THE ANTIOXIDANT AND PROOXIDANT EFFECT OF α–TOCOPHEROL IN HUMAN CELL

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Abstract

α-tocopherol, secara *in vivo*, memiliki aktivitas antioksidan paling kuat diantara kelompok tocopherols seperti β, λ and δ, karena kemampuannya dalam mentransfer ion hydrogen kepada radikal bebas, sehingga radikal bebas tersebut menjadi stabil. Selama oksidasi α-tocopherol dengan radikal bebas menghasilkan tocopheroxyl radical yang dianggap berperan dalam efek prooksidan dari α-tocopherol. Salah satu bentuk stabil dari α-tocopherol adalah α-tocopherol succinate.

Penelitian ini memanfaatkan sel manusia (WIL2-NS) untuk menguji efek prooksidan α-tocopherol succinate tersebut. Uji MTT dilakukan untuk menentukan efek toksisitas/ prooksidan α-tocopherol succinate. Dosis aman terhadap paparan α-tocopherol succinate (berperan sebagai antioksidan) selama 1 jam pada sel manusia (WIL2-NS) hingga 25 μM, sedangkan pada paparan yang lebih lama yaitu 24 jam dosis amannya adalah 2.5 μM.

Penelitian ini membuktikan bahwa meskipun α-tocopherol succinate dikenal sebagai antioksidan, tapi pada dosis tertentu akan berdampak negative pada sel (prooksidan).

Keyword: α-tocopherol succinate, antioksidan, prooksidan.

INTRODUCTION

Tocopherols, one of members of vitamin E, are essential dietary compounds which are only synthesized by plants. They originate from oil seeds, leaves and other green parts of higher plants (Kamal-Eldin and Appelqvist, 1996). Tocopherols including α, β, λ and δ are well known as lipophilic antioxidants. According to Kamal-Eldin and Appelqvist (1996) the antioxidants activity of α-tocopherol is due to its donation ability of phenolic hydrogens to lipid free radicals. Among tocopherols, α-tocopherol is considered to have the highest relative antioxidant activity *in vivo*, followed by β , λ, and δ–tocopherol.

Meanwhile, the potency of tocopherols as antioxidants agent in vitro does not only rely on their chemical reactivities toward hydroperoxy and other free radicals, but also many other possible side conditions, such as tocopherol concentration, temperature, light, type of substrate, and solvent.

The oxidation of α-tocopherol by reactive oxygen species, such as hydroxyl ($OH^{\bullet}$), is through indirect reaction, because of their low diffusibility. At the beginning, $OH^{\bullet}$ reacts with solvent (e.g. ethanol) to form solvent radicals, and after that the solvent radicals oxidize the α-tocopherol to generate tocopheroxyl radical (Kamal-Eldin and Appelqvist, 1996).

On the other hand, the existence of tocopheroxyl radical (TO•) in the oxidation reaction is supposed to deal with the prooxidant effect of α-tocopherol. This is based on the assumption that tocopheroxyl radical is present in high concentration in which it may initiate a number of undesirable reaction which increase the rate of oxidation (Kamal-Eldin and Appelqvist, 1996).

α–tocopherol succinate, which is an amphilic succinyl ester of α-tocopherol, is the more stable form of α-tocopherol, since the succinate group protects the hydroxyl group of the chromanol ring from oxidation (Fukuzawa et al., 2004; Lee et al., 2006). As reported by (Badamchian et al., 1994) that α–tocopherol succinate is a naturally occurring compound first isolated from green barley extract.

This study utilized WIL2-NS cell line that is a human β-lympoblastoid cell line derived from spleen of a 5 year male Caucasian.

The purpose of the current study was to investigate the prooxidant effect of α–tocopherol succinate on human cell line (WIL2NS).

MATERIALS AND METHODS

α-tocopherol succinate (α–TOS): α–TOS was derived from Sigma (USA). This was dissolved in ethanol (90%) solvent to make a stock solution and then stored at -20°C.

Human cells. This research utilized WIL-2NS cell line which was obtained from the ATCC (CRL-8155™, USA). WIL2-NS cells are a human β-lymphoblastoid cell line which is isolated from spleen of a Caucasian Male **(ATCC, 2000)**.

MTT assay: The current research used MTT assay to assess the cytotoxicity of the dietary compounds (α-tocopherol succinate). This assay conducted was based on Mosmann (1983) and Young *et al.* (2003) and involved plating the cells in 96-well round bottom microplates. An MTT standard curve was constructed by making 1:2 serial dilutions from concentration of 40,000 cells/well to 1,250 cells/well. Each treatment condition had six replicates which were surrounded by media (without cells) as negative controls. The cells then were incubated with 0.5 mg/ml MTT solution at 37 °C (humidified 5% CO_2) for 18 hours. Following this, 80 µl of 20% SDS in 0.02 M HCl was added into each well to dissolve the purple formazan crystalline that was produced by the living cells. The microplates were then incubated in the dark at room temperature (RT) for 1.5 hours.

The absorbance of the purple formazan was read using a microplate reader with KC junior software at 570 nm wavelength with 630 nm reference wavelength. The values of absorbance of this reading, which is stated as optical density at 570 nm (OD_{570}), were plotted against the cell survival in cells/well plated. OD_{570} for treated experimental cultures were converted to cells/well using the standard curve. A standard curve was run for each experiment.

The percentage of cell survival of each treatment was determined by referring to untreated cells (control) as 100% value.

Statistical Analysis. Data obtained from the experiments was performed using microsoft office excel 2003 and expressed as mean ± standard error (SE). Furthermore, SPSS version 14.0 was used to statistically analyze the level of differences among variables of the final data by ANOVA. If it was found a significance difference ($P<0.05$) following the ANOVA analysis, a Tukey's post-hoc test was applied to determine the significant difference within the groups.

RESULTS AND DISCUSSION

As is illustrated by the Fig.1, there was no prooxidant effect of α-tocopherol succinate exposure for 1 hour to WIL2-NS by 25 μM of the doses to reach approximately 80%.

The dramatic decrease happened in the cell survival in the dose range of 25 – 250 μM where at the 250 μM α-tocopherol succinate doses the cell survival was only approximately 1%.

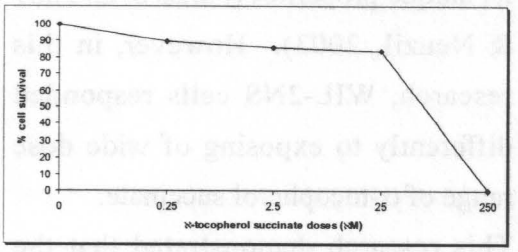

Fig. 1
Dose response of α-tocopherol succinate treatment for 1 h to WIL-2NS cell line ($0.5X10^6$ cells/ml).

In the same way to the 1 hour exposure, the increasing doses of α-tocopherol succinate for 24 hours exposure decreased the cell survival of WIL-2NS cell line (Fig. 2). However, as is shown in Fig. 2, at the 24 hours α-tocopherol succinate exposure, the cell survival was maintained close to 100% in the range doses of 0 – 2.5 μM, which was closer than 1 hour exposure. Afterwards, the cells survived tended to drop from the doses of 2.5 μM.

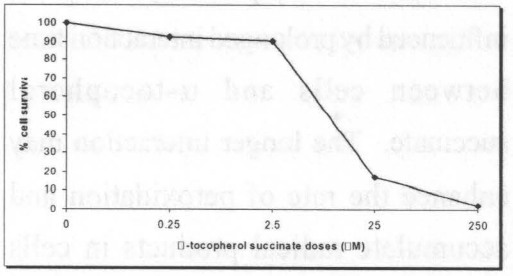

Fig. 2
Dose response of α-tocopherol succinate treatment for 24 h to WIL-2NS cell line ($0.5X10^6$ cells/ml).

The cells survived almost reached the bottom (approximately 2%) at the doses of 250 μM.

α-TOS have been shown to have antioxidant and anticancer ability due to its unique properties (Fariss et al. 2001 & Neuzil, 2003). However, in this research, WIL-2NS cells responded differently to exposing of wide dose range of α-tocopherol succinate.

This research demonstrated that the beneficial effect of α-TOS occurred at low concentration, 0 – 25 μM for 1 hour and 0 – 2.5 μM for 24 hours exposure (Fig. 1 & Fig 2). Thereby, the role of antioxidant is performed in the range of saves doses, since in those doses the oxidative stress has not occurred to result in cell damage and death (Halliwell et al. 1995).

However, higher concentrations of α-TOS showed detrimental cellular responses. The cell responses to α-TOS for 24 hours were more susceptible than 1 hour exposure (Fig. 1 and Fig. 2. respectively).

The more susceptibility may be influenced by prolonged interaction time between cells and α-tocopherol succinate. The longer interaction may enhance the rate of peroxidation and accumulate radical products in cells (Kamal-Eldin and Appelqvist, 1996).

In 1 hour treatment with α-tocopherol succinate, cell proliferation occurred at 2.5 μM.

The growth enhancement of cells at low concentration of α-TOS may be due to less radical levels accumulated and suitable environmental conditions (Kamal-Eldin and Appelqvist, 1996). At suitable condition and low radical disorders, cells reproduce themselves properly by cell division, initiated from G_1, S, G_2 and Mitotic phase (Campbell, 1996).

CONCLUSION

α-tocopherol succinate is considered as radical-scavenging antioxidants in cells (Fariss, 1990). It is suggested to be consumed in order to improve human health. However, some studies revealed that the existence of such compound in cells could generate adverse effects, such as cell toxicity (De Flora & Ramel, 1988). An inappropriate amount of dose provided may generate counter effects as expected. Thereby, the assessment of dose responses of those compounds is crucial to give general pictures of doses which determined safe doses as well as toxicity effect or prooxidant effect of it.

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