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Elucidating the Pathway of Apatone® Induced DNase II Reactivation During Autoschizic Cell Death

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Apatone® (IC-Medtech, El Cajon, CA), (Vitamin C (VC) and vitamin K_3 (VK_3) administered in a 100:1 ratio) exhibited potent antitumor activity against human cancer cell lines in vitro and in vivo by inducing auotschizic cell death. Autoschizis entailed the inhibition of the transcription factor NF-κB by Apatone which is followed by a cascade of processes that culminate in the sequential reactivation and release of DNase I and DNase II. DNase II is present in an inactive 1:1 complex with elastase and DNase II and is believed to be cleaved by cathepsins from the lysosomes into two active enzymes during cell death. Since, NF-κB is also involved in stabilizing the lysosomal membrane, we hypothesize that Apatone-induced reduction in tumor cell levels of NF-κB trigger destabilization of the lysosomal membrane and the release cathepsins, DNase II and other lytic enzymes which participate in autoschizis.

A microtetrazolium (MTT) assay was employed to evaluate Apatone induced tumor cell death. Transmission Electron Microscopy (TEM) was employed to study the ultrastructural changes in the lysosomes and other compartments of the tumor cells. Fluorescence microscopy, fluorescent bioassays and immuno-histochemistry were employed to coroborate lysosomal changes and monitor changes in DNase II and cathepsin B and L distribution and activity.

The MTT assay determined a 50% cytotoxic dose (CD50) of 2455 ± 28 VC alone, 12.9 ± 0.81 VK3 alone and 122uM VC/1.22uM K3 demonstrating the synergistic cytotoxicity of VC and VK_3 against DU145 prostate cancer cell lines. TEM confirmed that tumor cell death was due to autoschizis (Figure 1). Following 1hr of Apatone treatment, acridine orange staining revealed increased permeabilization of the lysosomal membrane (Figure 2). This activity was corroborated by increased cathepsin B and L and increased DNase II activity (Figure 3). These results confirm the importance of lysosomal permeabilization and DNase II reactivation in Apatone-induced Autoschizic tumor cell death.

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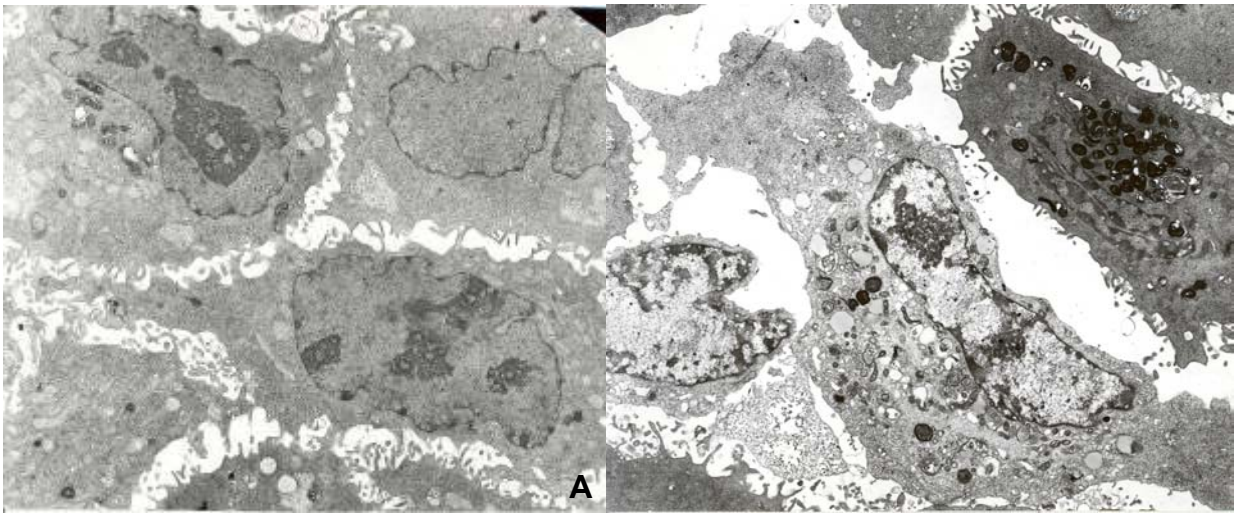

Figure 1 A) TEM of DU145 control cells B) DU145 cell treated with Apatone treated cells.

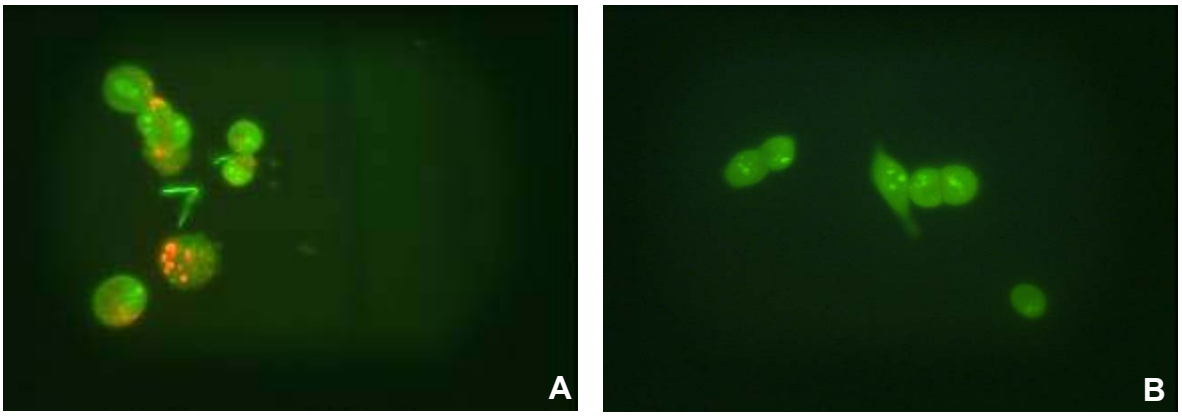

Figure 2 A) Control cells stained with acridine orange (AO). B) One hour Apatone exposure; lower AO levels in the lysosomes is consistent with lysosomal membrane permeability.

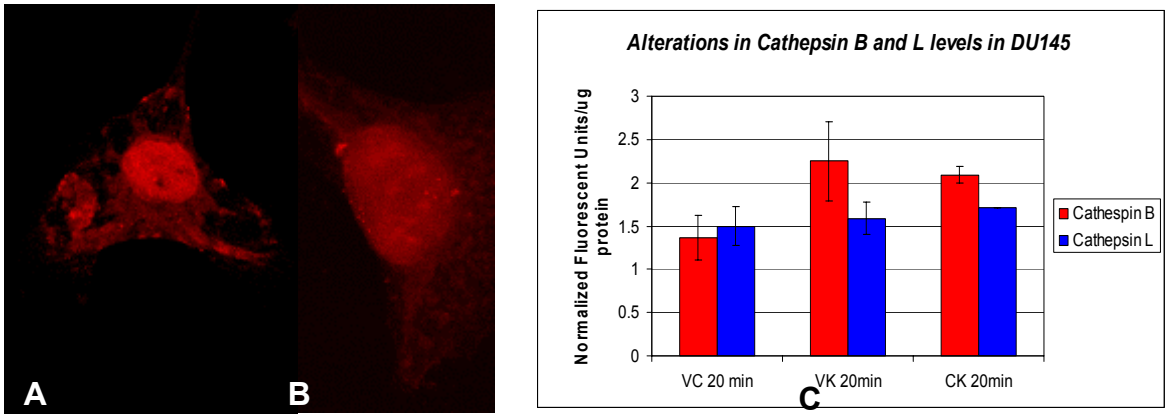

Figure 3 A) Disperse DNase II staining in a control cell. B) After treatment with Apatone for 15 minutes shows DNase II re-localization to the nucleolus. C) Increasing Cathepsin B and L levels verify permeabilized lysosomal membranes. Values are normalized to the control and expressed in Fluorescent Units per ug of protein.

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