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Review

Exploring plant lectins in diagnosis, prophylaxis and therapy

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Plant lectins are bioactive glycoproteins possessing carbohydrate-recognition domain which exhibit regiospecific, cell-surface specific and reversible interactions with glycoconjugates. Since, they can decode biological information, lectins play a pivotal role in mediating and triggering diverse cellular phenomena. They serve as powerful tools in immunological studies and can be employed as immunohistochemistry markers in diagnosis of cancer and profiling of cell surface types due to expression of aberrant glycans on diseased and transformed cells. Because of its affinity for mucin, lectin-mediated vaccine delivery seems to be a promising concept in the design of mucosal immunization via oral route of administration. Lectin-based glycotargeting provides site-specific and controlled release of drug from polymeric microparticulate systems and can be used for delivery to epithelial lining of gastrointestinal (GI) tract, eye, and pulmonary tract. Therefore, plant lectins are instrumental in diagnosis, prophylaxis and treatment of a wide array of diseases.

Key words: Lectin, agglutinin, immunohistochemistry marker, mucosal adjuvant, lectin-functionalized drug delivery system.

INTRODUCTION

Plant lectins are a class of predominantly multimeric, carbohydrate-binding proteins of nonimmune origin, possessing at least one catalytic domain, occurring in cereals and vegetables (Lei and Xhang, 2009; Gupta et al., 2009). They contain typically two or more carbohydrate–binding sites and multivalent binding to cell surface oligosaccharides can occur. They play a crucial role in plant defense and are being investigated extensively for their applications in human healthcare delivery and biotechnology, because of their multifarious involvement in several biological phenomena. Recognition and subsequent binding of lectins to cell surface glycans, which include monosaccharides, oligosaccharides, polysaccharides and their conjugates, is specific and reversible without producing any structural alterations. Existence of a toxic substance, ricin, with ability to agglutinate human and other animal blood cells was first noted by Stillmark (1888) in the crude extracts of castor beans (*Ricinus communis*) which has been now recognized as type 2 ribosome inactivating proteins (RIP) (Hamid et al., 2013). However, the term lectin was first coined by Boyd and Shapleigh (1954) from the Latin verb *legere* (to select) owing to their selective hemagglutination potential and this is why they are also called hemagglutinating proteins (Becer, 2012; Texeira et al., 2012; Melnykova et al., 2013). Isolation of Concanavalin A (Con A) from jack bean (*Canavalia ensiformis*) seeds proved to be the stepping stone in the field of lectinology

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(Granell et al., 2010). The network of communication based on carbohydrate-lectin interactions is at the focus of different biological processes like lymphocyte transformation, cell growth, inflammation, cancer metastasis, precipitation of certain polysaccharides and glycoproteins and host-pathogen interactions. Participation of lectins in such a vast array of intra- and inter-cellular phenomena suggests their applications for therapeutic purpose. The ability of plant lectins to decipher the code in glycoconjugates and recognize complex and aberrant glycans makes them appealing candidates for a wide range of diagnostic and prognostic applications in cancer and other diseases. They are resistant to digestive proteolytic breakdown and hence, can be used in design of oral dosage forms. Lectins can act as new generation mucoadhesives, because of greater specificity where they bind to receptors and not the mucus itself. This characteristic feature encourages the development of lectin-based targeted mucoadhesive drug delivery systems ensuring improved bioavailability and reduced toxicity. Presence of membrane-anchored specific glycoconjugates on cell surface enables lectins to be used for development of targeted drug delivery systems. Strong mucosal immunological responses and better bioavailability can be achieved by encapsulating antigens into lectin-grafted multiparticulate carrier systems. This approach enhances the binding of particles to the epithelial cells of the mucosa-associated lymphoid tissue. In order to enhance their scope and utility and reduce their toxicity, plant lectins have been modified by subjecting them to site-directed mutagenesis (Melnykova et al., 2013; Diesner et al., 2012; Khan et al., 2011; Maenuma et al., 2009).

Plant lectins are mostly distributed in the seeds of members of the Leguminosae family and they are structurally similar with affinity for both lactose and mannose. Examples of such lectins are *Phaseolus vulgaris* phytohemagglutinin (PHA), soybean lectin (SBA), *Ulex europaeus* lectin (UEA-1), peanut agglutinin (PNA), *Pisum sativum* (pea) agglutinin (PSA), *Lens culinaris* (lentil) agglutinin (LCA), chickpea seed lectin and jack bean Con A (Granell et al., 2010; Khan et al., 2012; Islam and Khan, 2012). However, another commonly known lectin, wheat germ agglutinin (WGA) is extracted from wheat and belongs to the family Gramineae. Lectins like tomato lectin and potato lectin are obtained from the Solanaceae family (Islam and Khan, 2012). Mannose-binding lectins have been isolated from Amaryllidaceae family (*Hippeastrum sp. hybrid*) and snowdrop (*Galanthus nivalis*), Alliaceae, Amaryllidaceae, Orchidaceae, Liliaceae, Iridaceae and Araceae. They interact with mannose and mannose-containing N-glycans. Another group of mannose-specific lectins related to jacalin have been screened (Gupta et al., 2009; Lu et al., 2012).

Structurally, plant lectins may be classified as (1) merolectins like hevein from the rubber tree *Hevea brasiliensis,* wheat germ agglutinin, potato lectin and tomato lectin (Granell et al., 2010), (2) hololectins which include most of the plant lectins, (3) superlectins e.g. tulip bulb lectin and lastly, (4) chimerolectins which include ricin and abrin (Texeira et al., 2012).

Knowledge of structural classification of plant lectins is essential for a better understanding of their affinities for specific cells. This knowledge will enable their proper utilization in various aspects of disease management.

A BRIEF INTRODUCTION TO CELLULAR SPECIFICITIES OF SOME WELL-KNOWN PLANT LECTINS

Mistletoe lectin

Mistletoe lectin (ML), isolated from European mistletoe (*Viscum album*) exhibits immunoadjuvant activity, produces cytokines from immune-related cells and enhances the activity of natural killer cells. Depending on carbohydrate-specificities, three types of mistletoe lectins have been identified. ML-I consists of an A chain (molecular weight: 29 kDa) which is similar to the A chain of ricin and inhibits the elongation step of protein biosynthesis. The B-chain (molecular weight: 34 kDa) is specific for D-galactose. The two chains are connected by disulfide linkages. The B-chain binds to the glycan moieties on the surface of cancer cells and facilitates the entry of the A-chain which is highly cytotoxic and induces killing of the tumor cells. ML-II interacts with both galactose and N-acetylgalactosamine, whereas ML-III has a high affinity for N-acetylgalactosamine only. Lectin extracted from Korean mistletoe (KML-C) possesses two dissimilar lectins with identical carbohydrate-binding domain and which are different from that of European mistletoe lectin and may be used in the treatment of autoimmune disorders and malignancies (Souza et al., 2013; Jung et al., 2011).

Con A

Con A interacts specifically with D-mannopyranoside or D-glucopyranoside ring and identifies pentasaccharide core of oligosaccharides. It can exist in dimeric and tetrameric form (at neutral pH). Its binding sites are 72 Å apart from each other. It is involved in T cell activation, up-regulation of Toll-like receptors (TLRs), production of nitric oxide and proinflammatory cytokines and regulation of calcium ion influx into human neutrophils. The dimeric form is less potent in inducing neutrophil oxidative metabolism (Becer, 2012; Diesner et al., 2012; Gupta et al., 2009; Cohen et al., 1980).

Wheat germ (*Triticum vulgaris*) agglutinin

Wheat germ agglutinin is a 36 kDa homodimeric dietary

lectin composed of two identical glycine- and cysteine-rich subunits connected by 16 disulphide bridges. It recognizes N-acetyl-D-glucosamine and sialic acid present on the cell membrane of human colonocytes and prostate cancer cells. Each of its four carbohydrate-binding sites is present at the interface of two inter-catenary domains and at a distance of 14 Å. It has been revealed that all eight binding sites are simultaneously functional. It can be transported across the intestinal wall and reach the systemic circulation in functionally-intact form. It also has an affinity for enterocyte-like Caco-2 cells where it enters the cytoplasm (Becer, 2012; Beckmann et al., 2012; Vaz et al., 2011; Zhang et al., 2005; Rieux et al., 2006).

Tomato lectin

Tomato lectin (TL) binds to N-acetyl-D-glucosamine, present in the glycoproteins of rat intestinal brush border mucosa and is resistant to enzymatic degradation (Zhang et al., 2005; Rieux et al., 2006).

U. europaeus agglutinin

Lectin or agglutinin from *U. europaeus* (UEA 1) interacts only with murine α-L-fucose residues. It possesses high affinity for highly specialized microfold (M) cells, present in the follicle-assisted epithelium (FAE). Therefore, it can promote macromolecular transport across intestinal epithelial barrier (Zhang et al., 2005).

P. vulgaris agglutinin

PHA binds reversibly to bisected bi- and tri-antennary complex N-glycans of villous and crypt epithelia and exhibited no interaction with goblet cells (Souza et al., 2013; Gabor et al., 2004).

PLANT LECTINS AS MEDIATORS OF CELLULAR PROCESSES

Lectins are instrumental in mediating and triggering diverse cellular phenomena, because of their role as cell recognition mediators whereby they decode glycocode. They may therefore be involved in different cell-cell communication events as subsequently explained.

Immunoregulation

Lectins serve as important tools in immunological studies owing to the crucial role played by carbohydrate-protein interactions in the immune system. They exhibit immuno-modulatory properties which are initiated by their interaction with cell surface glycans, which may trigger signal transduction producing cytokines. This ultimately results in elicitation of immune responses against tumors or microbial diseases. They can induce both Th1 and Th2 immunity. Several plant lectins may act as Toll-like receptor agonists (Souza et al., 2013). They are known to inhibit stimulated T cells and immobilize Fc and epsilon receptors (Chandra et al., 2006). Both the A- and B-chains of mistletoe lectin I exhibit immunomodulatory activity. The A-chain promotes release of interleukins from human lymphocytes. Immunomodulatory activity of B-chain is due to its stimulation of immune cells through the activation of iNOS pathway (Sung et al., 2013). Garlic lectin caused an increase in NO production which resulted in higher levels of IFN-γ in humans (Clement and Venkatesh, 2010). Korean mistletoe lectin has been found to modulate cytokine expression in murine splenocytes and can produce a shift in Th1/Th2 cellular immune response (Lee eta al., 2009). Con A, PHA and other plant lectins with different carbohydrate specificities can affect neutrophil agglutination, migration to different body cavities, degranulation, regulation of oxidative metabolism, release of cytokines, phagocytosis and apoptosis (Pereira et al., 2012; Cohen et al., 1980).

Hemagglutination

The role of lectins in agglutinating erythrocytes was the first noted physiological activity which has been studied extensively. Novel lectin isolated from the bulbs of *Pinellia ternata* (PTL) also possesses hemagglutinating potential (Zuo et al., 2012).

Induction of mitosis

Con A and PHA can initiate mitosis in resting cells and induce T-cell blastogenesis (Lei and Chang, 2009). The A-chain of European mistletoe lectin I has been shown to possess mitogenic activity.

ROLE OF PLANT LECTINS IN DIAGNOSIS, PROPHYLAXIS AND TREATMENT OF DISEASES

As markers in disease diagnosis

Lectins are used in investigation and understanding of alterations manifested during physiological and patho-logical processes. Since lectins are highly selective with respect to their saccharide-binding potential and can identify aberrant glycoconjugates, they find extensive applications in histochemical techniques for detection, isolation and characterization of cell surface glycol-proteins. Different plant lectins have been screened to exploit their marker functions in various diseases like peanut agglutinin in identifying hematopoietic cell sub-populations, soybean agglutinin in preparation of proper

cell fraction for bone marrow transplantation (Lu et al., 2012). There are reports of lectins being used as markers for characterization of normal and transformed tissues in cases of mammary, uterine and cerebral neoplastic tumors (Beltrao et al., 2003).

As targeting ligands for oral mucosal vaccination

Plant lectins may be employed as potential antigen-delivery mucosal adjuvants in oral vaccine development. Oral route of immunization can trigger both systemic and mucosal immune responses by entering the gut-associated lymphoid tissue (GALT) through the M cells. The major physiological obstacle in development of this otherwise meritorious strategy is the susceptibility of the antigens to acidic and enzymatic degradation before reaching the target site. Immunological barrier imposed due to interference by the lactogenic immunity, such as neutralizing antibodies and milk factors forms another hurdle. Moreover, the success rate is highly limited by the low availability of M cells as well as poor uptake at the Peyer's patches. Transcytosis of the antigens by M cells, subsequent uptake by the dendritic cells, followed by translocation to the underlying lymphoid tissue and interaction with the immune cells like T- and B-cells form the basis of generation of immune responses in case of mucosal vaccination. M cells are characterized by the absence of an extensive mucus layer and reduced enzymatic activity. Expression of specific surface markers, like α-L-fucose-specific lectin on mouse M cells is also known. Therefore, screening for substances with safe and effective mucosal adjuvant activity by augmenting uptake by the M cells can widen the scope of induction of antigen-specific immune responses. This requirement seems to be fulfilled by the introduction of plant lectins, which are stable in low pH condition and resistant to action of pepsin, trypsin, pancreatin and elastin, when studied *in vitro* (Kim et al., 2012; Azizi et al., 2010; Gabor et al., 2004; Devriendt et al., 2001). Plant lectins may be targeted to specific locations in the intestinal tract since they can recognize regioselective as well as cell-specific carbohydrate molecules.

Since the optimal balance of the immune reaction is dependent on the pathogen in question, induction of the desired type of immune response should be tailored for each specific vaccine and vaccine adjuvant, which can be achieved by the use of proper delivery systems. Encapsulation of lectin adjuvants in microparticulate polymeric systems is a viable option to deliver the vaccines to specific target sites and the encapsulated vaccines are protected from the metabolizing enzymes present in the mucus layer (Banerjee et al., 2013). Covalent coupling of UEA-1 with liposomes and polystyrene microspheres led to adherence, rapid endocytosis by murine M cells after oral administration, in contrast to BSA-coated nanoparticles. UEA-liposomes also enhanced IgA and IgG levels and production of IL-2 and IFN-γ in spleens indicating a Th1-dominant immune response. CTL (cytotoxic T-lymphocytes) responses are augmented in mice immunized with poly-L-lysine conjugated to UEA (Ulex europaeus agglutinin) and complexed to a plasmid encoding the HIV envelope (Souza et al., 2013). Grafting of WGA (Wheat germ agglutinin) to liposomes and nanoparticles through carbodiimide chemistry demonstrated increased *in vitro* uptake as well as receptor-mediated endocytosis or transcytosis by murine intestinal epithelial cells, porcine and human enterocytes and Caco-2 monolayers as compared to EGylated ones and UEA-grafted ones. Similar results were obtained with Con A-grafted polystyrene nanoparticles and peanut agglutinin nanoparticles. Since, tomato-derived lectin binds to rat, porcine and human enterocytes *in vitro*, are efficiently taken up from rat gut when coupled to nanoparticles. Acidic denaturation of mistletoe lectin was prevented by stabilizing lectin with alginate/chitosan microcapsules coated by a biodegradable polymer wall. These studies indicate the targeting potential of lectin-coupled nanoparticles. Triggering of mucosal immune responses and potentiation of cell-mediated immunity through enhanced expression of Th1 polarizing cytokines by stabilized, cell-targeted lectinised nanoparticles opens up a novel approach for oral antigen delivery (Hamid et al., 2013; Diesner et al., 2012; Granell et al., 2010; Rieux et al., 2006; Chen et al., 2011; Li et al., 2011).

As antiviral agents

Among diverse applications of plant lectins in different types of disease management, antiviral activity demands considerable attention. High glycoprotein content on the viral envelopes is the target for action of plant lectins. Mannose-binding plant lectins showed high affinity for corona viruses, impeded viral attachment in early stage of infection and thus can be used in the treatment of severe acute respiratory syndrome (Hamid et al., 2013). Several plant lectins have been found to possess antiretroviral activity e.g. anti-HIV activity of snowdrop lectin, *Hippeastrum* hybrid lectin, Con A, WGA, LCA, PSA and a jacalin-related lectin from banana fruit (BanLec) (Hamid et al., 2013; Granell et al., 2010). They bind to high mannose-glycosylated envelopes of HIV (e.g. BanLec binds to gp120), inhibit the syncytium formation between HIV-infected cells and uninfected CD4 cells and therefore, prevent the entry of virus into CD4 cells. Intranasal immunization with inactivated HIV encapsulated in Con A-immobilized polystyrene nanospheres in macaques and mice offers partial to almost complete protection against the viral infection (Gupta et al., 2009; Gavrovic-Jankulovic and Prodanovic, 2011). Extra long autumn purple bean lectin displayed anti-HIV activity by inhibition of HIV-1 reverse transcriptase (Hamid et al., 2013). Intranasal immunization via UEA-1-conjugated PLGA microparticles encapsulating HIV peptides resulted in enhanced and prolonged antibody titers in mice,

compared to rectal, oral and intramuscular routes of vaccination (Rajapaksa and Lo, 2010).

In protein and peptide delivery

Reduction in blood glucose levels in diabetic mice or rats by oral administration of WGA-modified insulin liposomes and solid lipid nanoparticles (SLN) shows the possibility of lectins in transporting protein and peptide drugs. Drug delivery system consisting of Con A-glycosyl-polyethylene glycol-insulin complex has demonstrated a pulsatile, and reversible release pattern for insulin in response to altered blood glucose levels (Gupta et al., 2009). Pharmacological efficacy of calcitonin has been improved by Carbopol-based nanocarriers whose surface was modified by WGA (Diesner et al., 2012).

In cancer management

Cancer research has been specifically enriched by the advancements in the field of lectinology since lectins are equally effective in diagnosis and treatment of cancers. Alteration in protein glycosylation and increased sialylation are hallmark features of cancer cell surfaces which serve as targets for lectin-based markers in histochemical studies and therefore, lectins can be used to reveal the stages of carcinogenesis. For example, ovarian cancer biomarker (CA125), breast cancer biomarker (Her2/neu) and prostate cancer biomarker (prostate specific antigen) are all glycoproteins which can be readily recognized by lectins with selective sugar-binding potential. Plant lectins, like Con A and UEA 1 have been exploited in immunohistochemical assays as markers for pancreatic cancer, parotid gland mucoepi-dermoid carcinoma with low, intermediate, and high grade dysplasia. PHA-coupled bionanocapsules have been employed for *in situ* cancer imaging (Gupta et al., 2009; Zhao et al., 2006; Texeira et al., 2012). Histological subgroups of meningioma have been identified due to diversity in lectin-binding pattern by use of peanut agglutinin (PNA), Con A, UEA-1, *Dolichos biflorus* (DBA) lectin and WGA. WGA and UEA-1 can distinguish fibroblastic meningioma whereas *P. pendula* lectin (PpeL) recognizes meningothelial tumor. Senile changes in the brain can also be manifested by lectin-based markers, because age changes monosaccharide distribution and biochemical metabolism in brain (Beltrao et al., 2003).

Plant lectins have been explored in cancer manage-ment as anti-cancer agents. Anti-cancer activity is attributed to their ability to adhere to cancer cell membrane, to cause apoptosis-induced cytotoxicity in mitochondrial-dependent pathway, to increase the content of reactive oxygen species (ROS), to trigger autophagy or necrosis in cancer cells. Death of cancer cells by lectins like Con A may also proceed through stepwise activation of macrophages for up-regulation of Toll-like receptors (TLRs), enhancement of cytokine production and finally the participation of the recruited lymphocytes. Natural killer cells may also take part in Con A mediated anti-tumor effect. Con A has been designated to be a novel class of endogenous cancer vaccine immunotherapy. It is capable to generate tumor-cell specific immune response at the later stages of tumor eradication and induce autophagic activity. It has been found to be less toxic to normal murine hepatocytes in tumor-bearing mice as compared to those in naïve mice. In addition to its dose- and time-dependent cytotoxic action, it can also inhibit the tumor nodule formation. Moreover, Con A may trigger caspase-dependent apoptosis in human melanoma A 375 cells. Similar mechanism has also been postulated for another lectin (SFL) isolated from leguminous plant, *Sophora flavescens.* Korean mistletoe lectin is reported to inhibit tumor metastasis in murine cells which has been attributed to activation of NK cells and macrophages. Induction of apoptosis and telomerase inhibition results in anticancer effect on human A253 cancer cells (Lee et al., 2009). Legume lectin, extracted from *Phaseolus coccineus* seeds promoted apoptotic death of murine fibrosarcoma L929 cell line.

Del Monte banana lectin and dark red kidney bean hemagglutinin exhibited anti-proliferative effect on leukemia L1210 cells. Small glossy black soybean lectin retarded proliferation of MCF 7 cells. Anti-hepatoma effect has been demonstrated against HepG2 cell lines by extra long autumn purple bean lectin, Del Monte banana lectin as well as soybean lectin. Anti-neoplastic effect of Korean mistletoe lectin has been investigated on B16-BL6 melanoma cells, human A 253 cancer cells. Mistletoe lectin is already popular in Europe as adjuvant in breast cancer therapy. Among the three lectins isolated from the plant, ML-I was found to be most toxic towards human MV3 melanoma cells *in vitro*. PSA and LCA have demonstrated inhibitory action against hepatic tumor nodule formation in BALB/c mice (Hamid et al., 2013; Texeira et al., 2012; Lei and Chang, 2009). PTL inhibited growth of Sarcoma 180, HeLa and K562 cell lines in a dose-dependent fashion (Zhuo et al., 2012). Agglutinin from *Pinellia pedatisecta* causes death of cancer cells through interaction with methylosome (Lu et al., 2012). Cytoadhesive and cytoinvasive WGA has been found to be therapeutically active in bladder cancer and it enhances absorption of poorly available drugs by opening up a receptor-mediated pathway. WGA has been found to possess high degree of affinity for four metal-based anticancer agents-cisplatin, Pt porphyrin and two gold porphyrins (Bogoeva et al., 2012; Hamid et al., 2013; Plattner et al., 2009; Gabor et al., 2004). Two photo-sensitisers with anticancer activity, Fe porphyrin and Pd porphyrin have been found to exhibit high degree of affinity for WGA. Since WGA can recognize transformed cancer cells, this interaction has the potential to target drugs to specific cancer cells during photodynamic therapy. Similar observations have been obtained with

snake gourd lectin, Con A, pea lectin, etc (Bogoeva et al., 2011). Covalent linking of a porphyrin to a plant lectin (Moringa G) was able to recognize tumor-associated T and Tn antigens. The porphyrin-based photosensitiser was quickly taken up by the Tn-positive Jurkat leukemia cells and the cancer cells suffered phototoxicity (Pernot et al., 2013). Furthermore, the epidermal growth factor (EGF)-receptor plays a pivotal role in WGA-mediated drug delivery to cancer cells. Lectin from *Haliclona crater* and frutalin, a lectin extracted from *Artocarpus incisa* induced apoptotic cell death of HeLa cells in a time- and dose-dependent manner (Hamid et al., 2013; Texeira et al., 2012). Lectin-monoclonal antibody conjugates may exert cytotoxic effects by binding to malignant cells (Hamid et al., 2013).

LECTIN- FUNCTIONALISED SITE-SPECIFIC DRUG DELIVERY SYSTEMS

A crucial point in human therapy is to achieve a satisfactory balance between the toxicity and therapeutic effect of medicines. Site-specific delivery reduces toxic effect at non-target sites and enhances the efficacy of the therapeutic agent. Successful site-specific drug delivery depends on specific molecular-receptor interactions with specific cells and this criterion is most effectively met by the plant lectins having a definite structural configuration. In diseases, like inflammation and cancer, specific carbohydrate moieties are expressed on cell glycocalyx which play a pivotal role in biological recognition processes. Surface modification of nanoparticles with lectins allows their use as carrier systems toward specific targets. Lectin-coated formulations can target active molecules to α-L-fucose, present sparsely on the mucus-coated glycocalyx of M cells (Diesner et al., 2012). Con A-grafted PEGylated liposomes showed enhanced vesicle fusion and reduced non-specific adhesion and coalescence (Bakowsky et al., 2008). Lectin-functionalized silica beads have been shown to possess a strong affinity for Caco-2 cell monolayers (Cunning et al., 2008). Internalization of WGA within single cells of human 5637 bladder cancer cells demonstrated the presence of N-acetyl-d-glucosamine, sialic acid and alpha-l-fucose residues on the membrane surface. This indicated the potential of WGA to target drugs to bladder cancer cells (Plattner et al., 2008). Lectins can therefore be used in delivering drugs to the epithelial lining of different organs, pulmonary tract, etc.

Epithelial drug delivery

Lectin-based reverse glycotargeting is a novel approach where the carrier is decorated with exogenous lectins. Propensity of lectins to interact with mucins present on the absorptive epithelial cells leads to bioadhesion and enables development of second-generation specific mucoadhesive drug delivery vehicles which are less prone to mucus turnover rates. Once the lectin-grafted drug delivery system penetrates the mucus layer, the lectin interacts with the glycocalyx layer of each underlying cell. Presence of high concentration of drug in the vicinity of absorptive barrier accelerates the rate of absorption and simultaneously diminishes the effect of degradative luminal enzymes (Gabor et al., 2004). At neutral pH, the lectin-binding capacity of mucin was in the order of WGA >> UEA-1>> LCA = potato lectin >> peanut lectin > *D. biflorus* agglutinin. This indicates that the structure of the saccharide residues governs the intensity of interaction with lectin. Moreover, it has been observed that binding of WGA and tomato lectin to N-acetyl-glucosamine occurs throughout the whole small intestine, whereas binding to sialic acid was variable and less. Mannose-specific lectins bind to jejunal epithelial cells to low extent and M cells to moderate degree. Since, transformed cells as in cancer or cells during any pathological condition express very specific glycans or modified glycocalyx on the cell surface, lectins can be used as carrier molecules to target drugs and for anchorage at the sites of choice, like oral epithelia, airway epithelium. Specific binding of lectins to apical cell membrane promotes vesicular transport processes and enhances drug absorption. Surface modification of liposomes with lectins permits differentiation among intestinal epithelial cells, change in morphology and increase in the average diameter of the liposomes. Mucoadhesive polystyrene latex nanoparticles conjugated with tomato lectin facilitated strong and specific interaction with mucin. Site-specific treatment of *Helicobacter pylori* infections with clarithromycin could be achieved for a prolonged duration of 6 h by designing gastroretentive Con A conjugated microspheres. Antimicrobial effect of UEA-gliadin nanoparticles (GNP) and Con A-GNP was found to be higher as compared to naïve GNP in the treatment of *H. pylori* infections. Therefore, lectin-conjugated colloidal carriers such as nanoparticles or liposomes show great promise in enhancing drug bioavailability at desired sites (Gupta et al., 2009; Gabor et al., 2004; Zhang et al., 2005).

Another approach to lectin-based glycotargeting relies on the preparation of prodrugs where a spacer links the lectin targeting the glycoprotein and the drug which is the therapeutic entity. The spacer enhances the ability of lectins, immobilized on surface of colloidal carrier to interact with the biomembrane. Upon activation within the target cell, the drug will elicit pharmacological activity. In this regard, effect on colon cancer cells was studied by coupling WGA to doxorubicin via cis-aconityl spacer, where the drug molecule is released only after reaching the colon (Gabor et al., 2004).

Ocular drug delivery

Lectins have been found to bind on corneal and

conjunctival surfaces and can be utilized for their ocular bioadhesive properties. An interesting study revealed the utility of *Solanum tuberosum* (potato) lectin in targeting rat and rabbit precorneal tissues in *ex-vivo* experiments since the lectin binds specifically to N-acetyl-D-glucosamine. Thus, proper screening may find lectins to be used in the development of non-irritant and safe ocular drug delivery systems (Gavrovic-Jankulovic and Prodanovic, 2011).

Pulmonary drug delivery

Coupling of lectins like WGA, Con A and soybean agglutinin were found to improve the binding and uptake of liposomes containing amphiphiles to the by alveolar type II epithelial cells. Interaction between the sugar residues and Con A releases water which triggers the recognition system. Peanut lectin-mediated gene transfer proved to be a good non-viral vector for gene therapy of cystic fibrosis as it manifested selective binding to ciliated and non-ciliated cell populations of airway epithelium (Gupta et al., 2009; Gabor et al., 2004).

TOXICITY OF PLANT LECTINS

Application of plant lectins such as, PHA, Con A, WGA and red kidney bean lectin in vaccination is marred by their toxicity and anti-nutrient effect, as observed experimentally in rats and pigs, even at nanomolar concentrations. PNA interacts with glomeruli and abnormal IgA to cause IgA nephritis and it is reported to be responsible for bowel cancer. WGA has been reported to induce toxicity in human pancreatic cells in a dose-dependent manner (Kilpatrick, 2008). Con A is experimentally used to induce T cell mediated hepatic injury in animal model. Though, proteolytic stability is a common feature of most of the plant lectins, potato lectin is not resistant to such degradation. It is reported that lectin from red kidney bean is responsible for diarrhea, malabsorption, growth suppression and overgrowth of mannose-sensitive *E. coli* (Gupta et al., 2009; Granell et al., 2010; Gabor et al., 2004; Devriendt et al., 2001). Moreover, they have been found to be highly immunogenic to varying degree, to induce inflammatory response and cause GI irritation. Immunogenicity may reduce the benefits of lectin-functionalized formulations. In sensitive individuals, lectins can induce severe intestinal damage disrupting digestion, provoke IgG and IgM antibodies responsible for food allergies and in extreme cases, can cause anemia, because of hemagglutination. Lectin-based drug delivery systems should therefore, never be used in persons with reported food allergies (Vaz et al., 2012; Rieux et al., 2006).

However, recent attempts have been made to reduce the toxicity of lectins like UEA and mistletoe lectin by producing recombinant lectins possessing same binding affinity (Diesner et al., 2012).

Conclusion

Lectins have a plethora of physiological and immunological implications. Plant lectins have been studied as powerful biorecognition weapons, because of existence of a wide array of oligosaccharide-binding specificities. Immunohistochemistry has been profusely enriched with detailed investigation on various aspects of plant lectins, leading to their utility as markers in diagnosis of different types of pathological conditions. Most of them are stable to gastrointestinal conditions and are able to enter into systemic circulation. They constitute an important class of oral immunomodulators. The uptake of lectins via the Peyer's patches stimulates secretory IgA-mediated mucosal immune response and transcellular transport across the enterocytes favors systemic IgG-mediated systemic immune response. Lectins, being multifunctional, can be used as drugs themselves or can be used for targeting of other therapeutically active molecules. Potential selective lectin-sugar interaction can be exploited for targeting and intracellular trafficking of drugs in specific cells and tissues, receptor-mediated bioadhesion and enhancing vesicular transport processes in the GI tract. Since, lectins play a pivotal role in enco-ding biological information through cell-cell interactions and cell-routing, new perspectives in disease diagnosis, prophylaxis and treatment are being continuously explored in an attempt to identify novel and encouraging therapeutic approaches beneficial to mankind.

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