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PRECLINICAL STUDIES

Ring-substituted analogs of 3,3′-diindolylmethane (DIM) induce apoptosis and necrosis in androgen-dependent and –independent prostate cancer cells

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Summary We recently reported that novel ring-substituted analogs of 3,3′-diindolylmethane (ring-DIMs) have anti-androgenic and growth inhibitory effects in androgen-dependent prostate cancer cells. The objectives of this study were to confirm the ability of 4,4′- and 7,7′-dibromo- and dichloro-substituted ring-DIMs to inhibit androgen-stimulated proliferation of androgen-dependent LNCaP human prostate cancer cells using a non-invasive, real-time monitoring technique. In addition, their ability to induce apoptotic and necrotic cell death in androgen-dependent as well as -independent (PC-3) prostate cancer cells was studied. Prostate cancer cells were treated with increasing concentrations of DIM and ring-DIMs (0.3–30 μM) and effects on cell proliferation were measured in real-time using an xCELLigence cellular analysis system. Chromatin condensation and loss of membrane integrity were determined by Hoechst and propidium iodide staining, respectively. Apoptotic protein markers were measured by immunoblotting and activation of caspases determined using selective fluorogenic substrates. Intra- and extracellular concentrations of necrosis in LNCaP and PC-3 cells with 2–4 fold greater potencies than DIM. DIM and the ring-DIMs increased caspases −3, −8 and −9 activity, elevated expression of Fas, FasL, DR4 and DR5 protein, and induced PARP cleavage in both cell lines. The cytotoxicity of the most potent ring-DIM, 4,4′-dibromoDIM, but not the other compounds was decreased by an inhibitor of caspase −3. The 4,4′-dibromoDIM was primarily found in the extracellular medium, whereas all other compounds were present to a much larger extent in the cell. In conclusion, ring-DIMs inhibited prostate cancer cell growth and induced cell death in LNCaP and PC-3 cells with greater potencies than DIM; they also structure-dependently activated different cell death pathways suggesting that these compounds have clinical potential as chemopreventive and chemotherapeutic agents in prostate cancer, regardless of hormone-dependency.

Keywords Prostate cancer · Androgen-dependent · Androgen-independent · Diindolylmethane derivatives · LNCaP · PC-3 · Apoptosis · Necrosis · Caspase activity ·