Enhanced Antiproliferative Effect of Combination of D-fraction and Vitamin K_3 in Bladder Cancer Cells

Introduction and Objective: Bladder cancer is the second most common urologic malignancy next to prostate cancer in the United States. Although endoscopic transurethral resection (TUR) is a primary therapy, 50%-75% of patients will recur in 5 years and about 10% progress to invasive disease. Hence, the primary therapeutic aim is to prevent such multiple recurrences and progression to an advanced invasive stage. We were then interested in *combination* therapy as an alternative approach. D-fraction (DF) is a bioactive mushroom extract with antitumor activity and vitamin K_3 (VK_3) is also shown to have antitumor activity. Accordingly, we investigated if combination of DF and VK_3 would exhibit the better efficacy on bladder cancer cells *in vitro*.
Materials and Methods: Human bladder cancer T24 cells were treated with DF, VK_3, or combination of DF and VK_3, and cell growth/viability was assessed by MTT assay at 72 h. To explore the antiproliferative mechanism, cell cycle, Western blot, and DNA analyses were also performed.
Results: DF induced a ~50% growth reduction at the relatively high concentration (700 µg/ml), while VK_3 at any give concentrations (0-8 µM) had little effects on cell growth. However, when DF and VK_3 were combined at their specific concentrations (300 µg/ml DF and 4 µM VK_3), cell growth was drastically reduced by over 70%. Cell cycle analysis indicated that such an abrupt growth reduction was accompanied by a G_1 cell cycle arrest with a 51% increase and 63% decrease in G_1 and S phase cell numbers, respectively. Western blots also revealed the modulated expressions of G_1-specific cell cycle regulators (CDK2, cyclin E, and p27/Kip1) with DF/VK_3 combination. Moreover, DNA analysis of DF/VK_3-treated cells showed a discrete nucleosomal ladder formation, which was indicative of apoptosis.
Conclusions: The present study demonstrates that the combination of DF and VK_3 at their relatively low concentrations can be markedly potentiated to induce the significant (>70%) growth inhibition in T24 cells. Such an enhanced antiproliferative effect appears to be primarily associated with a G_1 cell cycle arrest, ultimately leading to apoptosis. Therefore, the DF/VK_3 combination may provide an alternative, improved therapeutic modality for superficial bladder cancer.