β-Caryophellene inhibits DNA-damage induced by tobacco smoke in mammalian cells

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Exposure to smoke induces damages in different organs and tissues, being the upper respiratory tract the first and continuously exposed target (Huang and Chen, 2011). A high incidence of precancerous lesions and malignancies has been also highlighted in smokers. In this context, inhibiting the smoke-induced damages by using chemopreventive agents could represent an effective approach for human health care. In the present study, the natural sesquiterpene β-caryophyllene (CRY) was studied for its ability to inhibit the DNA-damage induced by condensed smoke (CSC; obtained from standard 3R4F cigarettes) in human epithelial bronchial upper airway cells (.BEAS-2B and HepG2) The cytokinesis-block micronucleus (CBMN) assay (Di Sotto et al., 2010) was carried out in order to detect the presence of micronuclei (MN) in the cytoplasm of the cell exposed to the CSC, as markers of genetic damage. Furthermore, taking into account that an increase in the intracellular reactive oxygen species (ROS) has been associated with the acute toxic effects of smoke, the ability of the test substance to inhibit the CSC-induced oxidative stress was evaluated by the 2,7-dichlorofluorescein diacetate (DCFH-DA) assay (Duan et al., 2013). Finally, as cigarette smoke is reputed able to increase the activation of STAT3 protein in bronchial HBECs cells, so resulting in survival of damaged cells (Wu et al., 2014), we have evaluated the ability of CRY to inhibit the STAT3-phosphorylation induced by CSC, according to Chichiarelli et al. (2010). In our experiments, CSC (1-100 μg/ml) significantly increased the MN frequency in both cell lines already at the lower concentrations. In DCFH-DA assay, CSC induced an increase of the DCFH fluorescence with respect to the vehicle, so suggesting the presence of higher intracellular levels of ROS. Furthermore, an increase of the CSC-induced STAT-3 phosphorylation was found in both cell lines at different incubation times. When the smoke sample was tested in the presence of CRY (1-25 μg/ml), a significant reduction in the CSC-induced MN-frequency was found (maximum inhibition of about 60%). These results suggest a possible preventive role of the substance against CSC and agree with previous data obtained in bacteria (Di Sotto et al., 2013). The effect of CRY could be due to its lipophilic structure and to its high affinity for the phospholipid bilayers (Sarpietro et al., 2015). On the basis of these features, CRY could be able to induce a change in the cell membrane permeability so hindering the uptake into cells of the genotoxic species contained in CSC. CRY was also able to reduce the oxidative stress and to inhibit the STAT3 phosphorylation induced by CSC. Taking into account that the STAT3 protein has been found activated by an increased intracellular oxidative stress, the reduction of its phosphorylation induced by CRY could be strictly connected to the inhibition of the CSC-mediated pro-oxidant effects. STAT3-activation is involved in the progression of pre-neoplastic lesions induced by cigarette smoking: its inhibition could represent a possible mechanism for the protective properties of CRY against the precancerous events associated to the smoke exposure. On the whole, data obtained in the present research highlight protective properties of CRY and encourage further studies in order to evaluate its possible use as a chemopreventive agent against smoke damage .

References

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