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Title of PhD: **"Synergistic effect of nanoparticles and their payload on the DNA damage in Cancer Therapy"**

Abstract:

Nanotechnology refers to the ability to manipulate matter at minutest levels i.e one billionth (10^{-9}) of a meter in the range of 1 to 100 nm. The applications of nanotechnology are immense and varied with most promising application in the biomedical sciences. In biomedical sciences the polymeric nanoparticles both natural and synthetic, are important drug delivery vehicles. Cancer of the breast is a global health problem, and affects women worldwide with 4.68 million cases being added every year. Paclitaxel, a broad spectrum anticancer drug currently used in the treatment of patients with ovarian and breast carcinoma and is also effective in the treatment of cancers of lung, head and neck, bladder, and esophageal origins. Gelatin is the most exploited biopolymer for the biomedical application due to its biodegradable, nontoxic, nonirritant characteristics & has FDA approval as inactive ingredients.

The focus of the work undertaken was to examine the synergistic effect of void gelatin nanoparticles and Paclitaxel loaded Gelatin Nanoparticles (PLGNP) on cells.

The present study encompasses the following findings:

Gelatin Nanoparticles were prepared by modified protocol of Anita *et al*. (2005). The size distribution of the Gelatin nanoparticles was measured by Dynamic Light Scattering (DLS) and it was further confirmed by the Transmission Electron Microscopy (TEM). The size of the gelatin nanoparticles were in the range of 150-180nm with early correlation coefficient decay curve and poly-dispersity index (PDI) 0.137. The surface charge of gelatin polymer was -5mV and that of nanoparticles was approximately -31mV. The enhanced surface charge showed formation of nanoparticles and greater stability. The infrared spectroscopy of gelatin polymer, gelatin nanoparticles, Paclitaxel *per se* and paclitaxel loaded gelatin nanoparticles (PLGNP) was studied by KBr method. The disappearance of the peak at 1376.69 cm^{-1} signifies the formation of nanoparticles and the appearance of paclitaxel peak in PLGNP at 1070-3430 cm^{-1} signifies the entrapment of paclitaxel in the nanoparticles. The X - ray diffraction patterns of gelatin have a very broad intense peak (20.5^o, 22.52^o) while that of nanoparticles was sharp at 29.14^o. But when Paclitaxel was loaded in the nanoparticles it gave a highly intense peak at 29.08^o. The hiding of chemical shift in the ^{1H}NMR after 6ppm confirmed the interaction at aromatic, N-terminal amino and carboxylic group containing amino acid of the gelatin polymer while desolvation.

1. The entrapment efficiency of paclitaxel in gelatin nanoparticles was ~ 25%, as calculated by UV-Vis spectrophotometer. The cumulative release of paclitaxel from PLGNP was more at pH 7.4 than release at pH 5.8. At pH 7.4 paclitaxel release rate was pulsatile reaching a peak at 1 hr (14%), 48 hr (17%) and 96 hr (16%) and the average concentration of paclitaxel relesed from nanoparticles was in the total effective drug concentration of paclitaxel. The release of paclitaxel from PLGNP at pH 7.4 follows Korsmeyer-Peppas model while at pH 5.8 it follows Higuchi plot. The non-fickian release pattern at physiological pH was confirmed by the diffusion exponent value of release at physiological pH (0.5126).
2. The cellular uptake of FITC labeled gelatin nanoparticles was studied on propidium iodide stained MCF-7 and HEK cells. It was observed that nanoparticles were internalized more by cancer cell (MCF-7) than non-cancerous cell (HEK).

3. The survival of HEK and MCF-7 was inversely propotional to dose of paclitaxel. 80% survival was observed with 25 nM dose of paclitaxel loaded gelatin nanoparticle (PLGNP). With the increase of concentration of paclitaxel from 25 nM to 200nM the survival of MCF-7 cell decrease from ~85% to 49% in 24 hr while HEK cell exhibited decreased survival from ~ 78% to 40% in 24 hr. After entrapment in nanoparticles, the cytotoxicity was reduced and enhance cell survival by ~10-30% in both the cell lines(MCF-7 and HEK) was observed.

4. The elevated levels of nitric oxide and total peroxidase were directly proportional to cytotoxicity of paclitaxel but PLGNP shows decreased toxicity as well as reduction in total peroxidase and nitric oxide level. With the increase in concentration of paclitaxel *per se* (25nM & 50nM) TPA & NO was 100µM to 250 µM and 5nM to 20nM respectively. But the PLGNP has reduced these oxidative stresses from 35µM to 175µM and 3nM to 12nM TPA & NO respectively.

5. Single Cell Gel Electrophoresis (comet assay) with MCF-7 & HEK cell lines showed that the DNA damage observed was concentration & time dependent. It was observed that void nanoparticles did not show any DNA damage while PLGNP caused DNA damage. At 25nM & 50nM of paclitaxel *per se* the comet score of damaged DNA was 2 & 3 respectively while the PLGNP have comet score 0 & 1 only.

6. The effect of paclitaxel and PLGNP (25nM & 50nM) at genomic level was evaluated by the flowcytometric cell cycle analysis. Treatment with paclitaxel *per se* caused cell cycle arrest at the G2/M phase, but after entrapment in nanoparticles the cell were arrested at G1/G2 phase. Our results confirmed that the PLGNP prevents the cell from entering into the hyper proliferative stage. This may causes reduction in the number of dividing cells possibly arresting the growth of cancer.

7. (a) Immunocytochemistry analysis showed an adequate expression of p21 in PLGNP treated HEK cells but in MCF-7 cells the expression of p21 was significantly enhanced (~2 times). MCF-7 cells showed enhanced p21 activity in PLGNP treated cells when compared to paclitaxel *per se* too.

(b) Preliminary experiments were done to assess the effect of paclitaxel and PLGNP on the expression of p21 and p53 by real time PCR. The expression of p21 was very high in the cells treated with PLGNP .

Publication: Abhishek Chanchal et al. / Journal of Pharmacy Research 2012, 5(8), 4042-4044 *(*ISSN: 0974-6943)