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## Biological properties of a DHA-containing structured phospholipid (AceDoPC) to target the brain

Lagarde M, Hachem M, Bernoud-Hubac N, Picq M, Véricel E, Guichardant M.

Université de Lyon, Inserm UMR 1060 (CarMeN), IMBL/INSA-Lyon, Villeurbanne, France.  
Correspondence: michel.lagarde@insa-lyon.fr

### Abstract

1-acetyl,2-docosahexaenoyl-glycerophosphocholine (AceDoPC) has been made to prevent docosahexaenoyl (DHA) to move to the sn-1 position as it rapidly does when present in 1-lyso,2-docosahexaenoyl-GPC (lysoPC-DHA), an efficient DHA transporter to the brain. When incubated with human blood, AceDoPC behaves closer to lysoPC-DHA than PC-DHA in terms of binding to plasma albumin and lipoproteins, and DHA incorporation into platelets and red cells. In addition, AceDoPC prevents more efficiently the deleterious effects of the experimental stroke in rats than does unesterified DHA. Also, AceDoPC inhibits platelet-activating factor-induced human blood platelet aggregation. Overall, AceDoPC might act as an efficient DHA transporter to the brain, and as a neuro-protective agent by itself.

### Keywords

Metabolism; Blood-Brain Barrier; Stroke

### Introduction

Docosahexaenoic acid (DHA) is the main polyunsaturated fatty acid (PUFA) esterified in the mammal brain phospholipids, and almost the only omega-3 PUFA in this tissue (1). It is recognized as an important PUFA for the brain development and learning abilities, as well as in the retina for visual acuity (2). In addition to be an important endogenous component in these organs, DHA is a relevant nutrient from marine food, together with eicosapentaenoic acid (EPA), both being active to prevent cardiovascular events (3).

One important issue in the propensity of DHA to accumulate into the brain is the circulating form for its efficient crossing of the blood-brain barrier (BBB). Early work from our group suggested that PUFA esterified at the sn-2 position of 1-lyso,2-acyl-glycerophosphocholine were more efficiently taken up by rat brain than the corresponding non-esterified fatty acids (4). This observation is especially valid for DHA that shows the highest difference between the two forms, with a ten-fold preference for 1-lyso,2-docosahexaenoyl-glycerophosphocholine (lysoPC-DHA) than non-esterified DHA, when they were both bound to albumin (5).

This preference was confirmed by using an *in vitro* re-constituted BBB with a co-culture of brain capillary endothelial cells and astrocytes, whereas no preference was shown when those brain endothelial cells were replaced by aortic ones (6). In the latter case, lysoPC-DHA was esterified and integrated in astrocyte PC (more than 50% of the initial lysoPC-DHA) with virtually no remaining of the lyso form. This suggests that lysoPC-DHA was acylated into PC. Interestingly, this preference for lysoPC was significantly lower when 1-lyso,2-palmitoyl-PC was used instead (6). All these results are in favor of a specific passage of the BBB by lysoPC-DHA.

One question then arose: are 1-lyso,2-acyl-glycerophospholipids, such as lysoPC-DHA, stable enough to cross the BBB without isomerization into 1-acyl,2-lyso counterparts, a more stable form of lysophospholipids (lysoPL) (7). Indeed, the primary alcohol, present in 1-lyso,2-acyl-PL is more reactive for acylation than the secondary alcohol in 1-acyl,2-lyso-PL, which should facilitate the position isomerization. The measurement of human blood lysoPC, the

main lysoPL species, showed us a concentration of 150 nmol/ml in plasma, with one quarter being composed of PUFA (essentially linoleic acid (20%), arachidonic acid (4%), DHA (1%) (8). However, studies on the migration of linoleoyl residue from the sn-2 position of lysoPC to the sn-1 position showed that three quarters migrated after just 20 min incubation in plasma kept at 37°C and physiological pH (8). This prompted us to make a structured PC trying to mimic as far as possible 1-lyso,2-DHA-PC. Some results mentioned in this short review are published in patents but not yet in full articles.

### **Preparation of 1-acetyl,2-docosahexaenoyl-glycerophosphocholine (AceDoPC)**

Figure 1 shows the chemical structure of AceDoPC. In order to keep the structured phospholipid closest to lysoPC-DHA, especially for its polarity, we have acetylated 1-lyso,2-DHA-PC *via* an organic process (9). Although the lyso species was purified by HPLC immediately prior to acetylation, around 20% of 1-docosahexaenoyl,2-acetyl-PC was obtained, presumably due to DHA migration to the sn-1 position during the chemical process. We then decided to proceed through an enzymatic transacylation from a purified 1-acyl,2-DHA-PC (PC-DHA), issued from a marine biomass. AceDoPC was produced by using an immobilized triacylglycerol lipase acting upon pure PC-DHA in absence of water but presence of ethyl acetate/vinyl acetate as acetylation agents to replace the acyl moiety by acetyl at the sn-1 position. This transacylation process minimizes the migration of DHA, making AceDoPC an almost pure 1-acetyl,2-DHA-PC (10, 11). Figure 2 shows the purity of AceDoPC, either by radiochromatography when using the radiolabelled phospholipid (Fig. 2A), or by HPLC analysis of the unlabelled molecule (Fig.2B). Of note, AceDoPC exhibits polar properties closer to lysoPC-DHA than to DHA-containing PC as evaluated by chromatographic migration.

### **Behavior of AceDoPC in human blood**

To assess that AceDoPC could be a privileged carrier of DHA to the brain, we first studied its distribution compared to PC-DHA within different human blood compartments. To this goal, we synthesized <sup>14</sup>C-labeled-DHA in PC-DHA from the marine microalgae *Cryptocodinium cohnii* incubated with <sup>14</sup>C-labeled acetate, and prepared <sup>14</sup>C-labeled-DHA in AceDoPC using the transacylation process reported above (10). The labeled phospholipids were then incubated with human blood and the radioactivity distribution was measured in blood platelets and red cells, the latter compartment being accepted as an index of the brain DHA accretion. We found a greater incorporation of labelled DHA into red cells after incubation of whole blood with AceDoPC compared to PC-DHA. In contrast, this preference for AceDoPC was not observed with platelets (unpublished results). These results are in agreement with *in vivo* ones in which intake of DHA led to a slow but sustained rise in red cells, preferentially from lysoPC-DHA, while a faster but time-limited accumulation occurred in platelets from non-esterified DHA (12, 13). This means that AceDoPC may mimic lysoPC-DHA more closely than PC-DHA.

The analysis of the radioactivity distribution within human plasma indicated that AceDoPC was mainly bound to HDL and albumin (around 60 and 40%, respectively), whereas PC-DHA was mainly distributed within lipoproteins (around 85% within HDL, LDL and VLDL) and less than 15% within albumin (unpublished results). This suggests that AceDoPC behaves like lysoPC rather than PC. Overall, these results fit with the hypothesis that AceDoPC could be a privileged carrier of DHA to red cells and in turn to the brain.

### **Effect of AceDoPC on the ischemic stroke in rats**

As DHA has been shown to induce neuroprotection in rats that underwent a transient cerebral ischemia (14), we used this model for comparing DHA with AceDoPC. The external carotid

artery of rats was inserted with a coated monofilament for a one-hour transient occlusion, then non-esterified DHA or AceDoPC, solubilized in rat plasma to allow their binding to albumin and lipoproteins, was intravenously injected with 1ml of plasma containing 1  $\mu$ mol of each DHA form. Magnetic Resonance Imaging of the brain was performed as well as behavioral tests 24h after the injection. The lesion sizes due to the initial stroke were stable in rats receiving the plasma alone as a control, while they were decreased in rats receiving non-esterified DHA, and further decreased (twice as much) in rats receiving AceDoPC. Neuroscores tended to be improved ( $p=0.07$ ) in the AceDoPC group (15). It is interesting to note that the dose used was 2 to 4 times lower than those used in the Belayev's study (14), who stated that very high dose of DHA were inactive. Overall, our study makes promising the use of moderate injected dose of AceDoPC following stroke.

The mechanism of neuroprotection observed remains to be clarified. The measurement of isoprostanes (evaluated with 8-epi-prostaglandin  $F_{2\alpha}$ ) in the brain 24h after the injection of either DHA or AceDoPC showed that, although non-significantly decreased, a lower value was obtained after AceDoPC compared to DHA. Interestingly, 8-epi-prostaglandin  $F_{2\alpha}$  was significantly lower when both treatments were pooled in the statistical analysis, suggesting a possible reduction of oxidative stress. Whether the influx of DHA was higher in the AceDoPC group was not tested in this study, but an ongoing investigation using radioactively labeled molecules shows higher DHA accretion into normal rodent brains from AceDoPC compared to non-esterified DHA (Hachem et al., unpublished results).

### **Anti-PAF effect of AceDoPC**

Platelet-activating factor (PAF) has been characterized as 1-O-alkyl,2-acetyl-glycerophosphocholine (16). In addition to aggregate blood platelets, PAF is recognized as a pro-inflammatory molecule (17) as well as a broncho-constricting agent (18).

Apart from the O-alkyl moiety that differs from the docosahexaenoyl one, AceDoPC could be considered as a position isomer of PAF. The aggregation of human blood platelets isolated from their plasma, induced by PAF (hexadecyl,acetyl-glycerophosphocholine), was then evaluated in presence or absence of AceDoPC. An  $IC_{50}$  was observed with 10 $\mu$ M AceDoPC while a 15% inhibition could be observed with 1 $\mu$ M AceDoPC (19). Although this inhibition may be considered as modest with regard to the active concentrations, this biological property allows some extrapolation on the anti-inflammatory potential of the molecule in addition to its capacity to be beneficial to the brain.

### **Conclusions**

AceDoPC is a proposed DHA-containing structured phospholipid that mimics in a way lysoPC-DHA in terms of polarity and distribution within blood cells and albumin/lipoproteins. The acetylation of the sn-1 position keeps docosahexaenoyl at the sn-2 position that is the physiological one. Whether AceDoPC facilitates incorporation of DHA into the brain through its lysoPC-DHA moiety is under investigation.

On the other hand, AceDoPC proved to be able to prevent more efficiently deleterious effects of the experimental ischemic stroke than did non-esterified DHA. Also, it may exert anti-inflammatory activity by inhibiting PAF effects, putatively resulting from some antagonism due to the inverted position of the acetyl moiety but keeping the same polar head.

Altogether, AceDoPC might be considered as a dual structured phospholipid to provide DHA to the brain and exert anti-atherothrombotic effects.

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## Figure legends

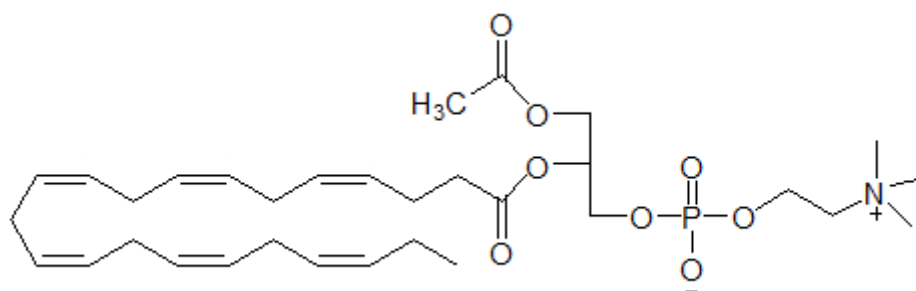
**Figure 1:** Structure of 1-acetyl, 2-docosahexaenoyl-glycerophosphocholine (AceDoPC).

**Figure 2:** Typical tracings showing the purity of labelled and unlabelled AceDoPC.

**2A:** Radiochromatogram of radiolabeled  $^{14}\text{C}$ -(DHA)-labelled AceDoPC (Thin-layer chromatography with chloroform/methanol/water (65/25/4) as eluent).

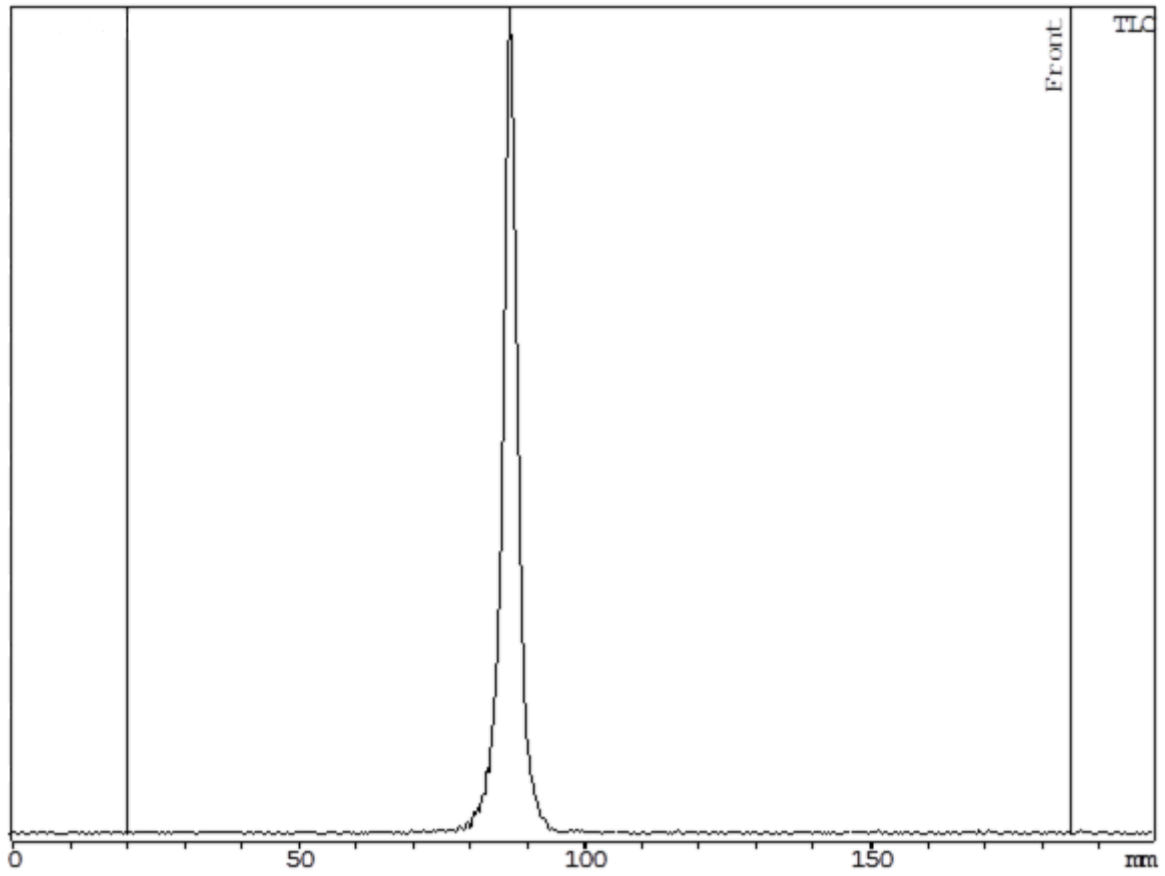
**2B:** Analysis of unlabelled AceDoPC by reverse phase high performance liquid chromatography (X Bridge  $\text{C}_{18}$  column) using a linear solvent gradient: solvent A and B were a mixture of methanol/water/acetonitrile (95/35/2.5 and 100/4/2.5, v/v, respectively) from 5 to 35 min (1ml/min). AceDoPC was detected at 205 nm after 27 min.

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