Phosphatidylcholine and PPARα: A relevant connection in liver disease?

Phosphatidylcholine et PPARα : une connexion appropriée dans une maladie du foie?

A. Lamaziere, C. Wolf

Mass Spectrometry group, faculté de médecine UPMC-Paris6, 27, rue Chaligny, Paris 75012, France

Available online 13 April 2010

Summary The nuclear receptors known as PPARs modulate metabolic and inflammatory pathways by responding to nutritional signals through ligand activation of transcription. They are targeted by drugs in use and in development for disease therapy. No endogenous PPAR ligand has been identified yet; the molecule that occupies the nuclear receptor-binding site in vivo while the receptor is actively driving transcription has been presently searched for by Chakravarthy et al. The group provides now a solid evidence that endogenous lipid synthesis generates a discrete phosphatidylcholine species, 1-palmitoyl 2-oleyl phosphatidylcholine (16:0/18:1 PC), that serves as an endogenous ligand for PPARα.

© 2010 Elsevier Masson SAS. All rights reserved.

Identification of a physiologically relevant endogenous ligand for PPARalpha in liver.’’
Chakravarthy MV, Lodhi IJ, Yin L, Malapaka RR, Xu HE, Turk J, Semenovich CF. Cell. 2009; 138(3):476–88. The nuclear receptor PPARα is activated by drugs to treat human disorders of lipid metabolism. Its endogenous ligand is unknown. PPARα-dependent gene expression is impaired with inactivation of fatty acid synthase (FAS), suggesting that FAS is involved in generation of a PPARα ligand. Here we demonstrate the FAS-dependent presence of a phospholipid bound to PPARα isolated from mouse liver. Binding was increased under conditions that induce FAS activity and displaced by systemic injection of a PPARα agonist. Mass spectrometry identified the species as 1-palmitoyl-2-oleoyl-sn-glycerol-3-phosphocholine (16:0/18:1-GPC). Knock down of Cept1, required for phosphatidylcholine synthesis, suppressed PPARα-dependent gene expression. Interaction of 16:0/18:1-GPC with the PPARα ligand binding domain and coactivator peptide motifs was comparable to PPARα agonists, but interactions with PPARγ were weak and none were detected with PPARγ. Portal vein infusion of 16:0/18:1-GPC induced PPARα-dependent gene expression and decreased hepatic steatosis. These data suggest that 16:0/18:1-GPC is a physiologically relevant endogenous PPARα ligand.
The identification of PPARα endogenous ligand has been wanted for a long time, omega 3 polyunsaturated fatty acids (FA), oxidized FA and phospholipids and various eicosanoids being among the numerous former candidates.

It is amazing that such a critical modulation of lipid and glucose metabolism is acted by a relatively abundant phospholipid species representing up to 11% of the phosphatidylcholine molecular species in the nucleus. However PPARα being the prominent activator in the liver of fatty acid oxidation, ketogenesis, lipid transport, and gluconeogenesis it makes sense eventually that the key modulator is an end product of FA and phospholipid synthesis.

The demonstration has required a thoroughly constrained animal model for genetics and the profiling of PC species bound to PPARα. PPARα null mice were used to eliminate the possibility of ligand competition between adenosinoly transformed PPARα and the endogenous PPARα. The expression of PPARα was reconstituted by adenoviruses infection encoding FLAG-tagged PPARα, which allows a convenient immunopurification of PPAR from which the extraction of bound lipids was done.

The crossing with FA synthase knockout mice which cannot synthesize fatty acids, neither 16:0 nor 18:1, has resulted in very low levels of the potential ligand. For quantification the variable amounts of 16:0/18:1 PC were compared with the prominent species of liver 16:0/18:2 and 18:1/18:1. These two later species maintain a constant level in the nuclear extract contrasting with the variable and regulatory role of 16:0/18:1 PC. Accordingly with the low fatty acid content of the ligand it was previously shown that FA synthase knockout mice have an impaired PPARα-dependent gene expression that is rescued after pharmacological activation of PPARα.1

The blockade of PC head group synthesis in choline-(ethanolamine)-phosphotransferase (Cept1) KO mice decreases also the PPARα-dependent gene expression. The full capabilities of tandem mass spectrometry have been applied presently to identify unambiguously the PC species bound to PPARα in FLAG-eluted hepatic nuclear extracts. Under a variety of conditions the variation of 16:0/18:1 PC was monitored. It was shown to parallel the biochemical phenotype expected for PPARα activity. For instance, wild type versus FAS knockout shows higher 16:0/18:1 PC but standard diet versus zero fat diet shows lower levels.

With relevance to nutritional and pharmacological studies the ligand binding was increased under conditions that induce FA synthase activity (zero fat diet) and was displaced by systemic injection of the PPARα agonist WY14643. Interestingly, the specificity of 16:0/18:1 PC for PPARα and hence for a pharmacologic application was comforted because the interactions with PPARγ were weak and none were detected with PPARγ.

Finally the activity of the potential ligand was tested in vivo. Liver fat content was decreased in control mice with portal infusion of 16:0/18:1 PC as compared to vehicle. The PPARα-dependent genes Acox1 and Cpt1α were increased by 16:0/18:1 PC in control mice but not in PPARα-deficient mice. These last observations are probably a weak point in the demonstration because it is difficult to understand according to the current state of knowledge how such a poorly soluble lipid molecule of MW 758 enters the hepatocyte and reaches the nucleus as an intact moiety.

PC are known to have cytoprotective properties in the biliary tree. The mdr2 KO mice model in which PC is virtually absent in bile, develop biliary inflammation, cholestasis, gallstones, biliary cirrhosis and liver cancer. In human there is also evidence that PC not only enters the hepatocyte and reaches the nucleus as an intact moiety.

Figure 1 The lipotropic activity of PPARα is preventive of steatohepatitis after the induction of PPRE (Responsive Element comprised of a series of multiple genes ruling the circulation of lipids distributed via the liver). PPARα is activated after binding in the nucleus of a by-product of fatty acids (FA) and phosphatidylcholine (PC) synthesized in the microsomes of liver cells, namely, the phospholipid ligand 16:0/18:1 PC. 16:0/18:1 PC is a PC species with palmitic 16:0 and oleic 18:1 fatty acid attached at position sn-1 and sn-2, respectively. Both of FAs are products of de novo synthesis but can have also a dietary origin. By contrast, the essential FA such as linoleic acid (18:2) can only be provided from dietary sources. Dietary 18:2 is incorporated in the prominent PC species of animal and human liver, 16:0 18:2 PC, which is abundantly co-secreted into the bile along with cholesterol and bile acids. Other participants of the “lipotropic” activity preventive of fatty liver are methyl group donors involved in the synthesis of PC headgroup (choline and betaine) and the assembly and export of VLDL (very low density lipoprotein) into the blood. During fasting period and under PPARα activation, FAs can be degraded by mitochondrial and peroxysomal oxidation to ketone bodies exported from the liver. The central role in the regulatory mechanism (green thick line) exerted by the endogenous ligand 16:0/18:1 PC on PPARα is displayed.

1 Chakravarthy et al., Cell Metab. 2005; 1309–1322.