DESIGN AND DEVELOPMENT OF SOLID LIPID NANOPARTICLES
OF TELMISARTAN FOR TARGETING PROSTATE CANCER

Pooja* and Rahul Sharma

*Hindu College of Pharmacy, Sonipat-131001 Haryana, India.

ABSTRACT
Recently, the anticancer action of telmisartan (TEL) has been discovered against prostate cancer. However, although good therapeutic profile, poor aqueous solubility and suboptimal oral bioavailability slow down the anticancer efficiency of Telmisartan. Consequently, in present research, Solid lipid nanoparticles of Telmisartan were prepared for targeting prostate cancer, PC-3 cells. The mean particle size of TEL-SLNs was measured to be 95.3 ± 3.4nm, Correspondingly, zeta-potential of SLNs was measured to be -23.6 ± 2.9 mV significantly lower than -38.6 ± 6.3mV of TEL loaded SLNs. The encapsulation efficiency of Telmisartan loaded solid lipid nanoparticles was estimated to be 89.6±6.5%. FT-IR and PXRD recognized the molecular encapsulation of the drug in amorphous state. In vitro drug release study was conducted to conclude the drug delivery potential of lipid nanoparticles. These data indicate that only 35% of TEL is released in 8 hours compared to the TEL loaded SLNs which releases significantly higher amount 80% of drug in the same time interval. The IC50 of Telmisartan was measured to be 15.9µM significantly higher than 7.5µM presented by TEL-SLNs in PC-3 cells. We elucidated that TEL- SLNs entered the PC-3 cells through receptor mediated endocytosis pathway and therefore exhibited greater cytotoxicity and greater extent of cellular uptake in PC-3 cells.

KEYWORDS: Telmisartan, prostate cancer, solid lipid nanoparticles, cytotoxicity, cellular uptake.

INTRODUCTION
Prostate cancer is the second chief reason of cancer death in men, exceeded by lung cancer and colorectal cancer.[1] It was the most ordinary cancer in males in 84 countries, occurring more frequently in the developed world. Prostate cancer (PC) comprises 32% of all cancers in
American men and is on the increase globally. According to current trends, the incidences of prostate cancer are growing in India by 1% every year. The global cancer burden is estimated to have risen to 18.1 million new cases and 9.6 million deaths in 2018. An estimated 164,690 new cases of prostate cancer will be diagnosed in 2018. Treatments may include a group of surgery, radiation therapy, hormone therapy or chemotherapy. In the untimely stages of the disease, patients are commonly treated through prostatectomy, radiotherapy, and brachytherapy.

Telmisartan has been found to have activity against a variety of cancers in vitro, including prostate, renal, colon, leukemia, and ovarian cancer. Recently, the anticancer action of Telmisartan (TEL) has been discovered against prostate cancer. TEL causes up-geration of peroxisome proliferator-activated receptor (PPAR)-gamma activation activity and therefore alleviated the growth arrest of cancerous cells via apoptosis. However, although favorable therapeutic profile, poor aqueous solubility and suboptimal oral bioavailability in humans slow down the anticancer efficacy of TEL. The dose-dependent side-effects are also related with Telmisartan action like renal dysfunction, myocardial infarction and cardiac dysfunction.

Solid lipid Nanoparticles (SLNs) have attracted increasing attention as a capable colloidal carrier system, particularly for lipophilic drugs. These are made of lipids, which are solid at room and body temperature and isolated in an aqueous medium. SLNs are composed of a high melting point lipid as a solid core, which is coated by surfactants. A apparent advantage of solid lipid nanoparticles (SLNs) over polymeric nanoparticles is the reality that the lipid matrix is made from physiologically tolerated lipid components, which decreases the possibility for acute and chronic toxicity.

2. MATERIALS AND METHOD

2.1 MATERIALS

Telmisartan (TEL) was purchased from Torrent pharmaceuticals Pvt. Ltd., Baddi, Himachal Pradesh, India. All other chemicals used were of highest analytical grade.

2.2 Determination of Solubility studies of Telmisartan

Semi quantitative determination of the solubility was made by adding solvent in glass tube containing correctly weighed amount of solute. The system is vigorously shaken and examined visually for any undissolved solute particles. The solubility is defined in terms of
ratio of solute and solvent. The solubility of Telmisartan was performed in methanol, ethanol, dichloromethane, distilled water, 0.1 N HCL, phosphate buffer solution pH 7.4, individually by keeping the drug containing test tube on vortex mixture.

2.3 Determination of melting point
For determination of melting point USP method was followed. Small quantity of drug was positioned into a sealed capillary tube. The tube was placed in the melting point apparatus. The temperature was slowly increased and the observation of temperature was noted at which drug started to melt and when the whole drug gets melted was noted.

2.4 Determination of partition co-efficient
The known quantity of Telmisartan was added into 20ml of octanol and it was mixed with 20ml of phosphate buffer pH 7.4 in a separating funnel. Then two phases was allowed to equilibrate at 37°C for 2 hours with intermittent shaking. The concentration of drug in the aqueous phase and organic phase was determined by UV spectroscopic method at \( \lambda_{\text{max}} \) 298 nm behind necessary dilution. The visible partition coefficient was calculated as the ratio of drug concentration in each phase by the following equation:

\[
K_p = \frac{C_{\text{organic}}}{C_{\text{aqueous}}}
\]

C organic is concentration of drug in organic phase.
C aqueous is concentration of drug in aqueous phase\(^8\)

2.5 Preparation of Telmisartan loaded solid lipid nanoparticles
Telmisartan loaded solid lipid nanoparticles (TEL-SLNs) were prepared by solvent diffusion method. Firstly, 120mg of stearic acid and 10 mg of TEL were dissolved in a mixture of 6ml of ethyl alcohol and 6ml of acetone. Next, this organic phase was dispersed in 100ml of distilled water, maintained at 70°C and continuously stirred for 30 min by employing a magnetic stirrer. Then this solution is centrifuged for the proper mixing of the solution and phase separation. After that the solution is dessicated or lyophilized to get the fine powder of Telmisartan loaded solid lipid nanoparticles (TEL-SLNs)\(^9\)

2.6 Characterization of TEL loaded Solid Lipid Nanoparticles (SLNs)
2.6.1 Particle size and Zeta potential
Nanoparticle samples were dispersed separately in phosphate buffer saline (PBS, pH~7.4) earlier than analysis. Malvern Nano ZS was used to determine the particle size and zeta-potential. A 150mV electric field was applied to measure the electrophoretic velocity of nanoparticles. All measurements were carried out in triplicate (n=3).

2.6.2 Transmission Electron Microscopy (TEM)
Particle shape and surface morphography were examined by transmission electron microscopy. In brief, an aqueous suspension of nanoparticles was separately drop casted onto a carbon coated copper grid, and the grid was air dried at room temperature before loading it into the microscope which was maintained at a voltage of 80 kV.

2.6.3 Fourier-transforms infrared (FT-IR) spectroscopy
FT-IR was performed to address the issue of any chemical incompatibility between drug and excipients. In brief, spectrum of TEL and TEL-SLNs was recorded.

2.6.4 Differential Scanning colorimetry (DSC)
Thermal behavior of Telmisartan, SLNs, physical mixture of TEL and SLNs was examined using a differential scanning colorimetry (DSC) thermal analyser.

2.6.5 Powder X–ray diffraction (PXRD)
The polymorphic position of the drug in lipid matrix was confirmed by X-ray diffractometer (X’Pert PRO, Panalytical Company, Malvern, UK) using Ni-filtered, Cu Ka-radiation, voltage of 60Kv and current of 50mA. The scanning rate was 1°/min over 10° to 60° diffraction angle (2θ) range. The crystal lattice of TEL, SLNs, physical mixture of TEL and SLNs, and TEL-SLNs was accounted.

2.6.6 Encapsulation efficiency and Drug Loading Capacity:
The encapsulation efficiency and drug loading capacity were determined by dissolving individually 50mg of Telmisartan loaded SLNs in 10ml of 0.05M NaOH and the sample was left untouched for 72 h at room temperature. Subsequently, sample was ultra-centrifuged at 40,000rpm for 2 h and the supernatant liquids were filtered off using 0.22µm membrane filter. The absorbance of the TEL was measured at 298nm by using a UV-Visible spectrophotometer (1700, Shimadzu, Kyoto, Japan) 45. All measurements were carried out in
triplicate (n=3). Encapsulation efficiency and drug loading capacity were calculated by using
the following formulas.[8]

\[
\text{% Encapsulation efficiency} = \frac{\text{Amount of drug recovered}}{\text{Amount of drug added}} \times 100
\]

\[
\text{Drug loading capacity} = \frac{\text{Amount of drug recovered}}{\text{Amount of nanoparticles}} \times 100
\]

2.6.7 In–Vitro drug release
In vitro drug release experiment was executed using dialysis membrane technique. In brief, an accurately weighed amount of TEL loaded SLN dispersion containing the drug equivalent to 3mg was poured into a dialysis bag. The bag then suspended separately in 900ml of simulated intestinal fluid maintained at 37°C. The dissolution medium is stirred at 50rpm, as suggested for dissolution testing of oral products. At different time intervals, 5ml of the sample was withdrawn and at the same time replaced with fresh dissolution medium to continue the sink conditions. The drug concentration was measured at 298 nm by using a UV-Visible spectrophotometer (1700, Shimadzu).[10]

2.6.8 Therapeutic efficacy testing of TEL-SLNs in prostate cancer cells
2.6.8.1 In vitro cytotoxicity assay
MTT assay (3-[4, 5-dimethylthiazol-2-yl]-2, 5 diphenyltetrazolium bromide) was employed for in vitro cytotoxicity assay. In brief, PC-3 cells were seeded in 200µl of serum DMEM medium enclosed in each well of a 96-wells microtitre plate. After 24 h of incubation period, the medium was replaced with serum free-DMEM. After that, seeded PC-3 cells were incubated with a gradient concentration of TEL and TEL-SLNs equivalent to 20-100µM of TEL for 72 h. At the end of treatment, 0.5mg/ml of MTT dye was added to each well and the plates were incubated for another 4 h at 37°C. After cell lysis, formazon crystals were obtained, which were solubilized using 100µl of dimethyl sulphoxide (DMSO). The absorbance was measured at 570nm using 630nm as reference wavelength by plate Reader.[11]

2.6.8.2 In vitro cellular uptake assay: Qualitative analyses
The cellular uptake assay was performed qualitatively. In brief, PC-3 cells were seeded in Lab-Tek II Chamber slide TM system. Using PBS (~7.4), dosing solutions of FTIC labeled TEL-SLNs (25µM-100µM of TEL) were prepared and diluted with DMEM. The cell
monolayers were rinsed thrice and pre-incubated with 1ml of DMEM at 37°C for 1h. Cellular uptake was initiated when 1ml of DMEM was replaced with 1ml dosing solution of FITC labeled TEL-SLN, respectively. The slide chamber was then incubated for 5 h at 37°C. Subsequently, the experiment was ceased by washing the cell monolayers thrice with ice cold PBS (pH~7.4) and lysing the cells with 1ml of 0.5% v/v Triton X-100 (25mM Tris-HCl pH 7.4, 150mM NaCl, 1% Triton-X 100, 1mM EDTA, 5% Glycerol). Cell-associated fluorescence was measured using a fluorometer (λex=553 nm, λem=574 nm).

After 5 h of incubation period, the DMEM medium was removed and the plates were washed thrice with sterile PBS (pH~7.4). After the final wash, the cells were fixed with 4% paraformaldehyde, and cover slips were individually mounted on clean glass slides with fluoromount-G mounting medium and scanned under confocal laser scanning microscope (CLSM) at λex~553 nm, λem~574 nm. 4, 6-diamidino-2-phenylindole (DAPI) dye was used for nucleus staining.[12, 13]

3. Results and Discussion
3.1 Solubility Analysis
Solubility studies are performed to determine the solubility of drug in different solvents. The solubility is expressed in terms of ratio of solute and solvent. Telmisartan was found to be Insoluble in water and Soluble in strong bases.

3.2 Melting point determination
Melting point of Telmisartan was found to be 261°C. A sharp transition took place from solid to liquid at this point indicated that the drug is completely melted.

3.3 Determination of partition coefficient
As the partitioning behavior of the drug molecule plays an important role in the lipid barrier transfer as well as drug loading in the lipid carrier systems, the partition coefficient of Telmisartan was found to be 3.2 which indicates that the drug has lipophilic nature.

3.4 Physiological characterization
3.4.1 Particle size and zeta potential
The mean particle size of Telmisartan loaded SLNs was measured to be 95.3 ± 3.4nm. The zeta-potential of SLNs was measured to be -23.6±2.9mV significantly lower than -38.6 ± 6.3mV of TEL loaded SLNs.
Fig No 1: (A) Particle size distribution of telmisartan loaded SLNs.

Fig No 1: (B) Zeta potential distribution of SLN and TEL loaded SLNs.
5.4.2 Transmission electron microscopy
The TEM micrographs of lyophilized Telmisartan loaded solid lipid nanoparticles suggest that the nanoparticles were smooth and spherical in shape. The photo micrographs indicated that centrifugal force and freeze drying factors did not affect nanoparticle texture. In this way, we can expect favorable cellular uptake of nanoparticles in cancer cells.

![TEM micrographs of Telmisartan loaded SLNs](image)

**Fig No 2: TEM of telmisartan loaded SLNs.**

5.4.3. Fourier transform infrared spectra (FTIR)
We characterize the nanoparticles by various spectroscopy techniques. FT-IR spectrum of Telmisartan, SLNs was recorded to examine the new linkage formed during the encapsulation of drug into the nanoformulation. The FT-IR spectrum of TEL demonstrated the characteristic peaks at 2830 cm\(^{-1}\) for aliphatic C- H stretching, 1697 cm\(^{-1}\) for carboxylic acid.

![FTIR spectra of Telmisartan and Telmisartan loaded SLNs](image)

**Fig. 3 FTIR spectra of Telmisartan and Telmisartan loaded SLNs.**
5.4.4 Differential Scanning Colorimetry (DSC)

The thermal behavior of the nanoparticles was compared to that of the original species by DSC measurements. DSC curve of TEL showed a sharp endothermic peak near 263.4°C and SLN shows a sharp peak at 121.5°C. The thermogram of the physical mixture of TEL with SLN indicated the presence of identical peaks of individual components at 264.1°C and 120.1°C. However, the thermogram of TEL Loaded SLN shows a complete disappearance of the endothermic peaks characteristic of TEL with a significant shift in SLN endothermic peaks to 118.12°C.

![DSC analysis graph](graph.png)

**Fig No 4:** Differential scanning calorimetry (DSC) analysis of Telmisartan, SLN, physical mixture of TEL and SLN and TEL loaded SLN complex.

5.4.5 Powder X-ray diffraction (PXRD)

PXRD was used to determine the crystalline geometry of the drug in lipid matrix. The PXRD pattern of Telmisartan showed peaks that were intense and sharp indicating its crystalline structure. In contrast, the peaks presented by SLNs were diffused and of low intensities indicating its amorphous state which is more soluble and less stable state. Correspondingly, the physical mixture of TEL and SLNs displayed a mixture of sharp peaks with diffused peaks. Subsequently, the diffused peaks displayed by TEL-SLNs were of little intensities indicating the amorphous state of TEL in lipid matrix.
5.4.6 Encapsulation efficiency

\[
\text{% Encapsulation efficiency} = \frac{\text{Amount of drug recovered}}{\text{Amount of drug added}} \times 100
\]

The encapsulation efficiency of telmisartan loaded solid lipid nanoparticles was estimated to be 89.6±6.5%.

5.4.7 Drug loading capacity

\[
\text{Drug loading capacity} = \frac{\text{Amount of drug recovered}}{\text{Amount of nanoparticles}} \times 100
\]

The drug loading capacity of telmisartan loaded solid lipid nanoparticles was estimated to be 7.6 mg/10mg of nanoparticles.

5.4.8 In-vitro drug release

In vitro release of TEL from nanoparticles was analyzed by dynamic dialysis method in PBS of pH~7.4. These data signify that only 35% of TEL is released in 8 hours compared to the TEL loaded SLNs which releases significantly higher amount 80% of drug in the same time interval.

Fig No 5: PXRD of Telmisartan, SLNs, Telmisartan loaded SLNs, physical mixture.
5.4.9 Therapeutic efficacy testing of TEL-SLNs in prostate cancer cells

5.4.9.1 *In-vitro* cytotoxicity assay

*In vitro* cytotoxicity analysis was done to analyze the therapeutic efficacy of the complex of TEL with SLN in human prostate cancer cells (PC-3) by dissolving the formulation in phosphate buffer saline (pH~7.4). The cytotoxic activity was evaluated using the standard MTT cell viability assay. The IC50 value of TEL loaded SLN was calculated to be 7.5µg/ml. However, TEL which is practically insoluble in phosphate buffer saline (pH~7.4) shows an IC50 value of 15.9µg/ml higher than the TEL loaded SLN in PC-3 cells.
5.4.9.2 In vitro cellular uptake assay: Qualitative analyses

We determined qualitatively the accumulation of nanoparticles in PC-3 cells by trafficking FITC labeled Telmisartan loaded solid lipid nanoparticles using a fluorometer and CLSM. The fluorescent nanoformulation was stable in cell culture medium. TEL loaded SLNs showed significantly higher fluorescence and cellular uptake in PC-3 cells due to greater endocytosis. We expect that cellular uptake of TEL loaded SLNs would have follow the non-receptor mediated endocytosis pathway.

![Cellular uptake of TEL-SLN in prostate cancer (PC-3) cells.](image)

4. CONCLUSION

Telmisartan was successfully loaded into solid lipid nanoparticles (TEL-SLNs) to increase its clinical efficacy against prostate cancer cells. Telmisartan loaded solid lipid nanoparticles was successfully prepared by using solvent diffusion technique However, its pharmacokinetic and pharmacodynamic properties are effected by low aqueous solubility. Our general and practical investigation of various synthesis parameters governing nanoparticles preparation showed that TEL- SLNs can be synthesized with a narrow particle size distribution. The prepared TEL-SLNs was evaluated by Fourier- transform infrared spectroscopy (FTIR), differential scanning calorimetry (DSC), Powder X-ray diffractometry (PXRD), Transmission electron microscopy (TEM). Therefore we have presented here TEL for the development of viable anticancer formulation. Our systematic and rationalized data suggested that TEL-SLNs may have the capability to prolong the drug release rate, cytotoxicity and apoptosis against PC-3 cells. Therefore, in vitro and cellular uptake study confirmed that TEL-SLNs may potentially be used for targeting prostate cancer cells.
5. REFERENCES


7. YiFan Luo, DaWei Chen, Jin Qin Chen Solid lipid nanoparticles for enhancing vinpocetine's oral bioavailability 2006 Elsevier B.V. All rights reserved, 2006; 106: 53-59


11. Ayen WY, Garkhal k, Kumar N. Doxorubicin- loaded (PEG) (3)–PLA nano polymerosomes: effect of solvents and process parameters on formulation development and in – vitro study. Mol pharm, 2011; 8: 446 - 478.
