Abstract

Blood-brain barrier (BBB) plays a vital role for maintaining homeostasis in brain by restricting the entry of most chemicals into brain due to its special tight endothelial structure. Such diffusion barrier protects the brain as a safeguard. In case of treatment of several diseases involving brain such as brain tumors, acute brain ischemic stroke, viral infection in brain caused by acquired immunodeficiency syndrome (AIDS), psychiatric disorders, etc., drug delivery into brain is often obstructed or difficult due to BBB and insufficient concentrations of drugs fails to provide proper therapeutic efficacy. At a very early stage of AIDS due to neuroinvasion, human immunodeficiency virus (HIV) infected circulated monocytes in blood stream can easily enter into brain and brain becomes a pool of HIV. Zidovudine (AZT), nucleoside reverse transcriptase inhibitor and first approved drug by US Food and Drug Administration (FDA) as a medication for AIDS, is incapable to reach brain across BBB due to its strong hydrophilicity. Highly water soluble drug AZT was utilized in this research work as a model drug to deliver into brain across BBB. It is a very tough and critical challenge for formulation scientists to deliver highly water soluble drugs into brain to produce proper therapeutic outcome. Delivery of highly water soluble drug into brain across BBB is too difficult due to its polar nature. Some physico-chemical characteristics such as ionisation, lipophilicity, size, etc. have impact on regulation of structure of BBB. Highly lipophilic nature along with small size or low molecular weight helps compounds to cross BBB. Nowadays, drug delivery through nanocarriers such as nanoparticles, nanoliposomes (NLs), micelles, dendrimers, quantum dots, and nanoemulsions, etc. is an emerging field of pharmaceutical research. NLs were selected as a nanocarrier to deliver AZT into brain though BBB due to its lipophilic nature and nanosize to obtain the desired outcome.

The main objective of this research work was to investigate the capability of a newly developed fatty acid combination (stearic acid:oleic acid:palmitic acid = 8.08:4.13:1) (ML) to act as base material for the development of nanocarrier to deliver highly water soluble drug

through BBB into brain. Further, the efficiency of ML-based nanocarrier was evaluated by comparing with that of soya lecithin (SL)-based nanocarrier.

Fourier-transform infrared spectroscopy (FTIR) was performed to check the interaction between the drug and the excipients. There was no chemical interaction was observed between the drug and the excipients, but some physical interactions were detected between the molecules of excipients as well as between the drug and the excipients. This physical interaction might help in formation of spherical nanostructure of the drug carrier along with the retention of drug in lipid layer and slower diffusion of drug through the membrane. NLs were prepared using lipid layer hydration technique by altering various process parameters such as temperature and speed of hydration, duration of sonication, time and speed of centrifugation, duration of freeze drying and ratio of the constituents. Depending on some physicochemical characteristics of formulations such as drug loading, particle size, polydispersity index (PDI), zeta potential, ML-based MGF and SL-based SYF were selected as two best formulations for further studies.

The drug loading of MGF was 5.7 ± 0.37 % whereas SYF had 7.00 ± 0.23 % drug loading. Yield of SYF was nearly about 6 % less as compared to MGF due to sticky nature of SL creating recovery problem of NLs. Both the formulations were of nanoscale size, but SYF had comparatively 38.49 % larger in size than that of MGF. Nearly same PDI values were observed for both of them. Similar zeta potential values (about -70 mV) were obtained for MGF as well as SYF which signify prolonged and better physical stability of both the formulations in colloidal suspension. From field emission scanning electron microscopy (FESEM) study it was determined that both the nanocarriers were spherical in shape along with smooth surface and were homogenously distributed. Nanosize unilamellar vesicles were found using cryo-transmission electron microscopy (Cryo-TEM) technique for MGF / SYF.

FESEM and Cryo-TEM data again also revealed that the vesicular size of MGF was smaller than that of SYF. The discrete particulate distributions were observed in atomic force microscopy (AFM) study for both cases. Drug release pattern was slower and more sustainable from MGF as compared to SYF in freshly prepared phosphate buffered saline (pH 7.4) and in 50 % human serum as drug release media. The variation of lipid composition might be the reason of this. AZT release pattern from MGF / SYF followed Korsmeyer-Peppas kinetic model in both the drug release media, which suggests AZT release from MGF / SYF followed the diffusion as well as erosion process. *In vitro* cellular uptake study of both the formulations was conducted in U-87 MG human glioblastoma cells by using the fluorescent NLs where fluorescein isothiocyanate (Isomer I) (FITC) was used as fluorescent marker. FITC-MGF was internalised by glioma cells in a time dependent manner whereas the concentration of FITC-SYF increased initially (at 1 h as investigated) and then decreased with time (at 3 h, total duration of the investigation) in U-87 MG human glioblastoma cells. It was confirmed from the stability study that 4ºC is the most suitable temperature for storage of MGF as well as SYF.

In case of *in vivo* studies, various experiments were performed such as gamma scintigraphy study, biodistribution study and plasma and brain pharmacokinetic study. The gamma scintigraphic investigation and brain pharmacokinetic study was organized to compare the capability of MGF and SYF to cross BBB. From gamma scintigraphic images it was determined that both the radiolableled NLs were capable to cross BBB and reached into brain. But radiolabeled ML-based formulation was retained in brain for longer time as compared to radiolabeled SL-based NLs. Very poor ability of radiolableled free drug AZT to cross BBB was observed through this study. In case of biodistribution study, it was confirmed from the brain / blood ratio data that radiolabeled MGF was capable to maintain its level in

brain persistently than that of SYF. So, MGF had ability to provide sustaining drug level in brain and blood better than SYF.

After intravenous administration in Sprague-Dawley rats, the plasma concentration of AZT from MGF was comparatively more than that of SYF after 4 h. MGF exhibited slow distribution as well as significantly ($P<0.05$) longer blood residence time as compared to SYF. Further, significantly more area under the first moment curve ($AUMC_{0-48}$) value of MGF could be due to less clearance and longer mean residence time (MRT) of MGF than that of SYF. In brain pharmacokinetic investigation, brain pharmacokinetic data showed less clearance, prolonged residence time, more bioavailability and sustained release of AZT from MGF in rats as compared to those data of the rats treated with SYF / AZT suspension.

In conclusion, nanosize unilamellar vesicular delivery systems of AZT were successfully developed using ML / SL to deliver the drug into brain across BBB. MGF as well as SYF had sustained drug release pattern. Though both the formulations sufficiently reached in brain across BBB, better pharmacokinetic profiles with respect to sustained drug release, prolonged blood residence time as well as brain residence time and increased half-life ($t_{1/2}$) value were observed for MGF as compared to SYF. Thus, ML may be utilized as a new and effective carrier material to deliver AZT effectively in brain. To develop various therapeutic strategies for delivering drugs into brain as well as in other organs, ML could be an emerging lipid pharmaceutical material. However, further studies are warranted in this arena.