World Journal of Pharmaceutical Sciences
ISSN (Print): 2321-3310; ISSN (Online): 2321-3086
Published by Atom and Cell Publishers
Available online at: http://www.wjpsonline.org/
Editorial

Lipoplex-mediated therapeutic gene delivery to liver parenchymal cells: Challenges and prospects

Prof. Mario Ariatti
Discipline of Biochemistry,
University of KwaZulu-Natal,
P.Bag X54001, Durban 4000,
South Africa

Professor Ariatti is a founder member of the Department of Biochemistry on the Westville campus of the University of KwaZulu-Natal in South Africa and has 35 years of lecturing experience at undergraduate and postgraduate level. During the course of his doctoral studies, which explored the peptidyl transferase step of protein biosynthesis, he developed a keen interest in the chemistry and biochemistry of nucleic acids. In the past 30 years much of his research has centered around the design and development of non-viral systems for the delivery of DNA and siRNA to mammalian cells, with particular focus on liposome-based vectors. Professor Ariatti continues to serve in an emeritus capacity since 2010 and co-supervises numerous postgraduate candidates. He has authored and co-authored over 75 peer reviewed articles, many of which are in the broad area of Pharmaceutical Nanotechnology and serves as ad hoc reviewer for several international journals. In 2010 Professor Ariatti was conferred the title: Cavaliere dell'Ordine Della Stella Della Solidarietá Italiana, by the Honourable President of the Republic of Italy. He is presently engaged in the assessment of novel hepatocyte-directed cationic liposome-based nucleic acid delivery vehicles that address hepatitis B and hepatocellular carcinoma.

The liver is the largest internal organ and is comprised mainly of hepatocytes (80 % by volume). These parenchymal cells are, in metabolic terms, the most active liver cell-type and perform important functions that include the production of numerous serum peptides, proteins and glycoproteins, lipid biosynthesis and lipoprotein metabolism, and the regulation of blood glucose. Therefore several congenital metabolic disorders are associated with this organ. They include α1-antitrypsin deficiency, familial porphyria, Wilson's disease, familial hypercholesterolemia (FH) and hereditary hemochromatosis. In addition to these and other inherited conditions, acquired conditions associated with hepatocytes, such as hepatitis B and hepatocellular carcinoma (HC) place a serious burden on society. Indeed hepatocellular carcinoma accounts for > 600 000 deaths annually and is the third most common cause of cancer-related mortality. Incidence of this tumor type is particularly marked in sub-Saharan Africa and Far East Asia, where hepatitis B is endemic. Here 80 % of patients present with prior or active hepatitis B infection. Treatment options for this malignancy are generally limited to transcatheter arterial chemoembolization (TACE), surgical resection and orthotopic transplantation. The prospect of applying gene-based approaches to the treatment of congenital and acquired conditions of the liver, which include those listed above, constitutes an exciting alternative and departure from conventional therapies. In principle, for example, the introduction of a fully functional corrective low density lipoprotein (LDL) gene into the hepatocytes of FH individuals would mitigate levels of LDL in circulation. Similarly, strategies for the transfer of suicide genes and the introduction of tumor suppressor genes into HC cells are being actively developed [1]. This process may be mediated by two major and diverse approaches. Thus viral-based procedures are generally very efficient but may be mired by concerns relating to immunogenicity, the possibility of insertional mutagenesis, recombination with wild-type viruses and limitations on the size of the genetic cargo carried. Nevertheless they account for two thirds of all recorded clinical gene therapy trials. Although the most extensively developed non-viral vectors are cationic liposomes, they constitute only about 6 % of vectors in clinical trials currently underway [2]. These vectors do not match the high transfection levels associated with viral vectors, but are less immunogenic, cheaper and simpler to manufacture

and may convey large nucleic acid constructs in the form of lipoplexes. It has long been established that the asialoglycoprotein receptor (ASGP-R), which is almost exclusively expressed on the sinusoidal surface of hepatocytes and on many hepatocyte-derived transformed cells provides a convenient target to which vectors may be directed. This may be achieved by decorating lipoplexes with high affinity ligands. Therefore β-D-galactopyranosyl moieties or asialoglycoproteins, which display these hexose sugar units at the distal non-reducing ends of their heteroglycan structures, have been deployed in this regard. The receptor-ligand association is followed by endocytosis of the complex through portals known as coated pits. However a number of obstacles must be overcome for this process to succeed as a gene delivery modality. Lipoplexes are quickly marked for elimination by the reticuloendothelial system (RES) after administration *in vivo*, a problem which has been partly resolved by the development of 'stealth' lipoplexes that are enveloped by a protective biocompatible hydrophilic polymer such as poly(ethylene glycol). This measure reduces opsonization and the binding of serum proteins, which would otherwise markedly change the aspect of vectors perceived by target cells. Moreover stealth lipoplexes, which must display their homing ligand prominently for ASGP-R recognition, are also required to extravasate in the liver sinusoids through endothelial fenestrations of approximately 150 nm diameter and incomplete pericyte coverage of tumors [3] to gain access to hepatocytes. Ligand density is also known to play a critical role in ensuring that complexes are selectively targeted to hepatocytes and not removed by non-parenchymal Kupffer cells (liver macrophages). In addition, lipoplexes exceeding 150 nm are too large for cellular endocytosis through coated pits. Upon cellular entry, lipoplexes must rapidly dissociate from the ASGP-R and escape from the acidic environment of the endosome to ensure that the cargo DNA avoids degradation in lysosomes following fusion events. At some point in its passage through the cytoplasm, the exogenous DNA is separated from its vector and penetrates the nuclear membrane to access the cell's transcription machinery. These stringent requirements have severely tested the ingenuity of liposome engineers, and numerous formulations have emerged to embody solutions to one or more of these issues in individual preparations. Thus 'proton sponge' accessories to facilitate endosomolysis, nuclear localizing signals to improve nuclear entry, variation of cationic headgroups in cytofectins to optimize electrostatic interaction between the DNA and cationic liposomes are a few of the measures which have been explored in the quest for efficient lipoplex-based gene delivery systems, a goal that has yet to be fully realized. Additional problems associated with HC include the variable expression of the ASGP-R observed with changes in the degree of cell differentiation, poor vasculature to large tumors and the need to distinguish between normal and transformed cells in delivery. A promising new direction may yet prove to be more fruitful in this respect. Phage display library biopanning has yielded a heptapeptide with high selective HC specificity [4], which may be appended to lipoplexes for the delivery of transgenic DNA to human hepatoma cells (HepG2) and not normal hepatocytes [5]. Peptides are synthetically very accessible yet display the degree of specificity normally ascribed to proteins. Perhaps this new focus area may benefit from the lessons learnt hitherto in the ongoing journey to realize lipoplex systems with the design capacity for application *in vivo*.

REFERENCES

1. Hwang LH. Gene therapy strategies for hepatocellular carcinoma. J Biomed Sci 2006; 13: 453-468.
2. Ginn SL et al. Gene therapy trials worldwide to 2012 – an update. J Gene Med 2013; 15: 65-77.
3. Perche F, Torchilin VP. Recent trends in multifunctional liposomal nanocarriers for enhanced tumor targeting. J Drug Deliv 2013; 2013: doi 10.11/2013/705265.
4. Zhang B et al. Screening and identification of a targeting peptide to hepatocarcinoma from a phage display peptide library. Mol Med 2007; 13: 246-254.
5. Tu Y, Kim JS. Selective gene transfer to hepatocellular carcinoma using homing peptide-grafted cationic liposomes. J Microbiol Biotechnol 2010; 20: 821-827.