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AceDoPC, a structured phospholipid to target the brain with docosahexaenoic acid

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Abstract – AceDoPC® is a structured phospholipid or acetyl-LysoPC-DHA made to prevent docosahexaenoic acyl migrating from the *sn*-2 to *sn*-1 position of the phospholipid, however keeping the main physical-chemical properties of LysoPC-DHA. As previously shown for LysoPC-DHA, AceDoPC® allows DHA crossing a re-constituted blood-brain barrier with higher efficiency than non-esterified DHA or PC-DHA. When injected to blood of rats, AceDoPC® is processed within the brain to deliver DHA to phosphatidyl-choline and -ethanolamine. When injected to rats following the induction of an ischemic stroke, AceDoPC® prevents the extension of brain lesions more efficiently than DHA. Overall, these properties make AceDoPC® a promising phospholipid carrier of DHA to the brain.

Keywords: AceDoPC® / acetyl-LysoPC-DHA / blood-brain barrier / carrier

Résumé – AceDoPC® est un phospholipide structuré correspondant à l'acétyl-LysoPC-DHA conçu pour empêcher la migration du radical docosahexaénoyle de sa position *sn*-2 à la position *sn*-1 du phospholipide, tout en gardant les principales propriétés physico-chimiques de la LysoPC-DHA. Comme montré précédemment avec la LysoPC-DHA, AceDoPC® permet au DHA de traverser une barrière hémato-encéphalique reconstituée plus efficacement que le DHA non-estérifié et que PC-DHA. Lorsqu'il est injecté dans la circulation des rats, AceDoPC® est métabolisé au sein du cerveau en apportant le DHA aux phosphatidyl-choline et -éthanolamine. Injecté à des rats ayant subi un accident vasculaire cérébral ischémique, AceDoPC® prévient l'extension des lésions cérébrales plus efficacement que le DHA. Globalement, ces propriétés font d'AceDoPC® un transporteur de DHA phospholipidique prometteur.

Mots clés : AceDoPC® / acétyl-LysoPC-DHA / barrière hémato-encéphalique / transporteur

1 Introduction

Docosahexaenoic acid (DHA) is the main polyunsaturated fatty acid (PUFA) of the brain lipid structures in which it is esterified at the *sn*-2 position of glycerophospholipids, and is recognized as an essential PUFA for the brain development and function (Crawford *et al.,* 1999). Its brain level has been reported to be lowered in neurodegenerative diseases such as Alzheimer and Parkinson diseases, which suggests that its replenishment in brain structures might help fighting those diseases (Alessandri *et al.,* 2004). Although DHA is known to derive from alpha-linolenic acid, the indispensable precursor of the omega-3 family PUFA, by a series of desaturations and elongations, however at a low rate in humans (Burdge and Calder, 2005), it cannot be produced in the brain, and must be taken up from blood plasma. It has been assumed that DHA, as well as another essential PUFA to the brain, arachidonic acid, are taken up from the blood when bound to albumin in their non-esterified form (Chen *et al.,* 2008). However, blood albumin also binds lyso-phosphatidylcholine (LysoPC), including those containing PUFA. The hypothesis that PUFA-containing LysoPC might provide such PUFA to the brain as well has been investigated. Indeed, PUFA, but not saturated fatty acids, esterified at the *sn*-2 position of LysoPC are better taken up by rat brains *in vivo* than the corresponding non-esterified PUFA (Thiès *et al.,* 1992). Among the studied PUFA, DHA from LysoPC-DHA was the most highly taken up (10-fold) compared with its non-esterified form, a preference that was not found for the uptake of DHA by other organs such as the heart, kidney and liver (Thiès *et al.,* 1994). Furthermore, the use of a re-constituted blood-brain barrier allowed to confirm that DHA provided in LysoPC was more efficient to cross this barrier than non-esterified DHA (Bernoud *et al.,* 1999). Interestingly, a very recent work has demonstrated that

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the blood-brain barrier expresses a specific protein that specifically binds LysoPC-DHA (Nguyen *et al.,* 2014), which may explain the preferential uptake of DHA when esterified in LysoPC. However, a likely important issue for the further metabolism of DHA is its position in LysoPC, if this lipid is processed within the brain after its uptake. Indeed, PUFA are physiologically present at the *sn*-2 position of glycerophospholipids, but they may easily migrate to the *sn*-1 position within LysoPC because of the higher reactivity of its primary alcohol. This has been found experimentally, and more *sn*-1 PUFA acyls than *sn*-2 have been measured in blood plasma LysoPC (Croset *et al.,* 2000).

2 Acetylation of the sn-1 position of LysoPC-DHA

In order to prevent the migration of PUFA from the *sn*-2 position of LysoPC to the *sn*-1 position, the latter has been blocked by the shortest possible acyl chain. In principle, the acylation of 1-lyso,2-DHA-PC with the short acetyl group must provide a diacyl-PC closest to LysoPC in terms of polarity, which is confirmed by the chromatographic behaviors of both molecules that are very close. So, a freshly prepared and purified 1-lyso,2-docosahexaenoyl-PC produced by triacylglycerol lipase treatment of 1-palmitoyl,2-docosahexaenoyl-PC has been acetylated (Polette *et al.,* 1999). The product, 1-acetyl,2-DHA-PC, has been named AceDoPC®. Recent investigation of the AceDoPC conformation by molecular modelling has confirmed that the acetyl group does not much alter the 3D of the LysoPC-DHA molecule (Hachem *et al.,* 2015).

3 Crossing of a re-constituted blood-brain barrier by AceDoPC

[^{14}C]-labeled DHA, either non-esterified, or esterified in 1-palmitoyl,2-docosahexaenoyl-PC (PC-DHA), or in AceDoPC were used. The three forms at 5 μM each were alternatively incubated in the culture medium of the upper phase of a transwell system consisting in a monolayer of capillary endothelial cells separated from glia cells by a lower medium. Then, crossing the endothelial cell monolayer allows the labeled molecules to face glial cells through the lower medium. The accumulation of labeled DHA in the lower medium and glial cells was then considered as the fraction crossing the blood-brain barrier. Under these conditions, the radioactive DHA which crossed the blood-brain barrier from AceDoPC® was significantly higher than from non-esterified DHA, which was significantly higher than from PC-DHA (Hachem *et al.,* 2015).

This means that AceDoPC behaves like LysoPC-DHA for crossing the blood-brain barrier. The distribution of [^{14}C]-DHA within endothelial and glial cell lipids after 4 h incubation onto the transwell system clearly showed that AceDoPC® was processed by the cells. The main [^{14}C]-labeled lipids were found in phosphatidyl-choline and -ethanolamine (around 60% in total), while the label in LysoPC plus AceDoPC® was 15% in endothelial cells and 10% in glial cells. The rest was in the neutral plus non-esterified fatty acid fraction (Hachem *et al.,* 2015). This means that AceDoPC® was highly metabolized after its preferential uptake.

4 Metabolic fate of DHA from AceDoPC® within brain lipids when injected in rats

To investigate whether AceDoPC could be a relevant delivery form of DHA to the brain, we performed an *in vivo* experiment using [^{14}C]-DHA in either AceDoPC® or its non-esterified form injected into tail rats, following by the analysis of the radioactivity associated with different organs. As we found in the past with LysoPC-DHA, [^{14}C]-DHA was higher in the brain when originating from AceDoPC® than from non-esterified DHA (at least double) at each time of analysis (1, 6, 24 and 48 h after injection). In contrast, no difference could be found in plasma, liver, heart and even eyes which are, like the brain, endogenously very rich in DHA (Hachem *et al.,* 2015). These results definitely confirm that AceDoPC® is an efficient vehicle of DHA to the brain.

Measuring the distribution of ^{14}C-label within the main lipid classes in the brain and heart grossly showed that [^{14}C]-DHA accumulated with time (1, 24, 48 h) in brain PC and PE while it decreased from AceDoPC, remaining constant in LysoPC but at a low level. In contrast, [^{14}C]-DHA remained constant in those heart lipid classes. A big difference was relating to [^{14}C]-DHA accumulation in neutral brain lipids plus the non-esterified fatty acid pool where [^{14}C]-DHA was quite low, while it constantly attained 40% in the heart (Hachem *et al.,* 2015).

5 AceDoPC was more active than DHA to decrease the post stroke lesions

It has been previously shown that non-esterified DHA, injected to rats after induction of an ischemic stroke, is able to improve the post-stroke status of the animals (Belayev *et al.,* 2009). Using a similar approach in the rat we compared the effect of AceDoPC® and non-esterified DHA injected 1h following the induction of stroke. Each rat was injected with one micromole of either DHA, or AceDoPC (both bound to rat plasma), as compared to the vehicle (rat plasma), or saline, one hour after stroke induction. The post-stroke brain lesions, assessed by magnetic resonance imaging, significantly decreased in both the DHA and AceDoPC® groups, compared to controls, but such a decrease was higher in the AceDoPC® group (Chauveau *et al.,* 2011).

On the other hand, the brain content in F_2-isoprostanes, as markers of oxidative stress, was measured by gas-chromatography coupled with mass spectrometry. When pooling the DHA and AceDoPC® groups it appeared that a significant lowering of oxidative stress occurred, although this was not significant for each group alone. However, the values in the AceDoPC® group were lower than in

the DHA group (Chauveau *et al.,* 2011). Altogether, these results show that AceDoPC® was more efficient than non-esterified DHA to protect the brain in a post-stroke situation. The mechanism of this is not clear, but recent findings suggest that AceDoPC®, in addition to enhance DHA delivery to the brain and consequently that of neuroprotective metabolites derived from DHA, could have additional effects through interaction with cyclooxygenase-2 (unpublished patent).

6 Conclusions

AceDoPC® shares the biological activity of LysoPC-DHA in terms of brain metabolism of DHA from blood plasma origin. Its advantage over LysoPC-DHA is to keep DHA at the *sn*-2 position, the physiological one, of the glycerol moiety in the carrier phospholipid, then favoring the brain DHA metabolism. In addition, the acetyl chain at the *sn*-1 position might have its own action.

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