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DIPARTIMENTO DI SCIENZE VETERINARIE PER LA SALUTE, LA PRODUZIONE ANIMALE E LA SICUREZZA ALIMENTARE

Relative bioefficacy of RRR-α-tocopherol versus all-*rac*-α-tocopherol in *in vitro* models

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Article

Introduction

In nature, Vitamin E is present under eight different forms, four tocopherols (α, β, γ, and δ) and four tocotrienols (α, β, γ, and δ). The α–tocopherol has the highest biological activity (Dersjant & Peisker, 2010). The natural form of α–tocopherol is composed of 100% RRR-α-tocopherol whereas the synthetic form (all-rac-α-tocopherol) consists of a mixture of eight stereoisomers at equal amount.

Previous studies suggest that the bioefficacy of the different α-tocopherol forms should be reconsidered (Vagni *et al*., 2011). At the cell level, α-tocopherol functions as an antioxidant (Baldi, 2005).

The main aim of this study was to investigate the in vitro relative bioefficacy of RRR-α-tocopherol (RRR- α-T) versus all-rac-α-tocopherol (all-rac-α-T) to counteract the cytotoxic effect induced by hydrogen peroxide (H2O2). To this aim, Bovine Mammary Epithelial cell line (BME-UV1) and Madine Darby Canine Kidney cell line (MDCK) were used since they represent suitable and well characterized in vitro models in relation to their particular susceptibilities to oxidative damage.

Material and Methods

BME-UV1 and MDCK cells were cultivated into 75 cm^3 tissue culture flasks in complete medium. Preliminary experiments were performed to determine H_2O_2 cytotoxicity and the LC50 were calculated in both cell lines.

Further a putative difference in the protective effect of the two forms of tocopherol against H_2O_2-induced stress was evaluated. Cells were trypsinized and seeded into wells of 96 wells -cell culture plates (seeding density: BME-UV1 2 x 10^5 cells/ml; MDCK 1 x10^5 cells/ml). Cells were pre-incubated for 3 h with selected RRR- α-T and all-rac- α-T concentrations and then exposed to increasing H_2O_2 concentrations ranging from 250 to 750 µM in BME-UV1 cell line and from 125 to 175 µM in MDCK cell line for the following 24h.

The effects of RRR- α-T and all-rac- α-T on both BME-UV1 and MDCK cell lines in counteracting H_2O_2 toxicity were determined by the MTT test and the LDH test.

At least three replicates at each incubation time were performed and all experiments were performed at least twice. Results are expressed as mean and SD. The effect of various treatments were evaluated by one-way analysis of variance using the GLM procedure of SAS (SAS institute Inc, NC, USA). Values significantly different from controls are indicated as $P<0.05$.

Results and Discussion

In BME-UV1, the proliferative effect induced by RRR-α-T form was higher at the lowest concentrations (1nM and 0.01uM) than all-rac α-T (about 30%, which is consistent with the current bioactivity conversion factors). In MDCK, no relevant effect on mitochondrial activity was observed (Figure 1). LC50 values determined in BME-UV1 and MDCK cells were 376µM and 140µM, respectively.

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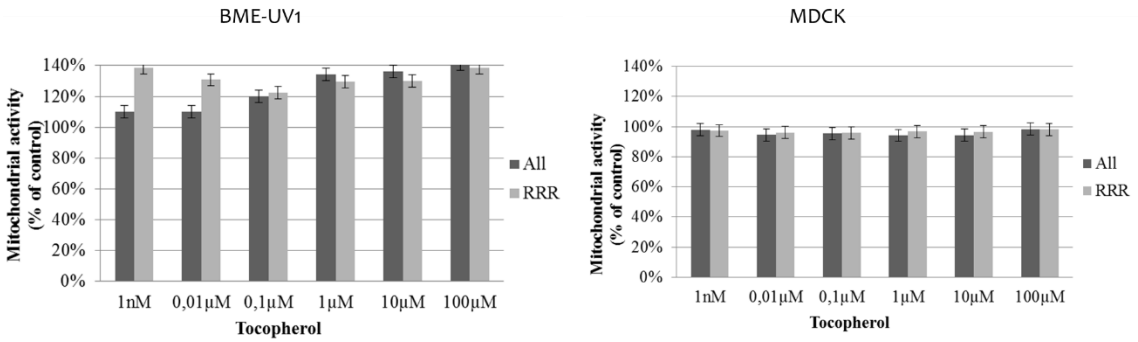

Figure 1: Evaluation of tocopherol treatments on cell mitochondrial activity

Pre-treatments with 100µM of RRR-α-T and 100µM all-rac-α-T were able to significantly (P<0.05) counteract the effect induced by 750µM of H_2O_2 in BME-UV1. In MDCK the pre-treatment with 1nM of all-rac-α-T was able to significantly (P<0.05) reduce the effect of 125 and 150mM H_2O_2 (Figure 2).

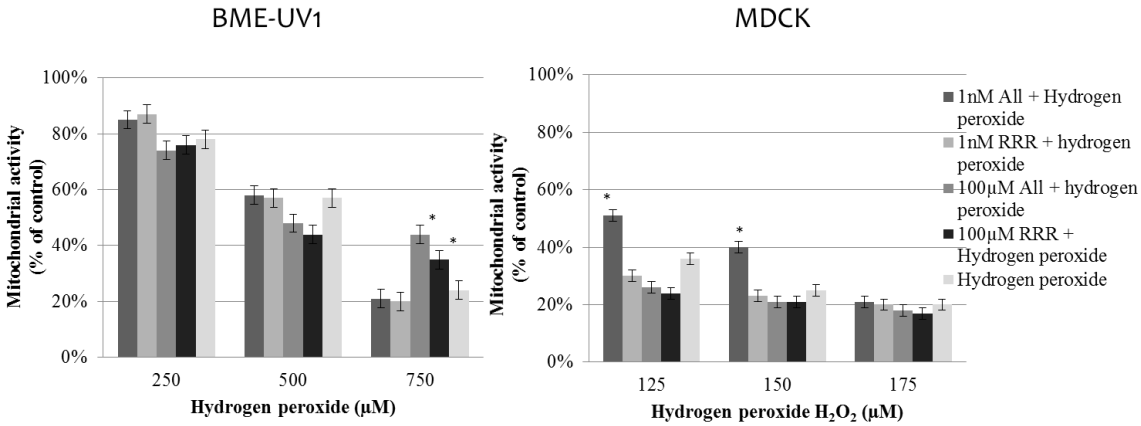

Figure 2: Evaluation of All rac-alpha-tocopherol (All) and RR-alpha-tocopherol (RRR) treatments against H2O2 citotoxicity

In MDCK cells, the pre-incubation with all-rac-α-T (1nM) determined a significant reduction of the membrane damage (LDH test), induced by 175 µM of H_2O_2.

Conclusions

The dose-response curve experiments shown that RRR-α-T and all-rac-α-T tocopherols were able to maintain (MDCK cells) and increase (BME-UV1 cells) the cell viability. It has been observed that adequate RRR-α-T and all-rac-α-T concentrations could reduce the oxidative damages induced by H_2O_2 in both BME-UV1 and MDCK cells. Differences detected in the two α-tocopherol forms, when present, were consistent with the conversion factors. However, in some cases all-rac was exhibited a higher antioxidant effect, compared with RRR-α-T. In conclusion, RRR-α-T and all-rac-α-T have shown the ability to counteract the oxidative effects of H_2O_2 in the cell lines considered. However, further investigation will help in describing their specific mechanism of action in vitro.

References

A. Baldi, "Vitamin E in Dairy Cows," Livestock Production Science, Vol. 98, No. 1-2, 2005, pp. 117-122. doi:10.1016/j.livprodsci.2005.10.004

Dersjant-Li, Y., & Peisker, M. (2010). A critical review of methodologies used in determination of relative bio-availability ratio of RRR-α-tocopheryl acetate and all-rac-α-tocopheryl acetate. Journal of the Science of Food and Agriculture, 90(10), 1571-1577

S. Vagni et al., (2011). "Vitamin E Bioavailability: Past and Present Insights", Food and Nutrition Science, 2, pp. 1088-1096

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