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Original Research Paper

Brain targeted delivery of paclitaxel using endogenous ligand

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ABSTRACT

The objective of the present investigation was to formulate nanoparticles constructed using PLGA polymer for the effective targeted delivery to brain via nasal route. The PLGA nanoparticles were optimized using novel design of experiment technique by 2^3 full factorial design. Drug: polymer ratio (X_1), surfactant concentration (X_2) and stirring speed (X_3) were identified as critical process parameters, and its impact on particle size (Y_1) and % entrapment efficiency (Y_2) was studied. The optimized nanoparticle formulation was conjugated with glutathione as an endogenous ligand by using carbodiimide chemistry using (1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide) (EDAC) as linker molecule. From Ellman's assay, it was found that a total of 691.27 ± 151 units of glutathione were conjugated upon each PLGA nanoparticle. The *in vitro* release studies as well as *ex vivo* studies revealed biphasic pattern of drug release with initial burst release followed by slow exponential release of drug over a period of 24 h. The *in vivo* biodistribution studies were conducted on rat following nasal administration of the nanoparticle formulation (conjugated and unconjugated) and were compared with plain paclitaxel suspension. The results clearly demonstrated that the brain targeting efficiency was enhanced with the glutathione conjugated formulation (387.474%) as compared to the unconjugated nanoparticle formulation (224.327%). Further, the *in vitro in vivo* correlation studies revealed good relationship ($R^2 > 0.95$) as obtained from the levy plot. Glutathione proves to be an efficient vector for the successful transport of poor bioavailable drug to the brain.

1. Introduction

Nanoparticulate delivery systems [1], such as those based on poly (lactic-co-glycolic acid) (PLGA) have been studied extensively for many years. For the past three decades, lot of researchers has explored PLGA to fabricate drug delivery systems for pharmaceutical and biomedical applications due to its biocompatible and biodegradable properties. PLGA, further, has the advantage of being well characterized and

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commercially used for microparticulate and nanoparticulate drug delivery systems [2]. PLGA polymer is one of the most common biodegradable polymers used for the controlled delivery of drugs due to its early use and approval as a compatible biomaterial in humans. Lewis (1990) reported that, by varying the molecular weight and lactide/glycolide ratio, the degradation time of the PLA and PLGA and the release kinetics of the active agent can be controlled [3]. In aqueous media, degradation of PLGA is triggered by hydrolysis of its ester linkages. Presence of methyl side groups in PLA makes it more hydrophobic than PGA, and hence lactide rich PLGA copolymers are less hydrophilic, absorb less water and degrade more slowly and control the release of drug for prolonged duration [4,5].

The major advantages of nanoparticles is improved bioavailability by enhancing aqueous solubility, increasing resistance time in the body (increasing half-life for clearance/increasing specificity for its associated receptors and targeting drug to specific location in the body. This is why nanoparticles are increasingly used in variety of applications that includes drug carrier systems and to pass organ barriers such as the blood–brain barrier, cell membrane, etc. The cellular uptake, biodistribution and circulating half-life are the key factors which are influenced by particle size of nanoparticles. Therefore, particle size becomes a primary concern while formulating a nanoparticulate system [6]. Moreover the particle size thus obtained should be uniform because more uniform the distribution of particles more consistent will be the biodistribution, cellular uptake and drug release [7].

Nasal route is one of the alternative approaches for delivery of drugs to brain, where the drug is absorbed into the systemic circulation through nasal mucosa. Anatomically, nasal mucosa is comprised of tight junctions, and molecules with smaller size (less than 10 nm) can be able to traverse the junctions to reach the systemic circulation. Normally, many hydrophobic drugs or formulations (200 nm or more) opt transcellular route for delivery across the nasal mucosa. Nanoparticles above 500 nm size fall prey to the mucociliary clearance or by triggering immune response. The lower the particle size of nanoparticles, the more will be the rate of permeation across the nasal mucosa via transcellular pathway [8,9].

Paclitaxel (PTX) is one of the most widely used chemotherapeutic agents against breast and lung cancer, however, the brain delivery of paclitaxel is restricted owing to several bodily defense mechanisms. However, P-glycoprotein, an ATP-binding cassette (ABC) transporter, acts as a barrier in cancer treatment by chemotherapy by multidrug resistance (MDR) at cellular/non-cellular level offered by the tumor cells [10]. Therefore, the absorption of paclitaxel is minimized due to rapid efflux of the drug out of the tumor cell because of the overexpression of the plasma membrane, P-glycoprotein (P-gp) [11,12]. Hence, nanoparticles consisting of Poly(Lactide-co-Glycolide (PLGA) have proven to possess potential delivery of insoluble drugs like paclitaxel to target areas, as they can be endocytosed/phagocytosed by cell resulting in the internalization of the encapsulated drug in the cell. PLGA, due to its biodegradability and biocompatibility, has attracted considerable attention for developing polymeric nanoparticles. PLGA possesses many advantages, such as able to encapsulate hydrophilic and hydrophobic drugs, improves interaction with biological matters and imparting stealthness, enhancing stability of drug, etc. [13,14].

Glutathione (GSH) is a hydrophilic endogenous tripeptide molecule that performs antioxidant function against reactive species and toxic metabolites of the cell [15]. Glutathione helps in transporting few endogenous substances across the blood–brain barrier (BBB) by interacting with the membrane proteins in the BBB [16]. Normally, P-glycoprotein (P-gp), the gatekeeper of BBB, helps in transporting the glutathione coupled compounds across the BBB [17]. This mechanism is exploited for the delivery of paclitaxel to the brain by coupling it with glutathione.

Paclitaxel, belonging to BCS class IV, exhibits limited absorption due to its poor solubility and permeability characteristics. Paclitaxel, due to its poor permeability to brain, is primarily indicated for breast cancer and small cell lung cancer. Hence, paclitaxel is a suitable candidate for formulating nanoparticles due to its safe and efficient targeting of drug to the brain. In view of pursuing the objective of fostering the development of size controlled nanoparticles with enhanced entrapment efficiency, novel quality by design concept was utilized. Further, the optimized nanoparticles were conjugated with glutathione and assessed the functionalization of nanoparticles with respect to its ability to transport drug across BBB.

The objective of the present investigation emphasizes the preparation of nanoparticles by using novel design of experiment concepts and subsequently conjugating with glutathione on the surface of nanoparticles. Hence, an attempt is being made in the current research to enhance the brain delivery of paclitaxel by loading PLGA nanoparticles conjugated with glutathione, which was further assessed by *in vivo* biodistribution studies.

2. Materials and methods

2.1. *Materials*

PLGA copolymer RG 502-H (lactide : glycolide ratio of 50:50, molecular weight 7–17 kDa), Resomer RG 504-H (lactide : glycolide ratio of 50:50, molecular weight 38-54 kDa) was obtained as gift sample from Evonik Industries AG, Germany. Poloxamer 188 was supplied as a gratis sample from Sandoz Pvt. Ltd, Mumbai. DCM (purity NLT 99% by GC), Paclitaxel was obtained as gratis sample from MAC-CHEM products Pvt. Ltd, Mumbai. Glutathione was purchased from SD Fine Chemicals, Mumbai. Dialysis membrane – Himedia LA401-5MT, Acetone (purity NLT 99% by GC), methanol (HPLC grade) were procured from Merck and Co., Germany. Double distilled water used was filtered through 0.22 μm filter from Millipore (Mumbai, India). All other cited chemicals used were of analytical grade.

2.2. *Animals*

Adult male Wistar rats weighing 200–240 g acquired from dedicated animal house of Institute of Pharmacy, Nirma University after receiving approval from the Institutional Animal Ethics Committee (IAEC) dated 15/06/2013 for studying the biodistribution potential of the formulation (Protocol No. IP/