Bufalin: Multifaceted Impact on Head and Neck Carcinomas Revealed through Cytotoxicity, Apoptosis, and Immune Modulation

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a) Background.

Head and neck carcinomas (HNC) are aggressive cancers, ranking fourth among men in Belgium. Late diagnosis often results in poor outcomes. Bufalin, an endogenous cardiotonic steroid derived from the Chusan Island toad, exhibits anti-cancer properties, inhibiting cell proliferation, inducing apoptosis, oxidative stress and potentially suppressing cancer growth and metastasis by influencing immune cells in the tumor microenvironment.

b) Aim

The research aimed to assess bufalin's effects on FaDu, 93VU-147T, and Detroit-562 head and neck cancer cells, examining its impact on cancer cell proliferation and migration, its action on the cell cycle, its influence on oxidative stress and its ability to induce apoptosis. Furthermore, the study investigated how bufalin regulates the polarization of M2 pro-tumoral macrophages into M1 macrophages with anti-tumor effects.

c) Methods

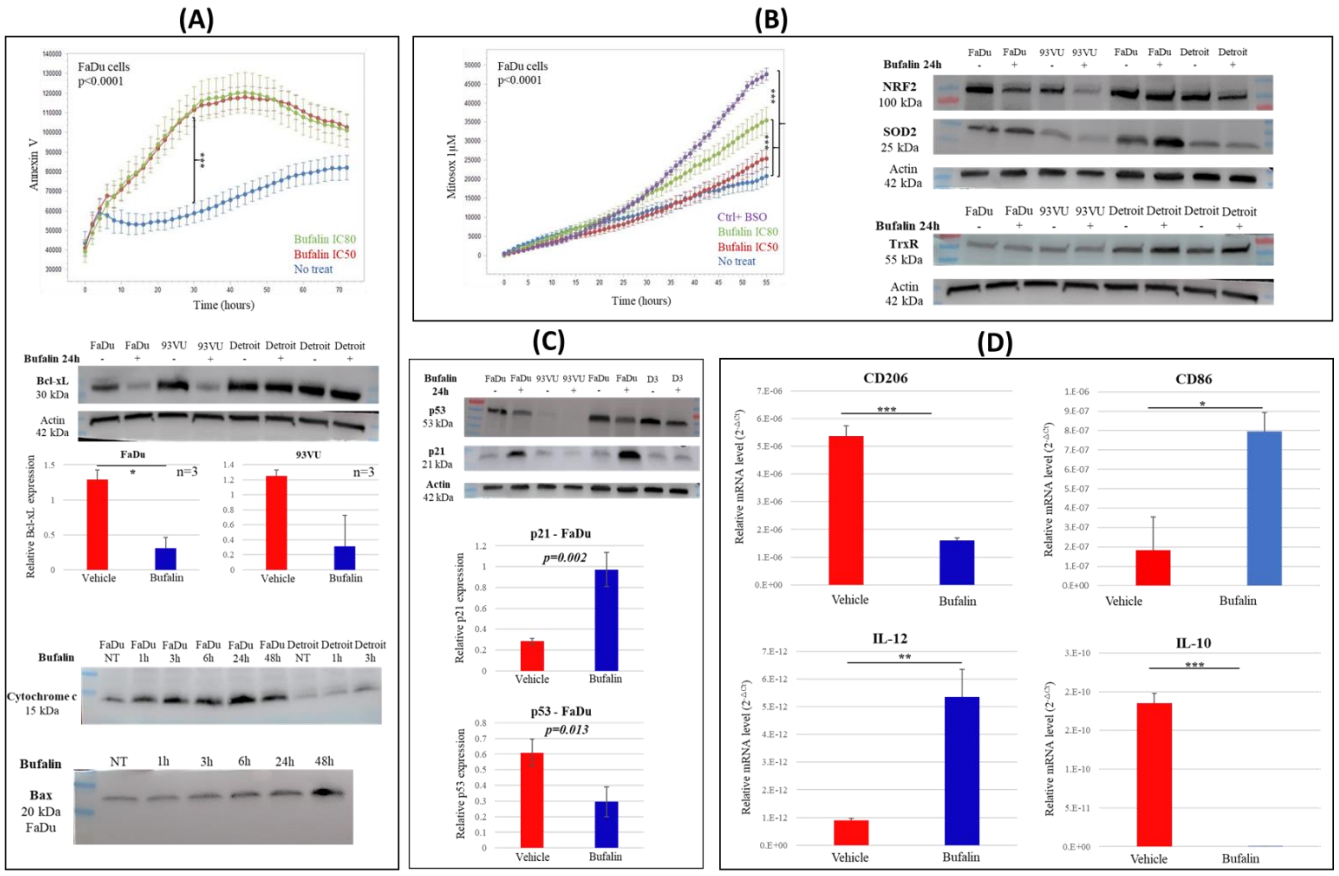
Bufalin's cytotoxic effects on cancer cells, assessing oxidative stress and apoptosis were evaluated with Incucyte S3 live-cell Analysis system. Flow cytometry (Muse) examined bufalin's impact on the cell cycle. Western Blot and RT-qPCR measured apoptotic, oxidative stress and cell cycle markers. Transwell chambers evaluated bufalin's migration effect. Monocytes were isolated from peripheral blood mononuclear cells (PBMC), M2 macrophages were generated, treated with bufalin and assessed for M1/M2 markers via immunofluorescence, Western Blot, and qPCR.

d) Results

Bufalin at IC80 induced cytotoxicity and increased apoptosis in FaDu and 93VU cancer cells within 24 hours. It downregulated anti-apoptotic protein Bcl-xL and upregulated pro-apoptotic proteins (Bax, Cytochrome c, and Apaf-1). Bufalin elevated reactive oxygen species (ROS) and nitric oxide (NO) levels while decreasing ROS-associated proteins (NRF2, catalase, and GSH reductase) and mitochondrial membrane potential ($\Delta\Psi m$). Additionally, it induced G2/M phase cell cycle arrest in 93VU cells by upregulating p21 and CDK1. Bufalin significantly reduced migration, modulated epithelial-mesenchymal transition (EMT) markers, increasing E-cadherin and decreasing Vimentin. Furthermore, it shifted tumor-associated macrophages from M2 to M1 phenotype via NF-κB signaling, confirmed by increased CD86 and decreased CD206 expression. Proinflammatory cytokine IL12 increased, while anti-inflammatory IL10 decreased, indicating M1 polarization after bufalin treatment. Bufalin also reduced macrophage migration inhibitory factor (MIF), known for recruiting TAMs to the tumor site.

e) Conclusion

Bufalin influences tumor development through diverse mechanisms, inducing cell death, disrupting the cell cycle, and reducing metastasis by inhibiting cell migration. Moreover, its role as an immune modulator highlights its antitumor effects, presenting a distinctive approach for cancer treatment.



(A) Figure illustrating Bufalin impact on Viability: Investigating apoptosis pathways. (BCL-XL, Bax, cytochrome c expression)

(B) Bufalin impacts on oxidative stress (NRF2, SOD2 and TrxR expression)

(C) Bufalin impact on cell cycle: p21 and p53 expression

(D) Bufalin effects on macrophage polarization