

3 MODE OF ACTION OF EPL

As already mentioned in chapter 2.1 "essential" phospholipids supply the organism with (3-sn-phosphatidyl) choline molecules with a very high standardized content of polyunsaturated fatty acids, in particular linoleic acid. Approximately 15 kg of soya beans are required to obtain a daily dose of 1.8g EPL.

3.1 EPL as Structural Elements for Formation and Regeneration of Biological Membranes

First of all, like phospholipids in general (see chapter 1), the "essential" phospholipids are high-energy basic structural elements of all biological membranes such as of cells, lipoproteins and the surfactant. Labelled material has been found in all the mono- and bilayer-membranes of the organism, predominantly in the monolayer of the lipoproteins (217, 283, 680, 764, 767, 524, 584, 765), in the bilayer of erythrocytes and hepatocytes (217, 403, 404, 767), in the spleen, muscle, lungs (404, 405) and in the gastric mucosa (442). The distribution of radioactivity in the organs of normal rats and 0-galactosamine-treated rats, 6 hours following i.v. administration of labelled EPL, is shown in table 11 (404):

<%Tab. 11:%>

The application and incorporation of one phosphatidylcholine molecule of the "essential" phospholipids spares the body to supply the energy quantity of 5.600 cal/Mol required for the cellular biosynthesis of phosphatidylcholine (391).

A stimulation of membrane formation and regeneration can also be seen on the biochemical and histological level, for example in a reduction of cellular enzymes in blood and of membrane deformation, and in an increased protein and membrane synthesis (37, 131, 280, 304, 361, 597, 695, 696, 754).

3.2 Membrane Fluidity and EPL

The main active ingredient in EPL is 1,2-dilinoleoylphosphatidylcholine, which is present to about 52 % of the applied mixture of phosphatidylcholine molecules (128). 1,2-dilinoleoylphosphatidylcholine is not physiologically present in the human body. Endogenous phospholipids are substituted by "essential" phospholipids, especially by the 1,2-dilinoleoyl-phosphatidylcholine, which are incorporated in all membrane-containing fractions (404, 405).

This means that the phosphatidylcholine molecules in the membranes with a saturated fatty acid at position 1 (718) are partly exchanged against those with a linoleic acid or a linolenic acid at this position; additionally, the amount of phosphatidylcholine molecules with a linoleic acid at position 2 is increased (524). In total the number of double-bonds in the group of phosphatidylcholine molecules in the membrane increases.

One of the important consequences of such a substitution of body-own phospholipids by these highly unsaturated phosphatidylcholines is a change of membrane fluidity.

The influence of EPL on membrane fluidity has been investigated and is continuously observed by measuring the

- cholesterol/phospholipid ratio (68, 311, 344, 466, 599),
- Arrhenius plots of enzymatic reactions (257, 353),
- regulation / stimulation of membrane-bound enzyme activities (49, 64, 92, 145, 285, 291-293, 295, 322, 384, 450, 539, 541, 584, 618, 644, 706, 730)
- and of receptor properties (515),
- influence on membrane permeability (46, 92, 705)
- decrease/normalization of aggregation of erythrocytes and platelets (46, 47, 68, 74, 110, 170, 345, 483, 520, 642, 705)
- and by Immunomodulation (36, 251, 513, 651, 750).

There is no doubt nowadays that EPL with its main active ingredient 1,2-dilinoleoylphosphatidylcholine maintains or promotes many membrane functions of different organs and tissues by influencing membrane fluidity.

3.3 EPL and the Activity of Membrane-Bound Enzyme Systems

Two groups of enzymes which can be stimulated with EPL, will be mentioned as examples: detoxifying enzymes and enzymes relevant for the lipid metabolism.

3.3.1 EPL and Detoxifying Enzymes

Changes in lipid composition in membranes of aging rats lead to a decrease in fluidity (661, 684).

W. Klinger et al. (354) studied enzymes for the biotransformation of lipid-soluble xenobiotics in aging rats. The microsomal electron transport chain slowed down with the consequence of lower activities of cytochrome P 450-dependent monooxygenases and also lower conjugation activities. However, EPL reincreased the ethylmorphin N-demethylation and ethoxycoumarin O-deethylation activities. The Arrhenius plots for the monooxygenase reactions indicated a higher fluidity of the microsomal membranes. UDP-glucuronosyl-transferase activities were also enhanced. Glutathion concentration was increased as well as H₂O₂ production, and lipid peroxide concentration lowered.

G. Benzi et al. observed in two experimental studies with old rats that EPL (49, 50)

- increases the activity of the anti-oxidative enzymes: cytoplasmic Cu-Zn-superoxide dismutase and glutathion reductase in some brain areas and,
- increases the glutathion redox index of the forebrain.

Reoxygenation after experimental hypoxia induced a decrease of activity of the superoxide dismutase in the myocard of rats while the activity remained increased when EPL was applied in parallel (246).

3.3.2 EPL and Enzymes of the Lipid Metabolism

The influence of "essential" phospholipids on the essential enzymes of fat metabolism was studied after oral and intravenous applications as well as after in-vitro incubation. C. Desreumaux et al. (145) obtained lipoprotein lipase (LPL) and hepatic triglyceride lipase (HTGL) from adipose tissue, myocard, lungs or the liver of rats and assessed enzyme activity after in-vitro incubation with either dipalmitoylphosphatidylcholine, egg-lecithin or EPL (tab. 12). The highest increase in activity was achieved with EPL. Similarly V. Blaton et al. (64) observed a rise in the activity of milk lipoprotein lipase with EPL.

<Tab. 12: Influence of the type of phospholipid on the lipolytic activity in different tissues of rats (n = 9)

The dependence of the degree of saturation is presented as an index of activity which is relative to EPL = 100 (according to 64). Phospholipid concentration /ml incubation medium: 0.35 μ mol/l

Several authors report an increase in the activity of cholesterol-esterase while the activity of acyl-CoA:cholesterol-acyltransferase (ACAT) (291, 292, 539, 541) was found to decrease after 2 to 6 months of oral or intravenous application of EPL. In addition, authors (155, 219, 285, 322, 344, 584, 644, 706) observed that EPL is a substrate for the lecithin:cholesterol-acyltransferase (LCAT) (supply of linoleic acid) and that it increases its activity.

The significance of these changes in the enzyme activities is that

- HTGL and LPL accelerate the breakdown of lipoproteins rich in triglycerides, and the reduction of serum lipoproteins;
- the decrease of the ACAT activity reduces the deposition of cholesterol esters in the cells;
- on the contrary, the cholesterol-esterase hydrolyzes cholesterol-esters in the cells, so that the free cholesterol can be transported to the blood and bound to the surface of HDL;

- LCAT transforms this mobilized free cholesterol to cholesterol esters, mainly cholesterol linoleate, for the uptake into the HDL particles.

3.4 Effects of EPL on Lipoproteins

O. Zierenberg and his group (764, 765) carried out comprehensive studies on the EPL pattern in lipoproteins after oral or intravenous administration to rats, rabbits and dogs. Depending on the individual species 50 to 80 % of the activity administered was traced to HDL and about 20 % to LDL and VLDL after intravenous administrations of radioactively labelled EPL. After oral administration 36 to 66 % of the activity was traced to the HDL. EPL-enriched HDL showed an increased capacity for the uptake of cholesterol from LDL and peripheral tissue and its transport to the liver (68, 244, 344, 515, 650, 765-767) (fig. 13), the so-called reverse cholesterol transport.

<Fig. 13: Normal (a) and increased (b) surface capacity of HDL3 for cholesterol uptake; normal (c) and enhanced (d) cholesterol ester content of HDL2>

3.5 Effects of EPL on Hemorrhology

Beside the more and more clear evidence that EPL influences the platelet and erythrocyte aggregation it improves the flexibility of the erythrocytes, especially by decreasing the cholesterol/phospholipid ratio in the membrane. This effect ameliorates the lifetime of these corpuscles, their functioning and the blood flow properties such as blood viscosity and microcirculation (63, 167, 311, 483, 597, 599, 641, 759).

3.6 EPL for Drug Transport and HDL Simulation

If solid phospholipids are treated with excess of water and detergents, for example with cholic acids we get the mixed micelles which are contained in the ampoules of Lipostabil and Essentiale.

On the other hand, an EPL product with small (mainly) unilamellar liposomes is under experimental and clinical evaluation (390,740).

Beside delivering polyunsaturated phosphatidylcholine molecules these particles can serve for intravenous and the latter one additionally for percutaneous drug transport (26, 222, 313, 740).

E.g. C. Werner and A. Wendel (740) found in mice a liver protection of EPL liposomes against paracetamol intoxication only when these particles contained vitamin E. Intravenously applied EPL seems to be an excellent carrier for vitamin E to the liver.

Additionally, after injection (3-sn-phosphatidyl) choline in the micellar/liposomal form may simulate, to a certain extent, the transport function of HDL by absorption of apoprotein A-I and cholesterol (359, 662, 706, 744, 745).

3.7 EPL, Carrier of Polyunsaturated Fatty Acid and of Choline

Due to its high amount of polyunsaturated fatty acids (especially linoleic acid and linolenic acid in the phosphatidylcholine molecules (404)) EPL is a splendid supplier of eicosanoid precursors (318, 443, 484, 520, 755).

Due to the substitution of body-own phospholipids in the membrane against EPL, the pool of these precursors is increased, which the organism uses on demand (486).

A.o. cytoprotection can be favoured (443) and, as already described, platelet aggregation decreased.

Additionally, EPL influences certain neurological processes (175, 455, 486, 609). Beside being a membrane constituent, important for neuron structure, neurotransmission, transduction of biological signals and for metabolic processes in neurons/glia cells, its main function seems to be to act as a choline donator (455).

3.8. Influence of EPL on Lipid Peroxidation

Lipid peroxidation is one of the earliest measurable events of damage on the molecular level (389). Growing importance has recently been attributed to the part played by lipid peroxides in cell membranes and lipoproteins, in the development and progression of atherogenic lesions in the vascular wall, in liver diseases, in acute pneumonia etc.

Therefore, it is very interesting to see, that EPL decreases parameters of peroxidation reaction such as of acyl hydroperoxides, of Schiff's bases, chemiluminescence, the diene/triene conjugates as well as malonaldehyde and the intensity of peroxidation-triggered hemolysis (46, 223, 242, 246, 333, 338, 363a, 394, 469, 571, 579, 609, 623, 639, 642, 653-655, 690, 717, 721, 722, 763).

As mode of actions are especially discussed a carrier-effect of EPL for vitamin E (740) or a direct influence on e.g. the peroxisomal membrane (471).

3.9 EPL as Fat Solubilizer

Beside some studies on the resorption of fat-soluble vitamins (363b), on lipid solubilization/resorption in the gastrointestinal tract (600) and on the stabilization of bile/influence on the lithogenic index (277, 598, 601, 655, 679, 704) the main focus on research in this field was and is on prophylaxis and therapy of fat embolism. As Lipostabil, intravenously applied, has, in this disease the character of a life-saving drug, its action has been intensively investigated. In particular its specific properties as a physiological surface-active emulsifier and stabilizer of blood lipids are essential criteria for the effect on lipid particles in human blood and the reduction of mortality in man (75, 87, 290, 298, 374, 375, 377, 379, 398, 400, 553, 627, 737):

- plasma fats are emulsified and stabilized,
- enzymatic degeneration of fats is increased,
- the number and size of fatty particles reduced in blood (and urine)
- fatty deposits in lungs and renal vessels decreased,
- blood flow properties improved and the risk of thrombus formation lowered, and
- microcirculation, especially in capillaries improved.

Figure 14a shows the possible influence of EPL on different indications in which these disorders/diseases are membrane-associated. Figure 14b summarizes the modes of action of EPL and their relation to each other. These actions are based on the assertion that EPL is a membrane therapeutic. The following chapters review the most relevant studies more in detail.

<%Fig. 14a: Potential influence of EPL on indications%>

<%Fig. 14b: Modes of Action of EPL%>