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Albumin nanoparticles for the delivery of gabapentin: Preparation, characterization and pharmacodynamic studies

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ABSTRACT

The study was aimed to prepare and evaluate gabapentin loaded albumin nanoparticles and to find out their effectiveness in treating epilepsy. Albumin nanoparticles of gabapentin were prepared by pH-coacervation method. The drug was administered into animals as free drug, gabapentin bound with nanoparticles, and gabapentin bound with nanoparticles coated with polysorbate 80. The polysorbate 80 coated nanoparticles increased the gabapentin concentration in the brain about 3 fold in comparison with the free drug. Moreover, the polysorbate 80 coated nanoparticles significantly reduced the duration of all phases of convulsion in both maximal electroshock induced and pentylenetetrazole induced convulsion models in comparison with free drug and drug bound with nanoparticle formulations, which indicates the ability of polysorbate 80 coated nanoparticles to enhance the gabapentin concentration in the brain.

1. Introduction

Drug delivery to specific sites using carriers such as nanoparticles, liposomes, dendrimers, polymeric micelles, viral vectors, antibodies and their conjugates and polymer–protein conjugates (Wilson, 2011) have been getting much attention recently by the researchers in this domain. Among these carriers, polymeric nanoparticles have been investigated extensively for the controlled and site specific delivery of drugs (Tröster et al., 1990; Wilson et al., 2010, 2011). This is due to their versatile nature and ability to overcome the problems which are related with the administration of a variety of drugs, vaccines, plasmid DNA, *etc.* (des Rieux et al., 2006). Nanoparticles, for drug delivery, are made of natural or synthetic or semisynthetic polymers. The size of nanoparticles range between 1 and 1000 nm, but particles with a size range of about 10–200 nm with hydrophilic surface have been used widely in drug delivery. Polymeric nanoparticles are more stable in biological fluids when compared with other colloidal carriers. Moreover, their polymeric nature controls the release of drug in a sustained and controlled manner for a longer time (Roney et al., 2005).

Nanoparticles have higher drug loading capacity and protect the incorporated drugs against degradation. If foreign particles enter the systemic circulation, they will be coated by opsonins and the coating makes them visible to the defense system (reticuloendothelial system). These particles are effectively taken up by Kupffer cells of liver. As a result, body considers nanoparticles as foreign particles and they are removed by reticuloendothelial system organs. Even though, it is useful to target (passive targeting) the drugs into the reticuloendothelial system organs, it acts as a barrier to target drugs to non-reticuloendothelial system organs (Illum et al., 1982). Nanoparticles surface can be modified/manipulated to avoid recognition by reticuloendothelial organs to enhance drug delivery to the target organ.

Nanoparticles can be prepared using various proteins such as albumin and gelatin. Among these, albumin is promising as a material for preparing nanoparticles, and a number of studies have been carried out by many researchers to study the effectiveness of albumin as a carrier (Michaelis et al., 2006; Wilson et al., 2012). In 2005, the first human serum albumin based nanoparticles containing paclitaxel, Abraxane® was approved by the FDA. It has been reported that the new formulation improves solubility of the drug with many advantages over the conventional paclitaxel therapy (Gradishar et al., 2005; Desai et al., 2006).

Epilepsy is a serious common chronic neurological disorder associated with recurrent seizures (sporadic symptoms). The other abnormalities include cognitive impairment, psychiatric disorders

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and social problems (Hermann et al., 2008). Epilepsy affects 0.6–0.8% of the people worldwide (Landmark et al., 2012). Epilepsy patients need to take medication daily for many years or for lifetime. The selection of drug(s) depends on the effectiveness of the drug(s) for treating specific type of epilepsy and their pharmacokinetics and side effects. Gabapentin is an antiepileptic drug used for the treatment of partial seizures and neuropathic pain. It is absorbed rapidly after oral administration from GI tract using L-amino acid transport system with a t_{max} of 2–3 h. Oral bioavailability of gabapentin decreases with increasing dose, which is probably due to saturation of transporters (Vollmer et al., 1988; Gidal et al., 2000). It has been reported that gabapentin concentration in the blood serum linearly increases upto a dose level of 1800 mg/day and at higher doses the increase is non-linear (Stewart et al., 1993). As per our knowledge, albumin nanoparticles containing antiepileptic drug gabapentin has not been reported. Hence, this study was aimed to formulate and evaluate gabapentin loaded albumin nanoparticles and to find out their effectiveness in treating epilepsy.

2. Materials and methods

Gabapentin and bovine serum albumin were purchased from Sigma–Aldrich and Himedia, respectively. All the other chemicals and solvents used for the study were analytical/HPLC grade.

2.1. Preparation of albumin nanoparticles

Albumin nanoparticles of gabapentin were prepared by pH-coacervation method (Lin et al., 1993). Briefly, specified amount of albumin was dissolved in 2 ml of 10 mM sodium chloride solution (Table 1). Gabapentin was dissolved in the albumin solution. The pH of the drug–polymer solution was adjusted between 7 and 8. Ethanol was added at the rate of 1 ml/min using a syringe under magnetic stirring until turbidity appeared in the solution. The nanoparticles formed were cross-linked by the addition of 100 μl of 4% glutaraldehyde solution and the stirring was continued at room temperature for 2 h. Thus, the nanoparticle suspension obtained was freeze dried after adding 1% anhydrous glucose as cryoprotectant (ModulyoD, Thermo, Milford, MA) to obtain free flowing powder particles. Five different batches were prepared by keeping the drug concentration constant and varying the albumin concentration (Table 1).

2.2. Characterization of nanoparticles

The surface morphology of gabapentin loaded albumin nanoparticles (F-4) was studied using scanning electron microscopy (JSM-6360LV, JEOL, Japan). Samples were subjected to gold coating prior to examination. Zetasizer Nano ZS (Malvern, UK) was used to determine the size of the particles (F-4). For this, samples were analyzed at 25 °C at an angle of 90°. Zeta potential (surface charge) of the particles (F-4) was also determined at 25 °C using Zetasizer Nano ZS (Malvern Instrument, Malvern, UK). Deionized distilled water was used to dilute the samples to obtain appropriate concentration.

Table 1
Formula for the preparation of gabapentin loaded albumin nanoparticles.

Ingredient	Batch				
	F-1	F-2	F-3	F-4	F-5
Gabapentin	10 mg	10 mg	10 mg	10 mg	10 mg
Serum albumin	10 mg	20 mg	30 mg	40 mg	50 mg
10 mM sodium chloride solution	2 ml	2 ml	2 ml	2 ml	2 ml
Glutaraldehyde solution (4% v/v)	100 μl	100 μl	100 μl	100 μl	100 μl

2.3. Drug loading capacity

Drug loading capacity was determined by calculating the difference between total amount of the drug used for the preparation and the amount of the free drug available in the nanosuspension (Varshosaz et al., 2010), by centrifugation (Remi Equipments Ltd., India) at 10,000 rpm for 20 min. The gabapentin concentration in the supernatant was determined by using UV spectrophotometer (Shimadzu 160A, Japan) at 230 nm.

2.4. In vitro *drug release*

The release of gabapentin from albumin nanoparticles was studied by dialysis bag method (Wilson et al., 2012). Drug loaded nanoparticles equivalent to 5 mg drug were suspended in 2 ml of pH 7.4 phosphate buffer (donor medium) in a dialysis bag (cut-off 5 kDa, Himedia, India) and was dialysed against 50 ml of pH 7.4 phosphate buffer (receptor medium). The medium was continuously stirred at 100 rpm and maintained at temperature 37 °C. Samples were (2 ml) drawn at predetermined time intervals and the same volume was replaced with fresh medium. The gabapentin concentration was measured using UV spectrophotometer (Shimadzu 160A, Japan) at 230 nm against blank.

2.5. Release kinetic studies

The data obtained from the release studies (F-4) were fitted with kinetic models to find out the best fit model as well as release mechanism. The models selected for this were zero order, first order, Higuchi and Korsmeyer–Peppas model (Costa and Lobo, 2001).

2.6. Animal studies

Healthy adult male rats of Wistar species in the weight range of 180–220 g were used for the study. The animals were maintained on a 12:12 h light/dark cycle and had free access to food and water. The protocol of the study was approved by Institutional Animal Ethical Committee. All efforts were taken to reduce the number of animals used and minimize animal suffering.

2.6.1. Nanoparticles effectiveness to deliver gabapentin into brain

To find out the effectiveness of nanoparticles to deliver gabapentin into the brain, the animals were divided into three groups each containing six rats. Group 1 received gabapentin free drug in solution form. Group 2 was administered with gabapentin loaded albumin nanoparticles (F-4) and group 3 received gabapentin loaded albumin nanoparticles (F-4) coated with 1% polysorbate 80. Prior to administration, for surfactant coating, 1% polysorbate 80 was added (relative to total suspension volume) and incubated for 30 min under mild stirring using a magnetic stirrer (Kreuter et al., 2003; Wilson et al., 2008a). The rats received a dose level equivalent to 5 mg/kg body weight. The formulations were injected by intraperitoneal route. After 1 h of injection, the rats were sacrificed and the brain was surgically removed quickly, washed with pH 7.4 phosphate buffered saline, weighed and homogenized in pH 7.4 phosphate buffered saline to give a final concentration of 20% w/v. The supernatant was collected and stored at −70 °C till analysis. The gabapentin concentration in the supernatant was analyzed by using Shimadzu LC 2010A HT HPLC.

2.6.2. Pharmacodynamic studies

The effectiveness of the prepared formulations (pure drug, nanoparticle bound drug (F-4) and nanoparticle bound drug (F-4) coated with polysorbate 80) to treat epilepsy was studied on rats