CHAPTER 1

Introduction

Effective chemopreventive and target-specific drug against several cancers is highly needful now a day. Regarding this, we have found Poly-L-Lysine (PLL) as an effective drug in combating EAC intoxicated cancer growth in BALB/c and Swiss albino mice model. PLL at a significantly lower dose (20 mg/kg and 40 mg/kg b.w.) was safe for normal cells but cytotoxic, anti-proliferative and apoptotic for EAC cells in BALB/c and Swiss albino mice model. The anticancer effect of PLL was assessed in BALB/c mice at 20 and 40mg/kg/b.w doses for 14 days for EAC liquid ascites carcinoma and 21 days for DAL, Sorcoma-180, B16F10 solid tumour model. PLL shows its efficacy at this dose by inducing EAC cell death through the mitochondrial-mediated intrinsic pathway. PLL had a cytotoxic effect on EAC, K562; A549; U937, MCF-7, Sarcoma-180 and B16F10 cancer cells. Significant decreases in liquid and solid EAC, DAL, Sarcoma-180 and B16F10 tumours and increased life span of treated mice were observed ($P<0.001$). Typical morphological changes, apoptosis bleb phenomenon and sub-G1 cell cycle arrests revealed that PLL promoted apoptotic cell death. Western blot and immune-histochemistry confirm PLL-activated apoptotic signalling cascades through Bcl-2 and CD31 protein down-regulation and up-regulation of Bax, caspase-3 and p53 proteins. The anti-angiogenic effects were also accompanied by decreased VEGF expression and reduced peritoneal angiogenesis and microvessel density. PLL modulated the Bax/Bcl2 level, activated caspase 3 and PARP molecules and induced the DNA damage in EAC cells, indicating apoptotic cell death. On the other hand, as PLL was found to upregulate generation of intracellular reactive oxygen species (ROS), PLL induced apoptotic pathway was assumed to be ROS-dependent.

PLL significantly decreased cell proliferation in in-vitro HUVECs and DAL cells without significantly affecting normal cell growth. PLL also induced alteration in cellular morphology in DAL cells. After that, in the BALB/c mouse model, PLL had noticeable inhibition in DAL-induced tumorigenesis. This inhibition was evident through reduced solid tumour

volume and weight in comparison to the control group. However, PLL promoted tumour apoptosis and suppressed cell proliferation and tumour angiogenesis. The amount of TdT in the nuclei of DAL cells in mice treated with PLL was significantly increased while, in contrast, decreases in anti-apoptotic protein Bcl-2 expression were observed. PLL also considerably upregulated the pro-apoptotic protein Bax and activated caspase 3. Measurable decreases of cyclin-D1 were observed through PLL treatments, indicating cell-cycle arrest. These studies also indicate PLL's induction and anti-proliferative effects by suppressing the c-Myc and Ki-67 proliferation indices. Additionally, PLL inhibited tumour-angiogenesis by suppressing VEGF and CD34 protein expression levels and reducing microvessel density compared to similar parameters in tumors from control mice.

Our findings also disclosed the significantly inducing apoptotic activity in *in-vivo* B16F10 cells and emphasised the anti-angiogenic action, comprising growth inhibition, in B16F10 melanoma tumour cell and angiogenesis suppression in the chicken embryos, which delivered sympathetic indication for the traditional use of PLL in cancer treatment. Furthermore, PLL conceivably stimulates the reticence of cell proliferation by inhibiting Ki-67 constructive cells and decreasing the degree of CD34 translation representation. PLL anti-angiogenic impacts by down-regulation of VEGF and VEGFR2 phosphorylation and via destruction of the VEGF/VEGFR-2 signaling pathways. That play a crucial role in controlling angiogenesis in B16F10 melanoma cells. These conclusions suggest that at least part of the anticancer impacts of PLL described prior might be incompletely attributed to the anti-angiogenesis mechanism identified here. PLL represents a fascinating candidate for expanding novel anti-angiogenesis treatments to treat cancer and other angiogenesis-related diseases. The effectiveness of such a chemopreventive molecule might be helpful when used more broadly in the near future.

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