Review Article

Current Pharma Research
ISSN: 2230-7842
CPR 2(1), 2011, 453-465.

Recent Modification in Vesicular Approaches for Site Specific Delivery of Disease-Modifying Anti-Rheumatic Drugs (Dmrds) and Synovial Replenishers.

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Abstract
Recent accepted regimen for rheumatoid arthritis, a chronic systemic inflammatory autoimmune disease recommends that NSAIDs should be used together with DMARDs. Although both these classes of medications are well tolerated for short periods, long-term administration may result in gastrointestinal ulcers. Vesicular carrier systems such as liposomes niosomes, fatty acid based vesicles and ethosomes are recently used for site specific delivery of anti-rheumatic drugs as they not only act as penetration enhancers, but simultaneously serve as a depot for the slow and controlled release of dermally applied active substance. Focus of present review is to highlight fatty acid based vesicles (e.g. oleic, linoleic acid) potential as an alternative carrier system for the topical delivery of Ant-rheumatic drugs.

Key Words
Rheumatoid arthritis, DMARDs.

Introduction
Today, clinical medicine possesses an extremely long list of different pharmaceutical products and very year many new drugs and products are added to the list with the understanding of molecular mechanisms of diseases. Scientists and physicians are never satisfied with the fact that drug provide favorable action against the disease under treatment. The task of avoiding undesirable drug actions on normal organs and tissues and minimizing side effects of the therapy is also considered equally important. Thus, screening of biologically active compounds became necessary, providing the choice of drug with selective action on the appropriate organs or tissues has become necessity. At the same time, many pharmacologically effective compounds cannot be used as drugs due to their undesirable action on normal tissues. Their specificity for the drug of choice is not based on their ability to accumulate selectively in the target organs. Normally, they are more or less evenly distributed in the whole body and to reach the target zone the drug have to cross many other organs, cells, intracellular compartments, etc., where it can be partially inactivated. To get desired concentration at target site, a high concentration of drug has to be administered, which has a potential to cause undesirable complications and is sometimes expensive. The anti-arthritic drugs have similar problem as discussed above. Presently these drugs are administered as conventional oral formulations like tablet, capsule and suspension dosage forms [1] which is a well-accepted means of administration but with a typical limitation of very poor delivery of drug at synovial joint along with high systemic side effects. Some other drawbacks of the present conventional therapy are such as GIT degradation and toxicity (e.g. With NSAIDs about 80% of patient experience gastrointestinal side effect including gastric ulcer, perforation and hemorrhage etc.), Poor solubility leads to high variability in oral bioavailability (e.g. Celecoxib, Colchicine have variable oral bioavailability from 24 to 88%), Lag time resulting in delay in onset of action (e.g. Celecoxib has 3-4 hr), Short biological half life (e.g. Colchicine has only 20 min.), Required frequent administration, Poor patient compliance, High systemic side effects (e.g. Rofecoxib showed cardiotoxic and renal side effects leading to its withdrawal from market, Methotrexate has shown prominent hepatotoxic and bone marrow depression), Very poor reach of drug at the site of action, High cost of treatment (e.g. Methotrexate and TNF-α) [2]. The ideal solution to such problems is the development and use of novel drug carriers like liposomes, elastic liposomes, niosomes, ethosomes, nanoparticles and solid-lipid nanoparticles which provide specificity. Among these carriers,

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transferosomes, ethosomes and fatty acid based vesicles show great potentials for effective delivery of drugs to the deeper layer of skin and in synovial tissues joint along with potential to develop sustained release [3]. When the aim is to deliver the drug through skin in a predetermined and controlled fashion it is well known that transdermal/ topical drug delivery system which were introduced more than 20 years ago avoids of first-pass liver metabolism, exposure to chemical and biological conditions of the gastrointestinal tract, reduction or avoidance of adverse events, improved patient compliance and the ability to provide a controlled delivery of drugs with short half-lives and/or narrow therapeutic windows were all attractive features that the pharmaceutical industry was looking for.

Rheumatoid arthritis (RA)

Rheumatoid arthritis is a long-term disease that leads to inflammation of the joints and surrounding tissues. It can also affect other organs. Rheumatoid arthritis is an autoimmune disease in which the body's immune system attacks itself. The pattern of joints affected is usually symmetrical, involves the hands and other joints and is worse in the morning. Rheumatoid arthritis is also a systemic disease, involving other body organs, whereas osteoarthritis is limited to the joints. Over time, both forms of arthritis can be crippling Fig. 1(A). Deformities distinctive to late-stage rheumatoid arthritis such as ulnar deviation of the bones of the hands, or swan-neck deviation of the fingers occur because muscles and tendons on one side of the joint may overpower those on the other side, pulling the bones out of alignment Fig.1 (B). The body's own immune system attacks a joint's synovial membrane, which secretes fluid and lines the joint. The synovium becomes inflamed, produces excess fluid, and the cartilage becomes rough and pitted Fig 1(C, D).

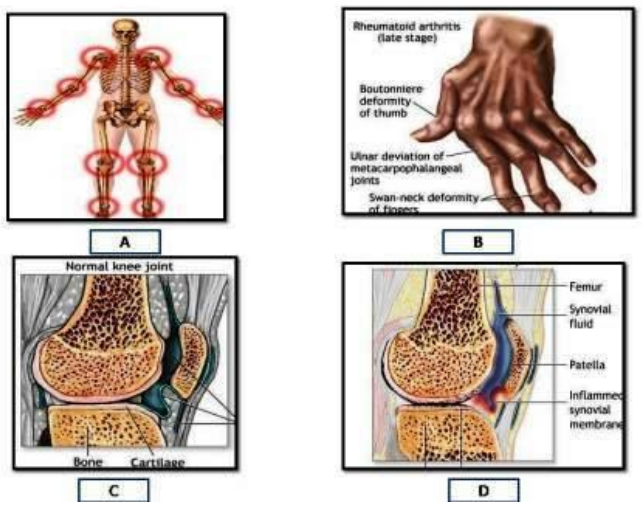

Fig.1: Arthritis.

Causes, incidence, and risk factors [4]

The cause of RA is unknown. It is considered an autoimmune disease. The body's immune system normally fights off foreign substances, like viruses. But in an autoimmune disease, the immune system confuses healthy tissue for foreign substances. As a result, the body attacks itself. RA can occur at any age. Women are affected more often than men. RA usually affects joints on both sides of the body equally. Wrists, fingers, knees, feet, and ankles are the most commonly affected. The course and the severity of the illness can vary considerably. Infection, genes, and hormones may contribute to the disease.

Symptoms

The disease often begins slowly, with symptoms that are seen in many other illnesses: Fatigue, Loss of appetite, Low fever, Swollen glands, Weakness, Eventually, joint pain appears. Morning stiffness, which lasts more than 1 hour, is common. Joints can even become warm, tender, and stiff when not used for as little as an hour. The joints are often swollen and feel warm and boggy (or spongy) to the touch. Over time, joints lose their range of motion and may become deformed. Other symptoms include: Chest pain when taking a breath (pleurisy), Eye burning, itching, and discharge, Nodules under the skin (usually a sign of more severe disease), Numbness, tingling, or burning in the hands and feet. Joint destruction may occur within 1 - 2 years after the disease appears [5].

Signs and tests

A specific blood test is available for diagnosing RA and distinguishing it from other types of arthritis. It is called the anti-CCP antibody test. Other tests that may be done include: Complete blood count, C-reactive protein, Erythrocyte sedimentation rate, Joint ultrasound or MRI, Joint x-rays, Rheumatoid factor test (positive in about 75% of people with symptoms), Synovial fluid analysis. Regular blood or urine tests should be done to determine how well medications are working and whether drugs are causing any side effects. RA usually requires lifelong treatment, including medications, physical therapy, exercise, education, and possibly surgery. Early, aggressive treatment for RA can delay joint destruction [6, 7].

Medication

Potential Disease-Modifying Rheumatoid Drugs (DMRADs) & synovial replenishers

Plaquenil (hydroxychloroquine) is a drug used to treat malaria. It was discovered that it worked for arthritis when people taking the drug for malaria reported improvements in their arthritis. The drug affects the immune system, although doctors do not know precisely how it works to improve rheumatoid conditions. Usually Plaquenil is used along with other DMARDs. It can be given along with steroid treatment to reduce the amount of steroid needed. It is also given to treat the lupus. Plaquenil is given by mouth daily. Side effects include low white blood cell counts, blood or protein in the urine, nausea, and skin rashes. High doses can rarely cause injury to the back of the eye (retina); therefore, patients on this drug should see an eye doctor every six to 12 months. Arava (leflunomide) helps calm the inflammation associated with RA. Arava interferes with the production of inflammatory cells, like those of the immune system. It can reduce signs and symptoms of RA, inhibit joint damage, and can also improve physical function. Arava is a tablet that is taken in a dose of 10 or 20 milligrams once a day. Arava can be taken on an empty stomach or with meals. Possible side effects include rash, hair loss, irritation of the liver, nausea, diarrhea, and abdominal pain. When taking Arava, it is necessary to have regular blood tests for liver function and blood count testing. Arava is not recommended for people who have liver disease, pregnant or nursing women, or people with immune systems weakened by an immune deficiency or disorder. Since Arava can cause serious birth defects, both men and women should use a reliable method of birth control while being treated with this medication. If a woman taking Arava wishes to become pregnant, she must stop the Arava. Then she must follow a drug elimination procedure to get all the Arava out of the body, and then have a blood test to prove that the drug is cleared. Less is known about the effects of Arava on men planning to father children. Men should consider stopping Arava use and following the drug elimination procedure before attempting to conceive. Cyclosporine is a tablet that's best known as a drug to prevent rejection of transplanted organs. It works by stopping an overactive immune system from attack. Therefore, it's effective in stopping joint inflammation and destruction caused by RA. The side effects include high blood pressure, headache, kidney problems, nausea, diarrhea, and heartburn. Regular blood count testing is mandatory. Azulfidine (sulfasalazine) is used for treatment of rheumatoid arthritis, arthritis associated with ankylosing spondylitis, and arthritis associated with inflammatory bowel diseases such as ulcerative colitis and Crohn's disease. It may be used alone or in combination with other medications. Persons allergic to sulfa drugs should not take Azulfidine. Side effects include rash, headache, changes in blood counts, and nausea or vomiting. Gold has been used as a medical treatment for centuries and was a mainstay of RA treatment from the 1920s to the mid 1980s. Gold works by decreasing inflammation in the joints, although doctors don't know how it does this. Gold is given orally or by injection into the muscle. The injection is more effective than the oral version. Possible side effects include skin rash, anemia, low white blood cell count, or liver and kidney problems. Imuran (azathioprine) is drug that has also been used for cancer and organ transplants. It can be effective for RA, particularly for complications such as vasculitis. It is an oral tablet. Side effects include nausea, vomiting, rash, mouth sores, liver and blood count abnormalities, and increased risk of infection. Regular blood test monitoring is mandatory. Cytoxan (cyclophosphamide) is a powerful immune suppression medication. Cytoxan is used only for serious complications of RA, such as vasculitis or inflamed lungs. Cytoxan can cause hair loss, oral sores, fatigue, bone marrow suppression, and increased risk of infection. Regular blood test monitoring is mandatory. Biologics e.g. Actemra, Cimzia, Enbrel, Humira, Kineret, Orencia, Remicade, Rituxan, and Simponi are among the newest treatments for rheumatoid arthritis, and are given by injection. They work by affecting the immune system's signals that lead to joint damage. They are often used in combination with methotrexate or other DMARDs. One side effect is the increased risk for potentially severe infections. These medicines can also cause skin reactions and affect blood counts, and they should be used with caution in patients with weak hearts (congestive heart failure). Other potential long-term effects won't be known until the drugs have been used by patients for many years. (www.webmd.com/rheumatoid-arthritis/modifying-medication).

Glucosamine is increasingly being used in synovial rebuilding with anti-arthritic therapy. Long term use of glucosamine may reduce radiographic progression of osteoarthritis of the knee suggesting it may be a chondroprotective, disease modifying agent in osteoarthritis of the knee. The daily oral dose requirement of glucosamine is 1500mg/day. Although rapidly absorbed from gastrointestinal tract, pharmacokinetic data show that, when administered orally glucosamine is subject to uptake and degradation by the liver and uptake into non joint tissues so that the dose reaching the articular cartilage is a fraction of a percentage of the oral dose. Oral bioavailability of drug molecule is just 26% [8,9]. Methotrexate a folic acid antagonist with antineoplastic activity is effective in controlling recalcitrant psoriasis when administered long-term by the oral or parenteral route. It has been shown to selectively inhibit DNA synthesis in psoriatic epidermal cells, thus decreasing mitotic activity. However, the systematic use of this drug may provoke any of a number of side effects, notably hepatotoxic effects [10]. To reduce these effects, clinical studies have been done with topical methotrexate. A major problem in topical administration of methotrexate is that the drug is hydrosoluble and is mostly in the dissociated form at physiological pH: its capacity for passive diffusion is thus limited.

Nonsteroidal Anti-inflammatory Drugs

NSAIDs are widely used to reduce pain and inflammation and improve function in RA patients [11]. The ability of different NSAIDs to inhibit COX, the enzyme that catalyzes the synthesis of cyclic endoperoxides from arachidonic acid to proinflammatory and other forms of PG, varies: some seem to be potent inhibitors of PG synthesis, whereas others more prominently affect non-PG-mediated biological events [12]. Two COX isoforms have been identified (COX-1 and COX- 2), and NSAIDs are now classified as nonselective or non-COX-2 selective NSAIDs and Coxibs (COX-2 selective agents). Gastrointestinal (GI) intolerability problems, including dyspepsia, abdominal pain, and nausea, are the most frequent adverse events associated with nonselective NSAIDs [13]. GI mucosal damage, such as ulcers, bleeding, perforation, and obstruction, also is common (1 to 2% ulcer complications, 2 to 4% peptic ulcer symptoms with ulcer complications and symptomatic ulcers) [14]. Nephropathy can occur with both COX-2 selective and nonselective NSAIDs. The risk factors for adverse kidney effects include serious hemodynamic compromise such as hemorrhaging, dehydration, moderate/severe congestive heart failure, excessive diuresis, and cirrhosis with or without ascites [15]. Older people with intrinsic renal disease are at greater risk of adverse renal effect due to NSAIDs [16].

Coxibs

The discovery of COX-2, the second isoform of COX, led to the development of NSAIDs with the same analgesic and anti-inflammatory activity as nonselective NSAIDs but without the inherent risk of gastroduodenal mucosal damage and impaired platelet aggregation mediated by COX-1 inhibition [17]. Both celecoxib and rofecoxib are as effective as the common NSAIDs in the treatment of a heterogeneous population of OA patients and, although they can cause dyspepsia, abdominal pain, and nausea slightly more frequently than placebo, they do so less frequently than nonselective NSAIDs [18]. Randomized controlled studies of large sample sizes have shown that rofecoxib 50 mg 4 times a day and celecoxib 400 mg twice a day, respectively, lead to 2 to 3 times fewer symptomatic ulcers and ulcer complications (e.g., bleeding, perforation, obstruction) than naproxen 500 mg twice a day or ibuprofen 800 mg 3 times a day [19]. The better GI tolerability of Coxibs in comparison with traditional NSAIDs also has been confirmed by a recently published large population based observational study: although Coxibs were prescribed to patients at higher risk of adverse GI events, the risk of their being hospitalized due to bleeding was significantly lower than that of the patients treated with conventional NSAIDs [20]. As platelet aggregation-mediated blood clotting is inhibited by COX-1 but not COX-2, the use of COX-2 selective NSAIDs also reduces the risk of GI bleeding [21]. Randomized clinical trials of both COX-2 selective NSAIDs (rofecoxib and celecoxib) have shown that, at the doses used to treat OA, the incidence of hypertension and peripheral edema was the same as for nonselective NSAIDs [22,23]. There have been concerns that COX-2 inhibition without 4 P. The inhibition of COX-1 may lead to an increased propensity for thrombosis in at-risk patients [24]. COX-2 selective inhibitors reduce the production of vascular prostacyclin, which has vasodilatory effects

and inhibits platelet aggregation and, unlike nonselective NSAIDs, they do not inhibit the production of thromboxane, an eicosanoid that promotes platelet aggregation [25,26]. Whether these effects may contribute to a prothrombotic environment is currently a matter of intense debate. In the Vioxx Gastrointestinal Outcomes Research (VIGOR) trial, there was a higher incidence of cardiovascular thrombotic events in the rofecoxib group than in the naproxen group: 1.67 versus 0.70 per 100 patient years [19]. However, a pooled analysis of rofecoxib studies found that the risk of a thrombotic cardiovascular event was similar when patients receiving rofecoxib were compared with those receiving placebo or non naproxen nonselective NSAIDs. These findings are probably at least partially due to the antiplatelet action of naproxen, which has been shown to be potent and sustained during a typical dosing regimen (500 mg twice daily in VIGOR). An attempt to define the question more clearly was made by a recently published retrospective observational study in which the cardioprotective effect of naproxen was disproved. There was no evidence of a negative effect on the cardiovascular system caused by other conventional NSAIDs or by celecoxib (at both therapeutic and overtherapeutic doses) and rofecoxib (at therapeutic doses of 25 mg/day) [27].These data are in line with what emerged from the Celecoxib Long-term Arthritis Safety Study (CLASS) in which there was no difference in cardiovascular event rates between celecoxib and the studied nonselective NSAIDs (which did not include naproxen) [19]; unlike the patients in VIGOR, those in CLASS were allowed to take low-dose aspirin. Despite the concerns raised by the results of VIGOR, other data (including those pooled from placebo-controlled trials) do not support the existence of a clinically relevant prothrombotic effect of COX-2 inhibitors. However, further placebo-controlled data relating to patients at high and low risk of cardiovascular events are warranted to clarify the cardiovascular effects of this class of agents.

Physical Rehabilitation

A variety of modalities have been investigated in the treatment of OA. Thermal therapies (heat, cold, ultrasound), transcutaneous electrical stimulation (TENS), pulsed electromagnetic field therapy, laser therapy, and electrical (Galvanic) stimulation are adjunctive interventions used in addition to exercise and medications [28-32]. Although there are limited scientific data demonstrating their efficacy in the treatment of OA, they are frequently prescribed. The Philadelphia Panel recently formulated evidence based guidelines for selective rehabilitation interventions in the management of low back, knee, neck, and shoulder pain [33]. TENS and exercise were recommended for knee RA.

Surgery

Occasionally, surgery is needed to correct severely affected joints. Surgeries can relieve joint pain and deformities. The first surgical treatment may be a synovectomy, which is the removal of the joint lining (synovium). At some point, total joint replacement is needed. In extreme cases, total knee, hip replacement, ankle replacement, shoulder replacement, and others may be done. These surgeries can mean the difference between being totally dependent on others and having an independent life at home.

Strategies to reduce side effects associated with DMRD'S and synovial replishners Vesicular approaches

For site specific topical delivery, formulation should be biocompatible; it should serve as a local drug reservoir, and should also be able to reduce systemic side-effects by decreasing the systemic absorption of drug(s). In treating skin diseases, the primary purpose of applying drugs to the skin is to induce local effects at or close to the site of application. It has been proposed that cutaneous absorption is desirable and preferred rather than percutaneous absorption. The conventional formulations such as creams, gels, and ointments suffer from the predicament of dermato pharmacotherapy, i.e. limited local activity. To enhance the penetration of bioactive moiety into the skin, and further to localize the drug at the site of action; a number of approaches have been explored including development of vesicular systems such as liposomes and niosomes [34-40]. For last two decades liposomes & deformable liposomes have been investigated as carriers for topical as well as transdermal delivery. Despite of extensive research in field vesicular approaches for site specific delivery, till today this concept remained controversial as most of liposomes or deformable liposomes reaches deep layer of skin i.e. dermis. The focus of present review is to highlight potential of fatty acid based vesicles as alternate carrier for topical delivery of bioactive.

Fatty acid vesicles composed of oleic& linoleic acid have properties to form vesicles in aqueous environment [41]. After about a decade of research saturated fatty acids with carbon atoms in the range of 8–12 were also found to under self-assemble into vesicles in a pH dependent manner [42]. These specially designed vesicles can partition into artificial as well as natural membranes quite rapidly as proved by [43]. Certain researchers reported that these vesicles not only act as penetration enhancers but they also enhances absorption of therapeutic molecules through GIT probably by forming mixed micelles or through chylomicron(s), thus increasing the bioavailability of the molecules [44,45]. Low oral bioavailability and long term therapy associated with RA'S poses a challenge for formulator to design a transdermal /topical delivery system which delivers drug site specifically at the targeted site. The ability of the soft malleable vesicles (ethosomes) to permeate intact tissue through the human skin due to high deformability make it a potential carrier for the delivery of proposed drugs into the deeper layers of skin and joint. It is suggested that the ethosomes , fatty acid based vesicles could reduce the amount of drug required to control the disease by facilitating transport of DMRD'S by minimizing its distribution to the other tissues. Fatty acid vesicles are colloidal suspensions of closed lipid bilayers that are composed of fatty acids and their ionized species (soap). They are observed in a small region within the fatty acid–soap–water ternary phase diagram above the chain melting temperature (Tm) of the corresponding fatty acid–soap mixture [46]. The formation of fatty acid vesicles was first reported by Gebicki and Hicks in 1973 and the following years for oleic acid (cis-9-octadecenoic acid) and linoleic acid (cis, cis- 9,12-octadecadienoic acid), and the vesicles formed were initially named "ufasomes": "unsaturated fatty acid liposomes" [41]. Later investigations have shown that fatty acid vesicles form not only from unsaturated fatty acids but also from saturated such as octanoic acid and decanoic acid [42,47]. Compared with diacylglycero phospholipid vesicles (conventional liposomes), fatty acid vesicles have some unique properties. One important feature is the dynamic nature of fatty acid vesicles owing to the fact that they are composed of single chain amphiphiles. The concentration of non-associated monomers in equilibrium with vesicles is considerably higher than in the case of double-chain phospholipids. For example, while the monomer concentration in equilibrium with 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC) bilayers is around 10−10 M [48], the monomer concentration of oleic acid in equilibrium with oleic acid vesicles is between 0.4 mM and 0.7 mM [49] (depending on the conditions, it could also be below 0.1 mm [50]. Therefore, the flip-flop of molecules between two monolayer leaflets of fatty acid bilayers and the exchange between fatty acid vesicles via monomers are expected to occur more rapidly than in the case of liposomes formed from double chain phospholipids. The second important feature is the formation of a range of fatty acid/soap aggregates just by changing the total concentration and the protonation/ionization ratio of the terminal carboxylic acid [47,51]. Fatty acid vesicles (Fig.-2) always contain two types of amphiphiles, the non-ionized, neutral form and the ionized form (the negatively charged soap), and their ratio is critical for the vesicle stability. Fatty acid vesicles are actually mixed "fatty acid/soap vesicles", but for the sake of simplicity. We just call them here "fatty acid vesicles". The formation of fatty acid vesicles is restricted to a rather narrow pH range (7–9), where approximately half of the carboxylic groups are ionized [47].

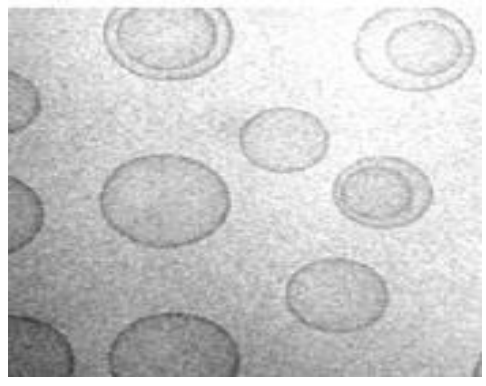

Fig. 2: Oleic acid vesicles.

The pH range for vesicle formation varies depending on the chemical structure of fatty acids. Fatty acids with a longer aliphatic chain tend to form vesicles at a higher pH, because the molecules can be packed more tightly in the membrane. It should be noted that the local pH value at the membrane surface can be substantially lower than the pH of the bulk solution (the measured pH) .These vesicles formed at lower pH regions compared with oleic acid vesicles. Micelles are the dominant aggregation species at higher pH (higher ratio of ionized to protonated

molecules), whereas oil droplets form in the low pH region. In dilute systems (N95 wt.% water), transitions from one type of fatty acid/soap structure to another can be induced easily by changing the pH

Dynamic formation and transformation of fatty acid vesicles

One of the most prominent features of fatty acid vesicles is the dynamic formation and transformation induced by changes of the environmental conditions. The fact that a range of fatty acid aggregates are formed just by changing the protonation/ ionization ratio of the terminal carboxylic acid and by changing the overall concentration provides an excellent opportunity to study physico-chemical properties of different aggregate structures. For example, the organization of the hydrophobic interior of fatty acid vesicles was compared with micelles in the case of the decanoic acid/sodium decanoate system by using fluorescence lifetime and anisotropy measurements of the incorporated chromophore perylene. Perylene embedded in decanoic acid vesicles experienced a somewhat less viscous environment (15 cP) than perylene solubilized in decanote micelles (21 cP). The value determined for the vesicles agreed with the value determined for vesicles from 1,2-dimyristoyl-snglycero- 3-phosphocholine (DMPC) below the main phase transition temperature, in the solid-ordered gel-state of the bilayers [52]. The comparison between vesicles and micelles is important also for clarifying thermodynamic and kinetic aspects of various aggregation states. Whereas micelles are generally regarded to be thermodynamic equilibrium structures, the thermodynamic stability of vesicles still remains widely debated [53]. The formation kinetics of micelles and vesicles from a saturated fatty acid/soap monomer solution was compared by dialyzing the fatty acid/soap monomers through a cellulose acetate membrane Starting from an asymmetric distribution of the fatty acid/soap molecules between two chambers separated by the dialysis membrane, one chamber containing aggregates (micelles or vesicles) and the other containing buffer solution only, the rate of attainment of equilibrium was monitored. An equilibrium state was readily obtained in the case of the micellar system (micelles formed in the diffusate chamber and the fatty acid/soap concentrations in both chambers became the same). In the case of vesicles, however, the attainment of an equilibrium state was severely hindered (the concentration in the diffusate increased very slowly after the solution was saturated with monomers). Vesicles are generally composed of a much greater number of amphiphiles than micelles. Recent experimental and molecular dynamics simulation studies on phospholipid-based systems suggest that the formation pathway of vesicles comprises the following steps: formation of disk-like micelles, successive slow growth of these micelles up to a critical radius, and their final closure to form vesicles [54,55]. The results obtained from the dialysis experiments with fatty acid vesicles suggest that the formation of fatty acid vesicles poses a much higher energy barrier compared with the formation of fatty acid (soap) micelles. One of the unique observations involving dynamic transformations of fatty acid vesicles is the so-called "matrix effect" [56,57]. A convenient way of fatty acid vesicle preparation is the addition of an alkaline soap solution to a buffer solution of intermediate pH. For example, a concentrated solution of sodium oleate micelles is added to a buffered solution at pH 8.5, and oleic acid/sodium oleate vesicles form spontaneously as a result of a partial protonation of the oleate molecules, caused by the drop in pH from about 10.5 to 8.5. Vesicles thus formed are polydisperse in size and lamellarity. However, vesicles formed after the addition of „seed vesicles" of a defined size (e.g. vesicles previously extruded through Nucleopore® track-etched polycarbonate membranes: had a much narrower size distribution, which was close to the size of the seed vesicles [56,57]. The same has been observed also if oleate was added to phospholipid vesicles [58]. Another example of the consequences of the dynamic nature of fatty acid vesicles is the rapid transformation observed on (or close to) a solid surface. Large unilamellar vesicles (LUVs) of oleic acid/oleate with an average size of ca. 100 nm transformed into giant vesicles (with diameters N1 μm) on a glass surface, if the surface contained small amounts of adsorbed hydrocarbon molecules such as squalane [59,60]. This observation was made for several fatty acids tested but not for phospholipids. Although the specific roles of the solid surface and the hydrocarbon olecules are not yet clear, the flexibility of fatty acids to form various aggregate structures should be one of the key factors for this experimental observation.

More detailed studies needed on fatty acid vesicles

For realizing applications based on fatty acid vesicles, one still needs a deeper understanding of their self-assembly process. In the case of oleic acid vesicles, a detailed physicochemical investigation by using a nitroxide-labeled fatty acid and electron spin resonance spectroscopy indicated, for example, that the vesicles may coexist with micelles (or non vesicular aggregates) also in regions of the titration curve in which originally only vesicles (bilayers) were thought to exist in equilibrium with monomers [61], compare with Fig. 1. This point needs to be clarified in future studies. Furthermore, there are only a few quantitative data on the permeability of fatty acid vesicles, one of the exceptions being a study on the permeation of sugars [62]. More data are needed, particularly if one likes to further explore fatty acid vesicles as protocell models or fatty acid-based vesicles as drug delivery or food additive systems. Although it is known that the presence of divalent cations like Mg^{2+} or Ca^{2+} leads to a precipitation of the vesicles [63], the interaction of fatty acid vesicles with other solutes, e. g. buffer ions.

Conclusion

It has been documented and reported that unsaturated fatty acids such as oleic acid and linoleic acid have a tendency to form vesicles in the aqueous environment [41]. After about a decade saturated fatty acids with carbon atoms in the range of 8–12 were also found to under self-assemblage into vesicles in a pH dependent manner [42]. Fatty acids being highly soluble tend to partition into artificial as well as natural membranes quite rapidly [43]. It has also been investigated that fatty acid vesicles enhance the absorption of therapeutic molecules through the GIT, probably by forming mixed micelles or through chylomicron(s), thus increasing the bioavailability of the molecules [44]. It has been reported that free fatty acids act as penetration enhancers for the bioactives through the stratum corneum [45]. However, skin permeation property of fatty acids varies with the chain length and branching. The penetration enhancement effect of fatty acid increases with an increase in the chain length; however, it follows the relation only up to C18. The skin permeation property of unsaturated fatty acids is higher than the corresponding saturated fatty acid. Further, fatty acid(s) containing Cis double bond exhibited higher penetration potential as compared to Trans form. The major limiting property of free fatty acids in their use as a penetration enhancer is their skin irritation characteristics. The problem of skin irritation, however, could be addressed by using fatty acid vesicles as drug bearing carriers. It has been shown that bilayer membrane possesses a fusogenic tendency due to its capability to lower the phase transition temperature of the lipids in the biological membrane. The vesicular membrane fuses with skin lipid bilayers, releasing its contents. Thus, it is hypothesized that fatty acid vesicles will act as a suitable carrier to enhance the penetration of bioactive agents through the stratum corneum with reduced toxicity. Moreover, fatty acid vesicles seem advantageous as they are easy to prepare as well as cost effective.

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Table 1: Details of the herbal medicinal products used for treatment of RA.

Botanical name	Plant part	Tradename	Constituent Marker	Marker mg/ day	References
Populus tremula Fraxinus excelsior Solidago virgaurea	bark, herb, leaf	Phytodolor	Total Flavonoids	0.34-0.56	[64,65]
Populus tremula	bark, leaf		Salicin	4.8-8.0	
Solidago virgaurea	Herb		salicyl alcohol	0.48-0.8	
Fraxinus excelsior	Bark		Isofraxidin	0.67-1.1	
Salix daphnoides	Bark	SM	Salicin	240	[66]
Tripterygium wilfordii Hook F	Root	SM	Triptolide, tripdiolide Triptonide, triptophenolide	0.194, 0.056, 0.0142, 0.746	[67,68]
Tripterygium wilfordii Hook F	Root	SM	Triptolide, tripdiolide Triptonide, triptophenolide	0.389, 0.112, 0.284, 1.472	[67,68]
Tripterygium wilfordii Hook F	Root	T2	Triptolide, tripdiolide Triptonide, triptophenolide	0.021, 0.041, 0.002, 0.002	[67]
Tripterygium wilfordii (local)	Root	Thunder God vine	not stated	not stated	[69]
Tripterygium wilfordii Hook F	Root	TwHF extract	Triptolide and tripdiolide	not stated	[68]
Withania somnifera, Boswellia serrata, Zingiberis officinale, Curcuma longa		RA-1	not stated	not stated	[70]
Clematis mandshurica, Prunella vulgaris, Trichosanthes kirilowii	root, flower, root; 1:1:2	SKI-306X	Oleanolic acid 4%, Rosmarinic acids 0.2%, ursolic acids 0.5%, hydroxybenzoic acid 0.03%, hydroxymethoxybenzoic acid 0.03%, transcinnamic acid 0.05%		[71]
Uncaria tomentosa	Bark	Krallendorn	pentacyclic oxindole alkaloids	0.88	[72]
Tanacetum parthenium	Leaf	SM	Parthenolide	2-3 micromol	[73]
Capsicum (local)	Fruit	Zostrix			[74]
	Fruit	Arlacel 165			[75]
Oenothera biennis	Semen	SM	gammalinolenic acid (GLA)	540	[76]
	Semen	SM	GLA	540	[77]
	Semen	SM	GLA	not stated	[78]
Ribes nigrum	Semen	SM	GLA	525	[79]
	Semen	SM	GLA	2000	[80]
Borago officinalis	Semen	SM	GLA	1400	[81]
	Semen	SM	GLA	2800	[82]
Ganoderma lucida (4g) San Miao San (Atractylodes macrocephala root, Phellodendron chinense cortex, Achyranthes Bidentatae root)	not stated	not stated	not stated	not stated	[83]

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