PRECLINICAL EVALUATION OF POTENTIAL SYNERGISTIC EFFECT OF NOSCAPINOIDS AND TAXOTERE FOR BREAST CANCER THERAPY

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by

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ABSTRACT OF THE DISSERTATION

Docetaxel (DOX) based combination therapy is a novel therapeutic strategy that attracts great interest in breast cancer treatment but its clinical utility got limited due to its side effects. In contrast, noscapine ($C_{22}H_{23}NO_7$), benzylisoquinoline alkaloid was discovered to bind tubulin, arrest dividing cells at mitosis and selectively induced apoptosis to cancer cells without changing the steady state monomer/polymer ratio of tubulin. It was also indicated that, unlike many other microtubule inhibitors, noscapinoids do not significantly promote or hinder microtubule polymerization; rather, they alter the steady-state dynamics of microtubule assembly. This is a unique feature over currently-available antimicrotubule drugs that either overpolymerize microtubules (*taxanes*) or depolymerize them (*vincas*) and hence cause various debilitating toxicities such as leucocytopenias, diarrhea, alopecia and peripheral neuropathies. In addition, noscapine has some other advantage properties as lead molecule: (1) retains activity against paclitaxel-resistant cell lines (1A9/PTX10, 1A9/PTX22) and epothilone-resistant cell line (1A9/A8); (2) a favourable pharmacokinetics (clearance in 6-10 hours); (3) a poor substrate for drug-pumps (polyglycoprotiens and MDR-related proteins) that comprise a major cause of drug resistance; (4) it does not show immunological and neurological toxicities and (5) inhibit tumorigenesis in vivo albeit at high concentrations (~ 300 mg/kg body weight). Although noscapine is cytotoxic in a variety of different cancer cell lines (NCI 60 cell lines panel), the IC_{50} values remain in the high micromolar ranges (~21.1 to 100 μM). To enhance its activity further, efforts have been focused on developing several derivatives of noscapine (we called as noscapinoids) by modification of its scaffold structure at various points.

The combination therapy of anti-microtubule agents is an undiscovered source of chemotherapeutic resources. Presence of multiple drug binding sites on the tubulin, suggests that a reasonable combination of two or more drugs of this class may increase the efficacy of

anticancer drugs and diminish toxic side effects, thereby improving the therapeutic index. In the current dissertation work, we embark upon an approach to rationally designed a novel derivative of noscapine and evaluate its additive effect with the clinically approved anticancer agent, docetaxel (DOX), to enhance the anticancer activity based on the molecular modelling, cellular study and tubulin binding activity.

Molecular docking of 9-vinyl phenyl noscapine (VPN) and DOX onto microtubule revealed a docking score of -4.82 kcal/mol and -6.67 kcal/mol respectively, while the docking score of VPN was changed to -3.23 kcal/mol when it was docked onto the co-complex of tubulin-DOX. Further, the binding free energy ($\Delta G_{bind,PBSA}$) of VPN and DOX with tubulin showed -24.04 and -18.65 kcal/mol respectively, while the binding free energy of DOX was increased further in combination with VPN ($\Delta G_{bind,\ PBSA}$ was reduced to -21.41 kcal/mol), denoting combination effect of both ligands. Similarly, a binding energy of -3.49 and -4.18 kcal/mol respectively was noted by the molecular docking of amino-noscapine and DOX on the microtubule. In contrast, the binding energy was improved significantly (-6.27 kcal/mol) when the DOX was docked to the co-complex of amino-noscapine and tubulin, indicating the combined effect of both the ligands. The individual predicted free energy of binding ($\Delta G_{bind,pred}$) for Br-Bn-Nos and DOX with tubulin was found to be -28.89 kcal/mol and -36.07 kcal/mol based on MM-GBSA as well as -26.21 kcal/mol and -34.65 kcal/mol based on MM-PBSA, respectively. However, the $\Delta G_{bind,pred}$ of Br-Bn-Nos was significantly reduced to -33.02 kcal/mol and -30.24 kcal/mol using MM-GBSA and MM-PBSA in presence of DOX on its binding pocket. Parenthetically, the $\Delta G_{bind,pred}$ of DOX was significantly reduced to -37.17 kcal/mol and -32.80 kcal/mol using MM-GBSA and MM-PBSA in the presence of Br-Bn-Nos on its binding pocket. The reduced $\Delta G_{bind,pred}$ in presence of Br-Bn-Nos and DOX together indicated a combination effect of both the ligands. The cell killing potential represented in terms of IC_{50} value amounted to 30.17 µM and 19.92 µM for VPN and 0.621 µM and 0.193 µM for DOX, respectively for 48 h and 72 h. The dose dependent cytotoxicity

of DOX has been reduced considerably with the combination dose regimen of VPN. The IC_{50} value amounted to 38.07 µM and 28.4 µM for treatment duration of 48 h and 72 h for amino-noscapine. Parenthetically the IC_{50} value was 0.61 µM and 0.08 µM for DOX respectively for the treatment duration of 48 h and 72 h. The cytotoxic effect of DOX was reduced significantly (to 0.05 µM) in combined treatment with amino-noscapine (20 µM). Further, isobologram analysis revealed the synergistic effect of Br-Bn-Nos and DOX in anti-proliferative activity using MCF-7 cell line at 48 h (sum FIC = 0.49) and at 72 h (sum FIC = 0.62). Apropos to the cytotoxic effect, noscapinoids and DOX induced apoptosis to cancer cell by interfering with the cell cycle progression. This study also revealed that the combination dose regimen of noscapinoids and DOX blocks the cell cycle progression at the G2/M transition phase and induced apoptosis to cancer cells more effectively compared to the single regimen. The combined interaction of both agents onto tubulin dimmer was also determined experimentally using purified tubulin, in which a combination regimen of noscapinoids and DOX reduced the fluorescence intensity of tubulin to a higher value (~55% to 68%) compared to the single regimen.

Female athymic nude mice were xenografted with MCF-7 cells and the efficacy of Br-TMB-Nos (150 mg/kg/day) by oral gavage, DOX (1.5 mg/kg/week, i.v.), and in combination (Br-TMB-Nos 300 mg/kg/day+DOX 1.0 mg/kg/week, i.v,) were determined. Tumor volume was reduced to 630 mm^3 with combination treatment, 960 mm^3 with DOX and 1145 mm^3 from the tumor size of 1630 mm^3 from the untreated control group on day 40 post tumour implantation. It is clear from these data that combination treatment of both Br-TMB-Nos and DOX reduces the tumor size quite dramatically compared to single regimen treatment of Br-TMB-Nos and DOX. Compared to untreated control mice, inhibition of tumor growth by the treatment of Br-TMB-Nos and DOX in single and in combination regimen was statistically significant ($p < 0.001$). Surprisingly, non-significant change in weight loss for Br-TMB-Nos, DOX and in their combination treatment, indicating that Br-

TMB-Nos and DOX have a favourable toxicity profile. Our in vivo results demonstrated that a combination has a synergistic effect on a murine breast cancer model induced by the MCF-7.

Taken together, a proof-of-concept has been developed that our study provides compelling evidence that the anticancer potential of noscapine derivatives may be substantially improved when it is used in a combined application with low dose of DOX, could produce synergistic effects on cancer therapies, which is highly promising for enhancing the therapeutic efficacy for preclinical and clinical research.

Keywords: Noscapine; Docetaxel; Apoptosis; Molecular dynamics simulations; Tubulin binding affinity; Tumor