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How Can Immunosuppression After Organ Transplantation Be Made More Effective and Safer? – A Review on Liposomal Formulations With Consideration of Archaeal Tetraetherlipid

Erni H. Purwaningsih[1], Anton Oertl[2,3], Seruni K.U. Freisleben[3,4] and Hans-Joachim Freisleben[1,2]*

[1]Faculty of Medicine, University of Indonesia, Jakarta-Depok.
[2]Faculty of Medicine, Goethe-University Frankfurt/Main, Germany.
[3]Asklepios Clinical Hospital and MVZ, Wiesbaden, Germany.
[4]Faculty of Natural Sciences, University of Indonesia, Jakarta-Depok.

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Immune-suppressive agents such as methylprednisolone and cyclosporine exert tremendous side effects, because of high dosage and long-term application required for immune suppression after organ transplantation. Major side effects of methylprednisolone include bleeding of the gastro-intestinal tract, hypertension, and osteoporosis, whereas cyclosporine is nephrotoxic. Liposomes are phospholipid particles that allow delivery of drugs preferentially to the reticuloendothelial system. They can be prepared from phospholipids, such as lecithin from soybean or egg yolk, other specific or modified lipids or from membrane-spanning tetraether lipid (TEL), which can be extracted and purified from archaeal cell membranes. One advantage in the use of liposomal application is reduced toxicity of many drugs. We report on various liposomal preparations of cyclosporine, methylprednisolone (L-MPL) and its palmitate derivative (L-MPLP). It has been documented that liposomal cyclosporine A (L-CsA), 1.75 mg/kg/day for seven days has potential for use as an immune-suppressive agent in rats with increased efficacy and decreased nephrotoxicity compared to commercially available forms of intravenous CsA. Liposomal methylprednisolone (L-MPL) 2 mg/kg, intravenously (IV), twice a week shows significantly prolonged cardiac allograft survival in rats and tissue-selective sequestration of the drug in comparison with the same dosage regimen of methylprednisolone in solution, administered daily. We report on organ distribution of L-MPLP in rats after intraperitoneal (IP) administration. Conclusion: **Liposomal preparations of immunosuppressants have significantly higher immune-suppressive potential and lower toxicity than non-liposomal preparations. Bipolar TEL can be extracted, fractionated and purified from archaea to form stable liposomes which are extremely resistant, even to gastric fluid. Hence, TEL liposomes allow (besides IV and IP) for oral administration of immunosuppressants after organ transplantation with pharmacological and toxicological advantages over common liposomal phospholipid bilayer preparations.**

Keywords: Liposomes; Cyclosporine; Methylprednisolone; Immunosuppressant; Toxicity; Organ transplantation; Allograft; Oral administration; Gastrointestinal stability; Absorption.

Organ transplantations are among the major health problems worldwide and especially in developing countries. The incidence of the organ rejection after transplantation is still high. Applying ineffective doses of immunosuppressive agents may be part of the problem. Immunosuppressive drugs such as methylprednisolone (MPL) and cyclosporine A (CsA) exert tremendous side

effects, especially because of high dosage and long-term application required for immunosuppression after organ transplantation. During the episodes of acute rejection, 500-1000 mg of MPL are given intravenously for several days. Because of the high dosage of glucocorticoids required for immunosuppression, side effects are common, such as bleeding of the GI tract, hypertension, and osteoporosis.[1-3] Moreover, long-term medication with MPL will disturb the endogenous corticosteroid regulation. Complications related to systemic immunosuppression are major causes of morbidity and mortality following organ transplantation. The major unwanted side effect of high dose and long-term application of CsA is nephrotoxicity.[1-3]

Incorporation of therapeutic compounds into liposomes depends on the physicochemical properties of both the compound and the lipid. The most effective way for the incorporation of sufficiently lipophilic compounds is to mix them with the lipids from the beginning of preparation, so that they are preferentially inserted into the liposomal membrane via hydrophobic interaction. Normally, therapeutic concentrations do not require amounts of a respective compound too high to be taken up into the liposomal membrane by approximately 100% with this method. This was also shown for methylprednisolone and cyclosporine; however, liposomes prepared without special stabilizers lack stability and exert rapid loss of incorporated compound(s). Hence, a special aspect of this review is the stabilization of liposomes with incorporated immunosuppressants by means of archaeal tetraether lipid. This part will be described in detail.

Cyclosporine

Cyclosporine A (CsA) is a cyclic peptide found in *Tolypocladium inflatum,* Gams. It is an extremely hydrophobic molecule. As an immune-suppressive agent, CsA has a highly selective ability to inhibit the activation of T-cells. Although its site of action has not been defined precisely, it inhibits an early cellular response to antigenic and regulatory stimuli, primarily in populations of Helper-T-cells. Cyclosporine also causes a general reduction in the production and release of lymphokines in response to an antigenic stimulus. At higher concentrations, CsA inhibits expression of receptors for IL-2. Although CsA can inhibit the activation of Helper-T-cells, it does not prevent the stimulation of their clonal expansion by IL-2. It is potentially significant that CsA allows the expression of suppressor cell activity at concentrations that inhibit the induction of cytotoxic T-cells.[4]

Liposomal Cyclosporine A

The first reports on liposomal CsA (L-CSA) go back to the eighties of last century.[5] In general, natural compositions of lecithin from soy beans or egg yolk were used, as well as defined lipid composition.[6-8] Liposomal CsA was reported less nephrotoxic.[9] In 1989, Vadiei *et al*.[10] compared two liposomal CsA formulations of dimyristoyl phosphatidylcholine (DMPC):stearylamine (SA) (7:1 molar ratio) and DMPC:dimyristoyl phosphatidylglycerol (DMPG) (4:1 molar ratio) for IV application, at optimal drug:lipid ratio of 1:20 (w/w) for both formulations. According to their evaluation, DMPC:SA is advantageous over the second formulation.[10]

Later on, liposomes for CsA delivery have been prepared from more complex composition, phosphatidylserine/phosphatidylcholine, cholesterol sulfate, and lyso-phosphatidylcholine in a molar ratio of 3:4:2.[11] The authors reported higher efficacy and lower nephrotoxicity. Further characterization and pharmacokinetic studies including oral administration and intermembrane exchange were conducted.[12-17]

The interaction and insertion of cyclosporine into biological and model membranes has widely been investigated and discussed.[18-21] Comparing the structure of cyclosporine, a lipophilic cyclic peptide with 11 amino acids with similar compounds that were investigated in bilayer and tetraether lipid (TEL) membranes,[22-24] valinomycin, a lipophilic cyclic peptide with 12 amino acids resembles cyclosporine most; however, alamethicin also exerts similar behavior, especially considering the interaction with cholesterol. Cyclosporine A interfered both with lateral lipid organization[19] and probably with cholesterol binding sites, because addition of cholesterol to liposomal membranes reduced their binding capacity from the maximum of one CsA molecule per 19 lipid molecules.[21] Although inserted deeply into the hydrophobic moiety of membranes, preferentially at the interface between fluid-crystalline and gel-analogous lipid domains, the single hydroxyl group of CsA may be oriented

to and interact with the polar head groups of lipid molecules[20] and thus, with cholesterol binding sites.[25]

Liposomal Preparations for Oral Administration

Liposomal preparations for oral cyclosporine delivery were investigated by several working groups.[26-29] Modified liposomes for the oral delivery of CsA have been tested in vitro and in rats.[30] Chitosan-modified liposomes (CS-Lip) were compared with Pluronic® F127-modified liposomes (PF127-Lip). Liposomes were prepared in a similar way as described above, finally extruded 6 times through polycarbonate filters of 200 nm pore size by means of a high-pressure homogenizer.[31-32] The composition was CsA, egg yolk phosphatidyl choline (EPC), and cholesterol at a 1:28:5 molar ratio. Pluronic® F127 (PF127), a non-ionic chemical polymer and cationic polysaccharide chitosan (CS), at a deacetylation degree of 94%, were added individually to yield hydrophilic non-ionic (PF127-Lip) and cationic (CS-Lip) polymer-modified liposomes.[30,33] The latter are known as mucoadhesive and the former as mucus-penetrating particles,[34] which both had been shown in animals to increase the intestinal absorption of loaded drugs *versus* the absorption of free drug and from unmodified liposomes.

Size stability of these particles was tested in artificially simulated gastric fluid (SGF, pH 1.2) for 2 hours and simulated intestinal fluid (SIF, pH 6.8) for 6 hours. Initial diameters were 165.25±9.28 nm of unmodified liposomes (Lip), 172.82±15.79 nm of PF127-Lip, and 207.81 ±12.21 nm of CS-Lip. In SGF, only PF127-Lip remained stable, whereas Lip significantly increased and CS-Lip significantly decreased in size. In SIF, Lip decreased in size, whereas PF127-Lip increased. Although significant these changes were still moderate within a range of less than 50 nm. On the contrast, CS-Lip changed tremendously in size, after 2 hours large aggregates precipitated and 40% of the liposome-loaded drug was released into the supernatant.[30] On the other hand, release profiles of all three preparations in ethanol-containing PBS within 24 hours were similar without burst phenomena indicating that CsA was well incorporated into the liposomes and not essentially influenced by the modifications. Entrapment efficiency was 85% in Lip, 82% in CS-Lip, and 90% in PF127-Lip, and release from all preparations was up to 85% of the incorporated CsA within 24 hours.[30]

For the determination of the penetration into intestinal mucus and tissue in rats coumarin-6 was incorporated into the liposomes as hydrophobic model substance which allows for fluorophotometric measurement. Fluorescence was measured in the duodenum, jejunum and ileum and was highest in the mucus with CS-Lip and highest in the tissue with PF127-Lip. In other words, CS-Lip get stuck in the mucus, typical of mucoadhesive particles and don't reach enterocytes in intestinal tissue, which means that they are not suitable for effective oral drug delivery. In contrast, PF127-Lip are able to effectively deliver their drug load into the intestinal tissue.[30]

Pharmacological Parameters of CsA and L-CsA

Oral bioavailability of CsA varies from 20 to 50 %. About 60-70 % of the drug in whole blood is contained in erythrocytes, 10-20 % contained in leukocytes. Cyclosporine has a plasma half-life of 5.6±2 hours and a plasma clearance of 5.9±1.0 mL/min/kg. The volume of distribution is 1.2±0.2 L/kg. Oral treatment with a dose of 15 mg/kg BW is initiated 4 to 24 hours prior to transplantation, once daily and continued for one to two weeks after transplantation. Thereafter, the dosage is reduced each week until a maintenance dose of 3 to 10 mg/kg/day is reached. Dosage is generally guided by signs of renal toxicity, as judged from changes in creatinine clearance.[4]

In the study of Chen *et al*.,[30] intestinal absorption and bioavailability of liposomal CsA after oral administration of 10mg/kg BW were determined by the measurement of C_{max} [μg/mL], T_{max} [h], and $AUC_{0\rightarrow t}$ [μg·h/mL] in the blood of Sprague-Dawley rats. The values of CsA in plasma differed between PF127-Lip and CS-Lip roughly by a factor of two; C_{max} (1.37 ± 0.15 *vs*. 0.79 ± 0.10) and $AUC_{0\rightarrow t}$ (11.59 ± 0.7 vs 6.30 ± 097) were significantly higher and T_{max} (1.68 ± 0.29 vs. 3.67 ± 0.58) significantly lower in PF127-Lip than in CS-Lip; the values with unmodified liposomes were in between.[30]

Cyclosporine encapsulation using glyceryl monooleate-poloxamer 407 nanoparticles (poloxamer 407 is identical with Pluronic® F127) yielded 85% encapsulation efficacy and higher relative oral bioavailability in beagle dogs

of about 178% *versus* Sandimmun Neoral as reference.[40] The relative oral bioavailability of L-CsA encapsulated in soy phosphatidylcholine (SPC) liposomes containing sodium deoxycholate instead of cholesterol was slightly more than 120% as compared with cholesterol-containing SPC liposomes or Sandimmun Neoral.[31]

If CsA cannot be administered orally, it must be infused slowly over a period of 2 to 6 hours or longer. The daily dose should be only one-third the oral dose (usually 5-6 mg/kg BW).[4] Two hours after IV injection of L-CsA in rats at a single dose of 2 mg/kg, the distribution was increased two-fold in liver and spleen tissues compared to CsA in saline. Intravenous administration of 10 mg/kg CsA in saline was demonstrated to exhibit exaggerated nephrotoxicity in renal ischemia induced by contralateral nephrectomy in rats, whereas serum creatinine in animals, which had received liposomal CsA, returned to control levels within 96 hours. In liver transplant models in rats, the dose of 1.75 mg/kg of L-CsA resulted in significantly prolonged survival rates for about 93 days as compared to the group, which had received the same dose of CsA in saline.[11]

Dexamethasone

Dexamethasone is a glucocorticoid, which has the capacity to suppress immune responses. Although considered to be immune suppressive, therapeutic doses of glucocorticoids do not significantly decrease the concentration of antibodies in the circulation. The immune response is initiated by the interaction of an antigen with macrophages and with antibodies on the surface of B-lymphocytes. Glucocorticoids interfere with the function of macrophages in several ways: firstly, they inhibit the action of MIF (migration-inhibitory factors), thereby promoting the emigration of macrophages from affected areas; secondly, they inhibit the processing and display of antigens by interfering with the facilitating actions of α-interferon; thirdly, they inhibit the synthesis and release of IL-1. More importantly, IL-1 participates in the activation of resting T-lymphocytes when they come in contact with processed and histocompatibility antigens displayed on the surface of activated macrophages. Hence, glucocorticoids suppress the activation of T-cells by several mechanisms. Recently, dexamethasone has come into the focus with SARS-CoV2 infections and COVID-19,[41] but this is not the topic to be discussed here.

Liposomal Dexamethasone

Tanaguchi *et al.*[42] compared the incorporation rates of dexamethasone (DM), dexamethasone acetate (DA), dexamethasone valerate (DV), and dexamethasone palmitate (DP) into liposomes at a ratio of 16 mM lipid and 0.5 mM steroid (or its respective derivative), which is equivalent to 3.125 mol% steroid. They determined the water solubility in physiological buffer pH 7.4 at 37°C as 80.5, 29.6, 1.3, and 0.052 mg/L, respectively. Chloroform/water partition coefficients (PC) were 9, 657, 8780, and not measurable for DP, which values are comparable to DM logP 0.95, DA logP 2.8, DV logP 3.9 and a higher, not measurable, value for DP.[42]

The authors used sonication and subsequent filtration through a polycarbonate filter with one μm pore width. Incorporation rates were increasing (slightly depending on sonication time between 2.5 and 20 min), 90.5-92.0 % (DM), 94.7-96.0 % (DA), 99.5-99.6 % (DV), and 100% for DP. Various lipid compositions comprised egg yolk lecithin (EYL), dioleoyl-phosphatidylcholine (DOPC), stearylamine (SA), diacetyl-phosphate (DCP) with similar incorporation rates; only dipalmitoyl-phosphatidylcholine (DPPC) had lower DM incorporation rates of 78.5%.[42]

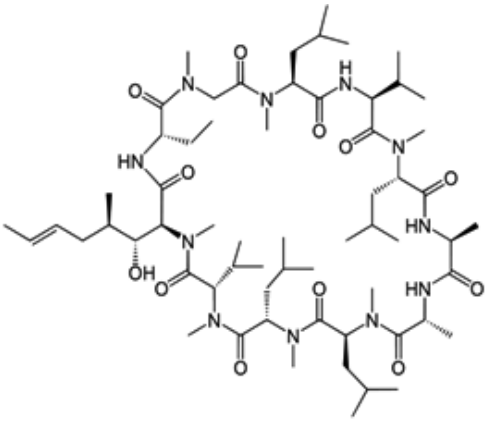
The authors also investigated the influence of cholesterol on the incorporation of steroids (DM and derivatives): cholesterol up to 4 mM concentration increased free steroid in the preparations concentration-dependently from 20 to 40 mg/L (DM); from slightly above 10 to 20 mg/L (DA); much less with DV (below 3.5 mg/L); and not at all with DP (0 mg/L).[42] It was concluded that steroids interact differently from DP with lipids in the membrane and that cholesterol competes with them.[43] Steroids may be oriented horizontally, so that the hydroxyls remain hydrated, whereas DP may be inserted into the membrane perpendicularly via its palmitate side chain, similar to cortisone-21-palmitate.[44,45]

Prednisolone and Methylprednisolone

Liposomal Prednisolone

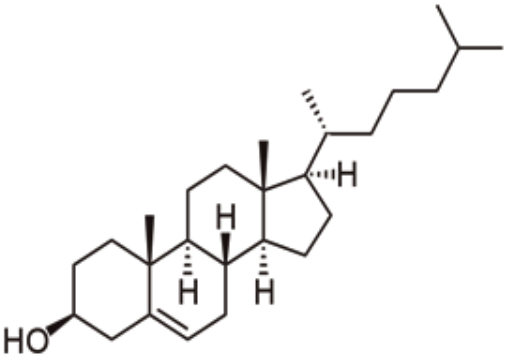
Prednisone and Prednisolone are mainly used for the treatment of rheumatic and atherosclerotic diseases. Liposomal preparations have been reported with prednisolone,[46-48] but

Cyclosporine



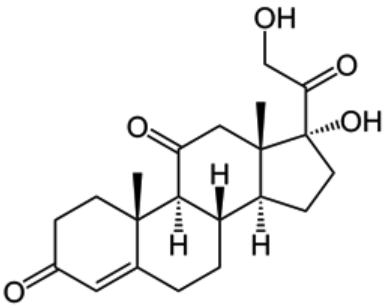
Cyclic undecapeptide
MF $C_{62}H_{111}N_{11}O_{12}$ MW 1202.6 g/mol
WS 6.6-106 mg/L O/W PC logP 2.8

Cholesterol



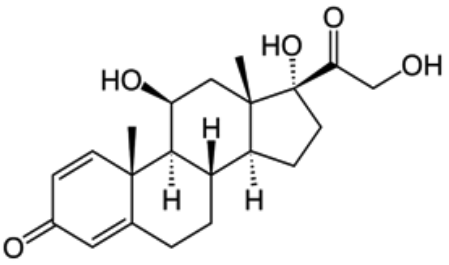
MF $C_{27}H_{46}O$ - MW 386.65 g/mol
WS 9.5e-05 g/L; 0.095 mg/L (30 °C)
O/W PC logP 3.7 (10.52)

Cortisone



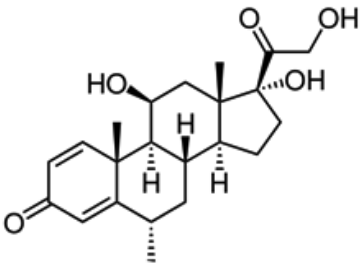
MF $C_{21}H_{28}O_5$. MW 360.4 g/mol
WS 7.77e-04 M; 280 mg/L (25 °C)
O/W PC logP 1.47

Prednisolone



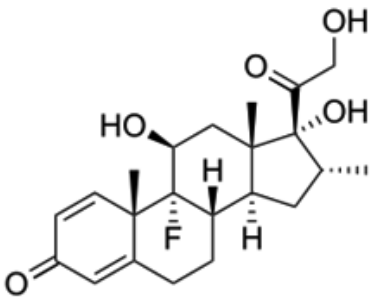
MF $C_{21}H_{28}O_5$ - MW 360.44 g/mol
WS 6.19e-04 M; 223 mg/L (25 °C)
O/W PC logP 1.62

Methylprednisolone



MF $C_{22}H_{30}O_5$ - MW 374.5 g/mol
WS 3.2e-04 M; 120 mg/L (25 °C)
O/W PC logP 1.5

Dexamethasone



MF $C_{22}H_{29}FO_5$ - MW 392.461 g/mol
WS 1.96e-04 M; 89 mg/L (25 °C)
O/W PC logP 1.83

Fig. 1. Chemical formulas of selected immunosuppressants; O/W PC, oil/water partition coefficient (data compiled from[35-39])

liposomal preparations have not been applied for immunosuppression and are therefore not discussed here.

Liposomal Methylprednisolone (L-MPL)

Methylprednisolone is a glucocorticoid with immunosuppressive activity similar to what was already described for dexamethasone. Mishina *et al*. considered the optimum formulation being EPC and phosphatidylglycerol (PG) at molar ratio of 9:1 and 5 mol% MPL.[49] The authors refer to[50-52] and consider 10% of negatively charged lipid in the EPC membrane best compromise for incorporation of MPL into and its retention in liposomal membranes. Liposomes were stable overnight, but lost 70% of MPL within one week.[49]

Therefore, it was decided to stabilize L-MPL with archaeal tetraether lipid. Liposomal MPL was prepared from EPC (= EYL) and phosphatidylglycerol (9:1 molar ratio) with 5 mol% of MPL.[53,54] Ethanolic solutions of these mixtures (20mg/mL) were evaporated under reduced pressure (250 mbar) in a round-bottom flask at 40°C on the water bath of a Büchi Rotavapor. The dry lipid film was stored at RT for 12 hours in a vacuum desiccator in order to remove residual moisture. Subsequently, the lipid film was suspended in PBS, pH 7.4, at an amount to result in 15-20 mg of lipid mixture per mL of buffer. This suspension was shaken by hand with two glass beads added into the flask. The resulting suspension of large multilamellar vesicles (LMV) was sonicated and extruded (5 to 7 times) through Liposofast® polycarbonate filters (pore size 100 nm).[55]

To separate free (not encapsulated) drug, the liposomal suspension was applied to an open Sephadex G-75 chromatography column. All liposomal preparations were routinely checked in a Malvern Particle Sizer and several representative preparations in addition by electron microscopy.[24] The polycarbonate filter extrusion resulted in unilamellar vesicles of roughly 100 nm in diameter, different preparations varied from above 50 to 120 nm, but each single preparation had to be uniformly size-distributed in order to be used for further investigation.

For the incorporation of MPL into liposomes**,** phosphatidylglycerol (PG) was admixed to EPC at 10 mol% or (instead of PG) TEL extracted and purified from *Thermoplasma acidophilum (T.a.*) at 2.5 or 10 mol%; in addition, pure TEL liposomes (100 mol%) were used. In all cases, 5 mol% MPL were applied for liposomal incorporation resulting in molar liposomal ratios of 9.4-9.5:0.5-0.6 (lipid:MPL), equivalent to incorporation ratio of about 100% in all cases and 0.5-0.7 mg MPL per mL of liposomal suspension. Since liposomes prepared according to this method lack stability (they must be freshly prepared for each test series), Oertl *et al*.[56,57] and Bräutigam *et al*.[58,59] suggested stabilization of L-MPL preparations with TEL according to Freisleben *et al*.[60] (Table 1).

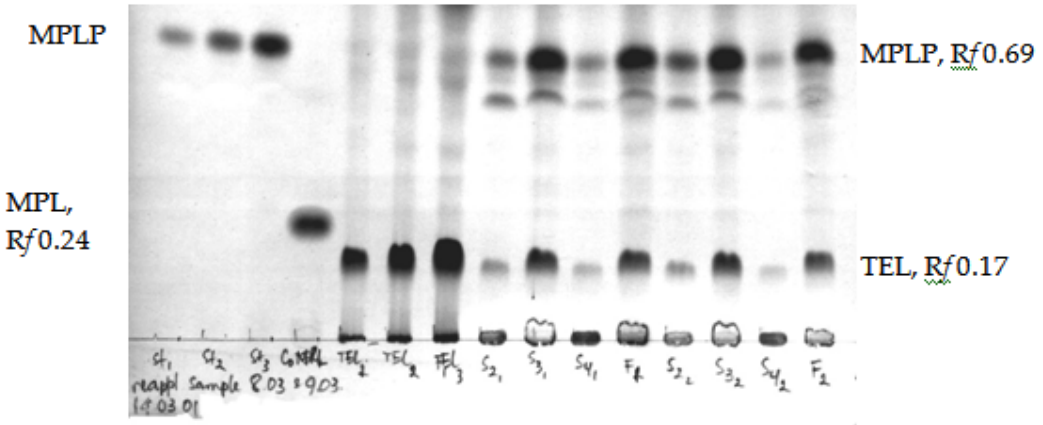


Fig. 2. Thin layer chromatography of GFC-fractionated liposomal samples (fraction volume 1 mL). Distribution of MPLP and TEL: lanes 1-3, MPLP at increasing concentrations; lane 4, MPL; lanes 5-7, TEL at increasing concentrations; lanes 8-10 and 12-14, fractions (S) 2-4; lanes 11 and 15, (F) = after extrusion, before fractionation; MPL, methylprednisolone; MPLP, methylprednisolone palmitate; TEL, tetraether lipid; GFC, gel filtration chromatography through a Sephadex G-75 column.[63,64]

The steroid MPL is effective when given orally; its bioavailability is 82 ± 13 %. In the plasma, 90% or more are reversibly bound to proteins (albumin and globulin) under normal conditions. MPL clearance is 6.2 ± 0.9 mL/min/kg.[4] MPL has a short plasma half-life, but the liposomal formulation L-MPL markedly prolonged plasma circulation time and led to sequestration of the steroid into the lymphatic tissues. Terminal half-life was dramatically extended from 1.5-2.5 hours for MPL to more than 30 hours for L-MPL (Table 2).[49] The distribution volume of L-MPL 1-2 hours after intravenous injection of a 2 mg/kg bolus in rats was significantly increased from 1.2 ± 0.2 L/kg of MPL in lymphatic tissues: in spleen 77-fold; in thymus 27-fold and in liver 9-fold. In liver and spleen MPL remained detectable for 26 days after L-MPL injection confirming the tissue-selective sequestration of the drug.[49]

Male Lewis RT1 rats served as recipients of cardiac allografts from Lewis x Brown Norway F_1 (LEWxBN)F1 hybrids.[53,54,56,57,61] Hearts were anastomosed to the abdominal great vessels using standard microvascular techniques. Ventricular contractions were assessed daily by palpation, and rejection was defined as the day of cessation of heartbeat.[53,56,57,59,61]

The dosage of L-MPL of 2 mg/kg BW, intravenously twice a week led to cardiac allograft survival of 20.8 ± 6.5 days (maximum 30 days), whereas with the same dose of non-liposomal MPL (from a conventional commercial preparation) the allograft was rejected after 7.8 ± 1.0 days, similar to the untreated control group (9.2 ± 1.2 days). The dosage of 4 mg/kg BW, intravenously once a week, also significantly prolonged cardiac allograft survival in rats to 17.5 ± 2.8 days, which was comparable with daily administration of 2 mg/kg BW of MPL in solution (17.0 ± 2.7 days). Only the daily injection of 4 mg/kg BW of non-liposomal MPL (i.e., seven times higher dosage) led to results comparable to the treatment with liposomal MPL.[53,61]

Bilaterally nephrectomized LEW rats were recipients of BN kidney transplantation (Tx).[54,58,59] L-MPL was administered IV, 2 mg/kg BW, twice a week or 4 mg/kg BW, once a week. Distribution of L-MPL was determined in liver, spleen and thymus between one and two hours after injection.[49] Renal allograft survival with 4 mg/kg BW of L-MPL (IV once a week) significantly prolonged allograft survival to 20.2 ± 7.4 days (comparable with daily administration of 4 mg/kg MPL in solution: 19.0 ± 6.8 days) vs. acute rejection of 8.5 ± 0.5 days ($p < 0.001$). MPL + empty liposomes injected separately once a week was ineffective (8.8 ± 0.5 days).[54]

Liposomal Methylprednisolone Palmitate (L-MPLP)

Methylprednisolone-palmitate (MPL-P) is a prodrug synthetized for the same reason as the derivates of dexamethasone (DA, DV, DP), in order to increase and stabilize their incorporation into liposomal membranes; in case of EPC alone MPL-P was incorporated at a maximum of 79%. The addition of 2.5 mol % of TEL from *Sulfolobus acidocaldarius* (TEL-*S.a.*) increased the incorporation rate to approximately 95% (range 94-97%) at concentrations of 4-10 mol% added to the original suspension. TEL itself was incorporated at a rate of 85-86% (Table 3).[62] In detail, the incorporation of 2.5 mol% TEL into EPC at various concentrations yielded in: EPC concentration 0.52 mM, TEL 0.011 mM, incorporation rate 84.6%; EPC concentration 2.03 mM, TEL 0.044 mM, incorporation rate 86.7%; EPC concentration 2.54 mM, TEL 0.054 mM, incorporation rate 85.0%.

Liposome suspensions were formed with input concentrations of 1.7 mM or 3.4 mM EPC, 2.5 mol% TEL, and 2-10 mol% MPLP. Best results were obtained with 4-5 mol% of MPLP. In liposome suspensions containing MPLP in 1.7 mM EPC, the highest amounts of EPC and MPLP were found in 1.0 mL fractions 2-4 after Sephadex G-75 column gel filtration chromatography (GFC) with MPLP distribution values in fraction 3 around 40-43% (43.1% at MPLP 4 mol% and 40.0% at MPLP 5 mol%) and in liposomal mixtures containing TEL, distribution value in fraction 3 was 47% at 4-5 mol% MPLP input[62] (for details see Materials and Methods section).

Particle size stability of the liposomes was determined; L-MPLP is stable at 20°C for 21 days, although the size tends to become larger and size distribution broadens. EPC-MPLP liposomes are less stable (Table 4, data collected from[63,64]).

Pharmacological Parameters

Organ distribution of MPL/MPLP was measured as µg/g tissue after IP injection of L-MPLP (data collected from[65]).

Suppression of TNFα levels was determined in in-vitro and ex-vivo lymphocyte cultures.[66] The percentages of TNFα levels in each group were compared to controls (= 100%); control groups: Tris buffer 5 ml/kg BW; MPL at 0.005, 0.05, and 0.5 mM MPL sodium succinate concentrations; L-MPLP at 0.005, 0.05, and 0.5 mM MPLP concentrations in liposomes; For the in-vitro cultures, the TNFα levels were measured using the splenic lymphocyte cultures. For the ex-vivo cultures, experimental CH3-mice were sacrificed 48 hours after the drug administration, spleens removed and used for lymphocyte cultures.[66]

DISCUSSION

Liposome Technology

Liposomes as vehicles or carriers for pharmaceuticals already brought about

Table 1. Stability of liposomal MPL preparations

Lipid composition	Molar ratio	Negatively charged lipid [mol %]	Stability: Loss of MPL within one week
EPC/PG	9:1	10	65-75%
EPC/TEL	9:1	10	14-17%
EPC/TEL	39:1	2.5	18-20%
TEL	-	100	20-23%

Footnote: PG, phosphatidylglycerol; EPC, egg phosphatidylcholine; TEL, tetraether lipid

Table 2. Pharmacokinetic parameters of MPL and L-MPL (data compiled from[49])

Parameter	MPL	L-MPL	Unit	Remarks
AUC	339	1093	ng x h/mL	area under the curve
Clearance (CL)	5.04	1.83	L/h/kg	apparent systemic clearance
CL_D	2.24	67.08	L/h/kg	distribution clearance
$t_{1/2}$	0.48	30.13	h	terminal half-life
MRT	0.42	11.95	h	mean residence time
MRT_t	0.14	11.73	h	mean residence time in tissues
V_c	1.39	0.39	L/kg	central volume of distribution
V_{ss}	2.1	21.87	L/kg	total volume of distribution

Table 3. Incorporation of TEL and MPLP into EPC liposomes (data compiled from[62])

Input MPLP	4 mol%	5 mol%	10 mol%
Input EPC 1.7 mM	Detected in EPC liposomes		
	1.09:0.028 mM*	0.86:0.034 mM*	0.73:0.013 mM*
Percentage of incorporation	MPLP	MPLP	MPLP
	64.2%	79.0%	17.8%
	Detected in EPC liposomes stabilized by 2.5 mol% TEL-*S.a.*		
Input	mM concentrations*	mM concentrations*	mM concentrations*
EPC:TEL	EPC:TEL:MPLP	EPC:TEL:MPLP	EPC:TEL:MPLP
1.7:0.04 mM	1.02:0.022:0.039	1.27:0.027:0.066	0.52:0.011:0.050
Percentage of incorporation	TEL 86.7%	TEL 85.0%	TEL 84.6%
	MPLP 94.1%	MPLP 97.1%	MPLP 95.2%

Footnote: EPC, egg yolk phosphatidylcholine, *measured by enzymatic reaction photometrically at wavelength λ = 490 nm; MPLP = methylprednisolone palmitate; TEL = tetraether lipid *measured from spots on TLC with scanner detection.

benefits to HIV infections or AIDS and otherwise immunosuppressed patients who suffer pulmonary or systemic mycoses[67] or protozoan diseases,[68] because the liposomal application of lipophilic antimycotic amphotericin B is much better tolerated and is more effective than IV administered non-liposomal preparations.[69] Other liposomal formulations are already successful with doxorubicin in cancer therapy.[70-73] Moreover, liposomes exert also immunoadjuvant properties for several antigens in vaccine design[74] in that they are capable to induce both lymphokine production and potent humoral response characterized by increased production of IgM and IgG2a.[75]

There are various mechanisms of interaction between liposomes and cells or cell membranes. The most promising and effective one appears to be intermembrane exchange,[18-21,76] but also fusion processes between liposomes and cell membranes and total uptake of liposomes via endocytosis play important roles in liposomal delivery systems. The surface of liposomes can be modified and adjusted to cell or tissue targeting or therapeutic aims.[22]

Liposomes from archaeal lipid are extremely stable towards acidic environment; hence, they could well be used for oral or local application in the GI tract,[60,77] or instillation into the urinary tract.[60] Archaeosomes are liposomal formulations based on natural archaeal[78] or synthetic TEL.[79,80]

Miscibility of TEL with Other Components

In differential scanning calorimetry (DSC) and differential thermo-analysis (DTA) experiments with highly purified TEL from *T.a*., it was found that – except for methyl-branched ether bond diphytanylglucosylglycerol which exerts unlimited miscibility[81] - it depends on the phase status of the lipids whether they are able to form uniform mixed phases or undergo some phase separation with domains of higher and lower contents of TEL.[82] TEL and bilayer-forming lipids with this kind

Table 4. Stability of liposomes consisting of EPC, TEL (2.5 mol%) and MPLP (5 mol%)

Temperature 20º C	Days of storage			
	0	3	9	21
EPC:TEL:MPLP	111 nm	132 nm	146 nm	162 nm
EPC:MPLP	91 nm	113 nm	185 nm	aggregation

Table 5. MPL/MPLP in organs [µg/g tissue]

Minutes	Liver	Thymus	Spleen	Bone marrow	Kidney left	Kidney right
30	10.66	1.23	3.28	0.65	0.81	3.25
60	13.48	5.35	5.88	1.01	3.49	6.71
210	16.33	8.22	2.52	2.70	1.40	5.47
Average	13.54	4.02	3.48	2.23	2.15	5.16

Footnote: MPL = methylprednisolone; MPLP = methylprednisolone palmitate; L-MPLP = liposomal methylprednisolone palmitate.

Table 6. MPLP in liver [µg/g tissue] after IP injection (data from[65])

Minutes	Liposome	MPL	MPLP	L-MPLP
30	0.28	0.30	0.01	0.88
60	0.24	0.08	0.50	0.84
90	0.27	0.21	0.20	1.60
150	0.30	0.32	0.44	1.39

Footnote: MPL = methylprednisolone; MPLP = methylprednisolone palmitate; L-MPLP = liposomal methylprednisolone palmitate

of limited miscibility are unbranched ether bond dihexadecyl-glucosylglycerol mixing similarly to dipalmityl-gucosylglycerol, unbranched ester bond dipalmitoyl-phosphatidylglycerol and dimyristoyl-, dipalmitoyl-, distearoyl-phosphatidylcholine, soy bean lecithin (SBL), and EPC (=EYL). The results were generally comparable to experiments with lipids and cholesterol, where different domains are found with higher (cholesterol-rich) and lower contents of cholesterol.[83] Mixtures of membrane-spanning TEL with varying degree of pentacyclization also exert complex phase transition behavior[84] with tendency to formation of metastable phases[85] which strongly depends on experimental conditions.[86]

Mixed phases of DPPC with TEL at higher ratios of DPPC:TEL appear more homogeneous than higher ratios of TEL:DPPC.[82] This is consistent with observations of the stability of mixed liposomes; higher ratios of EPC (= EYL) or SBL:TEL form more stable liposomes than higher ratios of TEL:lecithin.[87-89] It was postulated that TEL should not exceed 25 % in EPC/SBL liposomes, equivalent to 12 mol%.[60,90] Moreover, liposome stability increases with higher purity of TEL,[87-89] as was demonstrated with the polar

Table 7. TNFα levels of in-vitro and ex-vivo lymphocyte cultures.[66]

Administration of mg/kg BW	mM conc.	In-vitro % of control	Ex-vivo (= 100%)
MPL 2	0.005	70	100
MPL 8	0.05	50	95
MPL 16	0.5	35	98
L-MPLP 2	0.005	70	95
L-MPLP 8	0.05	35	70
L-MPLP 16	0.5	5	30
Liposomes	—	90	93

Table 8. Comparison of allograft survival in experimental animals

Drug	Non-liposomal	Liposomal	Organ/allograft	References
CsA	16.0 ± 2.3 days	30.4 ± 2.8 days	Heart	[17]
MPL	7.8 ± 1.0 days	20.8 ± 6.5 days (max 30 days)	Heart	[53,61]
	7.1 ± 1.0 days	19.1 ± 4.9 days	Heart	[54]
	8.8 ± 0.5 days + lip 19.0 ± 6.8 days*	20.3 ± 7.4 days (max > 80 days)	Kidney	
	10.5 days	27.5 days Dextran-MPL	Liver	[96]

Footnote: CsA, cyclosporine A; MPL, methylprednisolone; +lip, separate injection of MPL and empty liposomes; * daily injection of the same dose as once a week with the liposomal preparation

Table 9. Particle size [nm] of EPC and mixed EPC:TEL liposomes

Measurement	Liposomal components			
	EPC		EPC + TEL (2.5 mol%)	
	Sonication (-)	(+)	Sonication (-)	(+)
1	76 nm	26 nm	109 nm	37 nm
2	70 nm	25 nm	98 nm	36 nm
3	75 nm	26 nm	99 nm	36 nm

Footnote: EPC, egg phosphatidylcholine; TEL, tetraether lipid (*T.a.* purity 99%; data collected from[63]).

membrane fraction extracted from *T.a.* and main (glycol)-phospholipid highly purified from this fraction.[91]

Stabilization of lecithin liposomes with 2.5 mol% up to 5 mol% phosphatidic acid or other negatively-charged phospholipids has widely been used in liposome technology.[92] Ten mol% of negatively charged lipid was found best for the incorporation and retention of MPL in lecithin liposomes.[49] Similarly, 2.5% and 10% of negatively-charged TEL results in stable liposomes not differing much between each other.

Liposomal CsA

Many liposomal preparations and modifications have been tested in experimental animals for both, IV and oral administrations. So far, tetraether lipid has not been applied for liposomal encapsulation of CsA, but for oral delivery of other therapeutic peptides.[77] Hence, future studies on L-CsA should include TEL-stabilized liposomes.

Liposomal MPL

Optimal results were obtained with EPC, 2.5mol% or 10mol% TEL and 5mol% MPL, with respect to the incorporation rate and stability as measured through loss of MPL within one week. Stability as measured through size distribution was best with liposomes of highly purified TEL from *T.a*. according to Antonopoulos *et al*.[91] Since highly purified TEL is expensive, it was suggested to use 2.5 mol% for stabilization instead of 10 mol%. Highly purified TEL-*T.a*. from strain DSM 1728 was tested for its toxicity and mutagenicity and considered safe; it was neither cytotoxic nor mutagenic,[93] nor toxic to animals.[94] Archaeosomes

Table 10. Shelf stability of TEL liposomes at increasing storage temperatures

Storage temperature [°C]	Duration of storage [weeks]	Average diameter [nm]
4-8	109	155
20-25	109	160
37	109	165
60-70	10	150
80-90	10	155
100	10	177

Footnote: TEL, tetraether lipid (*T.a.* purity 99%; data compiled from[90]).

Table 11. Carboxyfluorescein release measured as % increase in fluorescence

Lipid	40°C	60°C	70°C
EPC	42%	64%	74%
EPC/TEL 2.5 mol%	10%	25%	32%
EPC/TEL 10 mol%	9%	18%	22%
TEL	2%	5%	7%

Footnote: EPC, egg phosphatidylcholine; TEL, tetraether lipid (*T.a.* purity 99%)

Table 12. Percentage distribution of various MPLP concentrations (mol%)

Fraction	Percentage distribution after GFC: EPC (input 1.7 mM) & MPLP							
	MPLP: 2 mol%		4 mol%		5 mol%		10 mol%	
	EPC	MPLP	EPC	MPLP	EPC	MPLP	EPC	MPLP
5	9.4	11.7	8.8	16.4	11.7	15.3	12.7	2.7
6	51.7	43.1	54.2	37.7	50.8	41.0	43.2	10.6
7	16.4	29.2	17.0	21.2	18.4	17.6	19.0	7.4

Table 13. Percentage distribution of EPC, TEL, and MPLP

Fraction	Percentage distribution after GFC (EPC:TEL:MPLP) mM ratios								
	3.4:0.08:1.7			3.4:0.08:3.4			1.7:0.04:0.17		
	EPC	TEL	MPLP	EPC	TEL	MPLP	EPC	TEL	MPLP
5	28.2	32.5	39.4	16.6	20.0	17.1	27.7	32.5	31,4
6	48.2	41.0	47.1	54.8	52.8	45.5	49.6	44.9	47.5
7	22.6	19.2	12.9	27.4	25.7	31.0	20.0	19.0	18.0

from total polar lipid extracts of archaea were also shown not to be toxic and well tolerated in mice.[95]

Liposomal MPLP

Addition of TEL-*S.a*. to stabilize EPC liposomes also improves the incorporation of MPLP. This MPL derivative inserts differently into liposomal membranes compared to the original compound MPL, the fatty acid residue functioning like an anchor into the hydrophobic depth of the membrane. The insertion into and orientation in the membrane resembles rather cholesterol. Among all liposomal formulations investigated in this study, the mixture of MPLP with EPC and TEL-*S.a*. resulted in the most stable pharmaceutical formula.[62-64]

Pharmacological Data

Table 8 compares liposomal and non-liposomal formulations of CsA and MPL including a dextran-MPL formulation in animal transplantation experiments.

Non-liposomal MPL was injected alone and combined with the simultaneous, but separate injection of empty liposomes in the same dosage (4 mg) and frequency (weekly) as the liposomal formulation. The survival time of kidney allograft was the same with or without the simultaneous injection of empty liposomes (8.8 ± 0.5 days). Only the daily injection of 4 mg MPL resulted in longer survival of the allograft (19.0 ± 6.8 days), however with the seven-fold dosage as compared with the liposomal preparation, which exerted allograft survival of 20.3 ± 7.4 days with a maximum survival of even more than 80 days.[54]

Summary and Highlights

In cell culture and animal experiments, liposomal preparations improve both pharmacokinetic and pharmacodynamic parameters.

• Liposomal MPLP reduces TNFα in lymphocyte cultures better than non-liposomal MPL, both *in vitro* and *ex vivo* experiments.

• The pharmacokinetic profile of L-MPL is essentially improved over non-liposomal MPL.

• In liver, liposomal MPLP reaches multi-fold higher concentrations than non-liposomal MPL or MPLP.

• Allograft survival is considerably prolongated with liposomal preparations as compared to non-liposomal drugs.

MATERIALS AND METHODS

Lipids and Liposome Technology

Since part of the original reports on MPL and MPLP in TEL-containing liposomes have been published in Indonesian language or are otherwise not easily accessible, materials and methods used in these experiments are presented here in detail.

Methylprednisolone and all other chemicals were purchased from Sigma-Aldrich, Singapore or Munich, Germany and from Merck-Darmstadt, Germany or from their Indonesian subsidiary, each at highest purity available. Methylprednisolone palmitate was synthetized by Bernina GmbH Munich, Germany.

Lipids were obtained from Sigma-Aldrich, Singapore or Munich, Germany: lecithin from egg yolk (EYL, 99%) and from soy bean (SBL, 99%), phosphatidylcholine, 1,2-diacyl-*sn*-glycero-3-phosphocholine (type XVI-E, 99%, from EYL, CAS 8002-43-5), phosphatidylserine, 1,2-diacyl-sn-glycero-3-phospho-L-serine (97%, from SBL), lysophosphatidylcholine, 1-stearoyl-sn-glycero-3-phosphocholine (LPC, CAS 19420-57-6), phosphatidylglycerol, 1-(3-sn-phosphatidyl)-rac-glycerol, sodium salt (99%, from EYL), phosphatidic acid sodium salt, 1,2-diacyl-sn-glycero-3-phosphate sodium salt (PA, 98%, from EYL), and sodium cholesteryl sulfate, cholesterol 3-sulfate sodium salt (CAS 2864-50-8).

Polar archaeal tetraether lipid (TEL) used for encapsulation of MPL and MPLP was obtained from different sources: i) from *Sulfolobus acidocaldarius* (*S.a*., Bernina GmbH, Munich), ii) from *T.a*. stem 1728,[97] purified yielding 99% pure 2,3,2',3'-tetra-O-dibiphytanyl-di-*sn*-glycerol-1'-gulosyl-1-phosphoryl-3''-*sn*-glycerol = gulopyranosyl-(*â*1-1)-caldarchaetidyl-glycerol.[91] In preliminary studies, TEL was also used from "crude" extract of the polar membrane fraction of *T.a.* mainly containing glycopyranosyl-caldarchaetidyl-glycerol after one column passage to remove brownish membrane dyes.[91,98]

The polar (liposome-forming) lipid fraction E (PLFE) from *S. a.* contains 90% of caldito-glycerocaldarchaeol (glycerol dibiphytanyl calditol tetraether, GDNT*) with phosphatidyl-myoinositol ester at the glycerol of one end and *ß*-D-glucopyranose at the calditol moiety of the other end and 10% of glycerol dibiphytanyl

glycerol tetraether (GDGT) with phosphatidyl-myoinositol ester at the glycerol of one end and *ß*-D-galactopyranosyl-*ß*-D-glucopyranose at the glycerol of the other end.[99-102] The number of pentacycles varies in all these tetraether lipids. (*Note: GDNT = glycerol dibiphytanyl nonitol tetraether was the original term, until it was proven that the chemical structure of this residue is not nonitol but calditol. However, the original abbreviation is still used in scientific work).[99-102]

Liposomes are spherical phospholipid particles that allow for drug delivery. These vesicles can be prepared from defined phospholipids in various formulations. Irrespective of the preparation method, the lipid mixture and the respective drugs were dissolved in chloroform/methanol (2:1 v/v). The solvent was removed by a rotary evaporator at about 40°C-50°C. The dry lipid film was subjected to a high vacuum (18-20 mmHg) in a desiccator at room temperature for at least twelve hours to remove chloroform, methanol and water residues. In the next step, the dry film was suspended in PBS (phosphate buffered saline, pH 7.4) by hand-shaking or vortexing to a final lipid concentration of 10-20 mg/mL.[92,103] To obtain smaller liposomes and more homogenous dispersion, in a subsequent step, the hand-shaken suspension was extruded through 100 nm polycarbonate filters[55,104] which served also as sterile filtration, because heat sterilization is limited to heat-stable TEL.

For experimental reasons, extrusion was conducted between 5 and 21 times. In addition, size reduction of the liposomes could be obtained by sonication[105] prior to extrusion. In general, the preferred preparation was extrusion through polycarbonate filter (without sonication) for 5-7 times, if not mentioned differently.

After preparation, the liposomal suspension was centrifuged at 3000 rpm for 10 minutes to remove non-liposomal material. The liposomes were contained in the supernatant which was subsequently passed through Sephadex G-75 column (gel filtration chromatography, GFC). In general, there were no significant differences between centrifuged and non-centrifuged preparations after GFC. Hence, the centrifugation step was skipped as a normal procedure and only carried out exceptionally, in case of suspected non-liposomal contamination.

Mixed liposomes were also prepared by various methods[24,92] in order to achieve optimal incorporation of the components, such as TEL, MPL, and MPLP. In all cases, liposomes were extruded through polycarbonate filters with 100 nm pore size. Subsequently, liposomes were passed and fractionated through Sephadex-75 (GFC) into 0.5 mL or 1.0 mL fractions. Table 9 presents three measurements of EPC liposomes and mixed EPC:TEL liposomes with or without sonication and with extrusion through a polycarbonate filter with 100 nm pore size.

Shelf stability of liposomes from egg phosphatidylcholine (EPC) = egg yolk lecithin (EYL), TEL and various mixtures from both had been compared,[24,90] at 20°C EPC liposomes are only stable for less than a week, at 4-8°C slightly longer, between one week and one month (depending on the conditions; apart from confluence to larger size EYL liposomes tend to be contaminated within this time), under very cautious germ reduced storage conditions at 4°C the longest observation of EYL liposomes with initially 159 nm in diameter was size stability up to 66 days (final size 149 nm).[24] Shelf stability of TEL liposomes of about 150 nm in diameter was 109 weeks at temperatures up to 37°C (size variation between 155 and 165 nm) and from 60-100°C TEL liposomes were stable for 10 weeks (size variation between 150 and 177 nm).[90]

Liposomal stability in terms of leakiness was also determined by carboxyfluorescein release within 24 hours at pH 7.4 and elevated temperatures (Table 11).[56,57,76]

Liposomal swelling rates, which can be considered as osmotic stability, were determined at 30°C by absorbance measurement at 570 nm, under addition of isotonic glycerol-containing buffer medium. Within 15 min, liposomes of TEL-*T.a.* had a swelling rate of 3%, EPC liposomes of 68%, and EPC liposomes containing 10 mol% of TEL-*T.a.* had a swelling rate of 16%.[106]

Incorporation of MPLP

Liposome suspensions were formed with input concentrations of 1.7 mM and 3.4 mM EPC, 2.5 mol% TEL, and 2-10 mol% MPLP. Best results were obtained with 4-5 mol% of MPLP. In liposome suspensions containing MPLP in 1.7 mM EPC, the highest amounts of EPC and MPLP were found in 1.0 mL GFC fractions 2-4 with the distribution value of MPLP in fraction 3 around 40-43% (43.1% at MPLP 4 mol%, and 40.0% at MPLP

5 mol%) and in liposomal mixtures containing TEL, distribution value in fraction 3 was 47% at MPLP input of 4-5 mol%.[63,64] Figure 2 shows thin layer chromatography (TLC) of TEL- and MPLP-containing mixed liposomes before and after GFC.

Loss of liposomal components through the polycarbonate filter extrusion was determined by 11-21 filter passages. Extrusion through the filter of 100 nm pore size and subsequent GFC through Sephadex G-75 column revealed that the amount of lipid, EPC (without TEL) and MPLP incorporated into the liposomal membrane decreased by 40-46%, while in the combination of EPC with 2.5 mol% TEL components were only reduced by up to 12% after 21 extrusion steps and after 11 extrusion steps even less than 10% (2.5-8%). Fractions collected after GFC were either 0.5 or 1.0 mL, each. The concentrations of MPL, MPLP and TEL were subsequently detected by TLC (Fig. 2), EPC was determined spectrophotometrically at wavelength λ= 490 nm after enzymatic reaction.[62-64] Percentage of MPLP distribution in EPC liposomes (Table 12) and EPC/TEL liposomes (Table 13) was determined in 0.5 ml fractions 5-7 after GFC fractionation.[62-64]

In liposomes containing EPC and TEL 2.5 mol%, the input composition of EPC, TEL, and 5 mol% MPLP was 3.4:0.08:0.17 mM concentration; after extrusion, 11 times through the 100 nm polycarbonate filter, the composition was reduced by 2.9% to 3.28:0.077:0.16 mM concentrations. Distribution after GFC fractionation was 48.2% for EPC, 41% for TEL, and 47.1% for MPLP in fraction 6 (fraction volume 1 mL).

In EPC:TEL:MPLP input composition of 3.4:0.08:0.34 mM, the content of each component was reduced by 8% to 3.13:0.074:3.128 mM after extrusion and the distribution of the components after GFC fractionation was EPC 54.8%, TEL 52.8%, and MPLP 45.5% in fraction 6. Halving the same ratio of components to 1.7:0.04:0.17 mM input concentrations reduced the liposomal components by 2.7% after 11 times of extrusion through the polycarbonate membrane. The distribution after GFC fractionation was EPC 49.6%, TEL 44.9% and MPLP 47.5% in fraction 6.[62-64]

Incorporation of TEL and MPLP in fractions with the highest amount of EPC (determined enzymatically) before and after GFC fractionation ranged for TEL before 0.05-0.08 mM, after fractionation 0.03-0.06 mM, with an incorporation rate of 50-82% and MPLP before 0.16-0.19 mM, after fractionation 0.12-0.18 mM with an incorporation rate of 73.6-96.8%. Incorporation could be improved to TEL before 0.1-0.14 mM, after fractionation 0.07-0.12 mM with an incorporation rate of 65-88%; MPLP before 0.48-0.54 mM, after fractionation 0.46 and 0.52 mM with an incorporation rate of 95.8-96.3%.[62,64]

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CONCLUSIONS

Various nanoparticles have been developed for effective delivery of hydrophobic immunosuppressants such as abovementioned chitosan particles or silica-coated iron oxide nanoparticles.[107] However, liposomes or modified liposomes appear the most frequently used formulations for modern delivery of immunosuppressive drugs.

Liposomal CsA, MPL, and MPLP were shown to exert pharmacological advantages in immunosuppression in lymphatic tissue cultures[66] and in experimental animals, in particluar after organ transplantation. Lymphatic tissue selectivity[49] plays certainly a major role in liposomal drug delivery formulations. For liposome-delivered MPL extended receptor binding adds to enhanced immunosuppression.[49] Liposomes by themselves appear to exert adjuvanticity.[75] Moreover, liposomal drug transfer to transplants (possibly even prior to transplantation in terms of preconditioning) could directly modify or shelter MHC and surface antigens in order to prevent immunogenic attack without general immunosuppression. Liposomal preconditioning might also be a possibility to prevent reperfusion injury in transplants.

Improved pharmacokinetics of TEL-containing liposomes or pure TEL-liposomes[59] have not yet been demonstrated versus freshly prepared EPC liposomes, but can be expected for oral administration of immunosuppressants,

which may also have a positive impact on the pharmacodynamic efficacy.[108] The already proven advantages of TEL-containing liposomes are better incorporation rates of MPLP[62] and higher stability with incorporated MPL[57,59] compared to EPC liposomes.

Author Contributions

Conceptualization, H.J.F.; methodology, E.H.P. and A.O.; literature search, analysis and validation, E.H.P., A.O., S.K.U.F. and H.J.F.; writing original draft preparation, H.J.F.; writing review and editing, S.K.U.F. and H.J.F.; supervision, H.J.F. All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest

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REFERENCES

1. Thiel G, Hermle M, Brunner FP. Acutely impaired renal function during intravenous administration of cyclosporine A: a cremophor side effect. *Clin. Nephrol*., **25**: S40-S42 (1986).
2. Haynes RC Jr. Adrenocorticotropic hormones, adrenocortical steroids and their synthetic analogs; inhibitors of synthesis and action of adrenocortical hormones. In *The Pharmacological Basis of Therapeutics,* 8th ed.; Goodman Gilman A, Goodman LS, Rall TW, Murad F., Eds.; Maxwell Macmillan International: New York, U.S.A., 1991; pp. 1431-1459.
3. Benet LZ, Williams RL. Design and optimization of dosage regimens: Pharmacokinetic data. In *The Pharmacological Basis of Therapeutics,* 8th ed.; Goodman Gilman A, Goodman LS, Rall TW, Murad F., Eds.; Maxwell Macmillan International: New York, U.S.A., 1991; pp. 1650-1735.
4. Handschumacher RE. Drugs used for immunosuppression. In *The Pharmacological Basis of Therapeutics,* 8th ed.; Goodman Gilman A, Goodman LS, Rall TW, Murad F., Eds.; Maxwell Macmillan International: New York, U.S.A., 1991; pp. 1868-1874.
5. Hsieh HH, Schreiber M, Stowe N, Novick A, Beodhar S, Bama B, Pippenger C, Shamberger R. Preliminary report: The use of liposome-encapsulated cyclosporin in a rat model. *Transplant. Proc*., **17**: 1397-1400 (1985).
6. Stuhne-Sekalec L, Chudzik J, Stanacev Z. Encapsulation of cyclosporine by phosphatidylinositol-cholesterol liposomes. *Transplantation*, **41**: 659-660 (1986).
7. Stuhne-Sekalec L, Stanacev NZ. Liposomes as cyclosporin A carriers: the influence of ordering of hydrocarbon chains of phosphatidylglycerol liposomes on the association with and topography of cyclosporin A. *J. Microencapsul*., **8**: 283-294 (1991).
8. Wiedmann TS, Trouard T, Shekar SC, Polikandritou M, Rahman YE. Interaction of cyclosporin A with dipalmitoylphosphatidylcholine. *Biochim. Biophys. Acta,* **1023**: 12-18 (1990).
9. Smeesters C, Grioux B, Vinet R, Arnoux R, Chaland P, Corman J, St-Louis G, Daloze P. Reduced nephrotoxicity of cyclosporine A after incorporation into liposomes. *Transplant. Proc*., **20**: 831-832 (1988).
10. Vadiei K, Lopez-Berestein G, Perez-Soler R, Luke DR. In vitro evaluation of liposomal cyclosporine. *Int. J. Pharm.,* **57**: 133-138 (1989).
11. Freise CE, Liu T, Hong KL, Osorio RW, Papahadjopoulos D, Ferrell L, Ascher NL, Roberts JP. The increased efficacy and decreased nephrotoxicity of a cyclosporine liposome. *Transplantation,* **57**: 928-932 (1994).
12. Venkataram S, Awni WM, Jordan K, Rahman YE. Pharmacokinetics of two alternative dosage forms for cyclosporine: liposomes and intralipid. *J. Pharm. Sci*., **79**: 216-219 (1990).
13. Alangary AA, Bayomi MA, Khidr SN, Almeshal MA, Aldardiri M. Characterization, stability and in vivo targeting of liposomal formulations containing cyclosporine. *Int. J. Pharm*., **114**: 221-225 (1995).
14. Fahr A, Holz M, Fricker G. Liposomal formulations of Cyclosporin A: influence of lipid type and dose on pharmacokinetics. *Pharm. Res*., **12**: 1189-1197 (1995).
15. Fahr A, Reiter G. Biophysical characterisation of liposomal delivery systems for lipophilic drugs: Cyclosporin A as an example. *Cell. Mol. Biol. Lett*., **4**: 611-623 (1999).
16. Shah NM, Parikh J, Namdeo A, Subramanian N, Bhowmick S. Preparation, characterization and in vivo studies of proliposomes containing Cyclosporine A. *J. Nanosci. Nanotechnol.,* **6**: 2967-2973 (2006).
17. Arnoux R, Dufourcq J, Houssin D, Perissat J, Smeesters C, Lalanne J, Deminiere C, Penin E, Clerc M, Poteaux L, Dartigues JF, Michel A. Effet immunosuppresseur de la cyclosporine A incorporée dans les liposomes [Immunosuppressive effect of cyclosporine A

incorporated into liposomes]. *Chirurgie (Paris),* **116**(8-9): 699-703 (1990).
18. Ouyang C, Choice E, Holland J, Meloche M, Madden TD. Liposomal cyclosporine. Characterization of drug incorporation and interbilayer exchange. *Transplantation,* **60**: 999-1006 (1995).
19. Soderlund T, Lehtonen JYA, Kinnunen PKJ. Interactions of cyclosporin A with phospholipid membranes: effect of cholesterol. *Mol. Pharmacol*., **55**: 32-38 (1999).
20. Lambros MP, Rahman YE. Effects of cyclosporin A on model lipid membranes. *Chem. Phys. Lipids,* **131**: 63-69 (2004).
21. Fahr A, van Hoogevest P, May S, Bergstrand N, Leigh MLS. Transfer of lipophilic drugs between liposomal membranes and biological interfaces: consequences for drug delivery. *Eur. J. Pharm. Sci*., **26**: 251-265 (2005).
22. Papahadjopoulos D, Allen TM, Gabizon A, Mayhew E, Matthay K, Huang SK, Lee KD, Woodle MC, Lasic DD, Redemann C. Sterically stabilized liposomes: improvements in pharmacokinetics and antitumor therapeutic efficacy. *Proc. Natl. Acad. Sci. USA,* **88**: 11460-11464 (1991).
23. Freisleben HJ, Blöcher D, Ring K. Calorimetry of tetraether lipids from *Thermoplasma acidophilum*. Incorporation of alamethicin, melittin, valinomycin and nonactin. *Arch. Biochem. Biophys*., **294**: 418–426 (1992).
24. Freisleben HJ. The Main (Glyco) Phospholipid (MPL) of *Thermoplasma acidophilum*. *Int. J. Mol. Sci*., **20**: 5217 (2019).
25. Blöcher D, Freisleben HJ, Ring K. Thermotropic properties of dispersions of cholesterol with tetraether lipids from *Thermoplasma acidophilum*. *Arch. Biochem. Biophys*., **290**: 224–228 (1991).
26. Aramaki Y, Tomizawa H, Hara T, Yachi K, Kikuchi H, Tsuchiya S. Stability of liposomes in vitro and their uptake by rat Peyer's patches following oral administration. *Pharm. Res*., **10**: 1228-1231 (1993).
27. Al-Meshal MA, Khidr SH, Bayomi MA, Al-Angary AA. Oral administration of liposomes containing cyclosporine: a pharmacokinetic study. *Int. J. Pharm*., **168**: 163-168 (1998).
28. Guo J, Ping Q, Chen Y. Pharmacokinetic behavior of cyclosporin A in rabbits by oral administration of lecithin vesicle and sandimmun neoral. *Int. J. Pharm*., **216**: 17-21 (2001).
29. Czogalla A. Oral Cyclosporine A – The Current Picture of Its Liposomal and Other Delivery Systems. *Cell. Mol. Biol. Lett.,* **14**(1): 139-152 (2009). DOI: 10.2478/s11658-008-0041-6
30. Chen D, Xia D, Li X, Zhu Q, Yu H, Zhu C, Gan Y. Comparative study of Pluronic® F127-modified liposomes and chitosan-modified liposomes for mucus penetration and oral absorption of cyclosporine A in rats. *Int. J. Pharm*., **449**(1-2): 1-9 (2013). http://dx.doi.org/10.1016/j.ijpharm.2013.04.002
31. Guan P, Lu Y, Qi J, Niu M, Lian R, Hu F, Wu W. Enhanced oral bioavailability of cyclosporine A by liposomes containing a bile salt. *Int. J. Nanomed*., **6**: 965–974 (2011).
32. Yuba E, Harada A, Sakanishi Y, Kono K. Carboxylated Hyperbranched Poly(glycidol)s for Preparation of pH-Sensitive Liposomes. *J. Controlled Release,* **149**(1): 72-80 (2011).
33. Mady MM, Darwish MM. Effect of chitosan coating on the characteristics of DPPC liposomes. *J. Advanced Res*., **1**: 187-191 (2010).
34. Li X, Chen D, Le C, Zhu C, Gan Y, Hovgaard L, Yang M. Novel mucus-penetrating liposomes as a potential oral drug delivery system: preparation, in vitro characterization, and enhanced cellular uptake. *Int. J. Nanomed*., **6**: 3151–3162 (2011).
35. Hansch C, Leo A, Hoekman D. Exploring QSAR - Hydrophobic, Electronic, and Steric Constants. American Chemical Society: Washington, DC, USA, 1995; pp. 173-178. HSDB https://pubchem.ncbi.nlm.nih.gov/source/hsdb/3385
36. Yalkowsky SH, Dannenfelser RM. Aquasol database of aqueous solubility, version 5, University of Arizona: Tuscon, AZ, USA, 1992.
37. Yalkowsky SH, He Y. Handbook of Aqueous Solubility Data: An Extensive Compilation of Aqueous Solubility Data for Organic Compounds Extracted from the Aquasol database. CRC Press LLC: Boca Raton, FL, USA, pp. 1179-1198 (2003).
38. O'Neil MJ., Ed., The Merck Index - An Encyclopedia of Chemicals, Drugs, and Biologicals. Royal Society of Chemistry: Cambridge, UK, 2013; pp. 1089-1432.
39. Lallemand F, Schmitt M, Bourges JL, Gurny R, Benita S, Garrigue JS. Cyclosporine A delivery to the eye: A comprehensive review of academic and industrial efforts. *Eur. J. Pharm. Biopharm.,* **117**: 14-28 (2017).
40. Lai J, Lu Y, Yin Z, Hu F, Wu W. Pharmacokinetics and enhanced oral bioavailability in beagle dogs of cyclosporine A encapsulated in glyceryl monooleate/poloxamer 407 cubic nanoparticles. *Int. J. Nanomed.,* **5**: 13-23 (2010).
41. Horby P, Lim WS, Emberson JR, *et al*. The Recovery Collaborative Group. Dexamethasone in hospitalized patients with Covid-19 - preliminary report. ÿþ *N. Engl. J. Med*., 1-11

(2020). Published online July 17, 2020. doi: 10.1056/NEJMoa2021436

42. Tanaguchi K, Itakura K, Yamazawa N, Mokisaki K, Hayashi S, Yamada Y. Efficacy of a liposome preparation of anti-inflammatory steroid as an ocular drug-delivery system. *J. Pharmacobiodyn*., **11**: 39-46 (1988).
43. Snart RS, Wilson MJ. Uptake of Steroid Hormones into Artificial Phospholipid / Cholesterol Membranes. *Nature (London),* **215**: 964 (1967).
44. Fildes FJT, Oliver JE. Interaction of cortisol-21-palmitate with liposomes examined by differential scanning calorimetry, *J. Pharm. Pharmacol*., **30**: 337-342 (1978).
45. Benameur H, Latour N, Schandene L, van Vooren JP, Flamion B, Legros FJ. Liposome-incorporated dexamethasone palmitate inhibits in-vitro lymphocyte response to mitogen. *J. Pharm. Pharmacol*., **47**: 812-817 (1995).
46. Hofkens W, Schelbergen R, Storm G, van den Berg WB, van Lent PL. Liposomal Targeting of Prednisolone Phosphate to Synovial Lining Macrophages during Experimental Arthritis Inhibits M1 Activation but Does Not Favor M2 Differentiation. *PLoS ONE,* **8**(2): e54016 (2013). doi:10.1371/ journal.pone.0054016
47. van der Valk FM, Schulte DM, Meiler S, Tang J, Zheng KH, Van den Bossche J, Seijkens T, Laudes M, de Winther M, Lutgens E, Alaarg A, Metselaar JM, Dallinga-Thie GM, Mulder WJM, Stroes ESG, Hamers AAJ. Liposomal prednisolone promotes macrophage lipotoxicity in experimental atherosclerosis. *Nanomedicine: NBM*; **12**: 1463-1470 (2016). http://dx.doi.org/10.1016/j.nano.2016.02.022
48. van der Valk FM, van Wijk DF, Lobatto ME, Verberne HJ, Storm G, Willems MCM, Legemate DA, Nederveen AJ, Calcagno C, Mani V, Ramachandran S, Paridaans MPM, Otten MJ, Dallinga-Thie GM, Fayad ZA, Nieuwdorp M, Schulte DM, Metselaar JM, Mulder WJM, Stroes ES. Prednisolone-containing liposomes accumulate in human atherosclerotic macrophages upon intravenous administration. *Nanomedicine: NBM,* **11**: 1039-1046 (2015). http://dx.doi.org/10.1016/j.nano.2015.02.021
49. Mishina EV, Straubinger RM, Pyszczynski NA, Jusko WJ. Enhancement of tissue delivery and receptor occupancy of methylprednisolone in rats by a liposomal formulation. *Pharmaceutical Res*., **10**: 1402-1408 (1993).
50. Tanaguchi K, Itakura K, Yamazawa N, Mokisaki K, Hayashi S, Yamada Y. Efficacy of a liposome preparation of anti-inflammatory steroid as an ocular drug-delivery system. *J. Pharmacobiodyn*., **11**: 39-46 (1988).
51. Tanaguchi K, Yamazawa N, Itakura K, Morisaki K, Hayashi SI. Partition characteristics and retention of anti-inflammatory steroids in liposomal ophthalmic preparations. *Chem. Pharm. Bull*., **35**: 1214-1222 (1987).
52. Devoisselle JM, Won-Dury J, Confort-Gouny S, Coustaut D, Cozone PJ. Liposomes Containing Fluorinated Steroids: An Analysis Based on Photon Correlation and Fluorine-19 Nuclear Magnetic Resonance Spectroscopy. *J. Pharm. Sci.,* **81**(3): 249-254 (1992).
53. Binder J, Mishina EV, Jusko WJ, Kupiec-Weglinski JW. Prolongation of cardiac allograft survival in rats by liposomal-encapsulated methylprednisolone. *Transplantation*, **58**: 633-635 (1994).
54. Binder J, Bräutigam R, Oertl A, Kramer W, Jonas D, Hancock WW, Kupiec-Weglinski JW. Methylprednisolone in Bilayer Liposomes Prolongs Cardiac and Renal Allograft Survival, Inhibits Macrophage Activation, and Selectively Modifies Antigen Presentation and T-Helper Cell Function in Rat Recipients. *Transplant. Proc.,* **30**: 1051 (1998).
55. MacDonald RC, MacDonald RI, Menco BPM, Takesita K, Subbarao NK, Hu L. Small-volume extrusion apparatus for preparation of large, unilamellar vesicles. *Biochim. Biophys. Acta,* **1061**: 297–303 (1991).
56. Oertl A, Adams J, Schuldes H, Freisleben HJ, Binder J. Neuentwicklung von Liposomen als Trägersubstanz immunsuppressiver Wirkstoffe [Novel development of liposomes as vehicle for immunosuppressive compounds] Deutsche Gesellschaft für Urologie, Heidelberg, Germany, June 1996.
57. Oertl A, Bräutigam R, Binder J, Jonas D. Liposomen in der experimentellen Organtransplantation. Entwicklung einer stabilen Methylprednisolonzubereitung [Liposomes in experimental organ transplantation. Development of a stable methylprednisolone preparation]. *Urologe A,* Suppl. 1997; Poster Session.
58. Bräutigam R, Oertl A, Binder J, Kramer W. Mischliposomen im Modell der akuten Abstoßung von orthotopen Nierentransplantaten in der Ratte [Mixed liposomes in a model of acute rejection of orthotopic kidney transplantation in the rat]. *Urologe A*, Suppl. 1997; Poster Session.
59. Bräutigam R, Oertl A, Binder J, Kramer W, Jonas D. Methylprednisolon in stabilen Monolayerliposomen in einem Abstoßungsmodell von Nierentransplantaten in der Ratte [Methylprednisolone in stable monolayer liposomes in a rejection model

of kidney grafts in rats]. Südwestdeutsche Gesellschaft für Urologie, Annual Meeting, 1999; Poster Session.

60. Freisleben HJ, Antonopoulos E, Bakowsky U, Rothe U. Tetraetherlipide und diese enthaltende Liposomen sowie deren Verwendung. [Tetraether lipids and liposomes which contain them, and their use], German/European Patent 19607722.2, 1996; PCT/EP97/01011, 1997.
61. Mishina EV, Binder J, Kupiec-Weglinski JW, Jusko WJ. Effect of Liposomal Methylprednisolone on Heart Allograft Survival and Immune Function in Rats. *J. Pharmacol. Exp. Ther.,* **271**: 868-874 (1994).
62. Purwaningsih EH, Freisleben HJ, Sadikin M. Peningkatan inkorporasi metilprednisolon palmitat pada liposom yang mengandung tetraeter lipid dari *Sulfolobus acidocaldarius* membentuk sediaan baru liposomal metilprednisolon palmitat [Increased methylprednisolone palmitate incorporation into liposomes containing tetraether lipid from *Sulfolobus acidocaldarius* constitutes a new preparation of liposomal methylprednisolone palmitate]. *Jurnal Farmasi Indonesia,* **1**(1): 24-30 (2002).
63. Purwaningsih EH. The Liposome's Diameter measured by the Computerized Program of Image Pro Express 4.5. *Majalah Kedokteran Nusantara,* **41**(3): 191-193 (2008).
64. Purwaningsih EH, Arozal W, Jusman SWA. Uji Stabilitas Fisik, Kimia dan Biologik terhadap Formulasi Terbaru Liposom Tetra Eter Lipid (EPC-TEL 2,5) Sebagai Pembawa Obat [Physical, chemical, and biological stability test of the novel formulation of tetraether lipid liposomes (EPC-TEL 2.5) as drug carrier]. *Makara Seri Kesehatan,* **11**(2): 84-89 (2007).
65. Purwaningsih EH, Sadikin M, Soeradi O, Rasad A, Freisleben HJ. Distribusi liposomal-metilprednisolon palmitat (L-MPLP) pada beberapa organ mencit setelah pemberian intraperitoneal [Distribution of liposomal methylprednisolone palmitate (L-MPLP) in several mice organs after intraperitoneal administration]. *Makara Seri Kesehatan,* **2**(8): 65-72 (2004).
66. Eff ARY, Suyatna FD, Purwaningsih EH. The Effects of Liposomal Methyl-prednisolone Palmitate on the Production of TNFα in Mice. Proceedings of the 1st Muhammadiyah International Conference on Health and Pharmaceutical Development (MICH-PhD), Jakarta, Indonesia, 10-11.08.2018; SCITEPRESS: Setubal, Portugal, 2018, pp. 77-83. DOI: 10.5220/0008239700770083
67. Schurmann D, De Matos Marques B, Grunwald T, Pohle HD, Hahn H, Ruf B. Safety and efficacy of liposomal amphotericin B in treating AIDS-associated disseminated cryptococcosis. *J. Infect. Dis*., **164**: 620 (1991).
68. Tamiru A, Tigabu B, Yifru S, Diro E, Hailu A. Safety and efficacy of liposomal amphotericin B for treatment of complicated visceral leishmaniasis in patients without HIV, North-West Ethiopia. *BMC Infectious Diseases,* **16**: 548 (2016). DOI: 10.1186/s12879-016-1746-1
69. Gregoriadis G. Engineering liposomes for drug delivery: progress and problems. *Tibtech Reviews* (Elsevier); **13**: 527-537 (1995).
70. Mayhew E, Goldrosen R, Vaage J. Effects of liposome-entrapped doxorubicin on liver metastases of mouse colon tumors 26 and 38. *J. Natl. Cancer Inst.,* **78**: 707-713 (1987).
71. Caponigro F, Comella P, Budillon A, Bryce J, Avallone A, De Rosa V, Ionna F, Comelia G. Phase I study of Caelyx (doxorubicin HCL, pegylated liposomal) in recurrent or metastatic head and neck cancer. *Annals Oncol*. **11**: 339-342 (2000).
72. Lao J, Madani J, Puértolas T, Álvarez M, Hernández A, Pazo-Cid R, Artal Á, Torres AA. Liposomal Doxorubicin in the Treatment of Breast Cancer Patients: A Review. *J. Drug Delivery,* 2013; article ID 456409: 12 pages. http://dx.doi.org/10.1155/2013/456409
73. Cronin B, Robison K, Raker C, Moore R, Granai CO, Dizon DS. Pegylated Liposomal Doxorubicin in Recurrent Ovarian Cancer: Is There a Role for Maintenance Therapy? *Clinical Ovarian and Other Gynecologic Cancer,* **6**(1/2): 17-20 (2014). http://dx.doi.org/10.1016/j.cogc.2014.06.007
74. Schwendener RA. Liposomes as vaccine delivery systems: a review of the recent advances. *Ther. Adv. Vaccines,* **2**(6): 159-182 (2014). Doi: 10.1177/2051013614541440
75. Shahum E, Therien HM. Liposomal adjuvanticity: Effect of encapsulation and surface-linkage on antibody production and proliferative response. *Int. J. Immunopharmac.,* **17**: 9-20 (1995).
76. Oertl A, Antonopoulos E, Freisleben SU, Freisleben HJ. Stable archaeal tetraether lipid liposomes for photodynamic application: Transfer of carboxyfluorescein to cultured T84 tumor cells. *Med. J. Indones*., **25**: 196–206 (2016).
77. Jacobsen AC, Jensen SM, Fricker G, Brandl M, Treusch AH. Archaeal lipids in oral delivery of therapeutic peptides. *Eur. J. Pharm. Sci*., **108**: 101-110 (2017).
78. Benvegnu T, Lemiègre L, Cammas-Marion S. New generation of liposomes called

archaeosomes based on natural or synthetic archaeal lipids as innovative formulations for drug delivery. *Recent Patents on Drug Delivery & Formulation,* **3**(3): 206-220 (2009).

79. Barbeau J, Cammas-Marion S, Auvray P, Benvegnu T. Preparation and Characterization of Stealth Archaeosomes Based on a Synthetic PEGylated Archaeal Tetraether Lipid. *J. Drug Delivery,* 396068: 11 pages (2011). doi:10.1155/2011/396068
80. Leriche G, Cifelli JL, Sibucao KC, Patterson JP, Koyanagi T, Gianneschi NC, Yang J. Characterization of drug encapsulation and retention in archaea-inspired tetraether liposomes. *Org. Biomol. Chem*., **15**: 2157-2162 (2017).
81. Blöcher D, Six L, Gutermann R, Henkel B, Ring K. Physicochemical characterization of tetraether lipids from *Thermoplasma acidophilum*. Calorimetric studies on miscibility with diether model lipids carrying branched and unbranched alkyl chains. *Biochim. Biophys. Acta,* **818**: 333–342 (1985).
82. Blöcher D, Gutermann R, Henkel B, Ring K. Physicochemical characterization of tetraether lipids from *Thermoplasma acidophilum*. IV. Calorimetric studies on the miscibility of tetraether lipids with dipalmitoylphosphatidylcholine and dipalmitoyl-phosphatidylglycerol. *Naturforschung,* **40c**: 606–611 (1985).
83. Blöcher D, Freisleben HJ, Ring K. Thermotropic properties of dispersions of cholesterol with tetraether lipids from *Thermoplasma acidophilum*. *Arch. Biochem. Biophys.,* **290**: 224–228 (1991).
84. Luthfa Z, Freisleben HJ, Saleh R. Temperature and pH-dependent molecular dynamics of *Thermoplasma acidophilum* tetraether lipid membrane in a computer-simulated model. *Int. J. Mater. Eng. Technol*., **13**: 161–185 (2015).
85. Blöcher D, Ring K. Mixtures of tetraether lipids from *Thermoplasma acidophilum* with varying degree of cyclization show a kinetic eûect for a metastable phase. *Chem. Phys. Lipids,* **58**: 233–239 (1991).
86. Ernst M, Freisleben HJ, Antonopoulos E, Henkel L, Mlekusch W, Reibnegger G. Calorimetry of archaeal tetraetherlipid - Indication of a novel metastable thermotropic phase in the main phosphotetraether lipid from *Thermoplasma acidophilum* cultured at 59æ%C. *Chem. Phys. Lipids,* **94**: 1-12 (1998).
87. Rudolph P. Herstellung und Charakterisierung von Liposomen aus Tetraetherlipid des Archaebakteriums *Thermoplasma acidophilum*. [Fabrication and characterization of liposomes from tetraether lipid of the archaebacterium *Thermoplasma acidophilum*]. Ph.D. Thesis, Goethe-Universität, Frankfurt am Main, Germany, 1993.
88. Rudolph P, Wiesner H, Engelhardt M, Freisleben HJ. Liposomes of the main phospholipid (MPL) from the archaebacterium *Thermoplasma acidophilum* - Size and stability. *Biol. Chem. Hoppe Seyler,* **374**: 145 (1993).
89. Wiesner HM. Herstellung stabiler Liposomen definierter Größe unter 600 nm aus dem Hauptphospholipid von *Thermoplasma acidophilum* unter Verwendung von Eilecithin. [Preparation of stable liposomes of defined size under 600 nm from the main phospholipid of *Thermoplasma acidophilum* in combination with egg lecithin]. M.D. Thesis, Goethe-Universität, Frankfurt am Main, Germany, 1995.
90. Freisleben HJ. Tetraether Lipid Liposomes. In *Membrane Structure in Disease and Drug Therapy*; Zimmer G., Ed.; Marcel Dekker, Inc.: New York, USA, 2000; pp. 127–152.
91. Antonopoulos E, Freisleben HJ, Mulyanto C, Krisnamurti DGB, Estuningtyas A, Ridwan R, Freisleben SKU. Fractionation and purification of membrane lipids from the archaeon *Thermoplasma acidophilum* DSM 1728/10217. *Separ. Purific. Technol.,* **110**: 119–126 (2013).
92. New RCC., Ed.; *Liposomes, a Practical Approach*; IRL Press: Oxford, UK, (1990).
93. Freisleben HJ, Neisser C, Hartmann M, Rudolph P, Geck P, Ring K, Müller WEG. Influence of the main phospholipid (MPL) from *Thermoplasma acidophilum* and of liposomes from MPL on living cells: cytotoxicity and mutagenicity. *J. Liposome Res.,* **3**: 817-833 (1993).
94. Freisleben HJ, Bormann J, Lehr F, Litzinger DC, Rudolph P, Schatton W, Huang L. Toxicity and biodistribution of liposomes of the main phospholipid from the archaebacterium *Thermoplasma acidophilum* in mice. *J. Liposome Res*., **5**: 215-223 (1995).
95. Omri A, Agnew BJ, Patel GB. Short-Term Repeated-Dose Toxicity Profile of Archaeosomes Administered to Mice via Intravenous and Oral Routes. *Int. J. Toxicol*., **22**: 9–23 (2003). DOI: 10.1080/10915810390181463
96. Chimalakonda AP, Montgomery DL, Weidanz JA, Shaik IH, Nguyen JH, Lemasters JJ, Kobayashi E, Mehvar R. Attenuation of Acute Rejection in a Rat Liver Transplantation Model by a Liver-Targeted Dextran Prodrug of Methylprednisolone. *Transplantation,* **81**(5): 678-685 (2006). DOI: 10.1097/01.tp.0000177654.48112.b6
97. Freisleben HJ, Henkel L, Gutermann R,

Rudolph P, John G, Sternberg B, Winter S, Ring K. Fermentor cultivation of *Thermoplasma acidophilum* for the production of cell mass and of the main phospholipid fraction. *Appl. Microbiol. Biotechnol*., **40**: 745-752 (1994).

98. Malik A, Santoso I, Yehuda A, Freisleben SKU, Wanandi SI, Huber H, Luthfa Z, Saleh R, Freisleben HJ. Characterization of *Thermoplasma species* cultured from sampling on Tangkuban Perahu, Indonesia. *Microbiology Indonesia,* **8**(1): 16-23 (2014). DOI: 10.5454/mi.8.1.3
99. Sugai A, Sakuma R, Fukuda I, Kurosawa N, Itoh YH, Kon K, Ando S, Itoh T. The structure of the core polyol of the ether lipids from *Sulfolobus acidocaldarius*. *Lipids,* **30**(4): 339-344 (1995).
100. Gambacorta A, Caracciolo G, Trabasso D, Izzo I, Spinella A, Sodano G. Biosynthesis of calditol, the cyclopentanoid containing moiety of the membrane lipids of the archaeon *Sulfolobus solfataricus*. *Tetrahedron Letts.,* **43**(3): 451-453 (2002). https://doi.org/10.1016/S0040-4039(01)02187-6
101. Chong PLG. Archaebacterial bipolar tetraether lipids: Physico-chemical and membrane properties. *Chem. Phys. Lipids,* **163**: 253–265 (2010).
102. Zeng Z, Liu XL, Wei JH, Summons RE, Welander PV. Calditol-linked membrane lipids are required for acid tolerance in *Sulfolobus acidocaldarius*, *Proc. Natl. Acad. Sci. USA,* **115**(51): 12932–12937 (2018). www.pnas.org/cgi/doi/10.1073/pnas.1814048115
103. Bangham AD, Standish MM, Watkins JC. Diffusion of univalent ions across the lamellae of swollen phospholipids. *J. Mol. Biol*., **13**: 238–252 (1965).
104. Olsen F, Heath CA, Szoka FC, Vail W, Mayhew E, Papahadjopoulos D. Preparation of unilamellar liposomes of intermediate size (0.1–0.2 μM) by a combination of reverse phase evaporation and extrusion through polycarbonate membranes. *Biochim. Biophys. Acta,* **601**: 559–571 (1980).
105. Huang C. Studies of phosphatidylcholine vesicles formation and physical characteristics. *Biochemistry,* **8**: 344–352 (1969).
106. Ring K, Henkel B, Valenteijn A, Gutermann R. Studies on the permeability and stability of liposomes derived from a membrane-spanning bipolar archaebacterial lipid. In *Liposomes as Drug Carriers*; Schmidt KH., Ed.; G. Thieme-Verlag: Stuttgart, Germany, pp. 100–123 (1986).
107. Hwang J, Lee E, Kim J, Seo Y, Lee KH, Hong JW, Gilad AA, Park H, Choi J. Effective delivery of immunosuppressive drug molecules by silica coated iron oxide nanoparticles. *Colloids Surf. B Biointerfaces*. **142**: 290-296 (2016). http://dx.doi.org/10.1016/j.colsurfb.2016.01.040
108. Freisleben HJ, Purwaningsih EH, Oertl A, Freisleben SKU. Perspective of novel liposomal formulation for immunosuppressants after organ transplantation. 3rd International Conference on Lipid Science and Technology. Rome, Italy, December 11-12, 2017**,** *Bioenergetics,* **6: 2(**Suppl) Lipids (2017). conferenceseries.com DOI: 10.4172/2167-7662-C1-003