

PEGylated-poly-l-lysine dendrimers for delivery of Chloroquine phosphate

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INTRODUCTION

The antimalarial drugs are used for prophylactic suppressive chemotherapy of malaria but various toxicity and resistance problems associated with such chemotherapy need some sustained delivery and safer carriers. Dendrimers are recently reported as carriers for various chemotherapeutic agents¹. Their structures are of regular repeated molecular architecture. Due to number of –NH₂ group and charged species like carbonyl groups available on dendritic structures there is huge possibilities for drug loading and drug delivery and further their chemical architecture increases stability of such carriers as compared to other conventional carriers like liposomes, nanoparticles and microparticles. The present work was undertaken to solubilize and load model drug Chloroquine phosphate (CP) and reduce drawbacks of simple drug administration.

MATERIALS AND METHODS

Synthesis of dendrimers and formulation

In the present study CP, was loaded in PEG-Lysine type dendritic peptide based macromolecular carrier for sustained and controlled delivery of the drug through i.v. route of administration. PEG-amine was taken as core in the proposed systems and various generations of dendritic nanoparticles were synthesized by Fluorenyl methoxy carbonyl (Fmoc) based liquid phase peptide synthesis process using dicyclohexylcarbodiimide-hydroxy benzo-triazole (DCC- HOBT)^{2, 3, 4}. CP was loaded in carrier by vortexing, shaking and incubation¹ for 24 h. The hydrophilicity and aqueous partitioning of the drug molecules facilitate this and hence drug is entrapped within the structure of the 4.0G and 5.0G PEGylated-poly-lysine types of dendrimers.

Evaluation of formulations

The confirmation of the reaction was done by IR, NMR and MALDI-TOF-Mass analysis. Also Kaiser tests proved the step-by-step progression of generations where half

generations were Fmoc-Protected giving yellow colour and Full generations Deprotected types gave purple Ruhemann's Blue Colour on heating at 100°C⁵. The drug entrapment and release rates were evaluated by dialysis method using spectrophotometric estimation¹. The stability of PEGylated drug dendrimers formulation (5.0G as representative formulation) was evaluated at various accelerated conditions of temperatures. The systems were then evaluated for hemolytic toxicity⁶, in-vitro macrophage interactions⁷, in-vivo blood level and hematological changes on chronic dosing.

RESULTS AND DISCUSSION

For the reactions PEG-amine-4000 was synthesized to prepare a stable complete amino terminated products. The reactions were carried out by the method suggested by Zalipsky et al⁸ and were monitored by IR and NMR analysis. Then for poly-lysine (PLL) synthesis, lysine was protected by Fluorenyl methoxy carbonyl (Fmoc) stable strategy so that there is uniform lysine linkage, not abrupt uncontrolled reactions, in any generation leading to Fmoc protected half generations and then piperidine 20% solution was used for deprotection to generate active amine terminations in full generations. The quantitative Kaiser test was used for each generations for determination of number of amine terminations at each full generations. NMR studies confirmed the ratios of PEG to Lysine groups by determination of peak intensity ratio of the ethylene protons of PEG segment ($\delta=3.7$ ppm) and the α -, β , and γ -methylene protons of PLL dendrimers ($\delta=1.4-1.8$ ppm). The theoretical ratios of the peaks matched the finally elucidated values of peaks in spectrum. MALDI-TOF Mass gave the mass of 3.0G, 4.0G and 5.0G as 6125, 8524 and 13184 D much nearer to theoretical.

TEM of dendrimers proved these systems as nano-sized spherical carriers. The data also shows increase in particle size with generations. The drug loading was determined for 3.0G and above generation indirectly after

dialysis of untrapped drug in external sink. There was lesser drug **entrapment** inside the lower generation of dendrimers as to higher generations, which were nearly 5 molecules in case of 3.0 G to upto 20 molecules for 5.0G dendrimers. This is due to the more compactness and increased availability of active groups in dendritic structures with generations. The loading of drug is by hydrogen bonding or hydrotropic solubilization by weak charge-charge molecular interactions. Release was uniform and prolonged from 1 day to 3 days with increase in generations. The hemolytic toxicity of the peptide dendrimers was about 2% per mg of formulations per ml in normal saline. The hemolytic toxicity of the drug was reduced by 10-20% due to the charge neutralization effects by dendrimers, which led to inhibition of interactions of RBCs with the charged quaternary ammonium ion of drug. The formulations were found to be most stable in dark, at room temperature. It was also found that more drugs were released from formulations stored in light than those stored in dark. This may be attributed to structural cleavage due to higher reaction kinetics at higher temperature. The dendrimeric formulations kept in refrigerated conditions have slightly lesser interaction with drug molecules takes place. Therefore the dendrimers have slightly less entrapment capacity and hence release free drug from its structure to a greater extent on storage as found by drug leakage studies after storage, which showed slightly increased drug leakage (10% w/w above normal release rates as compared to 25% w/w for formulations at 50°C).

Predialysed formulations followed sustained release characteristics *in vivo*. The blood level of the drug was found to have reached its steady state value by 3 h. The blood level of the drug was found to be lower in case of 5.0G dendritic carriers as to 4.0G systems, lesser release rate of the drug similar to the trend found *in-vitro*. The MRT was more than doubled to tripled as compared to plain drug on slow i.v, administration. The hematological toxicity profiles and resultant disturbance pattern also proved the systems for long-term usage. RBCs count was found to have been nearer to normal values in case of delivery of drugs by PEGylated systems as compared to long-term free CP delivery. This

was due to blood toxicity and cytotoxicity associated with CP administration. There was no stimulation of the macrophage level and WBC count by PEG-Lysine dendritic. Further tissue toxicity studies are on for determination of chronic and acute tissue toxicity by this proposed carriers.

CONCLUSION

The PEGylated drug-dendrimer formulations can be used suitably as long term circulatory i.v. nanoparticulate controlled drug delivery system for delivery of model antimalarial drug CP without any major side-effects.

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