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Prevention of rat liver fibrosis by selective targeting of hepatic stellate cells using hesperidin carriers

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ABSTRACT

Therapeutic efficacy of antifibrotic drugs can be improved by targeting hepatic stellate cells (HSCs). This study investigated the prospect of developing a carrier system for the effective delivery of hesperidin by selectively targeting HSCs in fibrotic rat model. Hesperidin-loaded surface modified liposome formulations were prepared with varying quantity of lipids to optimize physicochemical characteristics. Best liposome formulation was further conjugated with a homing ligand, recognized by HSCs, to achieve a carrier system that facilitates the targeting of hesperidin. This carrier system was characterized for various physicochemical properties. The effectiveness of the bioactive carrier to prevent liver fibrosis was investigated by carrying out biochemical, biodistribution, and histopathological analyses. The pharmaceutical properties demonstrated by the ligand conjugated carrier system were optimal to facilitate selective and preferential delivery to the liver. Steady and prolonged drug release behavior with zero order kinetics displayed by prepared carrier system established its prospect to increase efficiency and decrease dosing of hesperidin. The pharmacokinetic profile of the carrier system was very distinct and exhibited rapid plasma clearance. The bio-distribution data of formulated carrier system indicates higher uptake of hesperidin predominantly by fibrotic liver with insignificant amount in non-targeted organs, which is certainly beneficial due to low risk of toxicity and lower systemic distribution. In conclusion, this developed carrier represents a potentially beneficial approach for HSCs specific targeting of hesperidin in a rat model with liver fibrosis.

1. Introduction

Liver fibrosis is a major disorder that can increase the risk of hepatic cirrhosis, liver failure and hepatocellular carcinoma. Liver fibrosis is strongly associated with chronic liver diseases such as hepatitis viral infection and autoimmune hepatitis. It is characterized by impaired liver function and increased production of extracellular matrix proteins (Bataller and Brenner, 2005). Currently there is no effective treatment for liver fibrosis, although transplantation remains the last management option (Böttcher and Pinzani, 2017; Koyama et al., 2016). Thus, the successful pharmacological treatment of liver fibrosis remains challenging and investigation of new therapeutically relevant strategies is warranted. Several pharmaceutical actives have been evaluated for their potential to reduce the fibrogenesis in chronic liver disease (Bansal et al., 2016). The conventional therapy of antifibrotic drugs lack effectiveness due to poor tissue penetration and insufficient drug concentration in the hepatic cells (Cohen-Naftaly and Friedman, 2011). This is primarily because the drugs are neither liver specific nor fibrosis specific. Similarly, the tolerability and severe adverse effects during chronic therapy also limits the treatment of liver fibrosis. Several studies indicate that the antifibrogenic activities of many potent drugs that act on hepatic stellate cells (HSCs) have shown limited therapeutic effectiveness when experimented *in vivo*. This is essentially due to low uptake of these drugs by major cell type responsible for the liver fibrogenesis namely, HSCs (Schon et al., 2016). Therefore, there is a need for a safe, effective, sustained and targeted delivery of antifibrotic drugs to the HSCs in order to improve their therapeutic efficacy. In this perspective, hesperidin, a natural flavonoid abundantly present in citrus is known for its antioxidant, anti-inflammatory, and anti-apoptotic properties (Saiprasad et al., 2013). Oxidative stress, inflammation, and apoptosis have been suggested as targets for intervention to ameliorate the progression of liver fibrosis (Czaja, 2014; Wang, 2014). Hesperidin has considerable antifibrotic activity (Elshazly and Mahmoud, 2014; Pérez-Vargas et al., 2014), however, it has poor oral bioavailability and targeting efficiency. Thus, a drug delivery system that can effectively transport this drug to HSCs could be beneficial.

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The potential of conventional carriers (liposomes, nanoparticles, micelles, nanosuspensions, polymeric conjugates, niosomes, etc.) to alter the pharmacokinetic characteristics of drugs and provide passive liver targeting has been well documented (Rohilla et al., 2016; Wadhwa et al., 2012). Subsequently, the prospective of selective and preferential delivery of drugs to the liver has been designed and explored as an attractive approach to improve their efficacy as well as reduce uptake in non-target cells (Poelstra et al., 2012). Receptor-mediated drug targeting was achieved by making use of site directed ligands, which enable the homing of drug moieties to the most relevant target cells (Kawakami and Hashida, 2014). This active targeting approach utilized different ligands such as asialofetuin, fucose, galactose, lactobionic acid, mannose, mannose-6-phosphate (M6P), and peptides to develop cell specific drug targeting moieties. Moreover, these ligands provide selective binding to receptors found in all resident intrahepatic cells including hepatocytes, liver sinusoidal endothelial cells, Kupffer cells and HSCs, which are identified as prominent liver cells in various liver diseases (Kawakami and Hashida, 2014; Mishra et al., 2013; Rohilla et al., 2016). Among the conventional carriers outlined above, the prospective of liposomes to provide liver specific delivery of drugs, genes and oligonucleotides has been widely studied by coupling with various targeting ligands such as galactose, lactose, peptide, sterylglucoside, and vitamin A (Poelstra et al., 2012; Rohilla et al., 2016).

In general, targeting of potential antifibrotic drugs to the liver and in particular to the HSCs provides opportunity for successful treatment of liver fibrosis with reduced side effects (Li and Wang, 2009). The activated HSCs are mainly responsible for production of matrix proteins and play a crucial role in the initiation and propagation of liver fibrosis (Elpek, 2014; Higashi et al., 2017). Thus, the primary strategy for the treatment of liver fibrosis relies on the potential to inhibit the activation of HSCs. Subsequently, researchers explored the feasibility to achieve more specific targeting of antifibrotic agents to activated HSCs and binding sites that are highly expressed on these cells in fibrotic liver (Adrian et al., 2007a). Extensive studies in the past decade have engendered different HSCs selective systems with lower systemic distribution, greater efficiency, and therefore reduced toxicity to non-target site (Li and Wang, 2009). For instance, Beljaars et al. (2000) have demonstrated the HSCs targeting potential of albumin modified peptide moieties as a homing device that recognized the collagen type VI receptor. Similarly, the effective targeting of protein-based systems substituted with sugar moieties as M6P to the HSCs was used (Gary-Bobo et al., 2007). This neoglycoprotein, namely M6P-albumin, facilitates selective binding to cation-independent M6P/insulin-like growth factor 2 receptors (M6P/IGF2-R), which are highly upregulated on activated HSCs during liver fibrosis (Adrian et al., 2007a). Remarkable progress has been made and these M6P-albumin based drug carriers were further conjugated with liposomes, which made them an excellent delivery carrier for drug targeting (Li and Wang, 2009). Typically, these surface modified liposomes increase binding to M6P/IGF2-R and intracellular uptake by HSCs as well as provide high drug encapsulation efficiency (Adrian et al., 2006, 2007a). Thus, this liposome conjugated carriers open leads for new therapeutic interventions and has demonstrated their potential to function as an effective system to enhance the delivery of various antifibrotic agents (Dutta et al., 2017; Fiume et al., 2014; Patel et al., 2012). In this context, M6P-albumin conjugated to hesperidin-loaded liposome carrier system can improve its efficacy and attenuate liver fibrosis via targeting HSCs by providing sustained and cell-specific delivery at an optimal rate. Thus, the objective of the current study was to carry out a systematic investigation on the possible formulation of M6P-bovine serum albumin (BSA)-conjugated hesperidin-loaded liposomes carrier system for potential HSCs specific targeting to prevent rat liver fibrosis.

2. Materials and methods

2.1. Materials

1,2-Distearoyl-sn-glycero-3-phosphoethanolamine (DSPE), 1-(3-dimethylamino propyl)-3-ethylcarbodiimide hydrochloride, acetonitrile, bovine serum albumin, cholesterol, hesperidin, mannose, sebacic acid, soya lecithin, and Triton X-100 were purchased from Sigma-Aldrich Corp. (St. Louis, MO, USA). All the chemicals were of analytical grade and were used as received without any processing.

2.2. Quantification of hesperidin

Chromatographic separation and quantification of hesperidin was carried out using a high performance liquid chromatography (HPLC) system (Shimadzu LC-20AT, Tokyo, Japan) with a 20 µl sample loop coupled to UV–Vis detector (SPD-20A) set to 280 nm. The separation was achieved using a reversed phase Agilent TC-C18 column (4.6 × 250 mm, particle size 5 µm). Mobile phase consisting of methanol, acetonitrile and 5% acetic acid (35:50:15, v/v) was employed at a flow rate of 1 ml/min at room temperature. The method was validated by determination of linearity, precision and accuracy.

2.3. Formulation of liposomes

2.3.1. Preparation of DSPE-sebacic acid conjugate

The DSPE-sebacic acid conjugate was prepared in two steps (Fig. 1). Briefly, sebacic acid was reduced to sebacic anhydride by refluxing at 80 °C for 8 h in presence of phosphorus pentaoxide. The product was characterized by Fourier transform infrared (FTIR) spectrometer (8400S, Shimadzu, Tokyo, Japan). IR (KBr, cm^{-1}): 3430 (−OH), 2842 (−CH) and 1030 (−COO−). Then sebacic anhydride (2 mM) was dissolved in chloroform (5 ml), reacted with solid DSPE (1 mM) and trimethylamine (1 mM) in presence of acetic acid and evaporated to dryness to obtain DSPE-sebacic acid conjugate. The product was characterized by FTIR. IR (KBr, cm^{-1}): 3430 (−OH), 2842 (−CH), 1640 (O═C−NH) and 1547 (−COO−).

2.3.2. Preparation of hesperidin-loaded liposomes

Liposomes contain hesperidin were prepared (Fig. 1) by thin film hydration method reported earlier with some modifications (Adrian et al., 2007b). Briefly, required amount of hesperidin (10 mg) was weighed and dissolved in a binary solvent mixture of methanol:acetonitrile (1:1 v/v, 20 ml). Separately, required quantities of soya lecithin, cholesterol and DSPE-sebacic acid conjugate (23:16:1, 50–200 mg) were dissolved in the methanol:chloroform mixture (1:9 v/v) and added to the drug solution (20 ml) with constant mixing. Subsequently, the organic solvent was evaporated under reduced pressure to form thin lipid film. Liposomes were further size reduced by extrusion through polycarbonate membrane filters of pore size 200- and 100-nm using a high-pressure extruder. Untrapped free drug in the liposomal dispersion was separated by centrifuging liposomal suspension at 7500 rpm for 10 min. Liposomal suspension was decanted; drug pellet was separated and stored at 4 °C until use. Four different formulations (H1-H4) were prepared.

2.4. Characterization of hesperidin loaded liposomes

2.4.1. Percentage yield

The percentage yield of hesperidin-loaded liposomes was determined using the formula:

$$\% \text{ Yield} = \frac{\text{Practical yield}}{\text{Theoretical yield}} \times 100$$