Expert Opinion

Nanodisks: hydrophobic drug delivery vehicles

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Members of the class of exchangeable apolipoproteins possess the unique capacity to transform phospholipid vesicle substrates into nanoscale disk-shaped bilayers. This reaction can proceed in the presence of exogenous hydrophobic biomolecules, resulting in the formation of novel transport vehicles termed nanodisks (NDs). The objective of this study is to describe the structural organization of NDs and evaluate the utility of these complexes as hydrophobic biomolecule transport vehicles. The topics presented focus on two distinct water insoluble drugs, amphotericin B (AMB) and all *trans* retinoic acid (ATRA). *In vitro* and *in vivo* studies reveal that AMB–ND display potent anti-fungal and anti-protozoal activity, while ATRA–ND show promise in the treatment of cancer. The versatility conferred by the presence of a polypeptide component provides opportunities for targeted delivery of ND to cells.

Keywords: all *trans* retinoic acid, amphotericin B, apolipoprotein, nanodisk, phospholipid

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1. Versatility of reconstituted high density lipoproteins

Emerging from detailed studies of lipoprotein metabolism is the concept that the genesis of high density lipoproteins involves the formation of nascent discoidal particles that ultimately mature into spherical lipoproteins [1]. This process can be mimicked *in vitro* by taking advantage of the lipid surface-seeking properties of members of the class of exchangeable apolipoproteins (Apo) [2]. More than a dozen human plasma apolipoproteins have been characterized and shown to share the ability to generate disk-shaped particles *in vitro*. Reconstituted high density lipoprotein (rHDL) has been employed in studies of cholesterol esterification [3], cellular cholesterol efflux as part of the reverse cholesterol transport pathway [4], HDL maturation [5] and structure–function studies of apolipoprotein–lipid complexes [6-8]. This wealth of fundamental knowledge has generated interest in alternative applications of rHDL. An intriguing application involves the infusion of rHDL into human subjects to facilitate regression of atheroma [9,10]. The observation from population studies that a variant form of the major protein component of HDL, apolipoprotein A-I (apoA-I_{Milano}), confers cardioprotection [11,12] has led investigators to employ this variant in rHDL therapy. Likewise, others have employed rHDL-like particles as miniature membranes for solubilization and characterization of transmembrane proteins [13]. Finally, spherical rHDL have been studied as vehicles for drug delivery [14,15]. In the present context discoidal rHDL with unique structural and morphological properties, termed nanodisks (ND), have been characterized. Thus, for the purpose of this review we define NDs as disk-shaped rHDL that contain additional small molecule hydrophobic components such as antibiotics, bioactive lipids or drugs. The concept to be developed is that NDs can serve as vehicles for solubilization, transport and targeted delivery of otherwise insoluble, potentially therapeutic, compounds.

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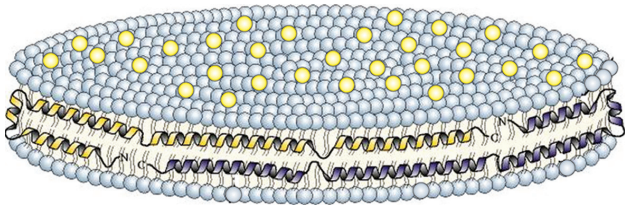

Figure 1. Generalized nanodisk structure. Phospholipid vesicles, bioactive agent and apolipoprotein are combined to form an ND. The ND particle structure is comprised of a disk-shaped phospholipid bilayer in which a given hydrophobic bioactive agent (dots) are integrated. The edge of the ND is stabilized through apolipoprotein binding to the disk perimeter.
Adapted from Redmond *et al.* [43].

1.1 Nanodisk structure

A model structure of a ND particle is depicted in **Figure 1**. The particles are comprised of a disk-shaped lipid bilayer whose edge is protected by interaction with amphipathic apolipoprotein molecules. The protein component of a ND circumscribes the perimeter of the bilayer disk, stabilizing the interface between the disk edge and the aqueous environment. A key feature of the apolipoprotein component is the existence of a series of amphipathic α-helices that align in a belt-like manner, with their hydrophobic face directed toward the phospholipid fatty acyl chains, while their polar face is directed toward the solvent. In this manner the apolipoprotein component defines the disk boundary and serves as a "scaffold" that maintains ND particle integrity. The third component of ND is variable and may be any hydrophobic molecule that can be stably integrated into the lipid milieu of the ND bilayer. Thus, NDs may be distinguished from conventional liposomes or vesicles in the following ways:

1. NDs do not possess an aqueous core.
2. Scaffold proteins constitute an intrinsic structural element of NDs.
3. ND diameters range from 8 – 20 nm versus 60 – 250 nm for liposomes.
4. Unlike liposomes, NDs are fully soluble in aqueous media.

1.2 Nanodisk particle formulation

Detailed studies of members of the class of exchangeable apolipoproteins have given rise to two general methods of ND formation: detergent dialysis and direct conversion. Whereas the detergent dialysis method [16] has the advantage that a broad spectrum of bilayer-forming phospholipids can be employed, a disadvantage relates to the potentially poor yield of incorporated bioactive agent due to loss during the extensive dialysis required to remove detergent from the system. While limited to relatively few phospholipid substrates, the direct conversion method does not employ detergents and can result in high bioactive agent solubilization/incorporation efficiency. The types of phospholipids that are commonly used in the direct method are synthetic saturated glycerophospholipids, such as dimyristoylphosphatidylcholine (DMPC) or dimyristoylphosphatidylglycerol (DMPG) or the sphingolipid, egg sphingomyelin [17]. These lipids generally possess a saturated fatty acyl chain with a gel to liquid crystalline phase transition temperature in the range of 23°C. Normally, the phospholipid substrate is hydrated and induced to form bilayer vesicles, either by membrane extrusion [18] or sonication. It has been found that incubation of such phospholipid vesicles with apolipoprotein at or near the lipid phase transition temperature results in ND formation (in this case empty ND). It is likely that this reaction proceeds most efficiently in this temperature range because defects in the bilayer surface represent sites for apolipoprotein penetration, bilayer disruption and transformation to ND. Among the apolipoproteins that have been examined for their ability to transform phospholipid bilayer vesicles into ND are apoA-I [19], apoE [20], apoA-IV [21], apoA-V [22] and apolipophorin III [23]. In addition, it is known that peptide fragments of apolipoproteins [23,24] or designer peptides [25] can substitute for apolipoproteins in this reaction and serve as the scaffold component of ND.

Thus, myriad combinations of phospholipid and 'scaffold' molecules can be employed to formulate unique ND particles. Empty NDs, lacking hydrophobic biomolecule/drug cargo, as well as drug-bearing NDs, are most readily characterized for particle size by non-denaturing native gel electrophoresis [26], while particle morphology can be evaluated by electron microscopy or atomic force microscopy [27].

2. Amphotericin B nanodisks

The prototype hydrophobic biomolecule employed in studies of NDs as drug delivery vehicles is the polyene antibiotic, amphotericin B (AMB). AMB is produced by *Streptomyces nodosus* and is a member of a large family of macrolide polyene antibiotics that display potent antifungal properties [28]. AMB is insoluble in water and functions by complexing with membrane sterols to form pores that permit leakage of cellular contents, which, ultimately, leads to cell death. Whereas AMB shows a preference for the fungal membrane sterol ergosterol, interaction with cholesterol in host membranes also occurs and is likely to be responsible for the toxic effects of this drug in patients [29]. Although it is widely used for treatment of systemic fungal infections, AMB administered as a deoxycholate micelle formulation displays dose-limiting nephrotoxicity [30]. In the past decade lipid formulations of AMB have been developed that significantly attenuate AMB toxicity, permitting higher doses to be administered with reduced toxicity [31]. Among these are a liposomal formulation (AmBisome®), an insoluble complex with DMPC and DMPG (Amphotericin B lipid complex; ABLC; Abelcet®) and a complex with cholesteryl sulfate that exists as a colloidal dispersion

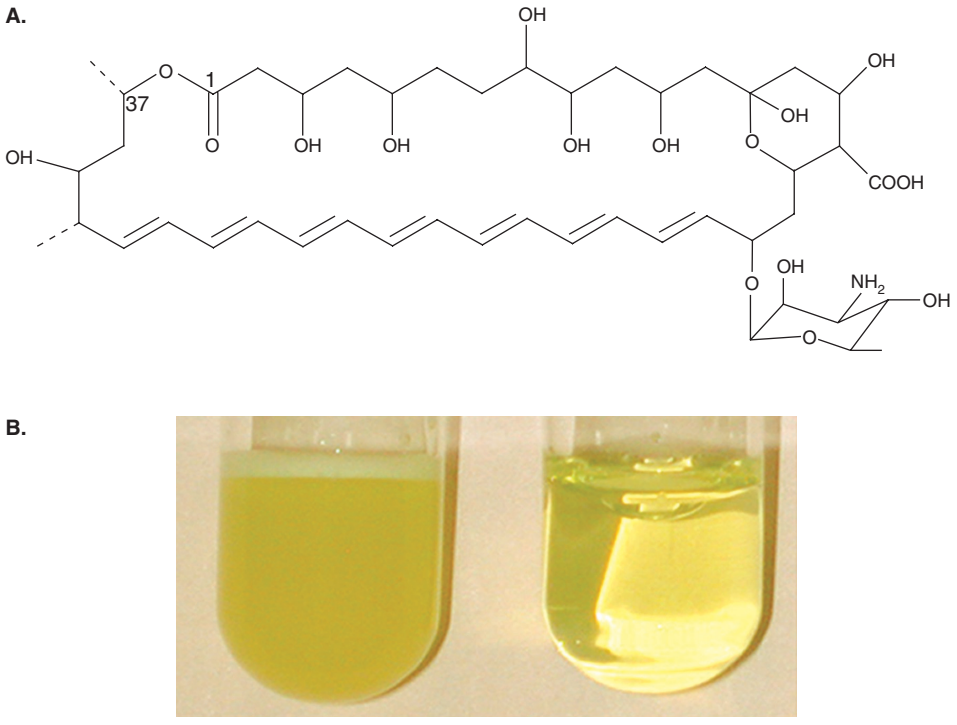

Figure 2. ApoA-I induced solubilization of AMB. A. Amphotericin B structure. **B.** Phospholipid vesicle/AMB mixture before (left) and after (right) the addition of apoA-I.
With permission from Oda *et al.* [32].

(Amphotec®). Although more expensive than conventional AMB–deoxycholate, these formulations have an improved toxicity profile. At the same time, existing lipid formulations of AMB are of relatively large size and are insoluble or poorly soluble in aqueous media.

To formulate AMB–ND, isolated recombinant apoA-I was incubated with a vesicle substrate comprised of DMPC/DMPG (70:30 w/w) in the presence of AMB [32]. This procedure results in stable incorporation of AMB into ND (**Figure 2**) with a solubilization efficiency of 98% [33]. Electron microscopy analysis of the product particles indicated the presence of disk-shaped complexes with diameters in the range of 10 – 25 nm [32,27]. On the basis of characterization studies including density gradient ultracentrifugation, it was concluded that the membranous environment of ND serves as a matrix for solubilizing AMB. This interpretation was confirmed by spectroscopic analysis, taking advantage of the intense yellow color of AMB and the unique spectral signature of the molecule as a function of self-association [33]. More recently, atomic force microscopy experiments showed that AMB induces compression of ND bilayer thickness, while infrared spectroscopy analysis revealed that the presence of AMB does not alter the mean angle of ND phospholipid fatty acyl chains, nor does its presence lead to a general lipid disorder [27]. Taken together, the results are consistent with AMB-induced bilayer interdigitation [34], a phenomenon that is likely to contribute to AMB-dependent pore formation in susceptible membranes.

Given that AMB–ND and ABLC are comprised of similar lipids, the ability of apolipoproteins to transform these insoluble, micron-sized lipid sheets into ND was evaluated [35]. Studies revealed that incubation of ABLC with apoA-I induced complete solubilization of ABLC components with formation of ND. Transformation of ABLC into ND preserved the potent biological activity of AMB, as well as the reduced toxicity of the ABLC formulation. ABLC-derived AMB–ND offers advantages over conventional ABLC in terms of solubility, storage and nebulization potential, while the presence of a protein component provides an intrinsic 'handle' for tissue-specific targeting via protein engineering (see below).

2.1 Scaffold protein versatility

Whereas most studies to date have employed apoA-I as the scaffold protein for AMB–ND, it is evident that other apolipoproteins as well as peptide mimics of apolipoproteins can also function to induce ND particle formation and stabilize the product particles. In studies designed to evaluate whether a peptide can be substituted for the function of the apolipoprotein component of ND with respect to particle formation and stability, an 18-residue

synthetic amphipathic α-helical peptide, termed 4F (Ac-D-W-F-K-A-F-Y-D-K-V-A-E-K-F-K-E-A-F-NH_2), was employed [25]. 4F solubilized vesicles comprised of DMPC at rates that were equivalent to rates observed with apoA-I. The inclusion of AMB in the vesicle substrate resulted in the formation of 4F AMB–ND and negative stain electron microscopy revealed that AMB–ND prepared with 4F possessed a disk-shaped morphology similar to ND prepared without AMB or prepared with apoA-I. In yeast and pathogenic fungi growth inhibition assays, 4F AMB–ND were as effective as apoA-I AMB–ND. Thus AMB–ND generated using a synthetic peptide in lieu of apoA-I retain potent biological activity. Given their intrinsic versatility, peptides may be preferred for scale up and clinical application of AMB–ND. Given that a defining characteristic of members of the class of exchangeable apolipoproteins is the ability to form disk particles, it is evident that any one of a host of well-characterized apolipoproteins may be used to generate AMB–ND.

2.2 *In vitro* and *in vivo* biological activity of AMB–ND

AMB–NDs are potent inhibitors of *Saccharomyces cerevisiae* growth, yielding 90% growth inhibition at < 1 μg/ml yeast culture [32]. In studies with pathogenic fungal species, similar growth inhibition characteristics were observed. Furthermore, compared with AMB–deoxycholate, AMB–ND displayed greatly attenuated red blood cell hemolytic activity and decreased toxicity toward cultured hepatoma cells. *In vivo* studies in immuno-competent mice revealed that AMB–ND are nontoxic at 10 mg/kg AMB, and showed efficacy in a mouse model of candidiasis at concentrations as low as 0.25 mg/kg [32]. These results indicate that AMB–NDs constitute a novel formulation that effectively solubilizes the antibiotic and elicits strong *in vitro* and *in vivo* antifungal activity with no observed toxicity at therapeutic doses.

In related studies, AMB–NDs were examined for efficacy in treatment of *Leishmania major*-infected BALB/c mice. Membranes of these protozoal parasites are known to be susceptible to AMB-induced pore formation [36]. When *L. major*-infected mice were treated with AMB–ND, remarkable efficacy was observed [37]. Mice administered AMB–ND at 1- or 5-mg/kg doses were significantly protected, displaying decreased lesion size and parasite burden, particularly at 5-mg/kg AMB (**Figure 3**). In stark contrast to liposomal AMB therapy, mice treated with AMB–ND had completely cleared the infection by 140 to 250 days post-infection, with no lesions remaining and no parasites isolated from infected animals. Furthermore, re-stimulated mixed lymphocyte culture cytokine responses (IL-4, IL-12, IL-10, NO and γ-interferon) were unchanged by AMB–ND administration compared to controls. The marked clearance of *L. major* parasites from a susceptible strain of mice without an appreciable change in cytokine response suggests that AMB–NDs represent a potentially useful formulation for the treatment of intrahistiocytic organisms.

3. All *trans* retinoic acid ND

All *trans* retinoic acid (ATRA) is a naturally occurring vitamin A derivative that functions as a regulator of gene transcription [38-40]. ATRA interacts with members of the hormone receptor superfamily including the retinoic acid receptor (RAR) and the retinoid X receptor (RXR) [41]. ATRA binding to these transcription factors modulates their interaction with 'retinoid response elements' on a wide range of genes that function in cell proliferation, differentiation and apoptosis. Specifically, when ligand-activated, RAR–RXR heterodimers dissociate from co-repressor proteins they recruit co-activators that lead to chromatin decondensation and activation of gene transcription [42,41]. Subsequently, pathways controlling growth, differentiation and cell death are activated. Upon recognizing that liposomal formulations of ATRA have been generated wherein this bioactive lipid intercalates between phospholipid molecules in the bilayer, it was hypothesized that ATRA could be incorporated in NDs. Such particles would constitute a novel formulation of this bioactive lipid with potentially desirable properties.

ND particles enriched with ATRA (phospholipid:ATRA molar ratio = 5.5:1) have been formulated wherein all reaction components are solubilized [43]. ATRA–ND migrate as a single band on native gradient polyacrylamide gel electrophoresis and ATRA, phospholipid and apolipoprotein co-elute from a Sepharose 6B gel filtration column, consistent with stable integration of ATRA into the ND particle milieu. Spectroscopic analysis of ATRA–ND in buffer yielded an absorbance spectrum characteristic of ATRA. ATRA–ND mediated time-dependent inhibition of cultured HepG2 cell growth more effectively than free ATRA.

Clinicians have used ATRA to treat various forms of cancer including leukemia, breast cancer and liver cancer [44,45]. In the case of hepatomas, retinoid therapy has successfully reduced both primary and secondary malignancies. This effect may be attributed to a defect in retinoic acid metabolism in patients with hepatocellular carcinoma, many of whom have significantly lower levels of circulating retinol [45]. At the same time, other cases may be related to RAR activation following integration of the hepatitis B virus [46]. For these maladies, it is conceivable that the nanoscale size of ATRA–NDs, combined with their ability to harbor a concentrated bioactive agent payload, suggest that NDs represent a potentially useful vehicle for solubilization and *in vivo* delivery of ATRA. Of interest in the case of hepatic disease is the potential for targeting via apoE as the scaffold protein [43]. ApoE is a known ligand for members of the low density lipoprotein (LDL) receptor family. For example, the prototype LDL receptor is highly

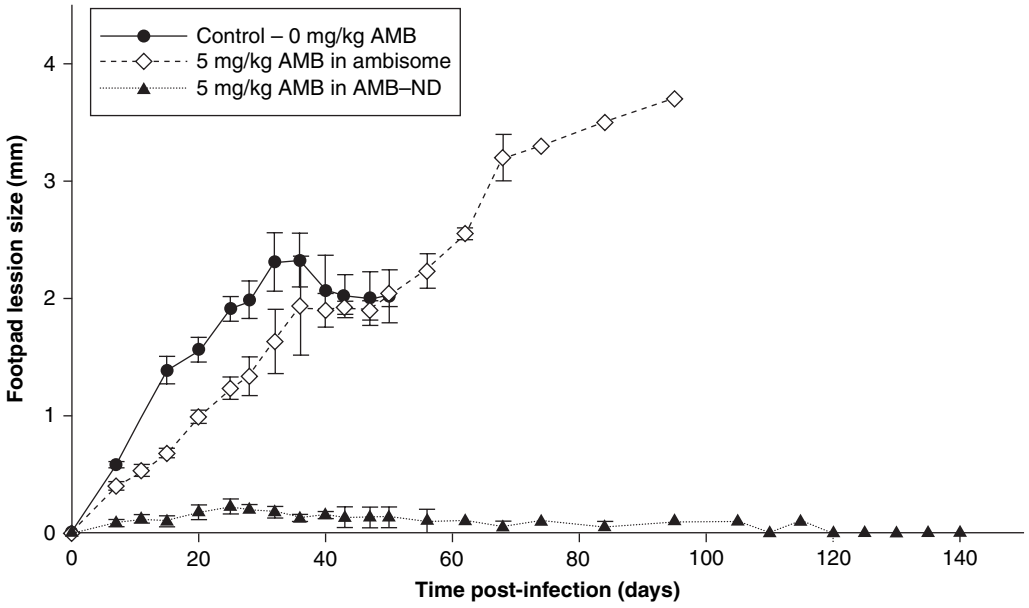

Figure 3. Effect of AMB formulation on footpad lesion size in *L. major* infected BALB/c mice. Groups of mice were infected with 1×10^6 LV39 *L. major* promastigotes and treated at 24 h, 48 h, 10 days, 20 days, 30 days and 40 days with PBS (control), 5 mg/kg AMB in AmBisome®, or 5 mg/kg AMB in AMB–ND. Footpad lesion size was measured at the indicated time points.
With permission from Nelson *et al.* [37].

expressed on hepatocytes and functions to internalize apoE containing lipid particles. Thus, it may be anticipated that ATRA–ND prepared with apoE may be preferentially targeted to hepatocytes. While this approach suffers from the fact that LDL receptors are widely expressed, as discussed below, the possibility of protein engineering of the scaffold moiety may facilitate specific targeting of ATRA–ND.

4. Conclusion

NDs provide unique advantages for targeted drug delivery that are yet to be fully realized. These include nanoscale size, solvent accessibility to both leaflets of bilayer and the presence of integrally associated protein. The intrinsic versatility of NDs permits formulations with a variety of phospholipids, apolipoproteins and hydrophobic biomolecules. The ability to engineer the protein component of NDs creates additional opportunities for targeted delivery of bioactive agents to tissues and cells, holding promise for enhancing therapeutic efficacy while decreasing toxicity and side effects. The future holds great promise that is limited only by the imagination and ingenuity of investigators.

5. Expert opinion

Based on results obtained to date with AMB–ND and ATRA–ND, it is reasonable to consider how these complexes may be further adapted to affect cell- or tissue-specific targeting. The unique presence of integrally associated protein on NDs presents opportunities for chemical modification or protein engineering that confers additional properties. For example, by engineering a receptor recognition sequence into the apolipoprotein component, NDs can be targeted to specific endocytic cell surface receptors. In the case of an internalizing receptor, this would be anticipated to enhance drug payload delivery and reduce non-specific toxicity. Two examples of this general concept with reference to current research activity are provided below.

5.1 Targeting AMB–ND to macrophage scavenger receptors

Considering that macrophages are the tissue site of *Leishmania* spp. amastigote proliferation, a strategy to target AMB–ND to these cells has the potential to deliver this antibiotic directly to the tissue site of infection. It is worth noting that the structural properties of NDs permit this strategy whereas liposomes or micelles do not have this advantage. Macrophages play a central role in host defense as well as normal physiological processes in tissue maintenance. Macrophages are present in most tissues, particularly those that function in filtration of blood or lymph, such as liver, spleen, lung and lymph nodes. A major characteristic of macrophages is their ability to recognize, internalize and destroy harmful endogenous and foreign substances. Macrophages contain scavenger receptors with a broad binding specificity that is used to discriminate between

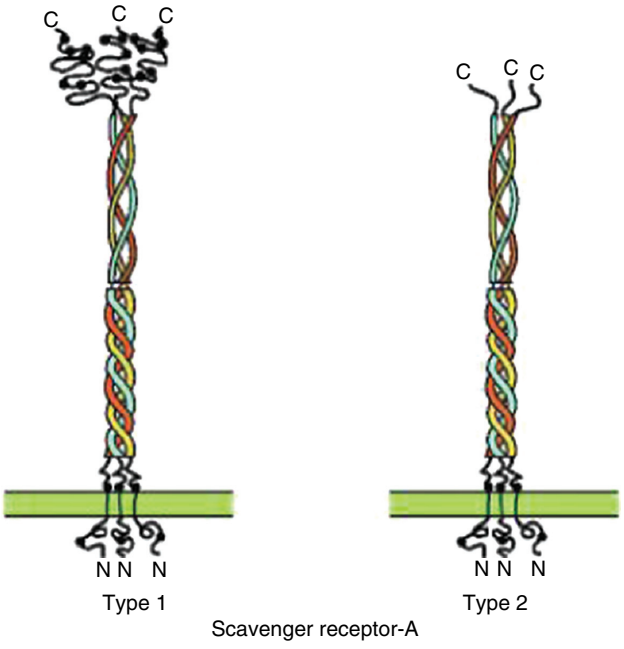

Figure 4. Structural models of Type I and Type II class A scavenger receptor.
Modified from Platt and Gordon [49].

self and non-self by nonspecific, antibody-independent recognition of foreign substances. Scavenger receptors are broadly defined as cell surface membrane proteins that bind LDL that has been modified by oxidation. Besides modified LDL, scavenger receptors are able to bind many other types of ligands. Indeed, scavenger receptors are now categorized into specific classes, depending on their structural and functional properties.

5.1.1 *Class A scavenger receptor ligand binding properties*

The prototypical class A scavenger receptor can be further categorized as Type I or Type II (**Figure 4**). Both Type I and Type II class A scavenger receptors (SR-A) are trimeric membrane bound glycoproteins with a short N-terminal intracellular segment. The larger extracellular portion contains a spacer segment, α-helical coiled-coil region and a triple-helical collagenous domain [47]. Type I SR-A also contains a cysteine-rich C-terminal domain. Interestingly, it is the collagenous region that is responsible for ligand binding. As such both Type I and Type II SR-A are functional endocytic receptors [48]. These receptors are present in macrophages throughout the body and exhibit an unusually broad ligand binding specificity. In general, SR-A binds chemically modified proteins as well as a variety of polyanions. The ability of SR-A to bind and endocytose large quantities of modified lipoproteins (e.g., acetylated or oxidized LDL) has implicated SR-A in mediating lipid influx into macrophages, a process that can result in their conversion to foam cells, a clinical sign of atherosclerosis. In addition, SR-A has been shown to play a role in cell adhesion processes related to macrophage-dependent host defense and inflammatory conditions [49].

The wide range of ligands that has been identified for SR-A is both perplexing and intriguing. On one hand, all known ligands share the property of being polyanionic or having concentrated polyanionic regions. On the other hand, not all polyanions bind to SR-A. To date, no consensus has been reached that provides a molecular explanation for the broad ligand specificity of this receptor. Pearson *et al.* [50] investigated polynucleotide binding to Type I SR-A in transfected CHO cells and concluded that the spatial distribution of negatively charged phosphates in polynucleotide quadraplexes presents a charged surface that is complementary to the positively charged surface of the collagenous domain of SR-A. These data are consistent with the observation that conferring a net negative charge to potential ligands by chemical modification or oxidation will confer SR-A recognition properties.

Haberland *et al.* [51] showed that chemical modification of lysine residues of the apoB component of human LDL by malondialdehyde, acetic anhydride or succinic anhydride, among others, conferred recognition by macrophage scavenger receptors. These authors concluded that receptor recognition depends on charge modification of specific, critical lysine residues resulting in a spatial cluster of anionic charge that comprises the recognition determinant. These results are consistent with the original proposal by Brown and Goldstein [52] that a cluster of negatively charged groups provided by the ligand is essential for interaction with the scavenger receptor. Zhang *et al.* [53] provided evidence that modification of various protein lysine residues with lipid peroxidation products is sufficient to confer scavenger receptor recognition. The ability of such oxidation products to induce recognition of unrelated proteins by the receptor was taken as evidence that lysine residue positive charge neutralization, rather than a charge modification-induced protein conformational change, is responsible for receptor recognition.

5.2 Engineering SR-A recognition properties into candidate apolipoproteins

It is recognized that an apolipoprotein engineered to possess SR-A binding activity must also retain its ability to transform phospholipid bilayer vesicles into NDs. It is also essential that the SR-A recognition properties of the apolipoprotein employed be manifest in its ND-associated state. Members of the apolipoprotein family constitute the only known proteins capable of generating ND. Types of modifications that may confer SR-A recognition include:

5.2.1 *Chemical modification*

Based of the documented success of chemical modification as a means to confer a variety of proteins with SR-A

binding ability, this approach holds promise for ND targeting. When considering the type of chemical agent to be used to modify candidate apolipoproteins, it is noteworthy that differences have been observed in the case of albumin. For example, maleylated albumin, but not acetylated albumin, is conferred with scavenger receptor binding capability [54]. The reason for this difference may be that derivitization of lysine residues with maleic anhydride introduces a change in net charge of -2 (neutralization of the positive charge on the lysine side chain plus introduction of a negative charge by maleic acid), while the corresponding reaction with acetic anhydride or malondialdehyde induces a charge change of -1. Thus, the ability of maleylation but not acetylation to confer receptor-binding ability to albumin appears to be related to charge density.

5.2.2 *Site directed mutagenesis*

Selective replacement of positively charged residues with neutral or negatively charged amino acids may result in acquisition of SR-A binding by apolipoproteins. Furthermore, it is possible that replacement of a given positively charged residue with an amino acid that possesses a negatively charged side chain may lead to charge repulsion. On the other hand, it is known that apolipoproteins are exceptionally resilient proteins that readily recover a native fold following denaturation [55]. Furthermore, numerous mutations have been introduced into apolipoproteins without deleterious effects on their lipid interaction properties. For example, upon introduction of three Arg residues into apolipophorin III, its lipid interaction activity increased [56]. Indeed, it has been proposed that perturbations that decrease the overall stability of the helix bundle conformation actually enhance the lipid-binding activity of these proteins [57,58]. It seems that a balance exits between the stability of the lipid-free conformation of apolipoproteins and their propensity to interact with lipid surfaces [59]. Thus, given that apolipoproteins are known to tolerate structural change, engineering a locus of negative charge that will mimic natural SR-A recognition sequences is likely to be a viable approach.

5.2.3 *Chimera construction*

As an alternative to site-specific modification of apolipoprotein sequences, one may also consider extending the *N*- or *C*-termini of candidate apolipoproteins with a specific sequence. To confer SR-A recognition properties using this approach, it should be possible to attach a 20 – 30 amino acid extension onto the apolipoprotein with retention of its ND formation ability. In fact, the chimera could be designed in such a way that the extension will contain a high concentration of negative charge in the context of a protein sequence that will not display lipid-binding affinity. In this manner, the putative SR-A recognition site should be solvent exposed and, conceivably, more accessible to the ligand binding site of the receptor. This strategy has the advantage of not disturbing the sequence of the apolipoprotein other than by addition of a *N*- or *C*-terminal extension.

5.3 Antibody targeting

An extension of the chimera approach is the use of an apolipoprotein–antibody fusion to direct the ND particles to a specific cell surface antigen. This general approach may be applied to cancer therapy. For example, certain B-cell lymphomas (e.g., Mantle cell lymphoma [MCL]), express CD20 as a cell surface protein [60]. Because of its rapid progression and unresponsiveness to treatment, MCL poses a major challenge to clinicians and researchers. Indeed, progression typically occurs within one year after diagnosis, with a median survival time of three to four years. Although a wide variety of therapeutic strategies have been pursued, there is a pressing need for innovative MCL treatments. One such approach is targeted delivery of cytotoxic drugs to MCL cells. The apolipoprotein–antibody fusion approach is feasible in this case because of the availability of single-chain variable fragment antibodies directed against CD20. Investigators have reported on the construction of engineered single-chain antibody fragments that retain the antigen specificity and binding activity toward CD20 expressing cells displayed by the parent monoclonal antibody [61,62]. Since CD20 is a major surface antigen on MCL cells, it provides a potential site for targeted delivery of cytotoxic agents. The fact that CD20-specific single-chain variable fragment (scFv) antibodies exist as a single polypeptide chain presents the opportunity to create a fusion protein containing the scFv linked to an apolipoprotein capable of forming ND. For example, it may be anticipated that a recombinant fusion protein comprised of apoA-I, a spacer sequence and anti-CD20 scFv will not only form NDs, but also possess CD20 recognition activity. Subsequent formation of ATRA–ND using the fusion protein will generate a targeted ND for specific delivery of ATRA to CD20-expressing cells.

Declaration of interest

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