Carcinogenesis vol.32 no.3 pp.368–380, 2011
doi:10.1093/carcin/bgq278
Advance Access publication December 22, 2010

Guggulsterone (GS) inhibits smokeless tobacco and nicotine-induced NF-κB and STAT3 pathways in head and neck cancer cells

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Understanding the molecular pathways perturbed in smokeless tobacco- (ST) associated head and neck squamous cell carcinoma (HNSCC) is critical for identifying novel complementary agents for effective disease management. Activation of nuclear factor-kappaB (NF-κB) and cyclooxygenase-2 (COX-2) was reported in ST-associated HNSCC by us [Sawhney,M. *et al.* (2007) Expression of NF-kappaB parallels COX-2 expression in oral precancer and cancer: association with smokeless tobacco. Int. J. Cancer, 120, 2545–2556]. In search of novel agents for treatment of HNSCC, we investigated the potential of guggulsterone (GS), (4,17(20)-pregnadiene-3,16-dione), a biosafe nutraceutical, in inhibiting ST- and nicotine-induced activation of NF-κB and signal transducer and activator of transcription (STAT) 3 pathways in HNSCC cells. GS inhibited the activation of NF-κB and STAT3 proteins in head and neck cancer cells. This inhibition of NF-κB by GS resulted from decreased phosphorylation and degradation of nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, alpha the inhibitory subunit of NF-κB. Importantly, treatment of HNSCC cells with GS abrogated both ST- and nicotine-induced nuclear activation of NF-κB and pSTAT3 proteins and their downstream targets COX-2 and vascular endothelial growth factor. Furthermore, GS treatment decreased the levels of ST- and nicotine-induced secreted interleukin-6 in culture media of HNSCC cells. In conclusion, our findings demonstrated that GS treatment abrogates the effects of ST and nicotine on activation of NF-κB and STAT3 pathways in HNSCC cells that contribute to inflammatory and angiogenic responses as well as its progression and metastasis. These findings provide a biologic rationale for further clinical investigation of GS as an effective complementary agent for inhibiting ST-induced head and neck cancer.

Abbreviations: CLSM, confocal laser scan microscopy; COX-2, cyclooxygenase-2; EDTA, ethylenediaminetetraacetic acid; EGTA, ethyleneglycol-bis (aminoethylether)-tetraacetic acid; HEPES, N-2-hydroxyethylpiperazine-N′-2-ethanesulfonic acid; HNSCC, head and neck squamous cell carcinoma; GS, guggulsterone; IL, interleukin; IkBα, nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, alpha; NF-κB, nuclear factor-kappaB; PBS, phosphate-buffered saline; SCC, squamous cell carcinoma; SDS, sodium dodecyl sulfate; STAT, signal transducer and activator of transcription; ST, smokeless tobacco; VEGF, vascular endothelial growth factor.

Introduction

Tobacco is used by >1.3 billion people worldwide (1); it kills >5 million people annually and the World Health Organization estimates this number to increase to 8 million by 2030 (2). Tobacco use is the single largest cause of cancer globally (3,4). The number of adult smokers has declined in the USA (3,4), but the use of smokeless tobacco (ST) products (e.g. chewing tobacco, khaini and snuff) is increasing worldwide, and these products can be a gateway for life-long addiction (1,5). ST consumption is emerging as a major risk factor for head and neck squamous cell carcinoma (HNSCC) (6,7), the most common cancer in men in many Asian countries and the sixth most common cancer in USA (8). The causal association between smoking and HNSCC has been unequivocally established (5,9–12). The evidence that ST causes oral cancer was confirmed by the International Agency for Research on Cancer and ST was classified as a human carcinogen, with nitrosoamines being one of the major carcinogenic constituents (13). In a recent study, 23 polycyclic aromatic hydrocarbons have been identified in ST (14). Nicotine, a major component of ST, besides causing addiction has been shown to regulate cell proliferation, angiogenesis and inhibit apoptosis induced by anticancer drugs (15). An indepth understanding of the molecular mechanism(s) underlying ST-related head and neck carcinogenesis will lead to the development of effective strategies to prevent and treat ST-related HNSCC.

Studies carried out in our laboratory and others have shown aberrant expression of genes involved in ST-associated HNSCC (16–20). ST has been shown to increase oxidative stress (21) plays a major role in activation of nuclear factor-kappaB (NF-κB) and pSTAT3 pathways, involved in inflammation, survival and proliferation of cancer cells (21–24). Our laboratory also reported exposure of oral premalignant cultures and cancer cells (AMOS III) to ST resulted in increased cell proliferation and activation of NF-κB; association of ST consumption with overexpression of NF-κB and cyclooxygenase-2 (COX-2) was observed in clinical oral squamous cell carcinoma (SCC) tissue samples also (23–25). The persistent activation of NF-κB can lead to elevated expression and secretion of interleukin (IL)-6. Interestingly, blocking NF-κB diminished the accumulation of active signal transducer and activator of transcription (STAT) 3 in HNSCC cells, suggesting existence of cross talks between NF-κB and STAT3 pathways (25). Recently our group observed nuclear pSTAT3 accumulation in clinical oral squamous cell carcinoma tissue samples was significantly associated with ST consumption habits (26), indicating a plausible link between STAT3 and ST. Therefore, it is important to identify agents that can abrogate these effects of ST in head and neck cancer cells for developing therapies to prevent and treat ST-related head and neck cancer.

Guggulsterone (GS), (4,17(20)-pregnadiene- 3,16-dione), derived from the plant *Commiphora mukul*, is widely used to treat obesity, diabetes, hyperlipidemia, atherosclerosis and osteoarthritis (27). GS suppresses inflammation by inhibiting inducible nitric oxide synthetase (28) and NF-κB induced by various carcinogens and tumor promoters (29). Our group and several other reports have shown that treatment with GS induces apoptosis and suppress proliferation of wide variety of human tumor cell types (28–33). GS inhibits invasion, angiogenesis and metastasis of tumor cells and reverses chemoresistance (31–33). GS has also been shown to inhibit both constitutive and inducible STAT3 pathways in head and neck cancer cell lines (34,35). Recently, Leeman-Neill *et al.* (35) showed antiproliferative effects of GS are partially dependent on STAT3 inactivation. They showed knocking down expression of STAT3 using small interfering RNA in head and neck cancer cells reduced GS-induced cell death in comparison with the no transfection controls. In addition, our group in collaboration with Dr Bharat Aggarwal's laboratory showed that GS inhibits inducible and constitutive STAT3 activation through the