Evaluation of Antigenotoxic Effects of Phytochemicals:

DNA Protection From Oxidative Stress and Improved DNA Repair Ability

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Introduction

In recent years, plant extracts and their phytochemicals have received increasing attention, especially in the field of pharmaceutical sciences and medicine, due to the potential prevention activity of a number of chronic and degenerative diseases including cancer and cardiovascular diseases [1].

The DNA is constantly subjected to alteration by cellular metabolites and exogenous DNA damaging agents. DNA damages can perturb the cellular steady-state and activate or amplify certain biochemical pathways that regulate cell growth, division and pathways that help to coordinate DNA replication with damage removal. The main damage to cells results from ROS-induced alteration of macromolecules such as polyunsaturated fatty acids in membrane lipids, essential proteins, carbohydrates, and DNA, leading to the breakdown of normal cellular metabolism [2].

DNA integrity is challenged by the damaging effect of numerous chemical and physical agents, compromising its function. An intricate network of DNA repair systems has evolved early in evolution, which efficiently maintains genome integrity. DNA repair pathways can be divided into five categories: direct repair, base excision repair (BER), nucleotide excision repair (NER), double-strand break repair, and repair of interstrand cross-links. Among these DNA repair mechanisms, BER is one of the most highly conserved. DNA base damage, causing relatively minor disturbances to the helical DNA structure, is repaired by BER [3]. NER is a highly versatile and sophisticated DNA damage removal pathway that counteracts the deleterious effects of a variety of DNA lesions, including major types of damage induced by environmental sources [4].

The yeast *Saccharomyces cerevisiae* is perhaps the best studied eukaryotic organism and exhibits striking similarities in molecular mechanisms such as transcription, translation, replication, and DNA repair with higher eukaryotes. For these reasons, it is a good model for studying the DNA damage and search for antigenotoxic effects of plant compounds. Therefore, the application of the comet assay to yeast cells may constitute a valuable tool for the evaluation of DNA damage. In this work we investigated the antigenotoxic activity of the most common flavonoids found in plants: quercetin, isoquercitrin, quercitrin, rutin and hyperoside. In addition, this work helps to elucidate the potential inducing activity of DNA damage repair.

Results

Pre-treatment of *S.cerevisiae* cells with phytochemicals protects DNA against oxidative damage by H_2O_2 and $KMnO_4$

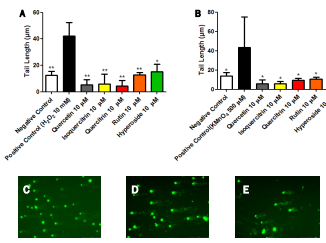

Figure 1. Yeast spheroplasts were suspended in 10 µM phytochemicals diluted in S buffer, incubated for 20 min, washed and subsequently incubated with 10 mM H_2O_2 (A) or 500 µM $KMnO_4$ (B) for 20 min. The negative control reflects the amount of DNA damage in cells without exposure to H_2O_2 or $KMnO_4$. DNA damage was analyzed by the comet assay method. Mean ± standard deviations (SD) are from three independent experiments (* represents $p < 0.05$ and ** represents $p < 0.01$ in relation to positive control). C: photomicrograph from negative control, D: photomicrograph from positive control (cells treated with 10mM H_2O_2), and E: photomicrograph of pre-treatment with quercetin 10 µM .

Pre-treatment of *S.cerevisiae* cells with quercetin protects DNA against oxidative damage by H_2O_2

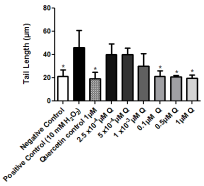

Figure 2. Yeast spheroplasts were suspended in $2.5x10^{-4}$, $5x10^{-4}$, $1x10^{-3}$, 0.1, 0.5 or 1µM quercetin diluted in S buffer, incubated for 20 min, washed and subsequently incubated with 10 mM H_2O_2 for 30 min. The negative control reflects the amount of DNA damage in cells without exposure to quercetin and H_2O_2. The quercetin control reflects the amount of DNA damage in cells only treated with 1µM quercetin. Mean± standard deviations (SD) are from three independent experiments (*represents $p < 0.05$ and ** represents $p < 0.01$ in relation to positive control).

Co-treatment of *S.cerevisiae* cells with quercetin and H_2O_2 attenuates DNA damage caused by H_2O_2

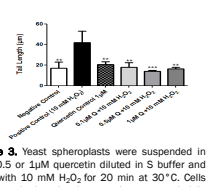

Figure 3. Yeast spheroplasts were suspended in 0.1, 0.5 or 1µM quercetin diluted in S buffer and also with 10 mM H_2O_2 for 20 min at 30°C. Cells were washed and subsequently resuspended in low melting agarose 1.5%. The negative control reflects the amount of DNA damage in cells without exposure to H_2O_2. DNA damage was analyzed by the comet assay method. Mean ± standard deviations (SD) are from three independent experiments (** represents $p < 0.01$ and ***represents $p < 0.001$ in relation to positive control).

Post-treatment of *S. cerevisiae* cells with low concentrations of quercetin improves DNA repair

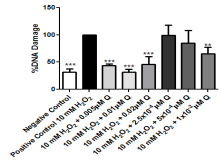

Figure 4. Yeast spheroplasts were suspended in 10 mM H_2O_2 for 30 min, washed and subsequently suspended in 0.005, 0.01, 0.02, $1x10^{-3}$, $5x10^{-4}$ or $2.5x10^{-4}$µM quercetin diluted in S buffer and incubated for 20 min. The negative control reflects the amount of DNA damage in cells without exposure to H_2O_2. DNA damage was analyzed by the comet assay method. Mean ± standard deviations (SD) are from three independent experiments (**represents $p < 0.01$ and *** represent $p < 0.001$ in relation to positive control).

Post-treatment of BER mutant yeast cells with quercetin does not improve DNA damage repair

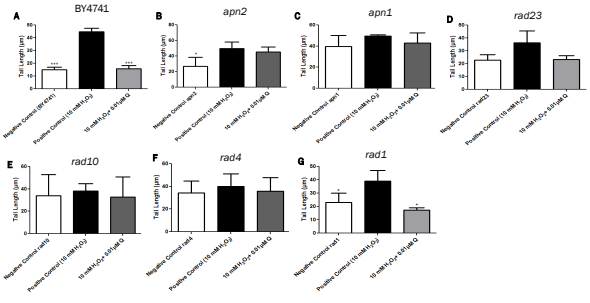

Figure 5. Mutant strains derived from strain BY4741 (A) are affected in BER (*apn2*, B, and *apn1*, C) or NER (*rad23*, D, *rad10*, E, *rad4*, F and *rad1*, G). Spheroplasts were suspended in 10 mM H_2O_2 for 30 min, washed and subsequently suspended in quercetin 0.01µM diluted in S buffer incubated for 20 min. The negative control reflects the amount of DNA damage in cells without exposure to H_2O_2. DNA damage was analyzed by the comet assay method. Mean ± standard deviations (SD) are from three independent experiments (* represents $p < 0.05$ and *** represents $p < 0.001$ in relation to positive control).

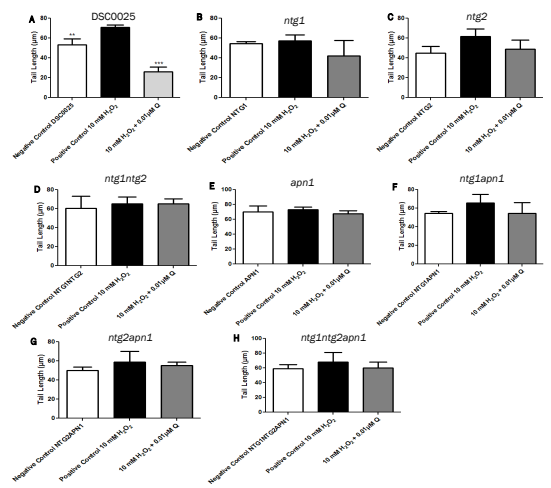

Figure 6. Mutant strains derived from strain DSC0025 (A) are affected in the BER pathway: *ntg1* (B), *ntg2* (C), *ntg1ntg2* (D), *apn1* (E), *ntg1apn1* (F), *ntg2apn1* (G) and *ntg1ntg2apn1* (H). Spheroplasts were suspended in 10 mM H_2O_2 for 30 min, washed, suspended in quercetin 0.01µM diluted in S buffer and incubated for 20 min. The negative control reflects the amount of DNA damage in cells without exposure to H_2O_2. DNA damage was analyzed by the comet assay method. Mean ± standard deviations (SD) are from three independent experiments (**represents $p < 0.01$; *** represent $p < 0.001$ in relation to positive control of DSC0025).

Conclusions

- Quercetin, isoquercitrin, quercitrin, rutin and hyperoside protect yeast cells against DNA damage by H_2O_2 and $KMnO_4$;
- Quercetin protects DNA against oxidative damage in a dose-dependent manner;
- DNA protection against oxidative stress in co-incubation experiments with quercetin suggests a mechanism of ROS scavenging;
- Quercetin improves DNA repair after damage caused by H_2O_2;
- Mutants affected in BER post-treatment with quercetin showed no reduction of comet tails, indicating that quercetin presumably could activate DNA repair enzymes from BER pathways.

References

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