

6. DISCUSSION OF THE PHARMACOLOGICAL AND CLINICAL RESULTS

6.1 EPL as a Membrane Therapeutic

The mentioned studies on EPL on various indication fields constitute a representative selection under the angle of membrane therapy. When all publications and reports, as far as known, are taken together their number will amount to over 2330.

It would not be admissible, however, to conclude that in the mentioned indications and others EPL was a preparation to be used in any case. In various fields detailed investigations are not yet available, e.g. on the possibility to influence membrane structure and function for many years, or large-scale double-blind studies to give clinical evidence of the hitherto observed properties, such as improved membrane fluidity and activation of membrane-dependent enzymes.

Yet it cannot be excluded that in the long run the organism adapts itself to membrane changes, e.g. by shifts in the phospholipid pattern, enhanced cholesterol incorporation, changed protein spectrum, or simply by metabolism of the incorporated phosphatidylcholine molecules. It is true that membranal changes, such as increased fluidity, are admitted by the organism to a certain extent only in order to maintain normal functions like membrane passage, barrier function, information transfer etc. This observation has already been described in the case of erythrocytic membranes (526).

A general statement on EPL as a membrane therapeutic is hampered also by the following facts:

1) an interaction takes place between the incorporated polyunsaturated phospholipids and other membrane components, which is still largely unknown;

2) it is not yet possible to clarify the effects of lipid composition on membrane function according to a consistent pattern; again the available information on membranes is still too fragmentary, and there is too much diversity in the existing data to permit generalizations (658);

3) the physiological content in linoleic acid, not to speak about other components, which is bound to the phosphatidylcholine is very different in membranes of different parts of the body (tab.48):

<%Tab. 48: Percentage of linoleic acid contained in the phosphatidylcholine in different parts of the human body (742)%>

The membrane composition is dependent of a fine regulation permitting only therapeutic "windows" for "essential" phospholipids.

The uncertainty about the actually obtained effects on the membrane level is still bigger if one considers that the liver alone has a membrane surface of approximately 33,000 square metres (389), and that the various compartments of the body take up phospholipids from blood circulation to different degrees. In principle, bioavailability could be demanded for each compartment. However, this is impossible not only because of the high number of compartments or of inconvenience to carry out such investigations in humans (an ethical and analytical problem), but it is doomed to failure also because there is no marker substance to control metabolism. The labelling must take account of the fact that the main active ingredient in EPL, 1,2-dilinoleoylphosphatidylcholine, is very similar to the large pool of endogenous phospholipids, otherwise, it cannot be detected any more in the organism.

At the moment another bioavailability study is ongoing in 3 probands with 1-[d6]-linoleoyl-2-linoleoyl-phosphatidyl-[Me-d9]-choline. Only for the manufacturing of this substance 2 new steps for the synthesis were required, which will be patented.

How is it possible then to judge if and, if yes, when the application of EPL as a membrane therapeutic is advisable?

We find a key to the answer when we make an overall assessment of the existing knowledge on mode of action, pharmacological models and clinical applications. A second approach would be to combine the huge number of findings (the total picture) with the results described in literature:

The most detailed investigations on membranes were carried out with blood cells, especially with erythrocytes, which are most easy to obtain and to analyse. In patients with liver disease, for example, abnormalities in the

composition of the plasma lipoproteins are associated with corresponding changes in the erythrocyte membrane lipid composition which can affect its shape and deformability and can decrease its permeability to sodium (531).

Apart from the observation that there are disease states in which the chemical composition of membrane phospholipids is altered significantly (761), also losses of phospholipids have been reported: e.g. K.R.Chien et al. (109) found out that the interruption of the blood supply to rat liver produces a progressive loss of phospholipid from the ischemic cells; whole homogenates and post-mitochondrial supernatants from liver, ischemic for 3 hours, showed a 40% and 55% decrease in phospholipids, respectively. Phosphatidylcholine and phosphatidylethanolamine were predominantly affected. It was suggested that the loss of phospholipids from the microsomal membranes results from the activation of endogenous, membrane-bound phospholipases.

Not only loss of phospholipids, but also loss of polyunsaturated fatty acids in phospholipids has been described, e.g. in severe chronic liver disease (323, 482, 597, 629).

Also the influence of a disease on changes in the membrane lipid composition of other organs has been described. It has been shown, for example by direct measurements, that membrane lipid abnormalities occur in the kidney of rats with experimental liver disease (337): the changes seen, cholesterol accumulation and arachidonic acid depletion, might well interfere with sodium transport and renal prostaglandin metabolism.

The observed abnormal lipid composition of the serum may reach a level which can override the intracellular homeostasis mechanisms and lead to a chronic change in all membrane lipid composition and fluidity (649). Therefore, a secondary outcome of various diseases and disorders, in which the composition of serum lipids is changed, could be a change in the lipid fluidity of all membranes. In most of these cases, the cell membranes become more viscous mostly due to an increase in cholesterol/phospholipid ratio or decrease in lecithin/sphingomyelin ratio. One example is an increased microviscosity of the lung surfactant as a characteristic of premature newborns (227, 648) or of allergic alveolitis in adults (356).

If membrane fluidity is decreased, there may be interference with a number of cellular processes. These include the inward and outward transport of various compounds including water, urea, electrolytes etc., the cellular response to drugs and hormones, the capacity of the cell for phagocytosis, endo/exocytosis and for membrane recycling and so on (482, 531).

According to M.Shinitzky (694) the obvious candidate for membrane fluidization both in vitro and in vivo is lecithin from natural sources, which can fluidize membranes either by extracting excess cholesterol or by incorporation into the membrane, either passively or by exchange.

The general examples given in literature on the behaviour of membrane changes in different diseases thus coincide with findings on EPL and confirm the therapeutic value of EPL.

In this context it has to be underlined that the processes at the membranes led gradually to taking into account the whole organism (holistic approach). The scientific interest in EPL has developed a dynamic which is reflected every year in many publications on various approaches of the mode of action and on a large field of application.

The continued scientific interest in EPL will now be shown with 3 examples:

- 1) It is very likely that the well-known drug withdrawal syndrome in addicts originates from a hyperviscous state of the brain membranes (261). On the other side, upon chronic intake of pharmacologically relevant doses of ethanol, anaesthetics and other drugs, a tolerance is developed. The tolerance is characterized by at least partial restoration of lipid fluidity by increase in cholesterol and saturated fatty acid content. In both situations the question rises whether EPL is able to attenuate withdrawal symptoms. A first indication can be seen in the recently published investigation by M.I.Khodzhaeva (346).
- 2) According to M.Shinitzky (649) the "fortification" of "weak" antigens, by manipulation of the membrane microviscosity, has the advantage of being non-

toxic, non-adversive and maintaining the overall texture of surface determinants; diagnostically, this method could facilitate the detection of buried antigens like the cold agglutinin in human erythrocytes; clinically, it opens up a new approach for augmentation of antigenicity as for cancer active immunotherapy, or for suppression of antigenicity as for tissue transplantation. According to the same author another interesting aspect of passive antigenic modulation relates to autoimmune diseases which originate from over-exposure of normal membrane constituents; as a result of an abnormal membrane microviscosity, haptenic residues which are naturally masked by the membrane become exposed and faultily recognized as non-self.

In this context has to be mentioned also the study by J. Neuberger et al. (513), who probably due to the EPL pretreatment of rabbits succeeded in changing the antigenicity of the subsequently isolated hepatocytes in a way so that the antibody dependent cell-mediated cytotoxicity did not exceed normal levels any more.

2) Also as a consequence of aging naturally masked haptenic residues become more exposed, when cell membranes are progressively rigidified. As this rigidification correlates well with increase in cholesterol, it is, therefore, expected that these antigens would become less mobile. These changes are not limited to antigenicity, but are also related with changes in accessibility and capacity of membrane receptors or certain enzyme activities (649). As in the case of antigen exposure, e.g. receptor exposure may increase and it may become vulnerable to enzymatic degradation or even to shedding. Therefore, the apparent increase in the number of accessible receptors may be actually associated with a decrease in total receptor pool stores in the membrane.

These changes in lipid composition with aging concern especially the relative increase of cholesterol, sphingomyelin, glycosphingolipids and saturation and EPL might be basically useful in counteracting these changes. The influences of EPL on decreased activities of membrane-dependent enzymes in the age has already been described (a.o. 49, 50, 257, 353).

Ongoing studies into these approaches are recommended and extended to receptors and immune expression.

It should also be studied until which age limit damages induced by membrane changes are reversible, or at which stage of age the protein population is altered irreversibly as a result of shedding, enzymatic degradation, (per-)oxidation, crosslinking or genome aberration (649).

It can be expected in any case that refluidization of older or aging membranes by EPL is of valuable help in gerontology and in geriatrics when the frequent diseases in old age, such as gastrointestinal inflammation, liver diseases and atherosclerosis are present.

6.2 Outlook on the Further Development of EPL

Every year appear about 30 new publications on EPL.

Steadily increasing knowledge on membrane function and the special effects of EPL extent and diversify the potential for its application as a membrane therapeutic (see also chapter 6.1).

These facts make demands on the preparation with respect to strategic trials, dose schemes adapted to each pathologic condition, galenic formulation, and composition, which will be discussed more in detail below.

6.2.1 The dosage

The majority of the existing clinical studies with EPL were carried out with daily doses of 1.5 g to 3 g orally, and 500 mg to 2.0 g intravenously. In isolated cases higher doses were applied (up to 45 g orally (566), see table 41.1.1), especially on the basis of deliberation such as that - not sufficient quantities of the phospholipid substance will pass the blood brain barrier;

- the major part of the applied material will be captured by other tissues or organs before reaching the target site (404).

No dose-finding studies are available which would indicate the optimum dose in a specific indication field under consideration of factors such as body weight, sex, age, activity and duration of the disease etc. Neither can such studies be expected because of the complicated nature of indications like liver disease, neurologic diseases or disorders of the lipid metabolism including atherosclerosis.

The mere fact that a large variety of liver diseases exist reflects the necessity of individual dosing:

- this indication field covers conditions ranging from simple disorders of liver function to hepatic coma and liver cell carcinoma;
- it may be of viral, toxic, metabolic, autoimmune or other origin;
- it is often associated with biliary, hemorrhologic or other accompanying diseases;
- and - let alone mixed forms - pure hepatoses, such as fatty liver, fibrosis or cholestasis have to be distinguished.

Under the consideration that EPL as a membrane therapeutic favours the regeneration of the liver (to stick to our example) we could take the regenerative potential of the organ as basis for the dose; this attempt, however, will fail as long as no adequate variable will have been found. Neither do help us experimental data since very different doses of EPL yielded beneficial results. Individual studies with increasing dose ranges are available which, as a general trend, describe a better therapeutic success with higher doses. From these results, however, cannot be deduced definite dose schedules for humans (see also tables 14 and 18).

Conversely, J.F.Flood et al. (184) stated in their publication that the dose-effect relation of cholinergic substances is similar to an inverted U-curve. For EPL this would mean an optimum dose effect. One or the other clinical study suggests such a phenomenon (62 and comparing the studies in table 41.2), being insufficient, however, to give evidence of this presumption. Knowing about this dilemma, the EPL doses will be oriented also in future along the clinical experiences collected by doctors and along the results obtained so far in trials. Evaluating the existing experimental and clinical studies, the optimum dose range amounts to 1.5 to 3.0 g orally (possibly even up to 4.5 g) and to 1.0 g to 2.0 g intravenously.

The Lipostabil and Essentiale products on the market have an EPL content of 175/250/300 mg in capsules, in the liquid form of 5%, (500 mg/10 ml), and in the parenterals of 5% (250 mg/5 ml, 500 mg/10 ml) and 10% (1000 mg/10 ml). The recommended daily dosage is 3 times 1 to 2 capsules (= up to 1.8 g), and 1-2 - in severe cases up to 4 - ampoules for injection or infusion (= up to 4.0 g).

6.2.2 The Galenic Preparation

Two main new galenic preparations are under investigation (164):

- a) Chewing tablets which allow to apply a daily EPL dose of up to 2.7 g (or even 3.0 g) in one unit;
- b) EPL lyophilisate consisting of 500 mg EPL as active ingredient and of 2000 mg maltose for cryoprotection, to be dissolved in 8.30 ml aqua dest.

The development of the chewing tablets has the advantage of allowing higher daily doses (see 6.2.1) with simultaneous reduction of the number of units to be taken. Compliance can thus be improved and the dosage can be chosen more individually by the physician according to the kind and the severity of the disease. This formulation was possible by mixing a melt of carbohydrate (palatinite = isomalt) with EPL, which results in a solid hard non-sticking material at room temperature. By freeze-milling and after adding some usual tableting excipients the EPL/carbohydrate powder can be compressed to tablets. These tablets show good chewing properties, they do not stick to the teeth and have a pleasant taste.

The reason for the development of EPL lyophilisate in vials was to obtain a parenteral EPL form without cholic acid as solubilizer and with improved stability. While the EPI ampoules nowadays available on the market have to be stored in the refrigerator, the new lyophilisate can be stored at room temperature. As long as the freeze-dried product remains dry it can be stored, most probably, for over 3 years. One pilot study by E.Kuntz (390) has already shown positive effects with these EPL vials in severe liver insufficiency, which are similar to those obtained with Essentiale ampoules. Moreover, the vials can also be used for the preparation of drugs with liposomes for parenteral application.

6.2.3 Future Studies

The research areas mentioned in chapter 6.1 indicate possibilities for EPL application but have not yet reached the planning stage. In two cases however, more concrete steps for studies have been made:

- Experimental studies which are aimed at providing further insight into the mode of action of EPL as a membrane therapeutic and, in the second run, studies which would confirm these experimental results, formulated as work hypothesis, in pharmacological trials.
- On the clinical level two strategic approaches are focussed: a large-scale, multi-centre, international double-blind study to find out whether EPL, apart from dietary effects, is able to positively influence hypercholesterolemia, also in the long-term application (total cholesterol > 250 mg/dl, LDL cholesterol > 175 mg/dl); and a second large-scale, multi-centre double-blind study to find out whether in long-term application EPL was able to inhibit or stop alcoholic fibrosis, or to reduce existing alcoholic fibrosis.

On the experimental field the following studies are presently ongoing:

- on the influence of the peroxidation processes of endothelial cells to prevent atherosclerotic changes;
- on modifications of fluidity in the erythrocytic monolayer by means of monolayer photometry and computer modelling;
- on modifications of the properties of immunocytes with EPL;
- on the chylomicron features after high-fat meals with and without EPL;
- on the activation of delta-6-desaturase, the key enzyme to catalyze the chain prolongation of the precursor acids to arachidonic acid and thus to eicosanoids.

Investigations on the peritoneal surfactant will be continued.

Furthermore, a series of experimental trials are discussed which will be described more in detail below:

On platelets:

As already described, EPL has been used in coronary heart disease to reduce the platelet cholesterol content and hence to normalize their hyper-reactivity and hyper-aggregability. In cirrhotic patients, however, the situation is opposite: the platelets are unreactive and difficult to aggregate, which may contribute to the major clinical problem of variceal bleeding. However, the conclusion that EPL-induced removal of platelet cholesterol might actually be detrimental is too simple for three reasons:

1) EPL infusion raises the arachidonic acid content of erythrocyte membranes (597); the low 20:4 content of cirrhotic patients may be responsible for impaired aggregation and override the stimulating effect of the high cholesterol/phospholipid ratio (C/PL) (530).

2) Laffi et al. (396) provided evidence that defective aggregation of cirrhotic platelets in vitro may partly reflect prior activation in vivo. This is an intriguing possibility since a raised platelet C/PL ratio may play an important role in this activation: by enhancing thromboxane formation (due to promoted release of arachidonate or its precursor fatty acids) and perhaps by prolonging splenic residence time through rigidifying the membrane. If so, the ability of EPL to counteract or reverse membrane cholesterol deposition as new platelets enter the circulation may be very important.

3) Abnormal apo E-rich HDL particles have recently been identified as a potent antiplatelet agent in cirrhotic plasma (144).

On HDL and LCAT:

The just mentioned abnormal HDL particles have direct apolipoprotein-mediated effects on cells in vitro which are not seen with normal HDL and which do not involve lipid transfer: they interfere with receptor-mediated endocytosis of LDL by cultured cells (532), they transform normal erythrocytes (discocytes) into echinocytes (533), they impair against induced aggregation of normal platelets (144), they inhibit mitogen-induced lymphocyte proliferation (289) and they fail to provide adequate protection against infarction by *T.b. brucei* (224).

We also know that HDL and other lipoprotein abnormalities in liver disease largely coincide with an LCAT deficiency.

Therefore, EPL infusion may improve HDL functioning in two ways: by stimulating endogenous LCAT activity, and by improving abnormalities in HDL lipid composition and thereby potentially reversing any deleterious alterations in apolipoprotein orientation or confirmation.

On renal dysfunction:

Renal cortical brush-border membranes in the biliary obstructed rat are cholesterol-enriched (and so have reduced membrane fluidity and an enhanced ability to cotransport Na^+ /glucose).

Because such abnormalities are secondary to LCAT deficiency and raised plasma lipoprotein C/PL (306), it is believed that kidney membranes in jaundiced patients will be similarly cholesterol-enriched and of reduced fluidity. If so, EPL infusion may lead to an improvement in renal functioning by normalizing the cholesterol content of kidney epithelial cells.

Finally, also clinical studies on pathological syndromes are planned. Besides secondary hyperlipoproteinemias and hepato-renal syndromes also multimorbidity of aged persons will be included (see also chapter 6.1); this multimorbidity is characterized, for example, by atherosclerotic changes of the vessels accompanied with senile dementia, liver fibrosis and reduced gastrointestinal mucosa. All these manifestations present membrane involvement.

6.2.4 Changes of the Composition

At the moment, EPL preparations under the name of "Essentiale" with and without vitamins of the 8 complex are on the market for the field of liver diseases. The presence on each market depends on the requirement of the national authorities to give evidence of the synergistic effect of the components of the preparation. This requirement on the individual B vitamins combined with EPL can hardly be met: it is principally extremely difficult to give evidence of the effectiveness of a mono-preparation in liver diseases when strict scientific criteria are taken as a basis.

Since on an international scale the authorities tend to make stricter demands on the approval of medical preparations and to require evidence of the synergism, the Essentiale combination forms will increasingly be reduced to EPL mono-forms. Vitamin E is an exception: it will be maintained either as a component or as adjuvant for antioxidative purposes.

This also applies to the use of EPL in disorders of lipid metabolism. The components of nicotinic acid (1 mg), vitamin B6 (2 mg) and adenosine-5-monophosphate (1 mg) contained in each ampoule, and 1.5 mg vitamin B6, 1.65 mg vitamin E-acetate and 30 mg theophylline in the Lipostabil simplex capsule, as well as 50 mg etophylline in the Lipostabil forte capsules will be taken out gradually. In addition to the argument of the lacking evidence of synergism also the underdosing of these components has to be taken into account; it has not been studied whether this underdosing is compensated in part by the carrier function of EPL.

From these changes in the composition will result that Essentiale and Lipostabil forms will be registered which will contain exactly the same

quantities of the actual active ingredient - EPL - and which will differ only by their names. This fact is conclusive with the fact that EPL is a membrane therapeutic.

Nevertheless, there are deliberations to give evidence of the synergism between EPL and other preparations. In Italy studies are being performed with a combination of silybinin (the main active component in SylimarínR) and phosphatidylcholine (IdB1016) in patients with chronic liver disease (93). Such investigations are justified by the fact that both liver preparations have different but complementary modes of action.

Similar reasons apply to the combination of EPL and glycyrrhizic acid (23, 448) since the latter is a reputed liver therapeutic in Asia and since it may serve as solubiliser for EPL.

Finally, a large-scale, double-blind study with Interferon + EPL has been initiated to give evidence of the synergism of effects, and to reduce the side-effects of Interferon.

Similar considerations let us think of studies (a) with lipid-lowering HMG-CoA reductase inhibitors (EPL might prevent their aggregation-inducing effects (247)) or (b) with tetrahydroaminoacridine (inhibitor of acetylcholinesterase activity) combined with EPL in the treatment of Alzheimer's disease (220).

In any case, combinations of EPL with one or several other preparations are of interest when the disease is caused by or related with membrane-associated damages.

Finally, the changes in the phospholipid composition of EPL should be pointed out. There are thoughts to enrich the fatty acids of the phosphatidylcholine molecules with polyunsaturated ones, which are expected to produce therapeutic effects, for example on the triglyceride level, such as eicosapentaenoic acid or docosahexaenoic acid (393). Other considerations aim at admixing other phospholipids, such as phosphatidylserine, to extend the range of action (60, 100, 410, 699).

For the time being however, neither of these 2 projects will be realized because of the increased manufacturing costs and of the demands of the authorities to document suitability and synergistic effects.