Berberine Synergistically Enhances Anticancer Activity of Vincristine *in Vitro* and *in Vivo*

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STATEMENT OF THE PROBLEM

Vinca alkaloids isolated from the periwinkle plant, Vinca rosea Linn. like vincristine (VCR) and vinblastine have proved to be the most active antitumor agents. VCR has been widely used in the treatment of many neoplastic diseases, including the non- Hodgkin's and Hodgkin's lymphomas, ALL, breast carcinoma, Wilms' tumor, neuroblastoma, and embryonal rhabdomyosarcoma, etc. The major antitumor effect of this agent appears to be related to its high-affinity binding to the basic protein subunit of microtubules, tubulin, which results in disruption of the mitotic spindle apparatus and arrest of cells in metaphase. VCR also binds to neuronal tubulin, disrupting axonal microtubules and resulting in neurotoxicity, therefore limiting the maximum clinical dose of VCR to 2.0 mg regardless of body surface area. Thus, vincristine neurotoxicity is major limitation in successful treatment of cancer that negatively impacts quality of life of cancer patients.

Berberine (BER), a botanical alkaloid, is purified from the roots and bark of the Berberis species. BER reportedly possesses multiple pharmacological properties, including anti-diarrheal, anti-fungal, anti-diabetic, hepatoprotective and cardioprotective effects. BER also suppresses tumor growth through the induction of apoptosis and cell cycle arrest in cancer cells. Berberine has potential as a chemotherapy adjuvant due to its low toxicity and anticancer properties. This study attempted to investigate the synergistic anticancer efficacy of vincristine-berberine combination *in vitro* and *in vivo* with the purpose of reducing chemotherapeutic dose of vincristine and thereby circumventing the neuropathy associated with it.

METHODOLOGY & THEORETICAL ORIENTATION

In vitro cytotoxic activity of VCR, BER, and their combination was tested on HL-60 cells. Cell viability was assessed by a colorimetric assay using MTT, and by using a fluorescent dye calcein-AM. The extent of apoptosis and effect of drugs on cell cycle was analyzed using propidium iodide stain on a flow cytometer. Effect on mitochondrial membrane potential was carried out using JC-1 stain and fluorescence was measured on a flow cytometer as well as the cell morphology was observed and captured under a fluorescent microscope. The intracellular ROS level was measured using DCFH-DA.

Anticancer efficacy of VCR-BER combination was tested in vivo in an experimental paediatric solid tumor model of Wilms' tumor i.e. nephroblastoma. Briefly, SCID mice were injected s.c. on the left shoulder with 1 x 107 SKNEP-1 cells/ml FBS. After visible tumor growth, animals were grouped and treated for 4 weeks. Vincristine was administered i.v. in the tail vein once a week and berberine was administered p.o. daily sterile intragastric tube 30 mins after vincristine injection. At the end of the study period, the animals were humanely sacrificed and the tumors, brain, spinal cord, and sciatic nerve were excised out, weighed, and used for histopathological studies. Tumor volumes, and mean survival time of animals were calculated.

FINDINGS

MTT assay showed that IC50 for VCR was 196.5- 553.2 pM and for BER 74.22-157.9 nM after 48 hours of drug treatment. Calcein AM assay further revealed that IC50 (Dm) value of VCR was 405.896 pM, and for BER it was 340.705 nM. VCR-BER act synergistically (CI<1) to induce cell death in HL-60 cells. VCR & BER treatment, individually & in combination showed a dose-dependent increase in number of sub-G1cells & arrested the cells in the G2/M phase of cell division. VCR & BER treatment, individually as well as combination, showed a dose-dependent depolarization of the mitochondrial membrane potential which was evident from a decrease in red:green fluorescence ratio. The intracellular ROS level was measured using 2, 7-dichlorodihydrofluorescein diacetate (DCFH-DA). VCR & BER treatment, individually as well as combination, showed a dose-dependent increase in ROS generation which may be responsible for their cytotoxicity. VCR-BER synergism was further substantiated in vivo in pediatric solid tumor model of nephroblastoma. Clinical dose (1 mg/kg) of vincristine was reduced to 500, & 250µg/kg and used in combination with BER (50 & 25mg/kg). VCR (250 µg/kg)-BER (25 mg/kg) resulted in highest tumor regression, and increased survival time of mice. (Histopathology results awaited to further corroborate these findings).

CONCLUSION & SIGNIFICANCE

Vincristine can successfully treat a variety of haematological as well as other solid tumors provided the therapy can be made more tolerable for patients. The adverse effects of vincristine can be evaded by reducing its therapeutic dose by using combination therapy or by introducing neuroprotectants for vincristine associated neurotoxicity. In our study, VCR-BER showed remarkable growth inhibition *in vitro* in human promyelocytic leukemia cells and *in vivo* in Wilms' tumor in SCID mice. Thus, berberine by the virtue of its antineoplastic and neuroprotective properties may prove to be a novel & safer adjuvant in chemotherapy in the clinical setting.

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