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Lectin-mediated drug targeting: history and applications

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Abstract

The purpose of this paper is to review the history of using lectins to target and deliver drugs to their site of action. The hour of birth of "lectinology" may be defined as the description of the agglutinating properties of ricin, by Herrmann Stillmark in 1888, however, the modern era of lectinology began almost 100 years later in 1972 with the purification of different plant lectins by Sharon and Lis. The idea to use lectins for drug delivery came in 1988 from Woodley and Naisbett, who proposed the use of tomato lectin (TL) to target the luminal surface of the small intestine. Besides the targeting to specific cells, the lectin–sugar interaction can also been used to trigger vesicular transport into or across epithelial cells. The concept of bioadhesion via lectins may be applied not only for the GI tract but also for other biological barriers like the nasal mucosa, the lung, the buccal cavity, the eye and the blood–brain barrier.
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Keywords: Lectin; Protein–sugar interaction; Bioadhesion; Drug delivery; Gene delivery; Transcytosis; Vesicular transport; Endocytosis

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1. Introduction

Lectins are proteins that recognise and bind to sugar complexes attached to proteins and lipids. They do this with very high specificity for the chemical structure of the glycan arrays. It is generally acknowledged by the lectin scientific community that "lectinology" began in 1888 when a young doctor, Hermann Stillmark, at the University of Dorpat (now Tartu in Estonia), presented a thesis describing the agglutinating properties of ricin, which had been extracted and partially purified from castor seeds [1]. It has been noted [2], however, that even earlier, in the 1860s, the agglutinating activity of certain snake venoms had been observed. The word 'agglutinin' was widely used to describe molecules and extracts that caused the clumping together or agglutination of erythrocytes and other cells. It was not until the 1950s that the word 'lectin' was coined to describe the substances from plants that recognised and distinguished the blood group substances on the basis of the different sugars expressed [3].

The modern era of lectinology might be considered to have started with the seminal paper of Sharon and Lis in 1972. Most lectins described at that time were isolated from plants and were being widely used as tools, particularly in histopathology; thanks to the high levels of specificity that lectins demonstrated for different cell types, both normal and pathological, as well as for subcellular structures. Sharon and Lis listed different lectins that had been purified and a further seven plant extracts known to agglutinate red cells and since then the number has increased dramatically [4]. In that 1972 article, a number of potential uses for lectins are mentioned, but 'drug delivery' is not one of them. Since 1972, a considerable number of lectins have also been identified from animal sources (for a recent review, see [2]).

2. The rationale behind lectin-mediated drug targeting

The rationale behind lectin-mediated drug targeting is very simple. Most cell surface proteins and many lipids in cell membranes are glycosylated and these glycans are binding sites for lectins. The combination of a small number of sugars can produce a vast range of different chemical structures. Different cell types express different glycan arrays and in particular, diseased cells, such as transformed or cancerous cells, often express different glycans compared with their normal counterparts. Therefore, lectins could be used as carrier molecules to target drugs specifically to different cells and tissues.

Apart from the concept of using the specificity of protein–sugar interactions for targeting to specific cells only, this kind of receptor-mediated bioadhesion

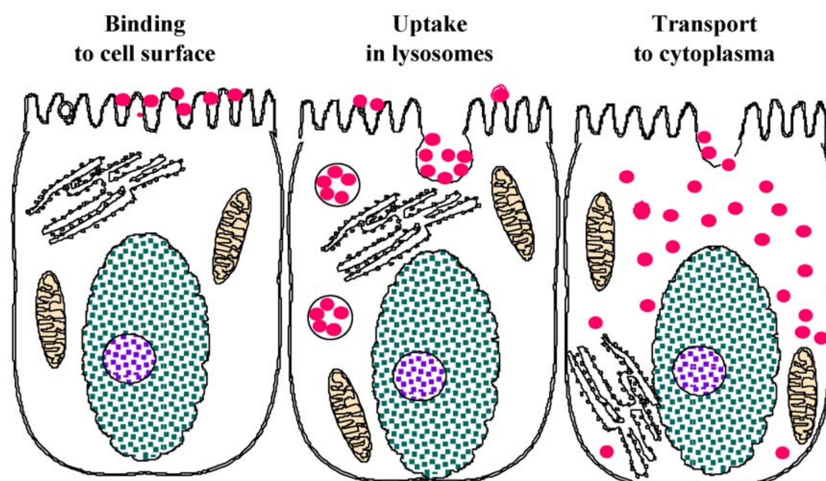

Fig. 1. The targeting potential of bioadhesive lectins at epithelial barriers: binding, internalisation and intracellular transport (e.g. into or bypassing acidic endosomal compartments) of lectins and lectin-conjugates depends on the protein–sugar interaction (from [79]).

may also be used to convey signals to cells in order to trigger vesicular transport processes into or across polarised epithelial cells. Haltner et al. [5] compared cellular binding and endocytosis of three *N*-acetylgalactosamine specific mistletoe lectins (ML I–III) with the *N*-acetylglucosamin specific lectins from stinging nettle (UDA), tomato (TL/LEA), wheat germ (WGA) and a succinylated derivative of the latter lectin (WGAs) to monolayers of human intestinal epithelial Caco-2 cells and in particular studied the temperature dependence of this process on FITC-labelled lectins. While the *N*-acetylgalactosamine-specific ML's showed identical binding curves at 4 and at 37 °C, the four *N*-acetylglucosamine specific lectins showed a marked difference in binding at the two temperatures. Temperature independent lectin binding suggests that those ligands only bind to the outer cell surface and are not internalised. If binding is reduced at 4 °C compared to 37 °C, which was observed for LEA and WGA, the lectins are likely to be endocytosed. Surprisingly binding of UDA and WGAs, was significantly reduced at 37 °C compared to 4 °C, which may be explained by cellular uptake and subsequent transport to acidic intracellular compartments, where the fluorescence of FITC was quenched. Together, such data suggest that depending on the lectin structure and sugar-specificity, lectins may or may not be endocytosed, and if so, may or may not be using an acidic endosomal pathway (Fig. 1). Thus, when coupled to macromolecular drugs or particular drug carriers, the selection of a suitable lectin may perhaps allow the cellular uptake and subsequent intracellular routing of such delivery systems to be controlled.

3. Lectin-mediated targeting to the gastrointestinal (GI) tract: the early studies

It was just 100 years after Stillmark's thesis presentation that Woodley suggested that lectins might be used to target the GI tract. In a paper presented at the 15th Annual Conference of the Controlled Release Society in Basle in 1988, he proposed the use of tomato lectin (TL) to target and bind to the luminal surface of the small intestine, that is the lectin would demonstrate bioadhesion [6]. Bioadhesion has been defined as the attachment of a drug carrier to a specific biological location [7]. The objective was to exploit the property of bioadhesion to slow down the intestinal transit time of oral drugs and thus enhance their bioavailability.

TL had been shown to have specificity for *N*-acetylglucosamine and derivatives such as its tetramer [8]. It was chosen because it was easy to purify, had been shown to bind to the intestinal mucosa of rats and was relatively resistant to degradation by intestinal enzymes. In addition TL was non-toxic to rats [9,10] and the fact that raw tomatoes were consumed by millions of people world-wide suggested it was not toxic to humans. Using radiolabelled TL, Naisbett and Woodley showed that it bound strongly to rat intestinal mucosa in vitro, targeting a number of the major glycoproteins of the intestinal brush border [11]. As a consequence of binding to mucosal cells, it was also transported across the mucosa in vitro in significantly higher amounts than other macromolecules [12]. The binding was inhibited by a number of sugars, which are specific binding targets of the lectin, confirming the true lectin nature of the interaction.

When the studies moved in vivo the results were less encouraging. The intestinal transit of orally administered radiolabelled TL was studied by administering the lectin into the stomach of rats and measuring the radioactivity in different sections of the small intestine at 1, 5, 10 and 24 h. The distribution was compared with the distribution of an inert polymer, polyvinylpyrrolidone (PVP) and albumin as a degradable protein of similar molecular weight. The TL showed resistance to digestion compared with the albumin, which was completely degraded during passage down the gut. However, while there was some retention of the TL in the upper parts of the GI tract, there was not a major difference in the transit between TL and the PVP [13]. The researchers concluded that the lectin was probably binding to the intestinal mucus, which is constantly being turned over and carried down the gut by peristalsis in a continuous flow. The mucus turnover time in rats has been estimated to be between 47 and 270 min [14]. Due to the mucus layer covering the intestinal epithelium, it is not evident that the cell surfaces are often completely exposed. Subsequently, the interaction between TL and mucin was confirmed by other researchers [15–17]. The work described by Naisbett

and Woodley was carried out with the soluble lectin, and it was clear that the next step was to investigate lectin-modulated bioadhesion using realistic pharmaceutical drug carriers, such as nanoparticles or liposomes, as described in the next section.

4. Bioadhesion studies with lectin-conjugated micro- and nanoparticles

Lehr et al. conjugated TL to the surface of polystyrene microspheres and demonstrated that they bound to isolated enterocytes. Inhibition of binding by tetra-*N*-acetyl glucosamine confirmed that the lectin was responsible for the interaction, and it was also observed that mucin reduced the binding [15]. As observed by Naisbett and Woodley, the transit time of the TL-conjugated particles down the gut was not significantly different from control particles [18]. Similar results were obtained with lectins specific for mannose (*Galanthus nivalis* agglutinin) or mannose/glucose (concanavalin A). Other lectins tested did, however, modify the intestinal transit profile: for example, kidney bean lectin (*Phasseous vulgaris* agglutinin) led to delayed transit of the microspheres and a broad distribution down the small intestine after administration. Irache and colleagues conjugated lectins, including TL, to polystyrene latex beads and demonstrated in vitro binding to intestinal segments and mucus [19]. The same group has also made lectin-conjugated nanoparticles. For example they conjugated a fucose specific lectin from *Ulex europaeus*, to nanoparticles made from gliadin, and demonstrated binding of these particles to mucin [17]. Montisci et al. have used radiolabelled degradable polylactide microspheres and conjugated to them, TL or the lectin from *Lotus tetragonolobus*, which is fucose specific [20]. Studies of the intestinal transit of the labelled microspheres orally administered to rats showed an overall delay in GI transit that was almost entirely due to retention of the lectin-conjugated microspheres in the stomach. Strangely this retention did not appear to be lectin specific as it was not reduced by preincubating the conjugates with the lectin specific sugars. Thus the use of lectin targeting to the GI tract to reduce the transit time of pharmaceutical formulations has to date had limited success.

5. Lectin-mediated drug absorption enhancement

The early studies of Woodley and Naisbett had shown that TL could cross the intestinal mucosa in vitro [12]. They used an improved everted rat gut sac to demonstrate that the lectin was endocytosed by small intestine into the enterocytes and that intact lectin could be detected on the serosal side of the gut. At a saturating concentration (15 μg/ml) the uptake into the cells was some 40-fold greater than that of the control inert polymer, PVP, and transfer across the mucosa some 8-fold greater. Using Caco-2 cells, Lehr and Lee [21] showed that compared with BSA as control, the cells took up/bound TL as well as phytohemagglutinin L and E. However, the transcytosis across the cell layer was low and the rate for lectins no higher than the control. On the other hand, using the same cell line, Russell-Jones et al. [22] were able to demonstrate translocation across the cell layer of nanoparticles which had been conjugated to the lectins wheat germ agglutinin (WGA), concanavalin A (Con A) and LTB, the binding subunit of heat labile toxin from *E. coli*. These in vitro studies suggested that conjugation of lectins to suitable drug formulations or the conjugation of drugs to lectins as carrier molecules might enhance drug delivery to the epithelial cells and/or to the systemic circulation. Florence and his colleagues at the London School of Pharmacy have pioneered studies on the intestinal uptake of microparticles in the face of prevailing dogma [23]. In 1997, they reported the results of an in vivo study in rats where they had conjugated TL to 500 nm polystyrene nanoparticles and fed them to rats over a period of 5 days. There was a systemic uptake of 23% of the dose compared with the controls, which were the same nanoparticles but in which the lectin active sites had been blocked with a competing sugar. If this scale of absorption could be achieved in man, then there is potential for an effective oral drug delivery system [24] (Table 1).

Another type of lectin interaction that has been used for similar purposes is that of bacterial adhesion and invasion factors, proteins that bind to cell surfaces. The 192 amino acid carboxy terminus of the invasin of *Yersinia pseudotuberculosis* has been shown to promote the binding and uptake of microparticles in both cultured mammalian cells and the rat intestine, causing them to enter the systemic circula-

Table 1
Lectins described in this article and their use in drug delivery

Lectin	Abbrev.	Source	Specificity	Use	Reference
Viscum album agglutinin	ML-I-III	Mistletoe	D-Gal; GalNac	targeting to GI	[5]
Lycopersicum esculentum agglutinin	LEA/TL	Tomato	$(GlcNAc)_3$	targeting to the lungs (alveolae, type I cells)	[49]
				targeting to GI and uptake (UDA, WGA)	[5-6], [8-13], [15-21], [24]
Urtica dioica agglutinin	UDA	Stinging nettle	GlcNAc		[5]
Triticum vulgaris agglutinin	WGA	Wheat germ	$(D\text{-}GlcNAc)_2$; NeuNAc		[5], [22],
				targeting to the lung	[51-52],
				targeting to the eye	[56],
				targeting to the blood-brain barrier	[58]
Galanthus nivalis agglutinin	GNA	Snowdrop	Manα3Man	targeting to GI (GNA, ConA, PHA, UEA-I, LTA)	[18]
Canavalia ensiformis agglutinin	ConA	Jack bean	α-D-Man, α-D-Glc		[18], [22]
Phaseolus vulgaris agglutinin	PHA	Kidney bean	Oligosaccharide		[18]
Ulex europaeus agglutinin	UEA-I	Gorse	L-Fucα1,2Galβ1,4GlcNAcβ		[17]
Lotus tetragonolobus agglutinin	LTA	Asparagus pea	Fucα(1-2)Galβ(1-4)Fucα(1-3)-GlcNac Fucose		[20]
Bandeireae simplifolia isolectin B_4	BSI-B_4	Griffonia	α-D-Gal	targeting to nasal mucosa	[34-36]
Maclura pomifera agglutinin	MPA	Osage-orange	GalNAc	targeting to the lungs (alveolae, type II cells)	[50]
Ricinus communis	RCA	Castor bean	D-Gal	targeting to the lungs (alveolae, type I cells)	[50]
E. coli heat-labile toxin B subunit	LTB	*E. coli*	Gal	targeting to GI (LTB, CT)	[22], [27]
Cholera toxin	CT	*Vibrio cholerae*	GM1 ganglioside		[27]

Gal, galactose; GalNAc, *N*-acetylgalactosamine; GlcNAc, *N*-acetylglucosamine; NeuNAc, sialic acid; Man, mannose; Fuc, fucose.

tion [25,26]. Additionally there are many bacterial and plant toxins, which gain entry into intestinal epithelial cells by receptor-mediated endocytosis. Amongst the bacterial toxins are the A-B5 toxin of *Vibrio cholerae*, the *E. coli* heat-labile toxin (LTB) and the toxin of *Shigella shigae*. The B-subunit of this toxin binds to glycolipids or glycoproteins on the luminal surface of the epithelial cells and triggers endocytosis [27]. Even if these adhesion/invasion factors are not lectins in the strict sense, these mechanisms of bioinvasion have definite potential for drug delivery.

6. The reverse situation: targeting to endogenous lectins

While the majority of lectins used and studied are from plant or microbial origin, it has become clear in recent years that there exist numerous animal lectins [2]. In the gut, it was known that certain bacteria expressed glycan containing molecules in their cell walls that bound to the epithelial surfaces via lectin interactions, indicating that there were endogenous lectins exposed on epithelial cell surfaces which could be targeted by sugar bearing drug formulations [28,29] In the 1980s, synthetic polymers bearing pendant sugar moieties were synthesised as potential drug carriers by Kopecek and his colleagues and these were tested for interaction with the GI tract. Different sugars gave different profiles of interaction with gut tissue, with galactose bearing polymers showing greater interaction in proximal regions of the gut, while fucose bearing polymers consistently showed the greatest interaction and were more specific for distal gut regions [30]. This work has been advanced in Kopecek's laboratory and is discussed later in this issue (Minko).

Another class of endogenous lectins which bind via specific protein–sugar interactions are the selectins. They have been recognised to play an important role

in inflammatory processes [31]. For instances, they are involved in the rolling and extravasation of leukocytes on the endothelial surface of blood vessels. As such phenomena are also relevant to transport processes at biological barriers in the context of controlled drug delivery, a particular chapter by Bakowsky et al. has been dedicated to the selectins in this issue.

7. Other lectin targeting possibilies in the GI tract: specific cell types and diseased tissues

Given the fact that different cell types, both normal and diseased, express different glycan arrays on their surfaces as well as demonstrated over the years by the use of lectins as histochemical tools; the idea of using lectins as targeting molecules for cell specific drug delivery is both attractive and feasible, and has generated considerable interest. In particular the targeting to the gut associated lymphoid tissue (GALT), manifested as the Peyer's patches, has clear possibilities for the development of more effective oral vaccines. Immunisation by the oral route has the considerable advantage of generating secretory antibodies in addition to systemic immunity. The Peyer's patches are covered by specialised cells called M-cells which endocytose and process macromolecules to stimulate a chain of immunological events. Lectin targeting to M-cells has been the subject of intensive studies as described in detail later in this issue in the article by Jepson et al.

The other possibility for lectin targeting is to diseased tissues, notably of the colon. Inflammatory diseases of the colon and cancer of the colon are both major medical problems. The possibility, therefore, of targeting drugs specifically to the diseased cells is very exciting and because of their specificity lectins may well have the potential to achieve this goal. For example, *E. coli* K99 fimbriae have been used to target the corticosteroid 6-methyl-prednisolone to the affected part of the GI tract of patients with Crohn's disease [32,33]. The group of Kopecek has been concentrating on the development of drug-bearing polymers with lectins to target to diseased cells. Again, these issues will be covered in more depth in the later articles by Minko and by Gabor.

8. Lectin-mediated delivery to the nasal mucosa

While the surface area of the nasal mucosa is relatively small (150 cm^2), it is highly vascularised and has a relatively permeable membrane. In addition, ease of access via the nasal cavity, the lack of first pass metabolism and rapid onset of action make it an interesting site for drug administration. The nasal cavity also contains the equivalent of the GALT in the form of the nasal-associated lymphoid tissue (NALT) covered by an epithelial layer of M-cells. Thus it is also a site of considerable interest for its potential, for the administration of vaccines, particularly as vaccination via the nasal cavity induces both systemic and mucosal immunity. On the other hand the main disadvantage of drug administration to the nasal mucosa via the nasal cavity is the low retention time due to rapid mucociliary clearance: the residence half life is between 15 and 30 min. This makes it a good candidate for the use of bioadhesive systems to increase the contact time between drug or immunogen and the sites of absorption. While there are many studies on the nasal administration of bioadhesive formulations, the emphasis has been on the use of polymers such as chitosan and carbopols, and to date there are few studies using lectins. Using the isolectin B4 from *Bandeiraea simplicifolia* 1 (BSI-B4) Giannasca et al. demonstrated lectin-mediated targeting of antigen to hamster M-cells, resulting in the production of specific serum IgG, and Kumar et al. have demonstrated that equine nasopharyngeal tonsillar tissue contains M-cells that react with a lectin from *Bandeireae simplicifolia*. [34,35]. This latter lectin (GS I-B4) has also been shown to be almost exclusively M-cell specific for rat NALT, in contrast to other lectins tested (UEA-1, DBA, WGA) and it suppressed the uptake of yeast particles by the M-cells [36]. Thus although studies on lectin targeting to the upper respiratory tract are still very preliminary, the possibilities for vaccine administration look interesting.

9. Lectin-mediated delivery to the lungs

Compared with the nasal cavity, the lungs have a very large surface area (75 m^2) and the thinness of the alveolar epithelium (0.1–0.5 μm) may facilitate rapid drug absorption. As with the nasal cavity, first pass

metabolism is avoided, and the relative lack of proteolytic enzymes (compared with the gut, for example) makes the pulmonary administration of peptides and proteins an attractive proposition.

Early histological data and more recent studies revealed the binding of lectins to tissues of the airway epithelium [37–39]. In addition it has been shown that several lectins that bound to the apical surfaces of lung cells in culture were actively taken up by the cells [40]. This binding and uptake of lectins by lung cells including carcinoma cells has particularly attracted the attention of gene therapists to enable them to target and enhance the uptake of DNA. Thus, for example, lectins have been shown to target a plasmid selectively to carcinoma cells [41] and to enhance the lipofection efficiency of different lung carcinoma cells [42].

The disease that has attracted most attention for gene delivery to lung cells is cystic fibrosis (CF), a relatively common disease (one in 2000–2500 Caucasian births) with several identified mutations in the gene coding for a membrane chloride transporter. In early studies, lectins were shown to enhance gene transfer of polylysine and/or histone plasmid complex to CFT1 cells, a human tracheal cell line derived from a CF patient homozygous for the ΔF508 mutation. More recently the reverse use of lectin targeting, i.e. targeting sugar bearing carriers to endogenous lectins has been exploited. Fajac et al. [43,44] have developed glycosolated polycations (glycofectins) and complexed them with DNA. These complexes were more efficient at transfection than naked DNA. The most successful were lactosylated complexes that promoted both cellular uptake and intracellular trafficking to the nucleus.

Further down the respiratory tract the alveolar epithelium constitutes the major barrier to macromolecular drug absorption into the pulmonary circulation [45,46]. The alveolar epithelium consists of two distinct epithelial cell types: the cuboidal type II cells, that produce the lung surfactant and serve as progenitor cells for the type I cells [47,48]. Type I cells cover 93% of the surface of the alveolae and appear as very thin cells with protruding nuclei providing a short diffusion path for gas exchange. Both cell types possess a different lectin binding specificity. While *Maclura pomifera* agglutinin binds specifically to type II cells, *Ricinus communis* agglutinin and TL show specific binding to type I cells [49,50]. Brück et al. (2001) showed the binding of lectin-functionalised liposomes to human alveolar cells in primary culture and the uptake of FITC-labelled dextrans encapsulated in these liposomes [51]. Nebulisation of these functionalised liposomes did not significantly influence their physical stability and cell binding capacity and led to a deposition of the liposomes in the lower parts of the lung. These results make the functionalised liposomes potential candidates as macromolecule-drug carriers for local and systemic administration [52].

10. Lectin-mediated drug delivery to the buccal cavity

The buccal cavity has a surface area of $\sim 50\ cm^2$ with a relatively poor permeable non-keratinised epithelium. Like the nasal cavity, due to the large flow of saliva, substances in the buccal cavity have a low residence time (< 5–10 min) and hence it is a prime site for the use of bioadhesive formulations, some of which are now marketed. Lectin targeting to this site is being actively investigated and is dealt with in detail in a later section of this volume (J. Smart).

11. Lectin-mediated ocular drug delivery

There are two major surface tissues of the eye facing the outside world, the conjunctiva and the cornea. The conjunctiva contains goblet cells secreting mucin, but there are no goblet cells on the cornea. Mucus is spread over both epithelia by the action of blinking, and bioadhesive polymers will attach to the conjunctival mucus. The turnover rate of the mucin is ~ 15–20 h, whereas normal tear turnover time is 16%/min. It has been suggested that ocular drug delivery may be prolonged by conjugation to lectins that adhere to the corneal and conjunctival epithelia, and this may enhance drug absorption across these epithelia. The conjunctiva has associated lymphoid tissues (CALT), although it is still unclear if the associated epithelium contains equivalents to the M-cells of the intestine [53]. The presence of lectin binding sites has been demonstrated on the corneal and conjunctival epithelia of humans and other spe-

cies [54,55]. Schaeffer et al. [56] showed that pretreatment of rabbit corneas with wheat germ agglutinin increased the binding of ganglioside-containing liposomes, and carbachol entrapped in these liposomes showed enhanced flux across the cornea. At the present time, there does not appear to be the development of major lectin-based systems for ocular drug delivery. Because the systemic absorption of ocularly applied drugs is relatively low, the risk of systemic side effects of the lectins should be relatively small.

12. Lectin-mediated delivery at the blood–brain barrier

The endothelial cells of brain capillaries lack fenestrations, have few pinocytic vesicles and form very tight junctions, which are responsible for the formation of the blood–brain barrier (BBM), which restricts the movement of most molecules from the blood to the brain [57]. The BBM is a formidable barrier to entry of many drugs into the brain, notably anticancer drugs, and a number of different approaches have been proposed to overcome the limited access of drugs to the brain. Fischer and Kissel [58] showed the binding of some plant lectins to primary endothelial cells isolated from porcine brain, especially WGA, which seems to be a good candidate for drug targeting to the blood–brain barrier due to its high affinity for the cerebral capillary endothelium compared with other lectins and its low cytotoxicity. In addition WGA has been shown to enhance the uptake of HIV-1 gp 120, usually slow at crossing the blood–brain barrier, without disrupting the barrier function [59].

13. Targeting the liver asialoglycoprotein receptor: drug and gene delivery

As already described for gene targeting to lung cells, genes can be targeted to their destination by conjugation to sugar moieties specific for animal or reverse lectins. The best-described example of such an animal lectin is the asialoglycoprotein receptor (ASGPr), a C-type animal lectin that is expressed on the surface of hepatocytes [60]. It plays a role in the clearance (endocytosis and lysosomal degradation) of deasialylated proteins from the serum [61,62]. The ASGPr recognises terminal ß-D-galactose or *N*-acetylgalactosamine-residues [63]. This receptor has been investigated as a site for drug targeting using carriers bearing galactose residues. The most advanced example of such systems is the *N*-(2-hydroxypropyl) methacrylamide (HPMA) anticancer polymer conjugates developed by Ruth Duncan and her colleagues [64]. These consist of a linear polymer backbone with drugs attached to the backbone via short peptide sequences that are hydrolysed by intracellular enzymes to release the drug. Targeting moieties can also be affixed to the polymer backbone. One of these conjugates (PK2, FCE28069) consists of the HPMA backbone with ~ 7.5 wt.% doxorubicin and ~ 1.5–2.5 wt.% galactose to target to the ASPGr. This compound has been studied in Phase I/II clinical trials and gamma camera imaging confirmed 15–20% of the dose targeted to the liver at 24 h following administration [65,66]. Other carriers to target the receptor have been proposed, notably liposomes. For example, Yu and Lin showed that asialofetuin-labelled liposomes enhanced the delivery of the hydrophilic molecule inulin into hepatoma cells and they are potential drug carriers for the intracellular delivery of membrane-impermeable drugs to liver cells [67].

The ASPGr has also been investigated for its potential in enhancing gene transfer into hepatic cells. The first approaches to use this receptor for the specific targeting of genes to liver cells have been undertaken by Wu et al. [68,69]. They used PLL-particles coupled to an asialoglycoprotein moiety to complex DNA. They were able to successfully transform rat and rabbit liver with this approach, but the transformation was only transient and with low efficiency [70,71]. To overcome these drawbacks, efforts have been made to make more stable and soluble particles with a well defined structure [72,73]. Lipoplexes are a non-viral gene delivery system formed when cationic liposomes are mixed with plasmid DNA. This system has also been optimised for gene targeting to the liver by the conjugation of modified galactolipids [74,75]. Hara et al. developed asialofetuin-labelled liposomes as a vector system that is effective in gene expression in vivo after intraportal injection in adult mice [76,77]. As far as the in vivo efficiency has been documented the major limitation

of most of these methods is the need for local administration or a partial hepatectomy. Recently, Arangoa et al. developed improved protamine-enhanced-asialofetuin-lipoplexes. Addition of protamine sulphate leads to smaller complexes that are better suited for efficient endocytosis. A nuclear localisation signal in the protamine sequence results in efficient targeting to the nucleus and obviates the need for partial hepatectomy [78].

14. Conclusions

From modest beginnings as potential tools for specific drug targeting and bioadhesion applications some 20 years ago, lectins are realising a number of important applications in the field, as reflected by the articles in this issue. Some of the problems associated with any macromolecular targeting system still have to be tackled, notably those of toxicity and immunogenicity. It is hoped that some of these problems might be overcome in the future by the application of biotechnology techniques to produce quantities of smaller fragments of lectins that will retain the high target specificity that these fascinating molecules possess, but will be easier to manipulate. The use of lectins in drug targeting is a fledgling subject that will surely grow in the years to come.

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