Cytoprotective Effect of White Tea against H_2O_2-induced Oxidative Stress *in Vitro*

Wen-Jye Yen[1], Charng-Cherng Chyau[2], Chia-Pu Lee[1], Heuy-Ling Chu[1], Lee-Wen Chang[1] , Pin-Der Duh[2]

1:Department of Food Science and Technology, Chia Nan University of Pharmacy and Science, 60 Erh-Jen Road, Section 1, Pao-An, Jen-Te District, Tainan, Taiwan, ROC.

2:Research Institute of Biotechnology, Hungkuang University, 34 Chung-Chie Road, Shalu County, Taichung, Taiwan, ROC.

Correspondent email: ipdduh@mail.chna.edu.tw

Summary

WEWT shows marked antioxidative properties and protects Clone 9 cells from oxidation induced by H_2O_2. In addition, WEWT demonstrates significant upregulation of GSH levels and the activity of enzymes. The upregulation of antioxidant enzyme mRNA expression accounts for the upregrulation of antioxidant enzymes. WEWT is most likely to exert an antioxidant action as a result of bioactive compounds that are present in WEWT. Therefore, this study provides a positive indication that the daily drinking of white tea is beneficial and may lessen oxidative stress induced by dietary toxicants.

Introduction

Tea is one of the most popular drinks because it has a pleasant taste. It has been used as a drug in China for thousands of years. White tea is made using very young tea leaves or buds covered with tiny, silvery hairs, which are harvested only once a year in the early spring. White tea is steamed and dried immediately after picking to prevent oxidation. In addition, because it is shield from sunlight during growth, the buds form less chlorophyll, giving it a white appearance and a light, delicate taste (Rusak et al., 2008). Some studies concerning white tea have been published. This study investigates the protective effect of white tea against oxidative stress in Clone 9 cells, induced by hydrogen peroxide.

Materials and methods

Each tea (50 g), including white tea, green tea and Pu-erh tea, was extracted with boiling water (500 ml) for 5 min, and the filtrate was freeze-dried, and then stored at 4 ℃for further use. The water extracts of white tea, green tea and Pu-erh tea were named WEWT, WEGT and WEPT, respectively. The followings were measured: trolox equivalent antioxidant capacity, reducing activity, DPPH radical-scavenging activity, antioxidant effect on liposome oxidation, protein oxidation, low-density lipoprotein (LDL) oxidation, Clone 9 cells viability assay, intracellular ROS measurement, glutathione and antioxidant enzyme activities in Clone 9 cells, total polyphenolics assay, total flavonoid content assay, HPLC analysis, and LC-MS/MS analysis.

Results and discussion

The antioxidant action of WEWT was compared with the effect of WEGT and WEPT using several assays. At a concentration of 1.0 mg/ml all of the samples are able to scavenge the ABTS radical cation. WEWT, like WEGT and WEPT, exhibits remarkable reducing ability, and thereby reduces the ferric ions. WEWT markedly inhibits lipid oxidation and stabilizes the protein against oxidation. In addition, the protective effect of WEWT and other tea extracts against the LDL oxidation. The levels of polyphenolic compounds in tea extracts are 236.65 mg/g, 320.23 mg/g, and 189.37 mg/g for WEWT, WEGT, and WEPT, respectively, while the levels of flavonoids are 25.75 mg/g, 20.96 mg/g, and 15.72 mg/g for WEWT, WEGT, and WEPT, respectively. WEWT contained the highest level of flavonoids. In order to further understand the antioxdative action of WEWT on an intracellular model system, Clone 9 cells were tested. WEWT and other tea extracts in concentrations of 0-200 μg/ml demonstrate not harmful effect on the viability of Clone 9 cells. As expected, the addition of WEWT to the medium protects the cells against H_2O_2-induced cytotoxicity. This observation clearly demonstrates that WEWT protects Clone 9 cells from cell damage induced by H_2O_2.

In order to further determine whether WEWT scavenges intracellular ROS formation in Clone 9 cells, the DCFH-DA model was used. Pretreatment of Clone 9 cells with WEWT and other tea extracts in concentrations ranging from 0-0.2 mg/ml results in a significant decrease in intracellular ROS formation. This observation demonstrates that WEWT and other tea extracts act as a scavenger of the ROS that is caused by H_2O_2 in Clone 9 cells. In other words, WEWT-suppressed oxidative stress may account for the effect of WEWT on the survival of H_2O_2-induced Clone 9 cells. WEWT significantly lowers TBARS, compared with the control, which indicates that WEWT may protect biomolecules from oxidative damage in cells. It is noteworthy that WEWT, WEGT and WEPT not only prevent the decline in GSH levels observed in H_2O_2 cells, but also significantly increase the GSH levels compared to the control. This finding suggests that increases in the GSH levels due to WEWT, WEGT, and WEPT may contribute to a reduction in the oxidative stress in H_2O_2-induced Clone 9 cells. Exposure to 0.2 mM H_2O_2 significantly decreases the GPx and CAT activity, compared with the control, but it increases CuZnSOD activity. Moreover, there is a slight decrease in the GPx and CAT mRNAs (0.93 and 0.94, respectively) of H_2O_2-treated cells, but a slight increase in the CuZnSOD mRNA (1.04). It is speculated that the stimulation of antioxidant enzyme mRNAs expression may be one of the palatial signaling pathways utilized by WEWT to exert a beneficial biological effects. The major compounds in WEWT were therefore analyzed using HPLC-DAD and HPLC-Ms/Ms. Sixteen phenolic compounds are identified. Apart from (-)epicatechin and its galloylated derivatives, tea extracts also contain substantial and physiologically relevant levels of gallic acid, caffine , rutin and the flavonols such as derivatives of quercetin, kaempferol, and myricetin (Gondoin, Grussu, Stewart, & McDougall, 2010), which may complement the (-)epicatechin and its galloylated derivatives. In other words, other characterized bioactive compounds in WEWT may occur due to the synergy between the compounds, thereby contributing to the protective effect of WEWT against oxidative damage in cells.

Acknowledgements

This research is supported by research grants from the National Science Council of the Republic of China NSC: 95-2313-B-041-009 and 96-2313-B-041-003

References

Gondoin, A., Grussu, D., Stewart, D., & McDougall, G. J. (2010). White and green tea polyphenols inhibit pancreatic lipase in vitro. *Food Research International, 43,* 1537–1544.

Rusak, G., Komes, D., Likić, S., Horžić, D., Kovač, M. (2008). Phenolic content and antioxidative capacity of green and white tea extracts depending on extraction conditions and the solvent used. *Food Chemistry*, *110,* 852–858.