

HIV: A Raft-Targeting Approach for Prevention and Therapy Using Plant-Derived Compounds (Review)

S.P. Verma*

Department of Public Health/Family Medicine, Tufts University School of Medicine, 136 Harrison Ave., Boston, MA 02111, USA

Abstract: It has been widely accepted that HIV-1 enters into and buds out from microdomains known as lipid rafts/caveolae of plasma membranes of infected cells. Since lipid rafts are recognized sites for budding and entry of HIV-1, and since lipids in rafts (including composition/dynamic structure) play a crucial role in modulating the functions of raft-associated signaling proteins and receptors, it has been consistently shown that modulating the composition/structure of lipid rafts have influenced the life cycle of HIV-1 inhibiting its replication. Since anti-retroviral multi-drugs treatment has severe side effects, one of the strategies could be to block the HIV-1 entry and its replication using natural compounds that can target lipid rafts. Dietary and plant-derived compounds have advantage over synthetic drugs exhibiting minimum side effects and are available in cost effective manner. Studies exploring the effects of dietary and plant-derived compounds targeting lipid rafts could be an evolving strategy to control the progression of AIDS. This article is intended to review: (i) composition/structure and conditions for the formation of lipid rafts in plasma membranes, (ii) interaction of HIV-1 with lipid rafts and (iii) to introduce a novel concept that dietary and plant-derived compounds, which can target lipid rafts, could have potential preventive/therapeutic values against the progression of AIDS. More emphasis has been given to the roles of omega-3 fatty acids and plant-derived various triterpenes, especially euphane-types of triterpenes extracted from Neem tree, targeting lipid rafts and its major component cholesterol.

Key Words: HIV, AIDS, plasma membrane, lipid rafts/caveolae, triterpenes, euphane triterpenes, omega-3 fatty acids, cholesterol, sphingolipids.

INTRODUCTION

Proteins and their encoding genes remain the main focus to understand the cellular activities and related disease states. The role of lipids has received comparatively less attention. During the past few years, however, it has been recognized that lipids and their assembly in plasma membranes directly play a significant and an essential role in normal cellular functions as well as in the progression of variety of diseases. There are compelling data suggesting that lipids assist numerous signaling proteins and receptors in facilitating their normal operation. Many of these molecules that are involved in picking up the exterior signals and transmitting them to the interior are located in the plasma membranes and more specifically within its "micro domains" known as lipid rafts and caveolae [1, 2]. These micro domains are resistant to extraction by non-ionic detergents at 4°C [3] and are also known as detergent-resistant membrane (DRM). Presently, the nomenclature is author's preference. However, at the Keystone Symposium on lipid rafts and cell function, the term 'raft' recently received a comprehensive extended definition. Signaling molecules and receptors located in or recruited by lipid rafts require a specific lipid composition and structure for their proper functioning and these bioactive molecules generally become inactive if composition and/or structure of lipids are perturbed [4,5]. Lipids, therefore, play

a crucial role in modulating the functions of raft-associated signaling proteins and receptors. The knowledge of lipid raft composition and associated signaling pathways are important to understand their role in health and influence on various disease states since many viruses including HIV, pathogenic bacteria, bacterial toxins and malaria parasite take advantage of lipid rafts to enter host cells [6]. Modulating the raft composition (e.g., depleting cholesterol) had been shown to influence the entry and budding out of viruses including HIV-1 virus [7]. Studies exploring the effects of dietary and/or plant-derived compounds targeting lipid rafts could be an evolving strategy to control the replication of HIV-1 and progression of AIDS. This article is intended to review; (i) basic structural/compositional information about plasma membranes and formation of lipid rafts, (ii) HIV-1 interaction with rafts and (iii) to introduce a novel concept that dietary and plant-derived compounds, which can target lipid rafts could have potential preventive/therapeutic values against the progression of AIDS.

COMPOSITION OF RAFT/CAVEOLAE

Rafts are microdomains of plasma membranes and are rich in two major classes of lipids, cholesterol and sphingolipids. Small amounts of other phospholipids such as phosphatidylcholine mainly with saturated acyl chains, phosphatidylethanolamine and phosphatidylserine are also present. Sphingolipids ganglioside 1 and 2 (GM1 and GM2) are generally used as markers for raft lipids. Caveolae are invaginations of plasma membranes and are a special sub-

*Address correspondence to this author at the Department of Public Health/Family Medicine, Tufts University School of Medicine, 136 Harrison Ave., Boston, MA 02111, USA; E-mail: surendra.verma@tufts.edu

class of rafts. Caveolae, microdomains in the plasma membrane, are identified by the presence of a family of structural proteins (caveolins1, 2 or 3) and a lipid composition that is high in free cholesterol and sphingolipids [8]. Caveolin-like lipid rafts contain flotillins operating as homologues of caveolins. Flotillins are integral membrane proteins and contribute to the organization and structure of lipid rafts. Flotillins include flotillin-1 and flotillin-2. Numerous signaling proteins and receptors are concentrated in or are being recruited by these domains. Foster *et al.* [9] have identified numerous raft associated proteins by proteomic analysis. Several of these lipid-modified proteins are involved in signal transduction, including heterotrimeric G-proteins, protein kinase C isoforms, eNOS, and estrogen receptors. Components of ras-MAPK pathway, Shc, src family tyrosine kinases, JAK/STAT signaling, nitric oxide synthase (NOS) and G-proteins are concentrated in lipid rafts [10]. Many of these signaling molecules have been shown to directly interact with flotillins through interacting with their scaffolding domain.

PHYSICAL PROPERTIES OF LIPID RAFTS

The size and nature of lipid rafts is still unclear and rather controversial. Recent evidence supports the existence of small raft size closer to 0.1 μm in diameter and that they can dynamically associate with each other forming larger transducing platforms [11]. Raft lipids are saturated acyl chains that pack tighter than unsaturated chains. Lipid rafts can diffuse laterally in the plasma membrane functioning as floating shuttles to bring together activated receptors and transducer molecules. Several important cellular activities such as organizing and modulating signal transduction pathways and membrane trafficking have been linked to lipids rafts/caveolae [12]. The physical properties of lipid rafts can be modified by temperature and by altering the composition and amounts of lipid and cholesterol. Knowledge about their physical properties such as phase behavior will help understanding their functions

WHY RAFT MICRODOMAINS ARE FORMED IN THE PLASMA MEMBRANES OF CELLS?

In spite of extensive research for many years and availability of sophisticated techniques, the dynamic structure of the plasma membrane and its microdomains is still not well understood. Since rafts are integral microdomain parts of plasma membrane, first we would like to present a simple overview of the composition and dynamic structure of plasma membranes. This knowledge will help in understanding the formation of raft, their composition and structure.

The most abundant lipids in plasma membranes are the glycerolphospholipids, phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylserine (PS), phosphatidylinositol (PI) and sphingomyelin (SM). PS bears one negative charge, PI carry one more negative charge. Sphingolipids have the same apolar moiety as SM, but their head groups consist of sugar residues, which may be uncharged or negatively charged, because of the presence of neuraminic residues in the case of gangliosides, or sometimes sulfate group. Sphingolipids are concentrated in the outer lamella of the plasma membrane, where their saccharide moieties extend into the extracellular space. Cholesterol is abundant in

plasma membranes. The hydroxyl group of membrane cholesterol lies in the polar region of the membrane and the rigid ring structure extends into the apolar core.

In the early seventies the structure of membranes was viewed as a uniform fluid lipid matrix in which proteins are floating [13]. Numerous modifications have been suggested since then and realized that plasma membrane structure is much more complex than to previously suggested [14-17]. Lipids in the plasma membrane are mostly amphipathic molecules with polar and/or charged head groups attached to apolar residues. Their segregation due to hydrophilic environment causes these molecules to aggregate in two dimensions, forming extended bilamellar membrane. Most of the lipids in plasma membranes occur in double-layered arrays and their acyl chains constitute a large part of the apolar membrane lamella.

Small changes in the environment can alter the dynamics of membrane lipids. Phospholipid and sphingolipids can exist in two major states, (i) a gel or crystalline phase where both the head groups and acyl chains are in a crystalline array, and (ii) a less ordered or liquid-crystalline phase, where the head groups are in a crystalline array, but the acyl chains form a fluid continuum. A shift between these two states known as "phase transition" can be brought about in pure systems by a small shift of temperature in the region of the critical melting temperature, T_c , of the acyl chains.

The phase transition behavior of lipids in biological membranes is much more complex [18-20] than in model systems for the following reasons. (i) The acyl chain composition and head groups in biological membranes are heterogeneous. Heterogeneous components of different acyl and head group mixtures may then form different gel and liquid-crystalline phase depending on their acyl chain length and the presence of number and position of double bonds in acyl chains. The lipid heterogeneity may cause the formation of crystalline islands in a liquid-crystalline continuum. Once this process initiated, the transition from liquid-crystalline to the crystalline state diffuses in a lateral direction within the lipid bilayer. In some membranes this can cause aggregation and segregation of penetrating proteins. (ii) The high concentration of cholesterol in plasma membranes, which modulates the order of acyl chains, makes phase transition less cooperative. The presence of cholesterol, therefore allows the mixing of dissimilar acyl chains. (iii) The interaction of surface and penetrating protein moieties with lipids can influence the organization of acyl chains and head groups. These and other factors are responsible for the complex phase behavior of biological membranes. Lipid acyl chains are in high order state in gel or liquid-ordered phase but rotational/ translational mobility is similar to the mobility of liquid-disorder phase. In the event of co-existence of these phases, phase separation may occur in the lipid bilayers and probably may form microdomains like lipid rafts. Plasma membranes at physiological temperatures exist in the liquid-crystalline state [21]. This state allows considerable diffusional mobility of lipid molecules within the plane of half a bilayer. The overall width of the plasma membrane is in the range of 7-10 nm, depending on the extent to which proteins (which generally make up two thirds of membrane mass) and carbohydrates protrude from the membrane surface.

WHAT IS THE ROLE OF SPHINGOMYELINS AND CHOLESTEROL IN THE FORMATION OF RAFTS?

Lipid rafts are specialized microdomains of plasma membranes enriched in cholesterol and sphingomyelin (SM). The role of SM and cholesterol in the formation of rafts can be better visualized by understanding their chemical structure and physical properties. This will provide useful and fundamental insights into understanding the structures likely to exist in lipid rafts. Molecular structures of sphingolipids and cholesterol are likely to play essential roles in regulating the lateral interactions among them. In mammalian cells, sphingomyelin is a long chain sphingolipid base an amide linked acyl chains of long (22:0, 24:0 and some cases 24:1^{Δ15(c)}) or intermediate (16:0, 18:0) chain length, a free OH group on C₃, the *trans*-double bond between C₄ and C₅ in *sn1* acyl chain and a phosphorylcholine head group. The hydroxyl and amide groups can be both hydrogen bond donors and acceptors, while the amide carbonyl in SM can act only as hydrogen acceptor. These properties of SM allow forming both inter- and fairly strong intra-molecular hydrogen bond. Generally, SM acyl chains are long and saturated that contribute to a high transition temperatures, which is characteristic of sphingolipids. Most natural SMs have transition temperature close to the physiological temperature. The composition of acyl chains varies between tissues and mixed population of acyl chain is common in natural sphingomyelins. These mismatch acyl chains can cause highly non-ideal mixing with other mostly unsaturated and nearly symmetric lipids in the plasma membranes of cells and may lead to the formation of lateral domains.

Cholesterol (cholest-5-en-3β-ol) contains a nonpolar tetracyclic hydrocarbon ring system. The rings of cholesterol are compact and have relatively planer conformational arrangement. The 3-OH is the only functional polar group in cholesterol that effects its orientation in lipid bilayers. Hydrogen bonding between 3-OH groups and amide groups of SM is more preferable. Cholesterol/SM interaction is stabilized by N-linked acyl chain of SM [22]. The nonpolar tetracyclic planer rings of cholesterol are adjacent to the hydrocarbon segments of sphingolipids allowing strong van der Waals stabilization contributed by planar cholesterol rings interacting with saturated acyl chains of SM. The polar/nonpolar interactions and the rigid planar structure account for the observed cholesterol preference to interact with SM over an acyl chain matched phosphatidylcholine [23].

IS CHOLESTEROL IMPORTANT FOR THE FORMATION OF RAFTS?

Physical behavior and structural features of cholesterol and sphingolipids suggest them as likely candidates for the formation of raft microdomains in model and possibly in plasma membranes of cells [24]. Sphingolipid acyl chains are rigid due to their gel crystalline character resulting into their confinement within the surrounding domain lattice and restricting diffusion of proteins and other molecules within, into or out of their domains. In the presence of high cholesterol concentrations (>25 mol%), the sphingolipids though would remain tightly packed but cholesterol would induce a liquid-crystalline state that would provide relatively a fluid environment within a restrictive lattice environment and fa-

ilitating the diffusion of GPI-anchored or other proteins in or out of such domains. Cholesterol-sphingolipid liquid-ordered state is suitable for dynamic association for several proteins and helps segregate the domains for functional purposes.

WHAT FACTORS CAN DISRUPT RAFT STRUCTURE AND FUNCTION?

(i) Depletion of Cholesterol

Depletion of cholesterol is now known to cause disruption of lipid raft structure and the functions of associated signaling molecules. Several compounds such as β-cyclodextrins, 3-hydroxy-3-methylglutaryl (HMG)-coenzyme A reductase inhibitors (statins, e.g. mevastatin), and cytochalasin (fumonisin B) are known to deplete cholesterol. The formation of ceramide in rafts can also deplete cholesterol. More than one hundred papers have been published suggesting that depletion of cholesterol can influence the functions of raft-associated signaling proteins and other molecules [Ovid search 2008].

(ii) Degradation of Sphingomyelin

The other major constituent of rafts is SM and its degradation can also lead to functional/structural disruption of rafts. Hydrolysis of SM caused by a variety of stimuli such as cytokines, antibody receptors, steroids, G-protein-coupled receptors and stress is known to generate ceramide and phosphorylcholine. Generation of ceramide is catalyzed by the enzyme sphingomyelinase (Smase). Several types of Smases have been identified that vary according to their pH optima (e.g., acid sphingomyelinase A-Smase), dependence on co-factors (e.g., Mg and Zn, neutral sphingomyelinase N-Smase) and cellular localization [25]. A-Smase has been identified in caveolae [26], suggesting that this enzyme is involved in generating ceramide at the plasma membrane. Ceramide is a small hydrophobic neutral lipid molecule that remains within the bilayer. Proteins must be in close proximity to the site of ceramide generation for their direct interaction. Ceramide is involved in multiple signaling pathways including apoptosis, senescence, growth arrest and differentiation. The generated ceramide can change the physical properties of plasma membrane, facilitating lipid raft coalescence and receptor aggregation in the outer leaflet [27, 28]. Natural and synthetic ceramides have been shown to displace cholesterol from lipid rafts while other lipids remain raft-associated [29]. Both ceramides and cholesterol have a small polar head group and both may compete for association with other lipids for minimizing their exposure to aqueous environment. Displacement of cholesterol by ceramides would alter the composition and the physical properties of raft and protein association with rafts specially those linked with cholesterol (e.g., hedgehog) [30] or interact with cholesterol (e.g., bacterial cytolysins and NAP-22) [31, 32].

IS RAFT LIPID STRUCTURE SIMILAR TO THE STRUCTURE OF HIV MEMBRANE?

The lipid membrane of HIV virus has a low lipid/protein ratio (wt/wt) and a high molar cholesterol/phospholipid ratio (C/P). High levels of lipid and elevated C/P ratio is similar to that found in lipid rafts suggesting the possibility that HIV

lipids may be derived from the host plasma membrane or lipid rafts. The lipid composition of HIV propagated in Hut 79 cells have been earlier found to contain C/P ratio of 0.88 and mol% of total phospholipids PC 23.8, PE 24.6, SM 28.3, PS 15.1, PI 2.1, PA 0.9 and other 5.0 [33]. The high C/P ratio and lipid distribution in HIV membrane is also comparable with those of erythrocyte ghost membranes [33]. Both HIV membrane and erythrocyte ghost membrane show similar fluidity at 37°C as studied by a spin labeled probe [33]. This similarity could be most likely due to their comparable lipid composition. As determined by Raman spectroscopy, erythrocyte ghosts have been shown to exhibit multiple broad phase transitions in the physiological temperature range [18, 19] similar to that shown by HIV membrane [33]. Erythrocytes have lipid rafts that are involved in the entry of malaria parasite [34]. The concept that HIV envelope lipid bilayer is derived from host plasma membrane has been widely accepted [35-39] and also from the evidence that lipid-metabolizing genes are absent in the HIV genome [40].

WHY HIV-1 REQUIRES LIPID RAFT TYPE OF DOMAIN FOR ENTRY AND BUDDING OUT?

The life cycle of HIV consists several viral stages between entry and budding out. For the entry stage, the concept is that the fusion is directed by the envelope glycoproteins (Env) of HIV-1. The fusion is mediated by interaction between HIV-1 envelope glycoprotein (gp41) and raft located surface receptor Cd4 and chemokine receptors such as CXCR4 or CCR5 (coreceptors). The widely accepted concept that HIV-1 enters into and buds out from lipid rafts of infected T cells is mainly derived from data showing that (i) lipid raft-associated molecules are found in purified virion [37, 41] and that (ii) the Env glycoproteins of HIV have been detected in rafts [37]. Lipid rafts to be a privileged site for virus must, therefore, provide physically, chemically and thermodynamically favorable conditions for fusion and the presence of HIV-1 receptor (CD4 receptor). Though there is ample evidence suggesting that cholesterol is critical for fusion and infection of cells [37, 38, 42] but the mechanism is not well established. First, the level of hydrophobicity in both raft and HIV membranes must be quite close. Second, the interacting proteins of HIV virus must have comparatively higher hydrophobicity. Acylated proteins (myristoylated, or palmitoylated or both) attain higher hydrophobicity and acyl chains generally facilitate the fusion. The structure of HIV envelope contains a lipid bilayer with loosely associated two glycoproteins; gp120 and gp41 resulting from proteolytic cleavage of the precursor protein gp160 during intracellular virus assembly. The transmembrane gp41 consists three domains, an ectodomain, a membrane spanning domain and a long intracytoplasmic segment [43]. It has been reported by several authors that the ectodomain of gp41 largely responsible for membrane fusion [44-48] since the formation of triple-stranded coiled-coil in gp41 allow the hydrophobic fusion peptide to insert into the plasma membranes of target cells. The higher hydrophobicity created by bending of the triple-stranded coiled-coil on itself and thus forming a six helix bundle brings the membrane of HIV into close proximity to the plasma membranes of target cells. This hydrophobically favorable condition probably allows formation of fusion pores [48, 49-52]. Phosphatidylethano-

amine, which comprises about 25% of lipid composition of lipid bilayers of both HIV-1 and target cells, is also vital in the process of HIV-1 fusion. The HIV-1 lipid bilayer has high cholesterol/phospholipid molar ratio (>1.00) (11), which is lower in host plasma membrane but similar to that of rafts. Higher hydrophobicity of gp41 and similarity in lipid composition of rafts and HIV-1 are attractive conditions for fusion. Lipids of the budding new HIV virions are comparable to the cholesterol-enriched lipid rafts [53]. These data strongly suggest that the lipid bilayer of budding HIV is derived from lipid rafts. Since lipid rafts play an essential role in the budding and entry of HIV-1, modulation in the composition and structure of rafts may influence HIV infection process and offer opportunities to block infection.

Acylation of HIV proteins is also very important factor for HIV infectivity. HIV-1 Nef (Nef), a myristoylated protein, that contributes to AIDS pathogenesis by triggering activation of T cell receptor cascade to facilitate virus spread. It has been reported that lipid rafts are instrumental for Nef-mediated pathogenesis [54]. Cholesterol binding is essential for Nef to increase the viral infection. Nef is responsible to increase cholesterol biosynthesis and its incorporation in lipid rafts and progeny virions. Other myristoylated proteins, matrix proteins (myr-MS) and myristoylated Gag protein of HIV-1 have been found in lipid rafts isolated from the infected cells [55].

Since cholesterol in HIV particles is strictly required for fusion and infectivity [56], removal of cholesterol from the virus bilayer by treatment with β -cyclodextrin resulted in a dose-dependent inactivation of the virus [57]. Since budding of HIV-1 acquire its lipids from lipid rafts, reducing the cholesterol levels in lipid rafts has been shown to reduce the infectivity [58]. Further, there are data suggesting that integrity of lipid rafts is important for virus entry and destabilizing rafts by either extraction of cholesterol or inhibition of glycosphingolipids synthesis inhibits virus entry [59, 60]. These and many other published results suggest that compounds, which can modify lipid composition and alter membrane fluidity (dynamic structure) of lipid rafts, will likely influence HIV replication.

It has been widely accepted now that HIV-1 enters cells *via* lipid rafts and sufficient progress has been made understanding the structure of viral proteins, their receptors and related mechanisms. Membrane fluidity is important for HIV-1 entry and for the formation of fusion-pores [61-63]. However, limited knowledge still exists about dynamic structural changes that occur in the membrane structure leading to the formation of fusion-pores that allow translocation of intact HIV-1 virus into cells.

CAN DIETARY COMPOUNDS INFLUENCE RAFT COMPOSITION AND FUNCTION?

Health benefits of omega-3 (or n-3) polyunsaturated fatty acids have been widely reported against many different human diseases such as cancer, heart disease, diabetes, obesity, kidney disease, malaria, multiple sclerosis, migraine headaches. Rheumatoid arthritis, lupus, dermatitis and many other disorders. The most studied bioactive omega-3 (also called n-3) fatty acid, docosahexaenoic acid, is a 22 carbon acyl chain having six double bonds and the final double bond

is located on the third carbon relative to the terminal methyl group of the fatty acid acyl chain (n-3 position) (DHA; 22:6n-3). The other n-3 fatty acid, eicosapentaenoic acid, has twenty carbon atoms and five double bonds (EPA; 20:5n-3). Although the chemical structures of omega-3 polyunsaturated fatty acids appears to be very simple (single acyl chain with multiple double bonds) but they surprisingly exhibit remarkably significant health benefits against numerous human diseases. DHA is the longest (22 carbon atoms) and most unsaturated (six double bonds) omega-3 fatty acid and possesses the most effective bioactivities against numerous human health problem and disorders. Since DHA has been shown to influence so many different biological systems and human health problems, it is assumed that the target of omega-3 fatty acids must be a common cellular site exhibiting a common fundamental mechanism [64]. Biophysical studies have shown that dietary DHA incorporates into the plasma membrane and recent studies have suggested its insertion into lipid rafts and affecting cell signaling [64, 65]. These fatty acids are also known to have effects on other metabolic functions. In this article we will only review their effects on lipid rafts.

There is a wealth of evidence demonstrating that n-3 PUFAs can alter the composition and function of membrane rafts in a variety of cell types [66-68]. The *in vitro* studies have suggested that insertion of n-3 PUFA in lipid rafts/caveolae of Jurkat T-cell line have caused modification of lipid composition, which in turn resulted into changing the functions of important signaling proteins (Src family kinases Lck and Fyn and LAT) associated with these microdomains [69,70] and LAT (the linker of activation in T cells) [71]. Similarly, Web *et al.* [72] have shown that EPA, DHA and arachidonic acid can inhibit the localization of Fyn in COS-1 cells. These *in vitro* data were supported by the *in vivo* studies of Fan *et al.* using a mouse model [73], where a diet rich in n-3 PUFA markedly altered fatty acyl lipid composition and sphingolipid content of lipid rafts. Ma *et al.* [74] have shown that feeding mice with fish oil enriched with EPA and DHA can markedly alter the lipid composition of colonic caveolae/lipid rafts in comparison to mice fed with n-6 PUFA (control). Interestingly, EPA and DHA selectively reduce cholesterol concentration from caveolae/lipid rafts without affecting total cellular cholesterol and selectively inhibit the localization of raft-targeted proteins. Polyunsaturated fatty acids (n-3) have been shown to change lipid composition and interleukin-2 receptor signaling in membrane rafts [75]. Dietary n-3 fatty acid DHA has been shown to alter raft composition and suppressing inflammatory processes [76]. There are numerous other papers suggesting that DHA acts *via* modifying the lipid composition of caveolae/lipid rafts thereby altering the important raft-associating signaling events [77].

These data apparently provide substantive evidence that (i) lipid rafts are likely molecular targets for n-3 PUFAs to influence cellular activities, (ii) n-3 PUFAs can selectively enter into lipid rafts, (iii) selectively displace cholesterol from rafts, and (iv) can selectively influence the activities of raft-associated signaling proteins. In spite of convincing evidence, research is surprisingly lacking to explore the role of n-3 PUFAs targeting lipid rafts for therapeutic and preventive purposes of controlling HIV-1 replication.

Published papers exploring the metabolic effects of dietary n-3 polyunsaturated fatty acids on the HIV replication and AIDS are also limited. Dietary n-3 PUFAs have been shown to lower elevated triglyceride levels in HIV-infected hypertriglyceridemic patients [78-80]. Dietary intake of n-3 fatty acids increases CD4 counts [81]. Das [82] has reviewed the useful role of fatty acids in controlling HIV proliferation and management of AIDS.

CAN PLANT-DERIVED COMPOUNDS INFLUENCE RAFT COMPOSITION AND FUNCTION OF HIV-1 INFECTED CELLS?

Limited information exists in the literature about the role of plant-derived compounds that can influence the composition and signaling properties of rafts. However, the influence of synthetic cholesterol depleting drugs on raft composition and function has been well studied. The concept that plant sterols (phytosterols), which are the important structural components of plant membranes as cholesterol of animal cells, could influence raft composition/function of HIV-infected cells, has been least evaluated. Phytosterols are similar in structure to cholesterol. Phytosterols like cholesterol in animal plasma membranes are also thought to stabilize plant membrane structure with an increase in the sterol/phospholipid ratio leading to membrane rigidification [83]. Phytosterols when consumed by humans are known to reduce serum cholesterol levels [84]. Furthermore, plant cell membranes also have cholesterol-rich raft domains [85] similar to that found in animal plasma membranes. We considered these similarities in structure and biological properties between phytosterols and cholesterol in proposing our novel concept. We propose that phytosterols (such as triterpenes) owing to their cholesterol like core structure can favorably insert into cholesterol-rich lipid-rafts resulting in (i) cholesterol depletion, or (ii) destabilization of raft structure, and (iii) influencing the activities of downstream signaling proteins. This concept could be an evolving strategy for searching new phytosterols targeting lipid rafts as HIV-1 preventive and therapeutic agents inhibiting the process of disease progression.

A diverse group of natural plant derived compounds known as phytosterols (sterols, sterolins, triterpenes), are fats of plants, fruits and vegetables. Plant oils contain highest amounts while fruits and vegetables contain the lowest amounts of phytosterols. Although plant fats are quite close chemically to the animal fats but their biochemical effects are significantly different in humans and animals.

During the past few years, the interest in phytosterols has been tremendously increased due to their wide versified health benefits specifically cholesterol lowering properties and availability in larger amounts. Anti-cancer activities of phytosterols against breast cancer [86], prostate cancer [87] and colon cancer [88] have been well reported. Recently, studies on anti-HIV activities have drawn considerable attention because of their low cost and greater availability. Early studies in mid-nineties were conducted on the effects on of a mixture of beta-sitosterol, a major phytosterols in higher plants, and its glycoside, beta-sitosterolin on the disease progression of HIV infected subjects [89-91]. These early studies show slowing of disease progression.

CAN TRITERPENE CLASS OF PHYTOSTEROLS MODULATE THE RAFT COMPOSITION, PHYSICAL PROPERTIES AND FUNCTION?

Triterpenes are a wider class of compounds and thousands of structures have been reported. The list increases every year. Hundreds of various classes of terpenes have been isolated and assayed for their anti-HIV activities during the last few years and the results are promising. The literature reviewed here is not comprehensive but rather focused in view of the proposed raft concept.

The *kaurane-type diterpene*, neotripterifordin, isolated from ethanol extract of *Tripterygium wilfordii* exhibited potent anti-HIV activity by suppressing HIV replication (EC_{50} value $0.025 \mu\text{M}$) in H9 lymphocytes [92]. *Lanostane-type triterpene* (tetracyclic triterpenes), suberosol, isolated from *polyalthia suberosa*, possessed anti-HIV replication activity in H9 lymphocytes (EC_{50} value $6.78 \mu\text{M}$) [93]. Other diterpenes such as cucurbitacins also exhibited anti-HIV activities *in vitro* experiments but these compounds are highly toxic ($IC_{50} = 0.31 \mu\text{M}$) [94].

The anti-HIV effects of pentacyclic *lupane-type triterpenes* have been widely studied. Among them, biochemical effects of betulinic acid [3β -Hydroxy-lup-20(29)-en-28-oic acid], a C-28 carboxylic derivative of the ubiquitous triterpene betulin, have been extensively studied [95, 96]. Betulinic acid, the oxidized derivative of betulin, shows anti-HIV, anticancer and anti-inflammatory activities. Betulinic acid ($C_{30}H_{48}O_3$) is a white crystalline solid and poorly soluble in water. An early investigation by Fujioka *et al.* [97] has suggested that pentacyclic triterpenes are capable of inhibiting HIV-1 replication in *in vitro* model systems. Among the compounds tested, betulinic acid was found to be more potent anti-HIV compound. Since betulinic acid was found to be a promising triterpene and readily available in sufficient quantities, several chemical modifications were made to enhance its anti-HIV activity. Mayaux *et al.* [98] reported that the derivative of betulinic acid, RPR103611, exhibits greatly enhanced anti-HIV activity (EC_{50} 0.05 in CEM and $0.04 \mu\text{M}$ in MT-4 cells) *in vitro* cell based assays. Studies on the effects of RPR103611 on the activities of HIV-derived reverse transcriptase, integrase and protease enzymes have shown that these enzymes are not the targets. Derivatization of betulinic acid was generally performed at C-3 3β -hydroxyl and C-28 COOH groups. Hashimoto *et al.* [99], performed several modifications of betulinic acid and found that the analogs with 3', 3'-dimethylsuccinyl and 3', 3'-dimethylglutaryl C-3 side chains exhibited dramatically enhanced anti-HIV activity (EC_{50} in the 0.35 and 2.3 nM range respectively). Studies on the anti-HIV activities of derivatized compounds of betulinic acid have suggested that the presence of 3β -hydroxyl is essential for the activity [100]. The activity is lost if 3β -hydroxyl group is replaced by 3α -hydroxyl, 3β -amino, ketone or 3-deoxy derivative. Also the activity is lost if the A ring is modified [100]. Importantly, anti-HIV activity is dramatically enhanced by the C-28 amide analogs ($\text{NH}(\text{CH}_2)_{10}\text{COOH}$ and $\text{NH}(\text{CH}_2)_7\text{CONH}(\text{CH}_2)\text{COOH}$) of betulinic acid [100]. Detailed anti-HIV activities of several other derivatized betulinic acid have been reported [95, 101, 102].

The mechanism(s) of action of betulinic acid and its analogs are still far from understood. Research has been largely devoted, however, to synthesizing new derivatives of betulinic acid to improve the anti-HIV activity of triterpenes but less consideration has been paid to understand mechanisms of action. Mechanism-based designing of new derivatives will help developing compounds having potential anti-disease activity. We propose a novel concept of lipid rafts to hypothetically explain the mechanism of bioactivities of betulinic acid and its synthesized derivatives. Betulinic acid and its analogs are hydrophobic molecules. There is structural similarity between triterpenes and cholesterol. The chemical structure of betulinic acid is quite similar to cholesterol, an essential component of rafts. Since betulinic acid and its active derivatives are hydrophobic molecules and possess a cholesterol type of chemical structure, they are likely to incorporate into the plasma membranes and more specifically in the lipid rafts. Since rafts are tightly packed microdomains of plasma membranes, the insertion of betulinic acid and its analogs may compete for the position of cholesterol in rafts and may possibly delete cholesterol. As written above the 3β -hydroxyl is essential for anti-HIV activity of betulinic acid. Since betulinic acid is a hydrophobic molecule, its alignment with raft lipids will depend on the alignment of 3β -hydroxyl or C-28 COOH groups with hydrophilic surface. As has been reported by Evers *et al.* [100] that the activity is lost if 3β -OH group is modified. This can be possibly explained if the modification of betulinic acid precludes its proper association in lipid raft membranes that could alter the structure/function of lipid rafts, which is possibly required for anti-HIV activity. The functions of lipid rafts-associated signaling proteins are likely to be altered by anti-HIV compounds since their activities are dependent on the dynamic structure of rafts and since bioactivities of some raft-associated proteins are cholesterol dependent. Betulinic acid/derivatives-induced deletion of cholesterol from rafts would result in a rigid structure of sphingolipid hydrocarbon chains. The rigid structure of rafts would confine the signaling proteins within the surrounding domain lattice allowing a slow rotational diffusion. Under these structurally rigid conditions, the activities of downstream signaling proteins will likely be reduced.

Our concept of triterpene-induced alteration of lipid rafts structure and function gets support from some indirect studies. Zieger *et al.* [103] have shown that the malaria parasite, *plasmodium falciparum*, could not enter in erythrocyte that was pre-loaded with betulinic acid and analogues with functional group that can donate a hydrogen bond (COOH, CH_2OH , CONH_2). Betulinic acid and its analogs may cause modifications of cholesterol-rich erythrocyte membrane rafts. Rafts of erythrocyte plasma membrane are known to play an important role in parasite vacuolization [104]. Although more than one hundred papers have been published studying the anti-HIV effects of triterpenes and tentative mechanisms have been proposed [105], but surprisingly there is no report about studying lipid rafts as triterpene targets in relation to their anti-HIV effects (Ovid search). Targeting lipid rafts by triterpenes may open novel evolving therapeutic and preventive strategies.

Several types of triterpenes extracted from a variety of plants have been shown to reduce serum cholesterol [83]. More than 200 different kinds have been reported in plant species. Their biosynthesis and health effects have been reported [83].

Oleanane-type triterpenes have been isolated from flowers, pith, leaves and fruit of *Tetrapanax papyriferus* by Ho *et al.* [106]. *Tetrapanax papyriferus* has been used as a traditional medicine in China to treat inflammation and dysentery. The detailed structural information about four newly isolated triterpenes is reported [106]. The anti-HIV activity of papyriogenin A tested in acutely infected H9 lymphocytes show EC₅₀ value of <0.8 µg/ml (concentration that inhibits viral replication by 50%) and IC₅₀ value of 8.91 µg/ml (concentration that inhibits uninfected H9 cell growth by 50%) [106]. Mechanism(s) of their action was not presented by these authors.

Surprisingly the anti-HIV effects of *euphane-types of triterpenes* have not been tested as yet. The bark of the Neem tree *Melia dubia* is a good source of tetranortriterpenoids [107]. Euphane types of triterpenes have been shown to possess anti-cancer activities [108]. The chemical formula/structure of euphane-types of triterpenes are different from the widely studied lupane-types of triterpene betulinic acid. Euphane types of triterpenes, e.g., meliastatins 1-5, and other related triterpenes have been isolated from the giant Neem tree *Melia dubia* and structurally characterized by Pettit *et al.* [108]. The structure of several bioactive euphane types of triterpenes such as meliastatins 1-5 (C₃₀H₄₈O₃), dubione A and B (C₃₀H₄₄O₄) have been reported [108]. The core structures of the triterpene meliastatins have fused rings A, B, C and D. C is fused to a five-member D ring. Cholesterol also has four rings A, B, C and D. C is fused to a five member D ring. Both meliastatins and cholesterol have planar structures. In meliastatins the A-ring exists in a chair conformation, the B ring exists in a twisted chair conformation, C ring exists in a twist-boat conformation. Ring C is transfused to ring D [108].

These compounds have shown anti-cancer activities against the P388 lymphocytic leukemia and a minipanel of human cancer cell lines [108]. However, their anti-HIV activities have not been explored to date. We speculate that euphane-types of triterpenes may have potential anti-HIV activities for the following reasons. Their stereo core structure is much closer to the stereo core structure of cholesterol than those of lupane types of triterpenes such as betulinic acid (Fig. 1). Due to the similarities in their stereo core structure, euphane-types of triterpenes would tend to align with cholesterol in rafts. In doing so the euphane-types of triterpene molecules must also satisfy the requirements of the two hydrophilic OH groups that are attached to C-16 in the ring D and the other OH is at C-25 in the side chain. The significant difference between structures of cholesterol and meliastatins is in the location of OH groups. The single OH group in cholesterol is attached to the ring A at C-3 and no OH attached at C-25 in the side chain in contrast to that found in meliastatins. Theoretically, both OH groups of meliastatins would like to compete with the OH of cholesterol because of their hydrophilic nature. Due to two OH groups and a cholesterol-like core structure, the insertion of meliastatins in the membrane may either delete cholesterol from rafts or raft

membrane feels the pressure to expand. In either case, raft structure will be perturbed resulting into modulating the functions of downstream signaling proteins. The role of lupane-types of triterpenes (betulinic acid and its derivatives) has been widely studied. The field of euphane-types of triterpenes and their influence on HIV infection and progression of AIDS is probably a subject for future research and the proposed concept of targeting lipid rafts by natural compounds may provide an evolving strategy for inhibiting HIV replication.

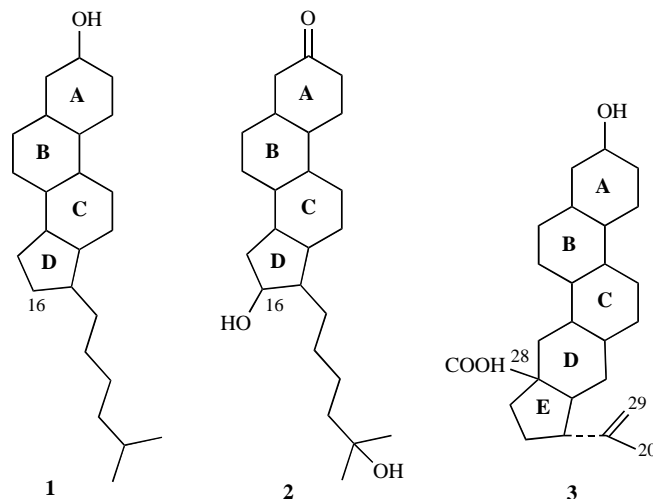


Fig. (1). Structures of cholesterol (1), meliastatins 1 (2) and betulinic acid (3). Cholesterol and meliastatins consist of a four-membered ring system, designated as A, B, C and D while betulinic acid consists five-membered ring system designated as A, B, C, D and E. Differences in the core structures are marked by numbers.

FUTURE STUDIES

Sufficient knowledge exists about the structure of HIV-1 proteins, their receptors, drugs that inhibit HIV-1 replication and related mechanisms. The role of lipid rafts in viral infection and budding is currently receiving substantial attention. We have limited knowledge how HIV virus modulate the dynamic structure of lipid rafts creating pores that allow translocation of the intact HIV into cells. We need to understand the mechanism of HIV interaction with lipid rafts. Mechanism based search for natural compounds can selectively influence the composition of lipid rafts of infected cells and could inhibit the progression of AIDS. Targeting lipid raft by plant-derived compounds will help in searching potential and cost effective anti-HIV natural compounds. The effects of euphane-types of triterpenes extracted from Neem tree on HIV-1 entry and budding from cells have not been tested to date. Euphane-types of triterpenes possess structures that are very close to cholesterol and their insertion in lipid rafts of infected cells may influence HIV proliferation and possibly inhibit AIDS progression. Other advantage of Neem triterpenes is that they can be prepared with lesser cost and in large quantities.

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