The Role of Polyenylphosphatidylcholine (PPC) for the Reduction of Fat Pads in Deoxycholate Containing Injectables

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Abstract

Increasing numbers of patients request lipolytic injection therapy for aesthetic indications instead of surgical procedures. Deoxycholic acid (DC) with or without phosphatidylcholine (PC) is widely used to reduce localized fat accumulation and lipomas. DC induces inflammation and reduces the adipocytes by necrosis, and the usefulness is controversially debated.

After the principal fat degrading process of injection adipolysis (IL) is described, and the special feature of PPC from soybean is characterized, the article summarizes its specific role for the transport of fat from peripheral tissue over blood to the liver, its influence on the activity of fat degrading enzymes, and on mitochondrial functions in the consent of IL. The interrelation between a strong inflammation by too quick adipocyte necrosis and mitochondrial dysfunction is considered to lead to decreased ß-fatty acid oxidation, while PPC improves mitochondrial structure and functioning. Besides a regulating influence of PPC on DC induced increased inflammation and its consequences on patient's symptoms, apoptosis of PPC on adipocytes is discussed as additional mode of action in IL. Finally, PPC reduces DC's side-effect profile and potential toxicity.

It is concluded that DC leads to fat cell necrosis, and PPC regulates this inflammatory process and adds apoptosis as second mode of action in fat tissue. Additionally, PPC reduces the side-effect and toxicity profile of DC.

Keywords: Injection adipolysis, injection lipolysis, fat reduction, necrosis, apoptosis, phosphatidylcholine, deoxycholate, PC/DC

1. Introduction

The use of the combination of polyenylphosphatidylcholine and deoxycholate (PPC/DC) for IL of fat pads started in 1995, when the dermatologist Patricia Rittes injected Lipostabil®, a drug used for the prevention and treatment of fat embolism, to lower lipid bulging due to prominent fat pads.[1] Rittes expected that the active ingredient PPC from soybean (also called essential phospholipids or EPL) was able to dissolve fat from local fat pads. Adam Rotunda was the first who put this hypothesis into question by focusing on the detergent effects of its solubilizer DC for localized fat dissolution.[2] Klein et al. and Chung et al. substantiated this assumption by own studies.[3,4] Since that time, DC is considered to be the main active ingredient for IL. However, many customers continue to treat their patients with combinations of PPC and DC, as they assume that they have a better efficacy / side effect profile than by using the secondary bile acid DC alone.

PPC/DC injectables have been approved for different indications. Additionally, PPC/DC injectables are produced by compounding pharmacies and used by aestheticians in countries, such as Germany, Austria, Switzerland, USA, Egypt, South Korea, Russia or People's Republic of China.

Based on an increasing number of papers with PPC in- and outside the context of IL – the discourse about its role for IL is intensified in order to position its administration on a more solid basis. The following questions are discussed:

Does PPC:

1. influence the inflammatory and necrotic process of the treatment with DC?
2. strengthen the fat degrading process of DC by additional apoptosis?
3. stimulate fat degrading lipases and support the fat transport from the adipocytes to the liver?
4. enhance the ß-fatty acid oxidation of the hepatic mitochondria?
5. reduce the potential toxicity and side-effect profile of DC?

2. From PC to PPC and to DLPC – the Description of the Molecule

The publications about IL most frequently use the general term phosphatidylcholine (PC). However, PCs from different animal or plant sources have different fatty acid compositions. PC has a broad range of application fields, such as for margarine and pastries, and also in medicine. In the latter, frequently a highly purified extract of polyunsaturated phosphatidylcholine (PPC) molecules from soybean without any remaining soy protein is used, especially for parenteral injection and infusion. PPC is composed of several, presumably polyunsaturated PCs[5] (Table 1).

Table 1: Composition of PPC from soybean

PPC	94-96 %
dilinoleoylphosphatidylcholine (DLPC)	40-52 %
palmitoyl-linoleoylphosphatidylcholine (PLPC)	23-24 %
oleoyl-linoleoylphosphatidylcholine (OLPC)	12-13 %
linolenoyl-linoleoylphosphatidylcholine (LLPC)	6-7 %
stearoyl-linoleoylphosphatidylcholine (SLPC)	6 %
palmitoyl-oleoylphosphatidylcholine (POPC)	3-4 %
stearoyl-arachidonoylphosphatidylcholine (SAPC)	1-2 %

The predominating quantitative and qualitative molecule of PPC is DLPC (Figure 1). This special molecule is normally only measured in trace amounts in the human body. It contains two bound linoleic acids with two double bonds each.

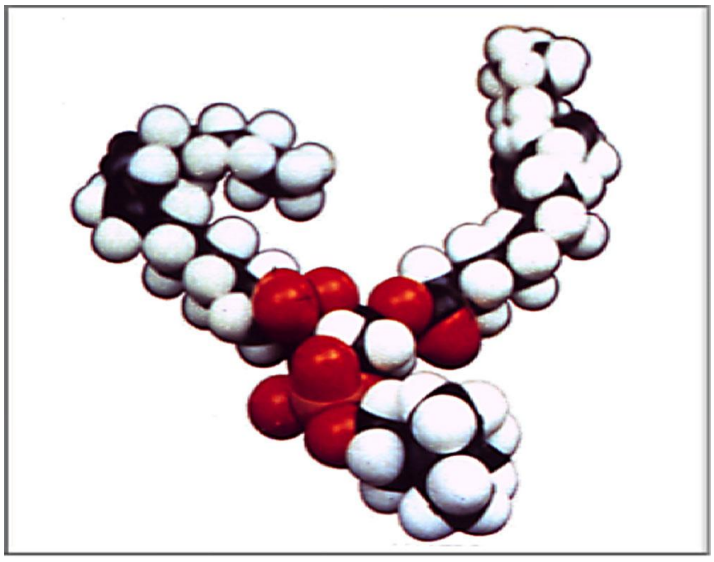

Figure 1: Stuart model of 1.2-dilinoleoylphosphatidylcholine

3. The Fat Degrading Process of Injection Adipolysis

Basically, each process of fat degradation follows the same mechanisms, such as through dietary change, lipolysis, or lipo-destruction. Lipases are involved in degradation of released fats, so the lipoprotein lipase (LPL), but also lipoproteins and here especially HDL, which transports fat back to the liver. Enzymes in the liver itself, such as the hepatic triglyceride lipases (HTGL) and phospholipases, ensure a further breakdown of the fats. The released free fatty acids are oxidized in mitochondria, producing energy rich molecules (ATP). Increased amounts of fat in peripheral tissue, blood circulation, and liver disturb the lipoprotein and lipid metabolism.

4. The Special Importance of PPC for the Transport of Fat from Tissue over Blood to the Liver

Desreumaux et al. showed already in 1979 that PPC significantly stimulates the lipolytic activity in heart and lungs (LPL), liver (HTGL) and adipose tissues of rats, and in adipose tissue of healthy subjects, compared to less unsaturated egg lecithin and the completely saturated dipalmitoyl-phosphatidylcholine.[6]

A second step is the uptake of triglycerides (TG), cholesterol (CH) and other fats into HDL for the reverse transport to the liver. Lecithin-cholesterol-acyltransferase (LCAT) plays a significant role in this procedure as it transfers an acyl group from PC to free CH so that it can be stored as CH ester (CH-E) in the inner part of the HDL for the transport to the liver. Already Rosseneu et al. showed with chimpanzees that PPC increases lysolecithin and CH-E in HDL_3, induced by activation of LCAT.[7] A complementary effect of the treatment with PPC was the decrease of the plasma concentration of TG and VLDL, and the increase in the unsaturation ratio of the TG. Zierenberg et al. reported 1981 *in vitro* a 55% increased CH uptake capacity of HDL after incorporation of PPC into the surface monolayer of the particles.[8] Additionally, a part of the LDL-CH was shifted to HDL. This change was in two experiments increased by 67 % and 49 % compared to normal HDL. Especially interesting was the increased fluidity of the monolayer of the HDL. Spann et al. added a further module to the mode of action of PPC.[9,10] They demonstrated with volunteers a significant increase of apolipoprotein A-I and A-II (Apo-A-I and Apo A-II) in the HDL after oral administration of PPC. Apo A-I is a structural protein of HDL and a cofactor of LCAT, improving the preconditions for the reverse cholesterol transport (RCT) with HDL to the liver. Jimenez et al. investigated this context more in detail with rats, which were given a fat diet.[11] Lipid metabolism during a fat rich diet should be about the same as during IL. The authors observed a significantly increased uptake of lipids from plasma into HDL. CH strongly reduced in VLDL, IDL, and LDL. The enhanced activity of LCAT above normal paralleled the significantly increased RCT to the liver after PPC administration. These results are supplemented by investigations from Verghese et al. who found that stimulation of lipolysis enhances the rate of cholesterol efflux to HDL in adipocytes.[12]

It can be summarized that PPC favored significantly the described transport of fat from the peripheral tissue to the liver, by that participating in the process of fat degradation in peripheral tissue.

5. The Effect of PPC on Hepatic Lipases

E. Kuntz summarized in 1991 the experimental and clinical results from 50 years research with EPL/PPC on liver diseases, and he named key functions of

these phospholipids, which are substantiated by diverse studies.[13] Among others, the phospholipids are imperative structural and functional elements of all cellular membranes, and essential for regeneration and new formation of biological membranes. They ensure the activity of membrane-linked proteins, such as of triglycerides degrading enzymes, of receptors and transport proteins, they regulate metabolic processes between the intra- and intercellular space, emulgate fat in the intestinal tract and bile, and they are structural and functional elements of all lipoproteins.

Regarding PPC and IL, one of the first interests is the influence of PPC on the activity of the hepatic lipase (HL). This protein is synthesized in and released from the liver in order to hydrolyze TG. Especially DLPC, the main active ingredient in PPC, stimulates the Apo-A-I and HL secretion from hepatocytes as has been shown by Chatterjee et al. *in vitro* with primary human hepatocytes and HepG2 cells.[14] The authors denominated the polyunsaturated phospholipids from soybean as HDL- and TG- regulating therapeutics. Assuming that the released lipids from adipocytes after IL behave as all lipids do, the activity of PPC on lipid degradation through HL has to be emphasized at this place.

6. The Effect of PPC on Cellular Membranes under Special Consideration of the Mitochondria

The membranes of all cells consist of a double layer of phospholipids, without which no cellular integrity would be possible. PC predominates quantitatively. The incorporation of the specific PPC-molecules from soybean into the cellular and subcellular membranes significantly increases their amount of DLPC.[5,15] DLPC with its two bound linoleic acids and its special steric configuration needs on one hand more space within the membranes, on the other hand it is more flexible at the double bond positions of the bound linoleic acids than most of the body own phospholipids. The consequence is increased membrane flexibility and fluidity. Correspondingly, PPC is not only useful for repair and regeneration of damaged membranes, but also for increasing membrane associated protein functions beyond normal. As all body-own cells and subcellular organelles are surrounded by a double layer of phospholipids as membrane, PPC acts as a membrane therapeutic.[16] The mitochondria are at this of special importance as they are surrounded by two phospholipid double layers as membranes in order to fulfill their essential functions utilizing free fatty acids, and as energy producers.

Additionally, the mitochondria are of essential importance for clinical symptoms, such as of tiredness, if membrane associated functions are disturbed or reduced. Examples are ageing processes or cancers with similar symptomatology. The results of a smaller pilot study of Ellithorpe et al. with 30 patients showed that PPC increases the activity of mitochondria so that on a long-term even weight reduction may be possible.[17] However, such a fat degradation with the help of PPC can only be regarded as an indirect evidence of increased activity of the mitochondria.

Nicolson et al. considered in 2014 this topic in a comprehensive review, and they described a part of the complex mechanisms, which takes place in mitochondria.[18] Today we assume that the mitochondrial membrane is a dynamic system with an interaction of transverse and lateral asymmetries of within the mitochondria synthesized and imported lipids in the form of precursors. Especially the mitochondrial inner membrane is associated with high complexity. Mitochondria use the oxidative phosphorylation via the tricarboxylic cycle and the electron transport chain to produce

energy. Additionally, they have numerous further functions so that Galluzzi et al. called their functions more general as "a gatekeeper for cell life and cell death."[19]

Far-reaching knowledge about the importance of inflammasomes as key signal platform for cellular stress, the formation of these through protein complexes, their damage through overweight with the help of oxidative stressors as well as their importance for the uptake, degradation and metabolism of fatty acids has been obtained during the last decade. The data show among others that we have to do it here with a central position of energy production, which can only succeed if oxidative stressors do not increase too much. However, exactly this can happen. If we destroy too quick a high number of adipocytes through oncosis / necrosis, which means an inflammatory process, we also influence the necessary functionality of the mitochondria outside of the adipocytes by stressors. Lipid substituting methods, such as with PPC, lead to changes of the composition of mitochondrial membranes and improve mitochondrial function.[20]

The importance of PPC for the activity of energy exchange of the mitochondria is shown. This leads to the hypothesis that the additional oral or intravenous administration of PPC with DC, which releases higher amounts of fat by cell destruction or lipolysis, reduces critical inflammatory effects in each therapy of IL with higher dosages.

7. PPC – is there an Apoptotic Pathway?

Duncan and Rotunda denied in their publication from 2011 that PPC alone has a cell destructing effect[21], but they did not discuss the possibility of oncosis (or necrosis) and apoptosis as two parallel or consecutive modes of actions. The authors did not take an additional significant apoptotic effect into account as the oncotic procedure overlaps the apoptotic process. How could an apoptotic effect be measured, when beforehand the adipocyte membrane is destroyed by necrosis? The investigations of Duncan and Rotunda were exclusively oriented to measure oncosis, an apoptosis assay was not used.

Li et al. were the first who specifically investigated the topic of PPC and apoptosis during IL.[22] They compared *in vitro* the effects of PPC, DC, and PPC in combination with DC on different adipocyte cultures. They observed among others that DC damaged all cell types, not only adipocytes, while PPC acted exclusively on adipocytes. The cells of muscles and others were not damaged by PPC. PPC not only induced the activation of p38 and the kinase JNK in 3T3-L1 pre-adipocytes but also the dividing of caspase-8, -9, -3 and poly ADP ribose polymerase (PARP), and in differentiated cells caspase-8. The authors led these indicators of an apoptotic pathway back to increased death receptor activations by PPC. They mentioned also other papers, in which PPC induced apoptosis of cancer cells of the bowel tract, vascular endothelial cells and macrophages. Furthermore, the here discussed PPC from soybean induced apoptosis in two studies from Sakakima et al. *in vitro* of the cancer cell types Hep-G2, Hep-3B, Alexander cells and HuH-7 cells.[23,24] Li et al. came in their own investigations to the conclusion that PPC alone does not induce an inflammatory but an apoptotic process.[22] Recently, Kim et al. observed that PPC specifically induced a decrease in mature adipocyte viability, but had less effect on 3T3-L1 preadipocytes, while the DC mediated cell death was non-specific to both preadipocytes and adipocytes.[25] PPC injections preferentially decreased gene expression in mature adipocytes, while a strong inflammatory response was elicited by DC injection. PPC treatment appeared to have a stronger action with respect to the decrease of lipid vacuoles, which is

caused by specific lysis of mature adipocytes, as demonstrated by a decrease in fatty acid binding protein 4 (FABP4) levels, a marker of mature adipocyte cells, after treatment with both PPC/DC and PPC alone compared to DC-only treatment, and by live images recorded.

8. PPC/DC versus DC alone – the Influence of PPC on the Toxicity and Side-effect Profile of DC during IL

Duncan and Rotunda discussed if and how PPC alone may have a cell destructing effect, and they denied it.[21] However, the authors indicated a buffering effect of PPC, which they claimed to reduce the potential toxicity of DC, without further expanding on that topic.

On the other hand, it is already known since 1969 that PPC significantly reduces the toxicity of DC.[26] Duncan replied 2013 to a letter to the editor of Hasengschwandtner and Gundermann that PPC reduces the immediate loss of cell viability.[27,28]

The double-blind trial of an Italian group from 2008 was one of the first, which investigated the effects and side-effects of IL with or without PPC.[29] The half-side comparison study showed no significant differences in the efficacy on fat reduction between PPC/DC and DC alone but differences in the sequence and severity of the observed adverse drug reactions. While some side-effects were of equal strength, such as swellings, burning or erythema, others, especially pain, duration of the side-effect appearance, and formation of hematoma were significantly more frequently observed or severe under DC alone in comparison with DC and PPC.

The Korean research group of Chung et al. confirmed with mice that the inflammation was caused by DC.[4] Comparing DC alone to DC with PPC and measuring IL-1ß, IL-6 and PGE_2, the authors observed that DC without PPC induced a much stronger inflammation. Additionally, histology was markedly different in patients treated with PPC/DC when compared to DC alone. Among others, a more organized fat necrosis was seen from PPC/DC versus DC alone.[30]

Further studies support the hypothesis that PPC plays an important role as a buffer.[31-34] Dial and Lichtenberger from the Department of Integrative Biology and Pharmacology of the University in Houston/Texas investigated the protective effect of PC on toxic substances, such as of bile acids, in the gastrointestinal tract.[35-38] Their proof of dose dependent reduction of toxicity of DC by PPC on hepatocytes and on bowel tract cancer cells confirms the reduction of toxicity of the secondary bile acid DC by PPC: „We showed that a submicellar concentration of the bile salt SDC (= sodium deoxycholate) was toxic to the hepatocytes and that this toxicity was overcome by mixing the SDC with PC before exposure of the cells.“[38] Furthermore, Tan et al. reported about the inhibiting activity of PPC on DC induced cytotoxicity using Caco-2 cells, and Ikeda et al. recently confirmed the protective effects of egg yolk PC on HepG2 cells and primary human hepatocytes from the cytotoxicity of bile salts – and here especially of Na-DC – by decreasing the cell-association of bile salts.[39,40] According to Dial et al., PPC is clearly more useful than body-own PC to protect bowel tract cells, red blood cells, and synthetic liposomes from DC's aggressiveness.[35] The mentioned papers show that PPC reduces the side-effects induced by DC: general inflammation, local pain and hematoma formation. PPC induces a shorter period of recovery, and inflammatory processes are quicker finished. PPC is able to reduce DC's toxicity and intensity of the inflammatory reactions. Consequently, a higher concentration of DC in PPC/DC injectables in contrast to DC alone

injection is possible. While, for example, the treatment protocol of the NETWORK-Lipolysis (a physicians' network) recommends 1,250 mg of DC in combination with PPC, the recommended amount of DC, approved in the USA for IL, is 100 mg at maximum for a single treatment session.[41,42]

Correspondingly, the authors observed that the differentiated adipocytes treated with PPC were apoptotic and released four-fold more triglycerides compared to adipocytes treated with the control ethanol, with a minimal effect on free fatty acids release, suggesting that its fat-reducing effect was mediated mainly through the induction of adipocyte cell death rather than lipolysis.

9. Discussion

DC is suggested to be the principal component acting on the reduction of adipose tissue, and the approval of DC as active ingredient for IL is regarded as a pragmatic solution, oriented on cause and effect association. However, its administration is limited to a low dose to avoid any severe side effects, and at present, the approval of pure DC is restricted to the small submental fat area in adults.

However, serial histological studies indicate that DC may be better tolerated and lead to more cosmetically favourable patterns of fat necrosis and fibrosis when co-administered with PPC.[30,43]

Additionally, PPC has not only relevant activities on lipid metabolism in general, as described in literature, but it appears to have a specific apoptotic influence on mature adipocytes, too, as demonstrated by a stronger decrease of lipid vacuoles of these adipocytes. While the reduction of adipose tissue by DC is caused by a non-specific destruction of the cells, the specific effect of PPC on adipocytes, and less on preadipocyte viability, suggests PPC to be a promising agent to selectively reduce adipocyte tissue mass.

Non-surgical fat reduction has significant advantages as it is cost effective, requires no general anesthesia or hospitalization, and does not have the risk of operative scar formation. However, safety of the treatment with the therapeutic agent is important. Considering that DC is a strong, non-specific detergent, disrupting cell viability, its application has been associated with inflammation with many adverse effects, such as pain, edema, numbness, and swelling, though transient and restricted to the local area.[44] Furthermore, patients who received DC complained about more pain than those treated with PPC/DC, and although PPC injection also appeared to induce local swelling in animal experiments, they were milder than those induced by DC.[25,29] The better side-effect profile of PPC/DC versus DC alone, and the reduced toxicity of DC when combined with PPC are additional reasons why the combination therapy may be justified.

What can the indication fields for IL be in the future to consider it as a useful standard procedure in the repertoire of the fat reducing therapies cryo-, ultrasound-, and laser-lipolysis? Its potential in several domains can be:

1. Small fat deposits, such as in the face.
2. Correction of treatments with liposuction.
3. Indications, which can only be treated to a certain extent by other therapies, such as lipoma.
4. Combination therapies, such as of PPC/DC with dual-frequency ultrasound.[45]
5. Combination therapies with cryo-lipolysis.[46]

6. Treatment of larger areas, if side effects and dosages make it possible, and if this becomes a condition for new drug registrations. Such drugs should at least contain the combination of DC and PPC.

7. Supplementary intravenous administration of PPC to support lipid metabolic processes in blood and liver, including mitochondria.

Additionally, other active ingredients should be searched for, either in combination with PPC – what would be a good possibility to see if the apoptotic pathway can be expanded – or by completely other substances. These substances should in any case improve the efficacy of the product or further reduce the side effects and eventual complications.

Further studies are recommended, which confirm the observed mode of action of PPC in general into direct connection with the injections into subcutaneous fat tissue. PPC is a highly purified extract of natural phosphatedylcholine molecules from soybean and innocuous to all cells but adipocytes, which makes it a promising agent not only for well investigated indications, such as dyslipidemia or fatty liver of different origin, but also for IL.[47,48]

10. Summary

Considering the modes of action and positive experimental and clinical effects of PPC, especially of the in PPC/DC solutions contained DLPC, on lipid metabolism and on the non-surgical process of IL, PPC seems to have its place value in the treatment of IL. It diminishes the local side-effects of pure DC, shortens the inflammatory processes, increases the activity of lipids-degrading enzymes, and enhances the reverse transport of TG and CH via HDL to the liver. Hepatic lipid metabolism and degradation, and the energy production in the mitochondria are augmented. First significant data point to an apoptotic process of PPC as a second mode of action in IL.

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