

Modulation of Androgen Receptor Expression by a Natural Product Amalgam, Zyflamend

¹D.L. Bemis, ¹J.L. Capodice, ¹E.-J. Lee, ¹A.E. Katz and ²R. Buttyan

¹Department of Urology, Columbia University Medical Center, NY, NY; Ordway Research Institute, Albany, NY

Introduction: Prostate cancer cells are driven to grow and divide by androgenic steroids whose actions are mediated by the intracellular androgen receptor (AR) protein. Several different herbal agents have been reported to down-modulate AR protein levels in prostate cancer cells and this activity is associated with a decrease in tumor cell growth and aggressiveness. Previously, we showed that a natural herbal amalgam with COX inhibitory activity known as Zyflamend was also able to reduce AR protein expression in prostate cancer cells and this effect was associated with a reduction in cancer cell growth in a manner similar to that reported for synthetic COX-2 inhibitors. Here we discuss our analyses of the effects of Zyflamend on effectors of AR expression in an attempt to better understand the molecular mechanism through which this herbal agent might have benefits for prostate cancer patients.

Methods: Human prostate cancer cells (LNCaP) were treated for 24 hr with 0.1 μ l/ml Zyflamend. High-throughput phosphoprotein screening assays were conducted to determine the effects of Zyflamend on the phosphorylation status of several cell cycle and apoptosis effector proteins including Akt and its downstream target MDM2 that regulates degradation of AR protein. Western blotting techniques were utilized to confirm the effects of Zyflamend on the phosphorylation of Akt (at Ser-472 and Thr-308) and MDM-2 (at Ser-166) proteins. A colorimetric protein phosphatase assay (Promega, Inc.) was utilized to determine the effects of Zyflamend or the COX-2 specific inhibitor, NS-398, on cellular protein phosphatases (PP2A, PP2B or PP2C) activity in these cells.

Results: The phosphoprotein screening assay showed that Akt phosphorylation at the serine and threonine residues was increased by Zyflamend treatment as was phosphorylation of serine on Mdm2. These changes were confirmed and quantified by Western blot analyses. Phosphatase activity assays showed that Zyflamend treatment significantly reduced the intracellular activity of the phosphatases PP2A, PP2B and PP2C that can dephosphorylate Akt or MDM2, whereas NS-398 selectively diminished PP2B activity.

Conclusions: We propose that Zyflamend's effects on AR protein in prostate cancer cells may be mediated by its ability to suppress the activity of intracellular protein phosphatases PP2A, PP2B and PP2C leading to increased accumulation of phospho-Akt and its downstream target, phospho-MDM2 that promotes AR ubiquitinylation and its destruction by the proteasome. Similar activity may mediate the ability of synthetic COX inhibitors to suppress AR expression.