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Title: The Effects of flubendazole and doxorubicin on the proliferation of LNCaP prostate cancer cells

Abstract:

Prostate cancer is the second most common malignancy in men in the world, and several factors, such as age, ethnicity, genetic factors, and diet, play a role in its development. Flubendazole, an antiparasitic drug from the benzimidazole family, has recently attracted the attention of cancer research, and its anticancer effects have been proven in various tumor models. The anticancer mechanism of flubendazole primarily involves disrupting the cellular microtubule structure, arresting the cell cycle, and activating caspase-3-dependent apoptosis pathways. Therefore, in this study, the anticancer effects of flubendazole and doxorubicin, as well as the combined effect of these two drugs on LNCaP prostate cancer cells, were investigated. For this purpose, prostate cancer cells (LNCaP) were cultured and treated with different concentrations of flubendazole and doxorubicin, and a combination of these two drugs. Cytotoxicity was assessed by trypan blue, MTT, and LDH assays. The incidence of apoptosis and necrosis was assessed using fluorescence microscopy and acridine orange and propidium iodide staining. The clonogenic assay determined the ability of cells to proliferate and form colonies after treatment. At the molecular level, the expression of apoptosis-related genes was quantified using the qRT-PCR technique. All data were repeated at least three times and analyzed using ANOVA and Tukey's test, with $P \leq 0.05$ as the significance level, to provide reliable results of the response of cells to the treatments. The results indicate that all three types of treatments, at specific concentrations, can induce a 50% reduction in cell viability (IC50). In particular, it was observed that doxorubicin alone showed the highest reduction in viability compared to flubendazole. The combination treatment resulted in greater cell killing compared to flubendazole alone. On the other hand, compared with doxorubicin as a single agent, the combination group exhibited higher efficacy, which may indicate the potential for reducing the dose and side effects of doxorubicin in future treatment protocols. This study showed that the combination of doxorubicin and flubendazole has a synergistic effect in inducing cell death in prostate cancer cells (LNCap), such that at higher concentrations, the combination group performed better than the monotherapies by increasing caspase-3 activity, increasing the BAX/BCL-2 ratio, increasing LDH release, and reducing colony formation. Doxorubicin alone was more potent than flubendazole in reducing cell viability and mitochondrial activity, while flubendazole was more effective in inducing caspase-3; However, the combination of these two drugs, by simultaneously activating apoptotic pathways, especially at higher concentrations, enhanced the anticancer effects and offered significant therapeutic potential for prostate cancer.

Keywords: Flubendazole, Prostate cancer, Doxorubicin