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MINI-REVIEW

Liposome mediated drug delivery for leukocyte adhesion deficieny I (LAD I): Targeting the mutated gene *ITGB2* and expression of CD18 protein

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Abstract **Leukocyte adhesion deficiency (LAD) I is a disorder caused due to mutations in a gene (*ITGB2*) located on chromosome 21 and encodes the β2 subunit of the leukocyte integrin molecules. This leads to defects in the adhesion of leukocytes on endothelial cells which further leads to recurrent microbial infections due to a decrease in the immune response. Base Excision Repair Mechanism (BER) is instrumental in repairing damaged DNA by removing mutated/ damaged bases. We have proposed a hypothesis for the treatment of LAD I by making use of the proteins/enzyme complexes responsible for base excision repair mechanism be introduced into the leukocytes via liposomes. This will target the mutated gene in the leukocytes (mostly neutrophils) and DNA repair will occur. The liposomes can be introduced into the patients via intravenous methods.**

Keywords CD18, β-integrin, LAD I, liposome, BER, ITGB2

Introduction

LAD I is an autosomal recessive disorder caused due to a mutation in a gene responsible for encoding CD18 subunit, which is common for leukocyte adhesion molecules, and as a result β-integrin is not expressed on the leukocyte (Zhang et al., 2000). The leukocyte adhesion molecules are cell surface expressed glycoproteins and include Mac-1 (complement receptor type 3), lymphocyte function-associated antigen-1 (LFA-1), and p150,95 (Anderson and Springer, 1987). This severely impairs the process of leukocyte extravasation, which is responsible for the leukocytes in the circulation to migrate to the site of infection (Kishimoto et al., 1989). The mutated gene which is responsible for LAD I is integrin beta-2 (*ITGB2*) gene, located on chromosome 21. The mutations in the genes are caused due to amino acid substitutions (Arnaout et al., 1990). Due to this affected patients are susceptible to bacterial and fungal infections, impaired pus formation as well as poor wound healing. (Anderson & Springer, 1987). Other symptoms observed are leokocytosis, severe periodontal disease, candidiasis. LAD I is found in every race and ethnicity and cases are found almost in equal number in men and women. As of April 2012, it has been reported in approximately 400 individuals out of which 75% have the severe form of LAD I (where there is less than 1% expression of CD18) (Stephen et al., 2012). However the number may vary due to LAD I being misdiagnosed or undiagnosed due to lack of familiarity.

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Base excision repair (BER)

BER also known as DNA damage repair pathway is a cellular mechanism that is used to repair or replace damaged DNA base throughout the cell cycle. It helps in the processing of small base lesions, which are majorly caused by oxidation and alkylation (Liu et al., 2007). During DNA replication spontaneous errors like mispairing, breaking of bonds may occur and also changes may occur in DNA due to mutation. BER helps in removing the wrong bases. BER mechanism comprises of basically five major protein enzymes that helps in the entire repairing process and the major enzyme used for initiating the mechanism is lesion-specific DNA glycosylases (Chaudhuri and Alt, 2004; Liu et al., 2007). This repair pathway can be sub divided into 2 different pathways,

Short-patch BER and long-patch BER. In short-patch BER only one nucleotide or base can be removed or repaired whereas in long-patch BER we can easily repair or remove 2–13 nucleotides at a time. Each sub pathway relies majorly on the formation of protein complexes that assembles at a particular site of the DNA lesion and side by side coordinates the repair in a coordinate fashion (Fortini and Dogliotti, 2007). BER mechanism comprises mainly of 4 major steps: removal of damaged DNA by DNA glycosylase, removal of deoxyribose phosphate from the backbone, replacement with the correct nucleotide and ligation of the new DNA strand (Liu et al., 2007). Enzymes involved in BER is DNA glycosylase, AP endonuclease, DNA polymerases and DNA ligase (Lehman, 1974; Steitz, 1999; Fromme et al., 2004; Marenstein et al., 2004; Liu et al., 2007).

Applications

BER plays an important role in the fields of pathogenesis of cancer, neurodegenerative diseases, chronic diseases and viral and bacterial infections. BER is a frontline repair system that is responsible for maintaining genome integrity and thus preventing premature aging, cancer and other human diseases by repairing thousands of DNA lesions and strand breaks continuously caused by endogenous and exogenous mutagens. Neurodegenerative diseases are generally caused by oxidative stresses. Some of these diseases are Alzheimer's disease and amyotrophic lateral sclerosis. These are associated with a lower DNA repair capacity in neurons, which may distort their function. Alzheimer's patients inherit 8-oxoguanine glycosylase (*OGG1)* mutations that impair the enzymatic activity. With the help of BER mechanism uracil is substituted in the DNA of the neurons (uracil DNA glycosylase (UNG) suppression causes neuronal apoptosis). This dramatically elevates their sensitivity to amyloid that helps in preventing Alzheimer's disease. DNA glycosylase helps in preventing diabetes and autoimmune diseases too. Recent findings implicate that DNA glycosylases in diabetes mellitus, which is accompanied by enhanced oxidative stress in cells of pancreatic islands of Langerhans can be easily cured by BER method. The OGG1 content in cells dramatically increases in type 2 diabetes mellitus which results in lower insulin sensitivity. With the help of UNG (deletion process) that excises uracil to produce an AP site, which on later stages is attacked by APEX nuclease, multifunctional repair enzyme 1 (APEX1) producing single stranded strands. These strands are later ligated with the correct nucleotide that prevents diabetes. It is possible that DNA glycosylases are also involved in some autoimmune disorders. In particular, the blood of patients with systemic lupus erythematosus (SLE) displays a high content of 8-oxoG in DNA immune complexes, characteristic of the disease and after using BER method it is observed that 8-oxoG monomers appear in the blood and urine just like that in healthy people (Zharkov, 2007; Sidorenko and Zharkov, 2008). In DNA repair, DNA glycosylases are actively studied as agents that are potentially capable of improving the human cell resistance to genotoxic stress (Zharkov, 2007).

Liposomal drug delivery—Interaction with leukocytes

A liposome is an artificially-prepared vesicle composed of a lipid bilayer. The liposome can be used as a vehicle for administration of nutrients and pharmaceutical drugs. Enzymes are protected from metabolism and inactivation in the plasma by liposomes, and due to size limitations in the transport of large molecules or carriers across healthy endothelium, the drug accumulates to a reduced extent in healthy tissues (Mayer et al., 1989; Working et al., 1994). Liposomes can also incorporate enzymes by entrapping them for their delivery into a cell (Finkelstein and Weissmann, 1978). Cationic liposomes can be prepared by using the positively charged DOTAP and the zwitter-ionic DOPE, as a helper lipid. They possess the ability to target the nucleus of a cell and are used in gene therapy (Ristori et al., 2005). Liposomes enter the systemic circulation after a subcutaneous injection. Following the subcutaneous injections liposomes are found in circulation (Allen et al., 1993). Cellular association of liposomal markers is used to evaluate the uptake of liposomes. Naturally also the leukocytes are capable of taking up liposomes (Kuhn et al., 1983). Initial binding, endocytotic internalizaiton, trafficking in the endosome/lysosome compartment, escape from the endosome/lysosome compartment, and transport to the nucleus are the processes the liposome undergoes once inside the cell. Liposomes will target the *ITGB2* gene and DNA repair will occur once the liposome content will be released.

Our hypothesis

We propose a method which integrates liposomal drug delivery of enzyme complexes and base excision repair method. It is known that the DNA glycolsylase along with the following proteins/enzymes are involved in BER mechanism: AP endonuclease 1, Polβ (DNA polymerase beta), Poly [ADP-ribose] polymerase (PARP 1 and PARP 2) DNA-repair protein XRCC1 and DNA ligase 3 (Akbari et al., 2004). PARP is responsible for detection of single strand breaks in the DNA and subsequently gives a signal to the enzymatic machinery involved in SSB (single-stand breaks) repairs (Schreiber et al., 2002). XRCC1 is a DNA-repair protein involved in the repair of single-strand DNA breaks in mammalian cells.

All these proteins (excluding DNA glycosylase) form a complex called BER complex (short patch). This complex is incorporated as it is, into a liposome. DNA glycosylase is

incorporated into a separate liposome. DNA glycosylase should be encorporated in a separate liposome, as it does not complex with any BER enzyme/protein. Liposome 1 (with DNA glycosylase) is injected first, followed by injection of liposome 2 (with BER complex). In this way DNA glycosylase is made to act first followed by the BER complex. Liposomes are injected into the body via subcutaneous injections. This will ensure that the liposomes enter the systemic circulation and thereby be available to interact with leukocytes. Once in the blood stream, the liposomes will encounter and subsequently be taken up by leukocytes (mostly neutrophils). Since the liposomes used are cationic liposomes, they have the ability to target the cell nucleus and thus deliver the enzymes into the nucleus where DNA repair of mutated *ITGB2* gene of chromosome 21 can occur. As a result CD18 can be expressed completely on leukocyte cell surface and can successfully undergo leukocyte extravasation (Fig. 1).

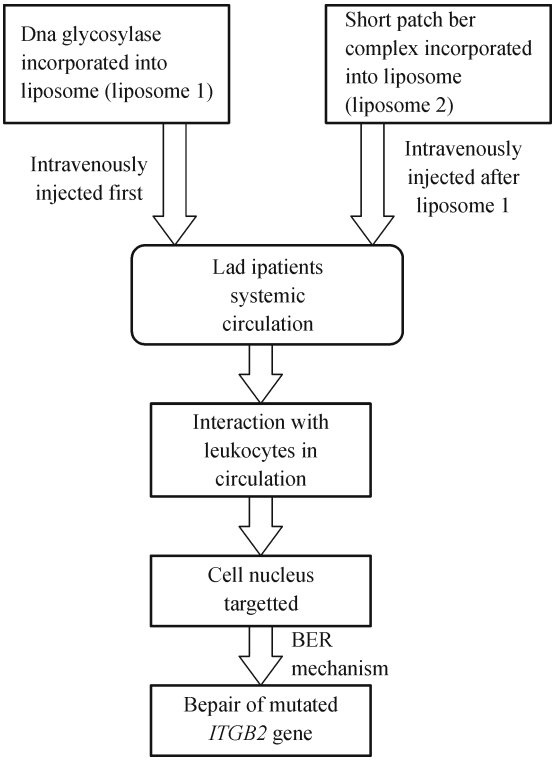

Figure 1 BER targeting the mutated gene *ITGB2*.

Current prospects

Bone marrow and other stem cell transplantation are the therapies of choice in leukocyte adhesion deficiency (LAD) and have a very high success rate. Usage of antibiotics to curb secondary infections, interferon-gamma drugs and leukocyte transfusions are also currently being pursued.

Limitations and precautions

The major problem limitation that can be faced with the proposed mechanism is that the liposomal integrity should be maintained till liposomes reach the leukocytes. Uptake of liposomes by the leukocytes *in vivo* and the interaction of the enzymes with the *ITGB2* gene should occur successfully and efficiently to bring therapeutic effects. For proper uptake of liposomes by leukocytes the liposomes should only target the mutated leukocytes. This can be ensured by coating the liposomes with monoclonal antibodies which are specific to the receptors on the mutated leukocytes in the blood circulation. During the large scale production of liposomes stringent quality control and assurance should be maintained else adverse effects can be seen (Wagner and Vorauer-Uhl, 2011). Cardiovascular and hematological changes can also be seen after administration of liposomes in vivo and can be explained by complement activation which can thereby hinder the mechanism (Szebeni, 1998).

Conclusion

By the use of base excision repair rectification of the damaged DNA can occur through cell cycles. The introduction of two cationic liposomes-one with the BER complex and the other with DNA glycosylase-into the circulation of the patient will ensure that the enzymes/proteins reach the leukocytes and cause therapeutic effects by repairing the damaged DNA and thus ensuring the patients a better, disease free life.

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Compliance with ethics guidelines

C. Subathra Devi, Kritika Kedarinath, Payal Choudhary, Vishakha Tyagi, Mohanasrinivasan. V declare that they have no conflict of interest.

This manuscript is a review article and does not involve a research protocol requiring approval by the relevant institutional review board or ethics committee.

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