Natural sesquiterpenes inhibit DNA-damage by tobacco smoke

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Tobacco smoke is one of the greatest threats to human health as it is responsible for malignancies and precancerous lesions in different organs and tissues (Huang and Chen, 2011). Although smoking cessation is the better strategy to avoid the development of cancer, former smokers continue to have an elevated risk for years after quitting. In this context, chemoprevention represents a highly sought-after approach to reduce the risk of smoking damage, and the identification of new chemopreventive agents is a very desirable goal. In present study, the natural sesquiterpenes β-caryophyllene (CRY) and β-caryophyllene oxide (CRYO) were studied for their potential antimutagenic activity against a condensed smoke from standard 3R4F cigarettes (CSC), in the bacterial reverse mutation assay on *Salmonella typhimurium* TA1535, TA1538, TA98, TA100 and on *Escherichia coli* WP2, WP2*uvr*A and WP2*uvr*A/pKM101 strains according to Di Sotto et al. (2012). Preliminary studies were carried out to identify the concentration of CSC to use in the antimutagenicity assay. Different experimental protocols (pre-, co- and post-treatment) were applied to distinguish a desmutagenic from a bioantimutagenic activity. In addition, taking into account that tobacco smoke has been shown to induce pre-neoplastic lesions also by stimulating the phosphorylation of the signal transducer and activator of transcription 3 (STAT3) (Liu, 2007), the ability of CRY and CRYO to inhibit, in MDA-MB-468 breast cancer cells, the EGF-induced phosphorylation of STAT3 protein was studied as a possible mechanism of action (Chichiarelli et al., 2010). In our experiments, CSC produced a mutagenic effect on TA98, TA100, WP2*uvr*A and WP2*uvr*A/pKM101 strains, only in presence of the exogenous metabolic activator S9, suggesting that CSC contains pre-carcinogenic species, requiring a bioactivation by CYP450 enzymes. In addition, the sensitivity of tester strains to these carcinogens seems to be due to the presence of pKM101 plasmide. At 700 μg/plate (265 μg/ml), the mutagenic effect of CSC was submaximal so this concentration was used for the antimutagenicity test. Both sesquiterpenes inhibited the mutagenicity of CSC, although with different potency and specificity. CRYO was the most potent compound, acting at concentrations about ten-times lower than CRY. The inhibition was not-concentration dependent and always strong (> 40%) in TA100 and WP2uvrA/pKM101 strains. The antimutagenicity was highlighted in all experimental protocols, being particularly strong in the co- and post-treatments. On the basis of these results, we hypothesize that aspecific mechanisms should be involved in the antimutagenicity of CRY and CRYO, being the substances effective in strains sensitive to different genotoxic damages (i.e. frameshift and base-substitution mutations, oxidative stress and DNA alkylation), and in all experimental conditions, so acting both as desmutagenic and bioantimutagenic agents (Kada and Shimoi, 1987). Furthermore, the western blotting analysis, carried out in presence of an antistat3-P-Tyr705 antibody, highlighted that only CRY was able to significantly inhibit the STAT3 phosphorylation in MDA-MB-468 cells. In conclusion, present results show that CRY and CRYO are capable to prevent the DNA-damage induced by the carcinogenic compounds contained in CSC. The effect seems to be due to aspecific mechanisms. The ability of test substances, particularly CRY, to inhibit the STAT3 protein could represent a new strategy for preventing the induction of pre-cancerous lesion in healthy normal tissue and encourages further investigation on these natural substances as chemopreventing agents.

References

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