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Telmisartan-poly (ethylene glycol) conjugate augmented drug dissolution and permeability in cervical cancer cells

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ABSTRACT

Telmisartan is currently reported for inhibiting cervical cancer cells. Despite favorable therapeutic profile, poor aqueous solubility and low oral bioavailability limit its therapeutic application in treatment of cervical cancer. Telmisartan was chemically conjugated to poly (ethylene glycol) through amide linkage to form telmisartan-PEG drug conjugate. Poly (ethylene glycol) with terminal $-NH_2$ was conjugated with telmisartan via amide linkage. Telmisartan-PEG conjugate displayed peak at 1690 cm^{-1} for C=O group of amide linkage. Furthermore, telmisartan illustrated the crystalline lattice as compared to amorphous nature of telmisartan-PEG conjugate. The in vitro dissolution testing indicated that telmisartan displayed only 22.6±3.8% drug release from dialysis bag as compared (Two-way ANOVA test, $P<0.05$) to 76.9±5.4% from telmisartan-PEG conjugate. The therapeutic efficacy of telmisartan and telmisartan-PEG conjugate was analyzed in cervical cancer, HeLa cells. The IC_{50} of telmisartan in HeLa cells was estimated to be 48.6±6.9µM as compared (Two Way ANOVA test) to 17.2±2.7-µM of telmisartan-PEG conjugate dissolved in aqueous phase. In conclusion, telmisartan-PEG conjugate must be investigated under a set of stringent *in vivo* parameters for pharmacokinetic and pharmacodynamic studies.

Keywords: Cervical cancer, telmisartan, poly (ethylene glycol), conjugate, dissolution, cytotoxicity

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INTRODUCTION

Telmisartan is currently used by several researchers for inhibiting cervical cancer cells. It acts by antagonizing endothelial growth factor (VEGF) production. In addition, it is also an angiotensin receptor II type 1 antagonist and displays PPARγ receptor activation. Despite favorable therapeutic potential, poor aqueous solubility (0.078 mg/mL) and low oral bioavailability (45-58%) limit its therapeutic application in treatment of cervical cancer. Several nanoscaled drug delivery systems including liposomes, nanoparticles were developed to improve the therapeutic efficacy of telmisartan in cervical cancer cells.[1-3] However, each drug delivery systems has its own pros and cons which limit their efficacy as a potent drug delivery system. Poly (ethylene glycol, PEG)-drug conjugates have been widely employed to improve drug permeability, dissolution and oral bioavailability of small and supramolecules.[4,5]

Therefore in current investigation, telmisartan was chemically conjugated to poly (ethylene glycol) through amide linkage to form telmisartan-PEG drug conjugate. Furthermore, telmisartan-PEG drug conjugate was characterized under a set of stringent parameters to exhibit its utility against cervical cancer, HeLa cells.

MATERIALS AND METHODS

Telmisartan (TEL) was a kind gift sample from Swiss Garnier Life Sciences, India. Poly (ethylene glycol) of molecular weight 2000 with terminal $-NH_2$ was purchased from Sigma-Aldrich, USA. Dicyclohexyl carbodiimide (DCC) and N-hydroxysuccinimide (NHS) were procured from Loba Chemie, Mumbai, India. All other chemicals used were of the highest analytical grade.

Synthesis of telmisartan-PEG conjugate

Poly (ethylene glycol) with terminal $-NH_2$ was conjugated with telmisartan via amide linkage.[6] Briefly, telmisartan (100 mg, 0.233 M) was solubilized in 10 ml of tetrahydrofuran

(THF) and subsequently 200 mg of DCC and 200 mg of NHS was added to the solution under constant stirring. The reaction was held for 6 h at room temperature. After that, 100 mg of PEG-NH_2 was incorporated to the mixture and stirring was continued for another 24 h at room temperature. Finally, the mixture was dialyzed against distilled water (MWCO~10 kDa) for 24 h to remove un-reacted components. The product was precipitated by adding 3 ml of distilled water and subsequently centrifuged at 10,000 rpm to get the final pellet. The pellet of telmisartan-PEG conjugate was washed twice and stored in vacuum dessicator.

Characterization of telmisartan-PEG conjugate

Fourier-transforms infrared (FT-IR) spectroscopy

The Synthesis of telmisartan-PEG conjugate was confirmed by employing FT-IR. FTIR spectrum of pure drug telmisartan and telmisartan-PEG conjugate was recorded at a scanning range of 400-4000 cm^{-1} with a resolution of 4 cm^{-1}. Each sample was prepared into KBR disc with hydrolic press at a force of 40 psi for 4 min.

Powder X-ray diffraction pattern

Polymorphic state of the drug into nano-size range self-assembled micelles was assessed by powder x-ray diffractometer (Ultima-4, Rigaku Company, Japan) with Ni-filtered, Cu K-radiation, a voltage of 60 kV and a current of 50 mA. The crystalline nature of pure drug telmisartan, physical mixture of drug and excipients, blank SAN and TEL-PEG-SAN were qualitatively analyzed by scanning powder sample over a range of diffraction angel between 5 to 50° at a rate of 1°/min (Aslam et Al. 2016; Puri et al. 2016; Sharma et al. 2017; Dixit et al. 2015)

Scanning electron microscopy

Telmisartan and telmisartan-PEG conjugate were examined by a scanning electron microscope (SEM) to visualize the surface topography. Samples were prepared by preparing the film on an aluminum stub. The stubs were then coated with gold to a thickness of 200–500 ˚A under an argon atmosphere using a gold sputter module in a high vacuum evaporator. The coated samples were scanned, and photographs were taken with a SEM (Jeol-1761, Cambridge, UK) camera.

In vitro dissolution study

Release pattern of pure telmisartan and relmisartan-PEG conjugate was quantitatively determined by dialysis tubing method in phosphate buffer solution, pH 6.8 at different time intervals.[7] An accurately weighed quantity 40 mg of telmisartan and telmisartan-PEG conjugate (~40 mg of telmisartan) was suspended separately into 5 ml of phosphate buffer of 6.8 pH and added in dialysis tubing. The dialysis bags were then dispersed in 900 ml of phosphate buffer 6.8 pH and maintained at 37°C and 50 rpm as recommended for dissolution testing of oral products. At predetermined specified time intervals, 5 ml of dissolution medium was withdrawn and replaced with equal volume to maintain sink conditions. The aliquots were filtered through 0.22µm membrane filter and absorbance of the filtrate was measured at 298 nm.[3]

Standard cell proliferation assay

A standard colorimetric MTT assay, as an in vitro cytotoxicity assay, was employed to investigate the cell cytotoxicity effect against HeLa cells.[7] In brief, HeLa cells was seeded in 200-µL of DMEM medium and incubated for 24 h. Post incubation period of 24 h, medium was replaced with serum free DMEM medium. The HeLa cells were incubated with a gradient concentration of telmisartan and telmisartan-PEG conjugate equivalent to 20-100 µM concentration of telmisartan for 72 h. After that, 0.50 mg/ml quantity of MTT dye was added to each well and incubated for a period of 4 h at 37°C. Subsequently, crystals were dissolved in 100-µL of DMSO. The absorbance of the mixture was determined at 570 nm and 630 nm as a reference wavelength by employing ELISA reader. All steps of the Study were carried out in triplicates.

RESULTS AND DISCUSSION

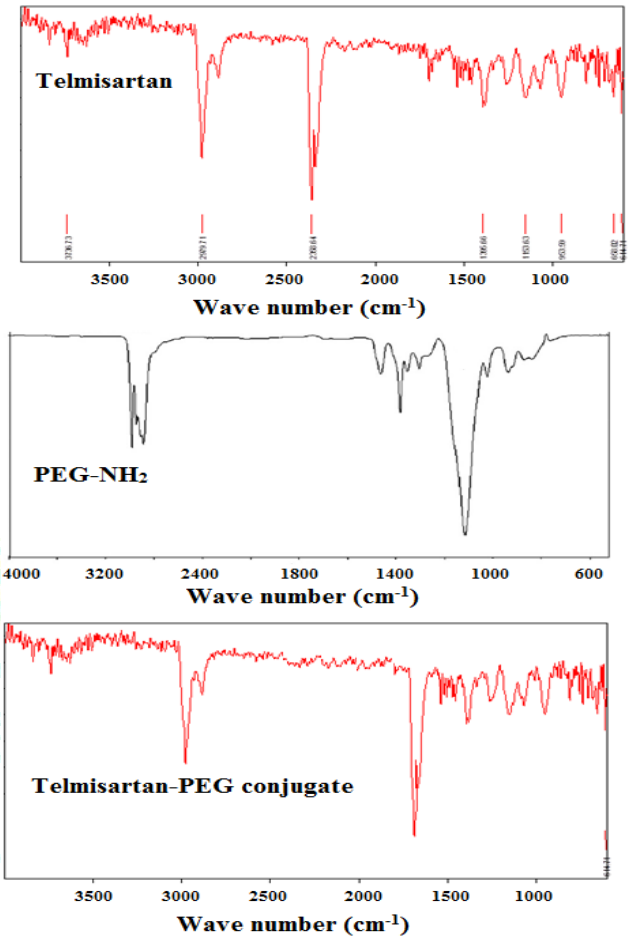

Figure 1: FT-IR spectrum of telmisartan, PEG-NH_2 and telmisartan-PEG conjugate.

In the current investigation, we have carried out the synthesis of telmisartan-PEG conjugate using covalent coupling chemistry. The carboxylic acid group of telmisartan was covalently coupled to amino functional group of poly ethylene glycol via amide linkage in order to improve dissolution profile and permeability in cervical cancer cells. Synthesis of telmisartan-PEG conjugate was verified by FT-IR spectroscopy (**Figure 1**). FTIR spectrum of telmisartan displayed characteristic peaks at 3335.13 cm^{-1}, 2991.31 cm^{-1}, and 1696.04 cm^{-1}, indicating the presence of functional aromatic C-H stretch, aliphatic C-H stretch, and carboxylic acid, respectively. Correspondingly, mPEG-NH_2 displayed peaks at 3180 cm^{-1} and 1530 cm^{-1} for N-H stretching and N-H bending of amino group. Telmisartan-PEG conjugate displayed peak at 1690 cm^{-1} for C=O group of amide linkage. The crystalline state of telmisartan and telmisartan-PEG conjugate was elected confirmed by PXRD technique. XRD diffractogram of telmisartan demonstrated sharp peaks of higher intensity illustrated crystalline structure. However, telmisartan-PEG conjugate led to transformation of sharp peaks of high intensity to less diffused peak with very low

intensity (**Figure 2**). This confirmed the amorphization and micronization of drug in telmisartan-PEG conjugate. This was also confirmed by scanning electron microscopy (**Figure 3**). Photomicrograph of telmisartan illustrated the crystalline lattice as compared to amorphous nature of telmisartan-PEG conjugate. The in vitro dissolution testing indicated that telmisartan displayed only 22.6±3.8% drug release from dialysis bag as compared (Two-way ANOVA test, $P<0.05$) to 76.9±5.4% from telmisartan-PEG conjugate (**Figure 4**).

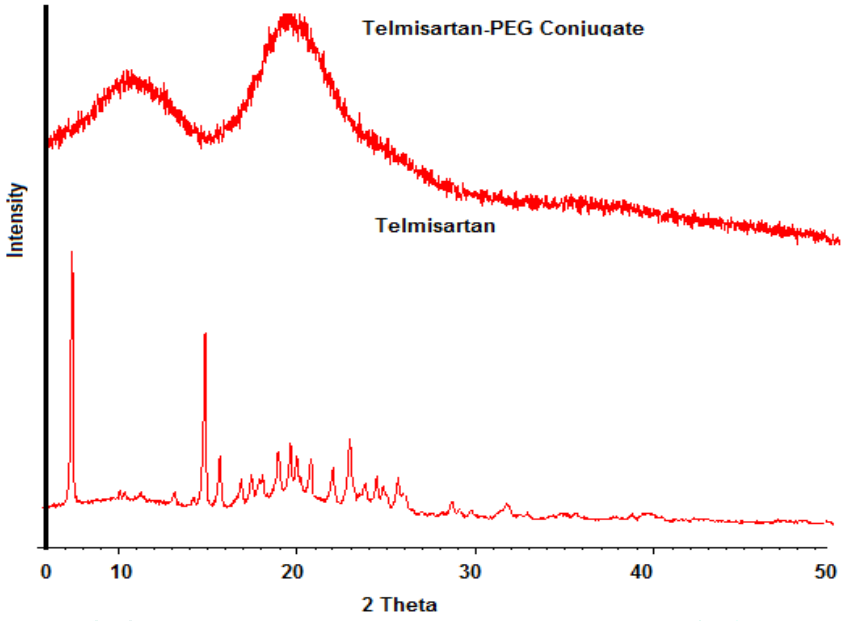

Figure 2: XRD pattern of telmisartan and telmisartan-PEG conjugate

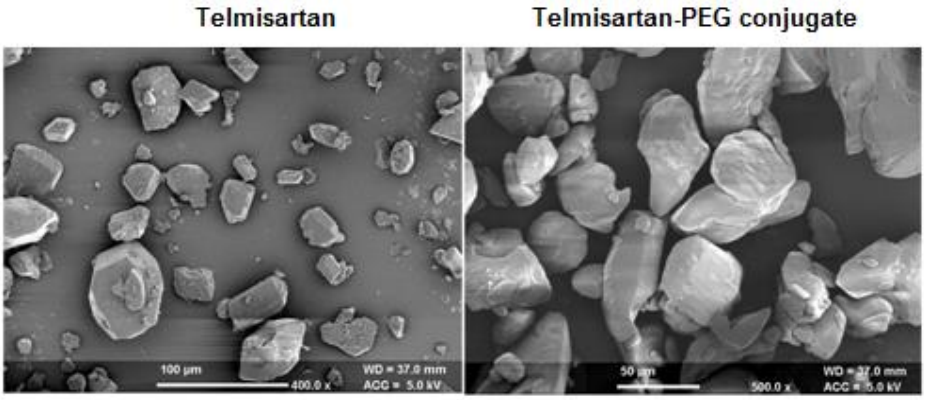

Figure 3: Scanning electron microscopy of telmisartan and telmisartan-PEG conjugate

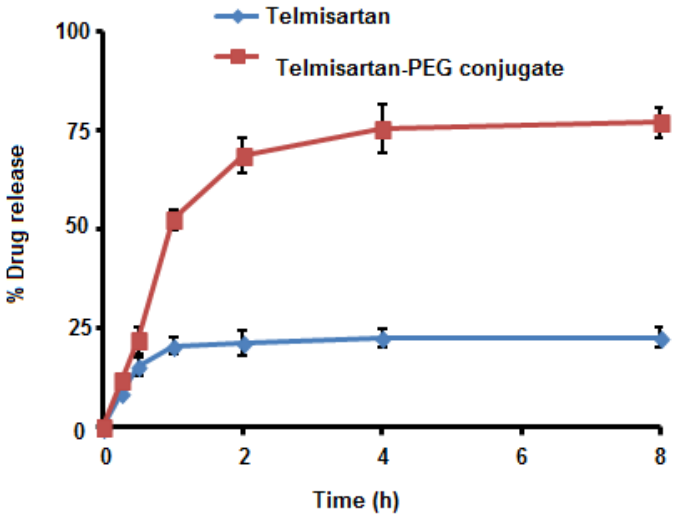

Figure 4: In vitro dissolution testing of telmisartan and telmisartan-PEG conjugate

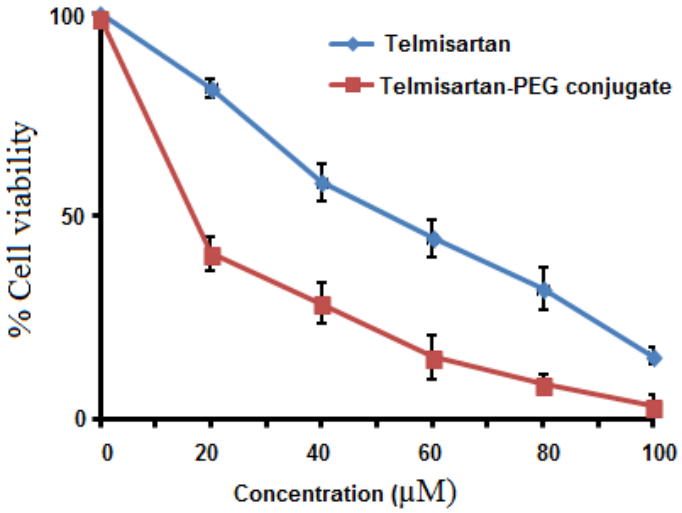

Figure 5: Measurement of IC50 value of telmisartan and telmisartan-PEG conjugate using standard cell proliferation assay against cervical cancer HeLa cells

The high dissolution profile may be attributed to high solubility of PEG analogue in aqueous phase that ultimately augmented dissolution profile. The therapeutic efficacy of telmisartan and telmisartan-PEG conjugate was analyzed in cervical cancer, HeLa cells. The IC_{50} of telmisartan in HeLa cells was estimated to be 48.6±6.9µM as compared (Two Way ANOVA test) to 17.2±2.7-µM of telmisartan-PEG conjugate dissolved in aqueous phase (**Figure 5**). The low IC_{50} value may be attributed to higher solubility and permeability of telmisartan-PEG conjugate as compared to lipophilic (Log P~3.2) character of telmisartan.[8-9]

CONCLUSION

In conclusion, telmisartan-PEG conjugate may be scaled up with high yield using minimum efforts. Further, telmisartan-PEG conjugate displayed high dissolution profile that may ultimately augment oral bioavailability and permeability across intestinal absorption window. Owing to desirable features, telmisartan-PEG conjugate exhibited low IC_{50} in cervical cancer cells. Therefore, telmisartan-PEG conjugate must be investigated under a set of stringent *in vivo* parameters for pharmacokinetic and pharmacodynamic studies.

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