7. SUMMARY

Phospholipids - Structural Elements

Phospholipids are essential components of all cellular and subcellular membranes. Phosphatidylcholine and phosphatidylethanolamine predominate quantitatively, substantially constituting the typical bilayer configuration. Phospholipide belong to the amphipathic molecules with a water-soluble and a fat-soluble component. In the bilayer configuration the hydrophilic groups are arranged at the outer and inner side of the membrane towards the surrounding medium; the lipophilic groups, in contrast, face each other at the inner side of the bilayer configuration.

Further important constituents of biological membranes are cholesterol, glycolipids as well as peripheral and integral proteins. The latter, in particular, act as receptors for biologically active substances and as transport proteins or enzymes.

The basic structure of biological membranes is thus a series of recurrent unities of lipid-protein-complexes. The membrane is asymmetric. The function of the external (cellular) and internal (subcellular) membrane systems depends on their composition and on the integrity of their phospholipid structure.

In addition to their presence in cell membranes, phospholipids constitute structural and functional elements of the surface monolayers of lipoproteins and of surfactants.

Fluidity

Of utmost importance for the function of biological membranes is their fluidity, which is decisively influenced by phospholipids. Besides the content of cholesterol, proteins and the nature and charge of the polar headgroups of phospholipids in the system, membrane fluidity depends on the length of the chains of the fatty acid residues in the phospholipid molecule as well as on the number and the type of pairing of their double-bonds.

"Essential" Phospholipids

The term of "essential" phospholipids indicates the highly purified extract derived from Glycine max. with a standardized content, as a rule, of 76% or 94% (3-sn-phosphatidyl)choline in the oral preparations, and of 94% in the intravenous forms.

"Essential" phospholipids supply the organism with non-toxic (3-sn-phosphatidyl)choline molecules with a high content in polyunsaturated fatty acids, in particular linoleic acid. Approx. 15 kg of soya beans are required to obtain the recommended daily dose of 1.8 g EPL The main active ingredient is 1,2-dilinoleoylphosphatidylcholine, which represents about 52% of the applied mixture of phosphatidylcholines.

1,2-dilinoleoylphosphatidylcholine is not physiologically present in the human body. Endogenous phospholipids are partly substituted by these "essential" phospholipids which are incorporated in all membrane-containing fractions and thus improve their fluidity.

Functions of 'Essential" Phospholipids

With the administration of "essential" phospholipids the following functions of phosphatidylcholines in general are combined with those of dilinoleoylphosphatidylcholine in EPL:

1) They are high-energy, basic, structural and functional elements of all biological membranes, such as of cells, blood corpuscles, lipoproteins and of the surfactant.

2) They are indispensable for cellular differentiation, proliferation and regeneration.

3) They maintain and promote the biological activity of many membrane- bound proteins and receptors.

4) They play a decisive role for the activity and activation of numerous membrane-located enzymes, such as sodium-potassium-ATPase, adenylate cyclase and lipoprotein lipase.

5) They are important for the transport of molecules through membranes.

6) They control membrane-dependent metabolic processes between the intracellular and intercellular space.

7) The polyunsaturated fatty acids contained in them, such as linoleic acid, are precursors of the cytoprotective prostaglandins and other eicosanoids.

8) As choline and fatty acid donators they have an influence in certain neurological processes.

9) They emulsify fat in the gastrointestinal tract.

10) They are important emulsifiers in the bile.

11) They co-determine erythrocyte and platelet aggregation.

12) They influence immunological reactions on the cellular level.

These multiple tasks of phospholipids are always related to the morphology of biological membranes; in each point this list of the multiple tasks of phospholipids, which is not exhaustive, is based on the incorporation into biological membranes, and thus on the intact character of the structures.

EPL as a Membrane Therapeutic

With its special ingredient dilinoleoylphosphatidylcholine EPL is theoretically of importance in all those diseases in which damaged membrane structures, reduced phospholipid levels and/or decreased membrane fluidity are present. This is a hypothesis which is supported by experimental and clinical investigations on various membrane-associated disorders and illnesses.

Active Principle and Indications

Studies on the active principle of EPL as well as pharmacological and clinical trials are available on the following disturbances and diseases related to membrane damages:

- In liver diseases the hepatocyte structures are always damaged, for example by viruses, organic solvents, alcohol, medicaments, drugs or too fatty food. As a consequence, membrane fluidity and permeability are disturbed, membrane-dependent metabolic processes are impaired as well as membrane-associated enzyme activites. Immunological and receptor properties may be changed. This may consi4erably inhibit the metabolism of the liver.
- In hyperlipoproteinemia with or without atherosclerosis various pathomechanisms, such as lipid peroxidation, decrease of lipid- metabolizing enzyme activity and modification of lipoprotein structure and function interact and provoke a rise in serum cholesterol and triglyceride levels and a subsequent accumulation of fat in the peripheral tissue, avoiding the receptor-mediated uptake of cholesterol. As it is relatively lowered, serum HDL takes up and transports less cholesterol from the periphery back to the liver.
- One of the hemorrheological disturbances is an elevated cholesterol/phospholipid ratio in the membranes of platelets and red blood cells (RBC) with concomitant changes in membrane function. This leads to an increased tendency of platelets and RBC to aggregate, which in turn influences blood flow properties and microcirculation.
- In neurological diseases the reduction of choline - a precursor of the neurotransmitter acetylcholine -, the deficiency in unsaturated fatty acids, or increased rigidity of neuronal membranes may influence metabolic processes and functions of the nerves.
- In gastrointestinal inflammation the mucosa quality, membrane structures, membrane-dependent immunological reactions and the local prostaglandin synthesis are altered.
- In lung diseases, such as infant or adult acute respiratory distress syndrome, the fatal outcome of the disease is triggered by a phospholipid deficiency in the pulmonary alveoli (surfactant).
- In kidney diseases phospholipid deficiency in the membranes is present involving impaired excretion and reduced prostaglandin synthesis.
- In chronic ambulatory peritoneal dialysis (CAPD) and in peritonitis the sharp fall of surface-active phospholipid material is striking. Disorders in the peritoneum and a reduction in ultrafiltration are the consequences.

- In the multitactorial picture of gestosis disorders of the Iipid metabolism and lipid peroxidation as well as impaired liver and kidney function can be observed.
- In skin diseases, such as psoriasis, the pathological mechanisms seem to be favoured, among others, by alterations of cell structures and of the fatty acid and phospholipid composition.
- In aging patients we are often faced with a combination of age-linked physiological changes and diseases, e.g. degenerative liver damage or atherosclerotic changes of the vascular wall associated with other degenerative or not degenerative diseases.

All these very different diseases may have comparable membrane disorders in common. With "essential" phospholipids such disorders may be positively influenced, eliminated or even improved beyond normal due to the high content in polyunsaturated fatty acids, for example:

1) HDL particles enriched with EPL are able to take up more cholesterol from LDL and tissues. More cholesterol can be transported back to the liver. This action on the reverse cholesterol transport is unique. All other lipid-lowering agents reduce either the cholesterol absorption in the body, or the cholesterol synthesis in the liver and its distribution to the periphery. These substances, however, do not physiologically mobilize the cholesterol already present in the periphery. Moreover, intravenously administered EPL micelles absorb apoprotein A-1 and may, to a certain extent, behave like HDL particles and take up cholesterol.
2) The stimulation of lipolytic enzymes, such as lipoprotein lipase and hepatic triglyceride lipase, favours the break-down of triglyceride-rich lipoproteins.
3) The cholesterol/phospholipid ratio in membranes, platelets and red blood cells decreases and membrane function is improved. Aggregability and blood viscosity decrease, microcirculation and life- span of the mentioned blood corpuscles increase.
4) Peroxidative reactions are reduced, damaged hepatocyte membrane structures restored, membrane fluidity and function stabilized, immunomodulation and cell protection improved, and membrane- . associated liver functions enhanced.
5) With the normalization of the cholesterol/phospholipid ratio also the bile is stabilized.
6) Due to its specific property as a surface-active emulsifier EPL solubilize fat in the gastrointestinal tract, and in risk and treatment of fat embolism.
7) The substitution with polyunsaturated fatty acids and choline may have a cytoprotective effect in the brain and activate neuronal processes.
8) Liposomes with polyunsaturated phosphatidylcholine molecules may act as drug carriers, such as of vitamin E.

Liver Disease

Experimental and clinical results support the assumption that the therapeutic application of EPL has protective and even curative and regenerative effects on the biological membranes of sinus endothelial cells and hepatocytes. The cytoprotective effect of EPL has been corroborated in 7 In vitro and in 55 in vivo experiments, in which 20 different models with 5 different animal species were used. Types of intoxication which are known to play a role in the etiology of liver disease have mostly been applied: chemical substances, medicaments, alcohol, cholestasis, immunological phenomena, exposure to radiation, etc.

The hepatoprotective effects of EPL have been confirmed as compared to the controls and were the more pronounced the earlier EPL was administered:

1) Structures of membranes were normal or largely normalized;

2) Fatty infiltrations and hepatocyte necrosis could be diminished or even eliminated;

3) Corresponding data were found for lipid peroxidation, transaminase and cholinesterase activity, and for serum lipids; liver cell metabolism increased;
4) The increase of RNA and protein synthesis and of the liver cell glycogen content indicated a stimulation of the liver cells;

5) Reduced collagen production, collagen/DNA ratio and liver hydroxyproline indicated a reduced formation of connective tissue.
Until end of 1989 78 open, 35 single-blind and 13 double-blind clinical trials in altogether 8334 patients were carried out; 74 of these studies were based on 3 groups of criteria, 41 on 4 groups and 5 on 5 groups of criteria (including electron microscopy). In 45 studies histological controls were performed.
The dosage of EPL ranged from 525 mg to 2700 mg/day when administered orally, and from 500 mg to 3000 mg/day in intravenous application. The duration of treatment lasted from some weeks to up to 30 months. The main liver indications were: acute hepatitis, chronic hepatitis, fatty liver, toxic liver damage, and cirrhosis of the liver.

The clinical findings, showing the efficacy of EPL, can be summarized generally as follows:

1) accelerated improvement or normalization of subjective complaints, of clinical findings and several biochemical values;
3) a better histological result as compared to the control groups;
3) a shortened duration of hospitalisation.
Two pharmacological, 11 open and 1 double-blind trials have shown the effectiveness of EPL in hepatotoxicity of anti-TB agents, which is the only representative clinical model of liver damage to assess effectiveness of a liver therapeutic in intoxication.

Renal Disorders and Chronic Ambulatory Peritoneal Dialysis
Eight pharmacological and 23 clinical studies give a first impression of EPL and its influence on renal disorders.
A significant rise of creatinine, urea and sodium clearances, correction of disorders of lipid metabolism, disappearance of proteinuria and hypoalbuminemia, and decrease of lysolecithin excretion range among the effects mostly seen.
Signs of intoxication could be improved in particular in nephrotic forms.
As main effects of EPL are considered the stabilization of renal cell membranes and a positive influence on cytoprotective prostaglandins.
At least in patients with diminished ultrafiltration EPL provoked a reincrease of the ultrafiltration rate in chronic ambulatory peritoneal dialysis after intraperitoneal and intravenous administration.
Quality improvement of the peritoneal surfactant is discussed as a possible mode of action.

Gestosis

A total of 684 patients in 13 studies recelved as adjuvant treatment 250-1000 mg EPL i.v. and/or 1.8 g EPL orally per day.

In these patients suffering from early or late gestosis the subjective symptoms, such as hyperemesis gravidarum, clearly improved or disappeared. This positive effect was also seen with respect to accompanying disturbances, such as lipid peroxidation, renal disorders, pathological liver function and hyperlipidemia.

Hyperilpoproteinemia/Atheroscierosis

To date, the influence of "essential" phospholipids on the lipid metabolism has been studied in 14 in vitro and in 95 pharmacological investigations, in which EPL was applied in different models or diets in 11 different animal species by the intravenous, oral, subcutaneous, intracardial or intraperitoneal route, prophylactically, simultaneously or curatively.
The results of these studies, reflecting the effects of EPL in lipid metabolic disturbances can be summarized as follows:
1) Increase of polyunsaturated fatty acids in cholesterol esters, phospholipids, triglycerides and lipoproteins, in serum and aorta.
2) Influence on enzyme activities in serum and aorta, such as on LCAT, HTGL, LPL, ACAT and phospholipase.
3) Lowering effect on serum lipid values.

4) Influence on lipoprotein structure and cholesterol content, especially on HDL-C/LDL-C ratio.
5) Antiatherogenic effect in prophylactic and therapeutic use.
6) Decrease of (lipid) peroxidation and platelet aggregation together with improved hemorrheology.

Until end of 1989 205 clinical trials, 12 out of them double-blind, and 53 controlled in a total of 7606 patients (save fat embolism) were carried out.

In the mean, EPL reduced total serum cholesterol by -8 to -30%, LDL cholesterol by -10 and -31%, triglycerides by -12 and -58%, and it increased HDL cholesterol by +10 to +45%.

In a pilot study in 15 patients with hyperlipoproteinemia and high-cholesterol plaques signs of reduced growth rate of minor plaques and a reduction of the size of larger high-cholesterol plaques were found.

Eleven studies demonstrated the influence of EPL on the erythrocyte morphology including improved cholesterol/phospholipid ratio in membranes, filterability and flexibility of red blood cells, erythrocyte aggregation, blood viscosity and capillary blood flow.

In 20 studies it was shown that platelet membrane composition improved, and that platelet susceptibility to ADP, PAF or collagen, and the thromboxane/6-keto-PGF1?> ratio of thrombocytes were normalized.

According to some authors, injected EPL micelles or liposomes simulate HDL function and improve reverse cholesterol transport.

Finally, in 18 experimental studies and in 23 clinical trials in altogether 4485 patients WB8 demonstrated that EPL has a prophylactic and therapeutic effect in fat embolism.

Gastrointestinal Inflammation

The following experiments, among others, were carried out:
- whole-body autoradiographs of rats after an oral dose of radioactively labelled 1,2-dilinoleoylphosphatidylcholine, visualizing the high EPL concentration in the gastric and intestinal mucosa even 24 hours after administration;
- mucosal PGE2 synthesis and release experimentally reduced by indomethacin application reincreased significantly after 60 and 120 minutes of simultaneous EPL administration.

Correspondingly, NSAID-induced gastric mucosal damage was reduced or prevented in experimental studies, when EPL was applied concomitantly.

Similar results were found in orientating clinical trials in gastroduodenal damage, especially due to NSAIDs, which, however, were dose-related.

These findings underline the significance of the surface-action of phospholipids for the hydrophobic properties of the gastrointestinal surface.

Neurology

Fourteen experimental studies have shown that:

EPL is taken up to a small amount intact into the brain endogenous phospholipid synthesis is stimulated - positive effects, such as raised choline content in the brain, improved cerebrovascular circulation and cerebral enzyme antioxidant system, and favourable actions on the dendritic material have been demonstrated.

35 clinical investigations in 1968 patients and 3 in 31 volunteers have also given clinical evidence of some positive effects in certain diseases, such as involutional dementias and multiple sclerosis. Improvements of subjective well-being, such as headache, dizziness, memory, concentration, endurance, irritability, insomnia, angina attacks, and walking time have been reported. However, final conclusions cannot yet been drawn since, for example, neither the exact EPL dose to be applied in different neurological diseases is known nor is the classification of the diseases with their sub-types clear enough.

Lung Surfactant

From 3 experimental and 8 clinical studies can be inferred that the therapeutic possibilities of EPL in this indication field consist first of all

in the prophylactic substitution of phosphatidylcholine molecules in the decisive pregnancy weeks to support the formation of surfactant in the unborn child. Another possibility is the compensation of membrane damages, e.g. in the presence of inflammatory processes in the lung, or in atherosclerotically changed blood flow properties and erythrocyte flexibility.

Psoriasis

Thirteen studies in 915 patients, treated for 1 month up to several years demonstrated the value of EPL as adjuvant therapeutic application in this condition. The positive effect is probably based on the correction of lipid values in the skin, primarily of fatty acid levels.

Geriatrics

In geriatrics, where age-related physiological changes in the organism are often combined with specific diseases, the membrane therapeutic EPL may prove to be useful. The effects described so far for various indications can be summarized as follows: enhanced memory performance of the aging brain, promotion of gastrointestinal function by mucosa restoration, activation of the liver metabolism and detoxication, activation of renal function, influence on the lipid metabolism and on atherosclerosis by cholesterol mobilization, improvement of the coronary, peripheral and cerebral blood flow and, finally, correction of the increased cholesterol/phospholipid ratio in cellular membranes in general. Since often multimorbidity is present In old age and the elderly mostly have to take different drugs, it is essential that geriatric disorders be treated with a preparation that does not provoke additional side-effects or which even alleviates the adverse reactions of the accompanying medication. "Essential" phospholipids are a phytotherapeutic product without noteworthy side- effects even in long-term application, and without contraindications.

Membrane Therapeutic

On the basis of the available data on EPL and its different modes of action, of pharmacological investigations and of a broad range of clinical trials it can be summarized that EPL acts primarily by its influence on membranes. As membrane changes and damages occur in many disturbances and diseases the therapeutic approach with EPL in man is a holistic one.

The possibilities of application reside, on the one hand, in the kind and severity of membrane damage and, on the other, in the influence on membrane fluidity and thus on membrane-dependent metabolic processes by the incorporation of the special molecule 1,2- dilinoleoylphosphatidylcholine. In addition, the membrane pool of substrates for the endogenous metabolism is increased.

The application of EPL as a membrane therapeutic seems to be limited by the attempt of the cell to maintain normal membrane homeostasis which allows only certain variations, although the cell is also active to eliminate damages.

From this we can infer that EPL can be applied (in general as adjuvant medication) in membrane-associated damages and diseases, and, in specific cases, to enhance membrane-related physiological processes.