamin E

Vitamin E is an essential substance which has reproductive health and antioxidant effect on human and wildlife health maintenance [9,16-19]. For example, lipid peroxidation was reported to be caused by vitamin E-deficient-induced rabbit liver mitochondria dysfunction, resulting in an increase in liver damage [18]. Also, in the gastrocnemius muscle and liver of rats fed to vitamin E-depleted diet for 48 weeks, malondialdehyde (lipid peroxidation indicator) was found to increase [20]. With regards to suppression of carcinogenic

and clastogenic damage by vitamin E, examples are shown below. Vitamin E pretreatment for 24 hours was found to suppress sodium chromate-induced chromosomal aberrations in Chinese hamster V79 cells [21]. Carcinogenic and clastogenic damage occurred in liver of transgenic mice carrying transforming growth factor- $\alpha$ /c-myc genes was reported to be inhibited by vitamin E [22]. Moreover, Vitamin E succinate (a form of vitamin E with most antitumor activity) was reported to suppress not only proliferation of MCF-7 and MDA-MB-231 cells derived from human breast cancers but also tumor growth of breast cancer in athymic nude mice implanted with MDA-MB-231 cells [23]. The same study [23] indicated suppression of vascular endothelial growth factor gene expression (a potent modulator of tumor growth and angiogenesis) in MDA-MB-231 cells by vitamin E succinate.

On the other hand, Brigelius-Floh [5] indicated that vitamin E does not always have beneficial effect on some of diseases such as cardiovascular diseases, cancer, diabetes and hypertension. Moreover, vitamin E in combination with the initiators azo compounds,  $\text{Cu}^{2+}$ , was reported to enhance oxidation of human low density lipoproteins [1-4]. These findings showed that the initiators are required for induction of vitamin E pro-oxidant activity [1-5].

As for one vitamin E pro-oxidant-initiator paraquat (PQ) [6-8], paraquat cation ( $\text{PQ}^{2+}$ ) is thought to be enzymatically reduced to blue-colored monocation radical ( $\text{PQ}^{+\bullet}$ ) in the presence of reducing agent such as NADPH, causing a chain reaction of free radicals including superoxide, hydrogen peroxide and lipidic peroxides, resulting in chromosomal damage [24-28]. PQ-induced structural chromosomal damage in the cultured leukocytes derived from *Rana ornativentris* frogs has been reported to be inhibited by addition of SOD mimetic substance Mn(III) tetrakis(1-methyl-4-pyridyl) porphyrin (Mn(III)TMpyP), hydrogen peroxide scavenger catalase and combination of the two free radical scavengers (more protective combination of the scavengers against ROS) [6,8] to the culture mediums. Furthermore, acetyl-L-carnitine (acylated form, ALC) has been found to suppress PQ-induced chromosomal damage [8]. ALC is thought to enhance transport of free fatty acids (FFAs) to inner mitochondrial membrane for  $\beta$ -oxidation, and thereby reducing an amount of lipidic free radicals resulting in apoptosis [29-33]. These findings have shown that PQ induces chromosomal damage through generation of reactive oxygen species superoxide, hydrogen peroxide and lipidic free radicals [6, 8].

Theoretical antioxidant function of vitamin E should show suppression of such PQ-induced chromosomal damage, because vitamin E has protective function against lipidic free radicals (for example, alkoxy radicals and lipid peroxy radicals) [10-11,13,15]. PQ plus vitamin E has been reported to enhance the PQ genotoxicity too markedly, however [6,8]. In addition, PQ plus vitamin E-enhanced chromosomal damage has been reported to be strongly suppressed by combination of Mn(III)TMpyP and catalase [6,8]. Thus, dual inhibitory action by Mn(III)TMpyP and catalase suppresses PQ plus vitamin E-enhanced superoxide and hydrogen peroxide generation leading to decreasing an amount of chromosomal damage in cultured anuran leukocytes [6,8]. These paradoxical findings have evoked an idea that  $\alpha$ -tocopheroxy radical generated from vitamin E at hydrogen transfer reaction donates electron non enzymatically to PQ [6,8].

## BHT

BHT is widely used as antioxidant for rubber, plastics, food packaging, cosmetics, hand-washing soap, pet foods, chewing gum etc.

BHT alone is believed to have little mutagenic and clastogenic potential, and has ability to suppress tumorigenic, carcinogenic and clastogenic damage induced by various chemical compounds [34-37]. For example, BHT was reported to suppress aflatoxin B1-induced hepato carcinoma formation in male rats [38]. Bleomycin (antitumor antibiotic)-induced chromosomal damage in cultured Chinese hamster ovary cells has been found to be inhibited by BHT [36]. On the other hand, chronic BHT treatments after exposure to carcinogens promote tumor development in some strains of rodents [39-44].

Another study showed that BHT fails to inhibit high dose-rate gamma (192Iridium) rays-induced chromosomal damage in Chinese hamster ovary cells [45]. Moreover, BHT in combination with PQ has been reported to enhance PQ-induced chromosomal damage in cultured anuran leukocytes despite the fact that BHT alone has no genotoxic effect on cultured leukocytes derived from *Pelophyrax nigromaculatus* (*P. nigromaculatus*) frogs [7-8]. The same studies [7-8] have reported that PQ plus BHT-enhanced chromosomal damage is inhibited by combination of Mn(III)TMpyP and catalase. This response of cultured *P. nigromaculatus* leukocytes to PQ plus BHT has been found to be very similar to that to PQ plus vitamin E [6, 8]. BHT has been further reported to induce  $\text{PQ}^{+\bullet}$  formation chemically in  $\text{PQ}^{+\bullet}$  formation-test [7-8]. These studies have indicated that BHT reduces  $\text{PQ}^{2+}$  chemically to  $\text{PQ}^{+\bullet}$ , leading to ROS generation, causing chromosomal damage.

Regarding preliminary study involved in induction of acute chromosomal damage in cultured anuran leukocytes through stimulation of BHT in combination with PQ, high level of nitrite was discovered in the tails of *Ranarugosa tadpoles* exposed to PQ plus BHT [8]. Tanaka [46] reported that nitrite derived from the NO releaser enhances PQ genotoxicity. Moreover, Hanada [8] has reported that sodium nitrite reduces  $\text{PQ}^{2+}$  chemically to  $\text{PQ}^{+\bullet}$  in  $\text{PQ}^{+\bullet}$  formation-test. PQ plus BHT-enhanced endogenous nitrite production has been therefore shown to have ability to induce remarkable free radical generation with concomitant acute increase in chromosomal damage.

## Concluding Remarks

Vitamin E and BHT alone are believed to have little toxicity [6-8, 9, 16-19, 34-37]. Whereas, vitamin E in combination with azo compounds and  $\text{Cu}^{2+}$  was reported to enhance lipid peroxidation in human low density lipoprotein, and further vitamin E in combination with paraquat enhances chromosomal damage in cultured anuran leukocytes [1-4, 6-8]. Information with regard to pro-oxidative function of BHT has been little reported until today except for PQ plus BHT-enhanced chromosomal damage in cultured anuran leukocytes. Regarding function disruption of the phenolic antioxidants, unpaired electron of antioxidant radicals generated from vitamin E and BHT may play a key role in the disruption, based on the findings obtained from cytogenetic and pharmacological studies using cultured anuran leukocytes. The unpaired electron is thought to activate PQ cation, and then produce PQ monocation radical. PQ monocation radical in the presence of molecular oxygen enhances generation of superoxide, which is converted into hydrogen peroxide ( $\text{H}_2\text{O}_2$ ) by superoxide dismutase. Accumulative  $\text{H}_2\text{O}_2$  induces hydroxyl radical by Haber-Weiss or Fenton reaction, resulting in increasing lipid peroxidation followed by high amount of chromosomal damage [6-8] (Figure 1). In addition, PQ-induced nitric oxide derivative, nitrite has capability to activate PQ which accumulates ROS in cultured anuran leukocytes acutely [7-8] (Figure 1).



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