Maxitarg-A Novel Targeting Approach For Hepatic Cancer

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ABSTRACT

“MAXITARG” is a novel drug delivery approach to maximize drug accumulation in hepatocellular carcinoma cells. Maxitarg relies on a novel targeting agent (NTA) with high affinity for asialoglycoprotein receptors, which are over expressed in the liver carcinoma cells. The present study reports application of Maxitarg in the design of a targeted nanoparticulate drug delivery system (DDS) of doxorubicin with the specific objective of enhancing liver uptake and decreasing dose related cardiotoxicity of the drug.

Alginic acid – sodium alginate nanoparticles (NP) loaded with doxorubicin and NTA were prepared by controlled gelation method using novel stabilizing agent Eudragit E100. Encapsulation efficiency (EE) of > 95% reflected minimum drug loss during processing. Average particle size of optimized nanoparticles (NP) ranged between 200-550 nm. NP’s showed sustained in vitro drug release over a period of 60 hrs in pH 7.4 buffer at 37°C.

Biodistribution study of NP revealed rapid and maximal uptake in the liver with significant drug accumulation up to 24 hrs, moreover low concentration of doxorubicin was evident in kidney, spleen and lung while negligible drug concentration was detected in heart up to 24 hrs. Plain doxorubicin however revealed high drug concentration in the heart at 1 hr detectable up to 24 hrs, moreover uptake by the liver was very low. Other tissues like lung, kidney, and spleen also showed presence of drug.

Key words: Maxitarg, nanoparticles, doxorubicin, hepatic cancer, targeting agent.

INTRODUCTION

Cancer is the second most dreadful disease after heart diseases. Around 22.9% of all deaths one caused mainly due to cancer. A major limitation of current cancer therapy is systemic spread and toxicity to normal tissues. The present approach, ‘MAXITARG’ maximizes the targeting of drugs to hepatocellular carcinoma cells (HCC) deals with the use of a NTA with high affinity for asialoglycoprotein receptors over expressed in HCC. Doxorubicin is widely used for various solid tumors and melanomas including hepatocellular carcinoma. Limitations of doxorubicin delivery include dose related cardiotoxicity and myelosuppression. The result of this is often a narrow therapeutic index, due to high levels of toxicity to healthy tissues. (1,2) The specific objective of the present study therefore includes enhancing liver uptake and decreasing cardiotoxicity and general systemic toxicity of the doxorubicin.

MATERIALS AND METHODS

1.0 Materials

Doxorubicin was supplied by RPG life sciences, India. Alginic acid, sodium alginate were obtained from Signet Chemical Corp., India. Surfactants samples viz. as tween, cetrimide were obtained form S.D. Fine Chem. India whereas Chremophore RH 40 was kindly provided by Colorcon Asia. Pvt. Ltd. India. All the other solvents and co solvents used in the study were of analytical grade.

2.0 Methods

2.1 Preparation of nanoparticles (NP):

Doxorubicin, sodium alginate and / alginic acid (70:30) were dissolved in 50ml of distilled water. NTA (0.1%w/v) was also dissolved in the same with stirring (rpm: 3000rpm) Calcium chloride (0.0002 mM) was gradually added with syringe (24 gauge) followed by addition of Eudragit E100(0.05%w/v in 0.1N HCl) , which also acted as a stabilizing agent. The NPs stirred for 1 hr at 3000rpm. The resultant NPs were isolated by centrifugation (20,000 rpm for 40min) and the supernatant analyzed for free drug. The NPs were freeze dried. All the solutions passed through 0.2µ filter prior to experimentation and procedure was carried out in sterile room.

2.2 Optimization for particle size and drug loading:

The drug: polymer ratio and surfactant (cationic, anionic and non- ionics) concentration and type was varied to optimize particle size and encapsulation efficiency (EE).

2.3. Physicochemical characterization

The mean particle size of the formulations were determined by Particle size analyzer (Beckman Coulter N+ Plus,
Wipro, India Ltd.) equipped with software N4 Plus. Every sample was appropriately diluted with 0.22 µm - filtered water and the reading was carried out at a 90º angle in respect to the incident beam. The FTIR spectra of gliclazide nanoparticles were taken in KBr pellet using Perkin-Elmer Fourier transformed infrared (FT-IR) spectrophotometer (spectrum 2000) instrument. The thermogram of nanoparticles, loaded and non-loaded, were obtained using differential scanning calorimetry (DSC) using a Perkin-Elmer DSC7 apparatus (Uberlingen, Germany) between 50°C and 250°C. The temperature gradient was 40°C/min.

2.4 In-vitro drug release studies

The in-vitro drug release studies were performed with some modification as described by Liu et al. Briefly, doxorubicin loaded NPs (corresponding to 10 mg of doxorubicin) were suspended in 50 ml of 7.4 phosphate buffer in glass vials. The vials were placed in constant temperature mechanical water bath maintained at 37°C and with a oscillation rate of 100 strokes/min. At selected time intervals, 5.0 ml of the samples withdrawn and replaced with fresh buffer. The samples were filtered through 0.2 µm membrane filter and doxorubicin concentration measured by spectrofluorimetry at an excitation wavelength of 480 nm and an emission wavelength of 590 nm concentration was extrapolated from a standard curve. The FTIR and DSC studies revealed no interaction between drug, polymer and other excipients.

2.5 Biodistribution study:

Male wistar rats from Haffkine Institute, India, with an average weight of 250–300 g, were used. Animals received standard laboratory chow and water ad libitum. Doxorubicin NP/Plain doxorubicin (10 mg/Kg body weight), was injected by intravenous bolus through the tail vein. Three rats were used for each time point (1, 2, 4, 8, 24 h). Blood samples obtained by heart puncture were collected in heparinized tubes to prevent clotting. Plasma was separated by centrifugation within less than 24 h after blood collection. The animals were dissected to remove the lung, liver, spleen, kidney, and heart, which were rinsed in 0.9% NaCl solution and stored at -20°C. The samples were homogenized in 0.075% HCl. Doxorubicin was extracted as described by Alminana et al. Briefly, 100 µL plasma or tissue sample was pipetted in an Ependorff test tube, to which 100 µL borate buffer (pH = 9) and 500 µL toluene:butanol (1:1, v/v) were added. After 1min of vigorous vortexing, the test tube was centrifuged for 5 min at 2500 rpm. The upper phase was discarded and 100 µL 0.2 M phosphoric acid was added. The mixture was vortexed for 1 min and centrifuged for 5 min at 2500 rpm. The upper phase was analyzed for drug by HPLC.

RESULTS AND DISCUSSION

The controlled gelation technique using Eudragit E 100 as a stabilizing agent resulted in NP with high EE and nanosize. Eudragit E 100 could therefore serve as a useful and inexpensive alternative to poly-l lysine as reported earlier. Different polymer: drug ratios were tried for optimization of particle size and encapsulation efficiency. The effect of polymer: drug ratio on drug entrapment and particle size (PS) were shown in Fig.1. Increase in drug concentration revealed decrease in the encapsulation efficiency (EE). Maximum decrease in particle size was observed at 1:1 drug: polymer ratio with EE of >70%. Hence 1:1 drug: polymer ratio selected for the further study. All the surfactants were evaluated at a concentration of 0.05%w/v. The cationic surfactant cetrimide revealed maximum decrease in particle size (150nm) whereas non ionic surfactant, tween 80 also showed somewhat decrease in the particle size. Similar findings have been reported by Trotta et al. in their study on solid lipid NP. (Fig.2)
Fig: 2 Effect of surfactants on % entrapment and particle size in NP, (Cet-cetrimide, Tw-tween 80, CR-40-Cremophore RH40, NP –NP with targeting agent).

NP’s without surfactants and with cetrimide revealed incomplete drug release over a period of 60hrs (maximum drug release-15%), (Fig.3) however NP’s with the non-ionic surfactant tween 80 revealed gradual increase in drug release and hence was selected further for in vivo study.

Fig: 3 In-vitro dissolution profile of doxorubicin loaded NP without and with surfactants (cet-cetrimide, TW-tween 80, CR40-Cremophore RH40)

Biodistribution study:
Biodistribution studies revealed maximal and rapid uptake in the liver from doxorubicin loaded NP. Drug levels varied from 28 mcg/g of tissue at 1 hr to 15 mcg/g of tissue at 24 hrs. However plain doxorubicin exhibited a maximum concentration of 6.24mcg/g of tissue in the liver. (Fig.4) Moreover no drug could be detected after 2 hrs. Drug levels in the heart was detected up to 24 hrs in doxorubicin treated rats. (Fig. 5). Moreover levels in the other tissues namely kidney (fig.6), lung (fig.7), spleen (fig. 8) were also seen to be higher and sustained for up to 24 hrs with plain doxorubicin as opposed to NP, suggesting decreased systemic exposure with NP’s. NP’s revealed sustained drug levels in the plasma upto 24 hrs while with plain doxorubicin no drug was detectable in plasma at 24 hrs. Though the t max of plain doxorubicin and NP’s was 1 hr, the AUC with plain doxorubicin was only 30.15 mcg. hr/ml compared to an AUC of 364.20 mcg.hr/ml with doxorubicin loaded NP (Fig.9).

It is evident therefore that plain doxorubicin i.v resulted in enhanced systemic exposure and relatively short duration of action as opposed to NP’s of doxorubicin that showed targeted uptake in liver, minimal systemic exposure and sustained effect.

Fig. 4: Comparative liver doxorubicin level after i.v. administration of doxorubicin and NP.

Fig. 5: Comparative heart doxorubicin level after i.v. administration of doxorubicin and NP.
CONCLUSION

MAXITARG using NTA has revealed the potential to enhance liver uptake of doxorubicin with decreased systemic spread and sustained effect. The MAXITARG therefore represents maximal efficacy and minimal toxicity approach in the therapy of Hepatocellular Carcinoma and could revolutionalize the therapy for hepatic cancer.

REFERENCES