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Cremophor-free Nanoparticle Based Drug Delivery System Improves the Uptake of Paclitaxel by Cancer Cells

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Background: The practical use of paclitaxel is sometimes limited by its poor aqueous solubility. Cremophor based formulation of paclitaxel (CP) is shown to be tolerated well in cancer patients, although it causes numerous side effects. Nanoparticle delivery systems are employed to improve the transport of paclitaxel to the target cells, and to increase the bioavailability inside the cells. Recently, a novel cremophor-free nanoparticle based paclitaxel (Nanoxel®, NP) formulation has been developed by Fresenius Kabi Oncology Ltd, India and made available in some countries. This study aimed to compare the cellular uptake of NP and albumin bound paclitaxel (AP) with CP by various human cancer cell lines.
Materials and Methods: Human cancer cell lines, A549, HBL-100 and PA-1 representing non-small cell lung cancer, breast cancer and ovarian cancer respectively were used in the study. The cells were treated with either drug (NP or AP or CP) corresponding to the final paclitaxel concentration of 10 μM or vehicle of NP and later on, studied under transmission electron microscopy (TEM) and high performance liquid chromatography (HPLC).
Results: HPLC showed that percentage of paclitaxel uptake was significantly higher in NP or AP treated cells compared to CP treated cells (Table 1). TEM showed that NP and AP were internalized into the cancer cells by the process of endocytosis whereas CP was internalized by non-endocytic mechanism. Similar to NP, the vehicle of NP also penetrated into the cells through endocytosis. In addition, TEM evidence of mitochondrial damage was found only in CP, but not in AP or NP, treated cells.
Conclusions: The penetration of NP and AP into the cancer cells was found to be significantly better than by CP. Furthermore, the evidence of cremophor-induced mitochondrial damage in CP, but not in NP and AP treated cells suggests that the NP and AP, may provide a benefit over CP by better delivering the drug to the tumour cells.

Table 1: Kinetics of uptake of paclitaxel in CP, AP and NP treated PA-1cells

Time points	% of paclitaxel uptake		
	CP	AP	NP
15 min	3.39	9.18*	8.81*
30 min	2.95	9.79*	9.67*
60 min	3.67	9.88*	10.95*

*$p < 0.001$, indicates % of paclitaxel uptake was significantly higher than CP CP-cremophor based formulation of paclitaxel; AP- albumin bound paclitaxel; NP-nanoparticle based formulation of paclitaxel.

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Turning Traditional Cytotoxic Drugs Into Tumour-selective Agents

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Background: Oligo-branched peptides, containing the human regulatory peptide neurotensin (NT) sequence, have been used as tumour-specific targeting agents. These peptides are able to selectively and specifically deliver effector units, for cell imaging or killing, to tumours that overexpress NT receptors. NT-branched peptide have been conjugated to different functional units: fluorophores, chemotherapeutics and also liposomes.
Materials and Methods: *In Vitro Cytotoxicity of drug-armed peptides*: HT-29, PANC-1 and PC-3 cells were incubated with different concentrations of NT-conjugated drugs and with NT-decorated liposomes carrying doxorubicin and compared respectively to free drugs and to non-decorated liposomes. Internalization and growth inhibition were evaluated. *Human Tissue Analysis*: Surgery samples were incubated with branched NT-biotin followed by avidin-FITC. Tumour and healthy tissue were compared. Images were taken with confocal microscope. *Inhibition of Tumour Growth in Xenografts*: CD-1 female nude mice carryng a tumour xenograft of HT29 were treated with branched NT conjugated to 5-fluorodeoxyuridine. Control groups were treated with 5-fluorodeoxyuridine.
Results: FACS and immunofluorescence analysis show that NT-functionalized liposomes have a clear advantage in comparison to pure liposomes in drug internalization in HT29 and TE671 tumour cells. Cytotoxicity of NT-liposomes loaded with doxorubicin is increased four-fold with respect to nude-liposomes. Cytotoxicity on different human cell lines from colon, pancreas or prostate carcinoma indicated that branched NT conjugated with MTX and 5-FdU are notably efficient and more selective than the free drugs. Fluorophore-conjugated peptides used to measure tumour versus healthy tissue binding in human surgical samples from colon, pancreas and bladder demonstrated that neurotensin receptors are very promising druggable tumour-biomarkers. Branched NT armed with 5-FdU was used for in vivo experiments in HT-29-xenografted mice and produced a 50% reduction in tumour growth with respect to animals treated with the free drug. An unrelated branched peptide carrying the same drug was completely ineffective. In vitro and in vivo results indicated that branched peptides are valuable tools for tumour selective targeting.
Conclusions: In vitro and in vivo results indicated that NT branched peptides are valuable tools for tumour selective targeting.

References

Falciani et al, ChemMedChem 2011; Falciani et al, CCDT 2010.

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Identification of a Polyoxometalate Inhibitor of the DNA Binding Activity of Sox2

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Background: Aberrant expression of transcription factors is a frequent cause of disease, yet drugs that modulate transcription factor protein-DNA interactions are presently unavailable. Sox2 belongs to a family of high-mobility group (HMG) transcription factors that play a key role in the maintenance of stem cell pluripotency and self-renewal of mammalian cells. Over expression of Sox2 is involved in tumorigenesis and is hypothesized to play a key-role in the self-renewal of cancer stem cells. Identification of a small molecule that can physically interact and directly inhibit the DNA binding activity of the Sox2-HMG domain can be very attractive as a therapeutic lead against cancer stem cells and as chemical agents to promote targeted differentiation of stem-cells into specific cell-fates.
Materials and Methods: A high-throughput fluorescence anisotropy screen was established to identify inhibitors of Sox2 binding to its cognate DNA element. The binding of Sox2 to a 22bp fluorescently labeled cis-regulatory *CCND1* DNA element constitutes the basis of the fluorescence anisotropy assay. The large dynamic range of the fluorescence anisotropy value separating Sox2-DNA complex and unbound DNA facilitated the upscaling of the assay into a robust high-throughput format to identify inhibitors that can disrupt Sox2-HMG DNA complex formation. Compound screening was carried out using the Mechanistic and the Challenge diversity libraries obtained from the National Cancer Institute using an automated sciclone liquid dispensing workstation in a 384 well microplate format. TROSY-NMR experiments coupled with computational docking studies using Autodock 4.0, were carried out to rationalize the mechanism of inhibition by the identified Sox2 inhibitor.
Results: The screening revealed a dawson polyoxometalate (Dawson-POM) as a direct and nanomolar inhibitor of the DNA binding activity of Sox2. The Dawson-POM was found to be selective for Sox2 and related Sox-HMG family members when compared to unrelated paired and zinc finger DNA binding domains. Based on TROSY-NMR and Autodock experiments, the Dawson-POM is proposed to inhibit Sox2-DNA complex formation by firstly causing a repulsion of the phosphate backbone of DNA and secondly, by inducing structural rearrangements of the N-terminal Sox2-HMG minor wing resulting in a conformation incompatible with DNA binding.
Conclusions: The DNA binding domains of transcription factors have previously been considered too impervious to be tackled as drug targets. Here we identified a Dawson-POM as an unconventional but potent compound to inhibit the DNA binding activity of Sox2. The inhibitory mechanism of Sox2 by the Dawson-POM demonstrated here could eventually spawn the development of modified classes of POM based drugs to specifically combat aberrant gene expression.