Published in final edited form as:

Future Lipidol. 2008 December ; 3(6): . doi:10.2217/17460875.3.6.599.

Therapeutic potential of phosphoethanolamine-bound C-reactive protein in atherosclerosis

Alok Agrawal

Department of Pharmacology, James H. Quillen College of Medicine, East Tennessee State University, Johnson City, TN 37614, USA

Three papers published in the year 2008 from my laboratory reported experiments and data indicating the therapeutic potential of phosphoethanolamine-bound C-reactive protein (PEt-bound CRP) in atherosclerosis. The small-molecule compound PEt, which binds to CRP, could in itself be of therapeutic value. The first paper described the capability of PEt-bound CRP to capture native low-density lipoprotein (LDL) cholesterol in human serum [1]. The second paper described the potency of PEt-bound CRP to bind to a modified form of LDL called enzymatically-modified LDL (E-LDL). Although CRP alone could bind to E-LDL, the PEt-bound CRP was much more potent than unbound CRP in binding to E-LDL [2]. This paper also reported a previously unknown function of CRP. CRP, once bound to E-LDL, prevented the formation of E-LDL-loaded macrophage foam cells. The third paper further emphasized the importance of PEt-bound CRP and presented a review of the literature on the connection between CRP and atherosclerosis [3].

CRP, also known as high-sensitivity CRP or hsCRP, is a serum protein which is used as a marker of systemic inflammation [see CRP reviews 3-7, and the references cited in 3, 4]. CRP is associated with risk of atherosclerosis; however, recent data suggest that the association of CRP with atherosclerosis may not be causal [8]. In 1982, it was shown that under certain experimental conditions CRP interacted with LDL [9]. Since then CRP was suspected to play a role in atherosclerosis. CRP was also found deposited and co-localized with LDL and macrophages in human atherosclerotic lesions. Discovering the functions of CRP in atherosclerosis quickly became a hot subject for research.

Atherosclerosis is a heart disease caused by the deposition and subsequent modification of LDL in artery walls. Atherogenic LDL, which includes modified forms of LDL produced by oxidation and proteolysis of LDL, enters macrophages to form foam cells. The LDL-loaded macrophage foam cells contribute to the development of atherosclerosis which leads to myocardial infarction. To prevent atherosclerosis, LDL can be targeted therapeutically at two locations: the native LDL found mostly in the circulation and the atherogenic LDL located mostly in the artery walls.

Targeting native LDL: Statins versus PEt-bound CRP

Statins, the inhibitors of a key enzyme in the cholesterol biosynthesis pathway, are used as cholesterol-lowering drugs and reduce the risk of atherosclerosis. Lowering of circulating LDL cholesterol levels is a good approach to prevent atherosclerosis, but many individuals receiving statins and with normal cholesterol levels also develop atherosclerosis [10]. Therefore, there is need for additional approaches to prevent atherosclerosis; for example, one strategy could be to prevent native LDL from becoming atherogenic LDL. If we could

prevent native LDL from becoming atherogenic, then levels in the circulation should not matter.

Under physiological conditions, native CRP does not bind to native LDL. Singh *et al* [1] exploited the property of CRP to bind to phosphocholine (PCh) and PEt, and blocked the PCh-binding site of CRP with PEt. They found that blocking of the PCh-binding site of CRP with PEt, but not with PCh, converted CRP into a potent molecule for binding to native LDL in whole human serum. Importantly, the PEt-bound CRP captured only native LDL, not HDL (high-density lipoprotein), in the serum. It is speculated that the LDL cholesterol circulating as CRPPEt-LDL complexes may not be modified into atherogenic LDL. Additionally, CRP-PEt-LDL complexes may be catabolized resulting in lowering the level of LDL cholesterol in the circulation. This is an assumption consistent with the finding that lipids rich in phosphatidylethanolamine isolated from natural gas-utilizing bacteria reduce plasma cholesterol levels [11]. Thus, the use of PEt-bound CRP might be an approach superior to the use of statins to target native LDL cholesterol.

The mechanism of action of PEt on CRP in converting CRP into a native LDL-binding protein is not known yet. It is hypothesized that PEt may be modifying CRP in some way because it has been shown that the modified forms of native CRP, such as monomeric CRP or the dissociated subunits of CRP, are capable of binding to native LDL. Interestingly, it has been demonstrated that when CRP is associated with a cell membrane (PEt is a constituent of cell membranes), the native pentameric CRP gets dissociated into monomers, and that monomeric CRP molecules are physiological and do exist *in vivo* [12].

Targeting atherogenic LDL

Native CRP is known to bind to atherogenic forms of LDL, such as E-LDL and oxidized LDL (ox-LDL), which are constituents of atherosclerotic lesions [13-15]. Singh *et al* [2] reported that the PEt-bound CRP was much more potent than unbound CRP in binding to E-LDL. The binding of CRP to ox-LDL was also dramatically enhanced by PEt (J. A. Thompson and A. Agrawal, unpublished observations).

Singh *et al* [2] further showed that, in contrast to free E-LDL, the CRP-bound E-LDL was inactive because it did not transform macrophages into foam cells as determined by the staining of accumulated lipid droplets made up of cholesteryl esters. The function of CRP in eliminating the activity of E-LDL to form foam cells by binding to E-LDL was not impaired by the presence of PEt in the CRP-E-LDL complexes. Other laboratories have reported that CRP does not inhibit uptake of modified LDL by macrophages [3, 16]. The contradictory findings are likely due to the differences in the experimental protocols. One such difference is the experiments performed with purified CRP-E-LDL complexes devoid of free E-LDL [2] versus the experiments performed with the mixture of CRP and modified LDL [reviewed in 3]. Further investigations are expected, although based on our findings we believe that the prevention of ELDL-induced formation of macrophage foam cells is one of the host-defense functions of CRP [2]. This activity of CRP can be made more efficient by PEt because PEt increases the potency of CRP to bind atherogenic LDL.

Human CRP is not pro-atherogenic but is atheroprotective in the LDL-rich *ApoB^{100/100}Ldlr^{-/-}* murine model of human-like atherosclerosis [17, 18], suggesting the possible role of CRP-LDL interactions in slowing the development of atherosclerosis. The findings that PEt-bound CRP could capture native LDL in the circulation and that PEt had a dramatic effect on CRP-E-LDL interaction raise the possibility of seeing improved atheroprotective effect in mice treated with PEt-bound CRP.

Since CRP binds to PCh on injured cells, CRP gets deposited at myocardial infarcts and activates complement to cause further injury [19]. CRP has been shown to increase the infarct size in rats undergoing experimental myocardial infarction. Accordingly, a PCh-based compound was developed to inhibit the deposition of human CRP at the infarcts by blocking the PCh-binding site of CRP [20]. However, such a compound may lessen the beneficial effects of CRP on foam cell formation and atherosclerosis, because PCh inhibits CRP-LDL interaction. A PEt-based compound would inhibit the deposition of CRP at the myocardial infarcts but would not lessen the beneficial effects of CRP. It would also allow CRP to capture and inactivate native LDL and E-LDL [1, 2].

Unanswered questions on PEt-bound CRP

The biochemical characteristics of PEt-bound CRP look promising. More functional tests of PEt-bound CRP need to be performed. As listed here, there are several unanswered questions:

1. Does the binding of PEt to CRP modify the structure of CRP to convert it into a native LDL-binding protein? Does PEt cause aggregation of CRP or does it cause dissociation of native pentameric CRP into monomers?
2. What is the structure and topology of the E-LDL-binding site of CRP and the native LDL-binding site of PEt-bound CRP? How does PEt sit at or near the PCh-binding site of CRP?
3. What is the effect of PEt on the binding of CRP to damaged or altered cells of the myocardial infarcts? What is the effect of PEt on CRP-mediated increase in infarct size in rat models of myocardial infarction? Is PEt-based compound as effective as PCh-based compound in preventing the increase in infarct size in experimental rats?
4. What is the effect of CRP mutants incapable of binding to LDL on the development of atherosclerosis in the LDL-rich *ApoB*^{100/100}*Ldlr*^{-/-} mice? What is the effect of PEt-bound CRP on the development of atherosclerosis in these mice?
5. What are the effects of PEt-bound CRP on the activities of cultures vascular cells?
6. What is the fate of PEt-bound CRP-LDL complexes *in vivo*? Can PEt-bound CRP cross the endothelium and enter the artery wall?
7. What is the effect of PEt on the binding of CRP to purified HDL? What is the effect of PEt on the binding of CRP to HDL in LDL-deficient serum?
8. Do CRP-ox-LDL complexes and PEt-bound CRP-ox-LDL complexes cause formation of macrophage foam cells? Does PEt-bound CRP capture ox-LDL in fluid-phase?
9. What is the mechanism for the inability of CRP-E-LDL complexes to form foam cells? Are the CRP-bound E-LDL not taken up by macrophages or the CRP-bound E-LDL complexes taken up but not processed to form cholesteryl esters?

Conclusions and future perspective

Combined data support the view that CRP is beneficial and, therefore, the side effect of statins to inhibit CRP gene expression in hepatocytes and lower CRP levels may not be advantageous [21-23]. It is reasonable to conclude that if CRP is present in sufficient amount in the arterial wall and if each LDL molecule retained in the arterial wall becomes CRP-bound, CRP may be capable of preventing foam cell formation *in vivo*. The administration of PEt or PEt-based compounds to target endogenous CRP to form PEt-

bound CRP or the administration of exogenously prepared PEt-bound CRP may be useful to capture native and modified LDL *in vivo* and to prevent binding of CRP to damaged cells at the myocardial infarcts. Overall, the biochemical properties of CRP and PEt-bound CRP suggest a new approach to prevent the development of atherosclerosis as an alternative to statin treatment.

Red wine has been shown to exert protective effects against heart disease [24, 25]. Red wine decreases LDL cholesterol levels and increases HDL levels and it has been proposed that wine may contain compounds responsible for these effects [26]. Although the mechanisms of the benefits of moderate wine drinking on atherosclerosis are unknown, it is interesting to note that the alcohol increases the production of PEt by the lactic acid bacteria present in the red wine [27].

Bibliography

1. Singh SK, Suresh MV, Prayther DC, Moorman JP, Rusinol AE, Agrawal A. Phosphoethanolamine-complexed C-reactive protein: A pharmacological-like macromolecule that binds to native low-density lipoprotein in human serum. *Clin. Chim. Acta.* 2008; 394:94–98. [PubMed: 18486609]
2. Singh SK, Suresh MV, Prayther DC, Moorman JP, Rusinol AE, Agrawal A. C-reactive protein-bound enzymatically modified low-density lipoprotein does not transform macrophages into foam cells. *J. Immunol.* 2008; 180:4316–4322. [PubMed: 18322245]
3. Singh SK, Suresh MV, Voleti B, Agrawal A. The connection between C-reactive protein and atherosclerosis. *Ann. Med.* 2008; 40:110–120. [PubMed: 18293141]
4. Agrawal A. CRP after 2004. *Mol. Immunol.* 2005; 42:927–930. [PubMed: 15829284]
5. Miller DT, Ridker PM, Libby P, Kwiatkowski DJ. Atherosclerosis: The path from genomics to therapeutics. *J. Am. Coll. Cardiol.* 2007; 49:1589–1599. [PubMed: 17433948]
6. Kushner I, Rzewnicki D, Samols D. What does minor elevation of C-reactive protein signify? *Am. J. Med.* 2006; 119:166, e17–28. [PubMed: 16443421]
7. Peisajovich A, Marnell L, Mold C, Du Clos TW. C-reactive protein at the interface between innate immunity and inflammation. *Expert Rev. Clin. Immunol.* 2008; 4:379–390. [PubMed: 20476927]
8. Kivimäki M, Lawlor DA, Smith GD, et al. Does high C-reactive protein concentration increase atherosclerosis? The Whitehall II study. *PLoS One.* 2008; 3:e3013. [PubMed: 18714381]
9. De Beer FC, Soutar AK, Baltz ML, Trayner IM, Feinstein A, Pepys MB. Low density lipoprotein and very low density lipoprotein are selectively bound by aggregated C-reactive protein. *J. Exp. Med.* 1982; 156:230–242. [PubMed: 7086355]
10. Brown MS, Goldstein JL. Lowering LDL: Not only how low, but how long? *Science.* 2006; 311:1721–1723. [PubMed: 16556829]
11. Müller H, Hellgren LI, Olsen E, Skrede A. Lipids rich in phosphatidylethanolamine from natural gas-utilizing bacteria reduce plasma cholesterol and classes of phospholipids: a comparison with soybean oil. *Lipids.* 2004; 39:833–841. [PubMed: 15669758]
12. Ji SR, Wu Y, Zhu L, et al. Cell membranes and liposomes dissociate C-reactive protein (CRP) to form a new, biologically active structural intermediate: mCRP_(m). *FASEB J.* 2007; 21:284–294. [PubMed: 17116742]
13. Bhakdi S, Torzewski M, Klouche M, Hemmes M. Complement and atherogenesis: Binding of CRP to degraded, nonoxidized LDL enhances complement activation. *Arterioscler. Thromb. Vasc. Biol.* 1999; 19:2348–2354. [PubMed: 10521363]
14. Chang MK, Binder CJ, Torzewski M, Witztum JL. C-reactive protein binds to both oxidized LDL and apoptotic cells through recognition of a common ligand: Phosphorylcholine of oxidized phospholipids. *Proc. Natl. Acad. Sci. USA.* 2002; 99:13043–13048. [PubMed: 12244213]
15. Taskinen S, Hyvonen M, Kovanen PT, Meri S, Pentikainen MO. C-reactive protein binds to the 3 β -OH group of cholesterol in LDL particles. *Biochem. Biophys. Res. Comm.* 2005; 329:1208–1216. [PubMed: 15766555]

16. Singh U, Dasu MR, Yancey PG, Afify A, Devaraj S, Jialal I. Human C-reactive protein promotes oxidized low density lipoprotein uptake and matrix metalloproteinase-9 release in Wistar rats. *J. Lipid Res.* 2008; 49:1015–1023. [PubMed: 18245817]
17. Kovacs A, Tornvall P, Nilsson R, Tegnér J, Hamsten A, Björkegren J. Human C-reactive protein slows atherosclerosis development in a mouse model with human-like hypercholesterolemia. *Proc. Natl. Acad. Sci. USA.* 2007; 104:13768–13773. [PubMed: 17702862]
18. Torzewski M, Reifenberg K, Cheng F, et al. No effect of C-reactive protein on early atherosclerosis in LDLR^{-/-}/human C-reactive protein transgenic mice. *Thromb. Haemost.* 2008; 99:196–201. [PubMed: 18217154]
19. Nijmeijer R, Krijnen PA, Assink J, et al. C-reactive protein and complement depositions in human infarcted myocardium are more extensive in patients with reinfarction or upon treatment with reperfusion. *Eur. J. Clin. Invest.* 2004; 34:803–810. [PubMed: 15606722]
20. Pepys MB, Hirschfield GM, Tennent GA, et al. Targeting CRP for the treatment of cardiovascular disease. *Nature.* 2006; 440:1217–1221. [PubMed: 16642000]
21. Voleti B, Agrawal A. Statins and nitric oxide reduce C-reactive protein production while inflammatory conditions persist. *Mol. Immunol.* 2006; 43:891–896. [PubMed: 16054696]
22. Kleemann R, Verschuren L, De Rooij B, et al. Evidence for anti-inflammatory activities of statins and PPAR β activators in human C-reactive protein transgenic mice in vivo and in cultured human hepatocytes in vitro. *Blood.* 2004; 103:4188–4194. [PubMed: 14976045]
23. Arnaud C, Burger F, Steffens S, et al. Statins reduce interleukin-6-induced C-reactive protein in human hepatocytes: New evidence for direct anti-inflammatory effects of statins. *Arterioscler. Thromb. Vasc. Biol.* 2005; 25:1231–1236. [PubMed: 15790934]
24. Klatsky AL. Alcohol, wine, and vascular diseases: an abundance of paradoxes. *Am. J. Physiol. Heart Circ. Physiol.* 2008; 294:H582–H583. [PubMed: 18083890]
25. Mukamal KJ, Jensen MK, Grønbaek M, et al. Drinking frequency, mediating biomarkers, and risk of myocardial infarction in women and men. *Circulation.* 2005; 112:1406–1413. [PubMed: 16129796]
26. Chung HS, Hong HS, Do KR, et al. MooPoong (Gye Young Jeong) increases HDL-cholesterol but decreases LDL cholesterol and body-weight. *Immunopharmacol. Immunotoxicol.* 2004; 26:225–232. [PubMed: 15209358]
27. Teixeira H, Goncalves MG, Rozès N, Ramos A, San Romão MV. Lactobacillic acid accumulation in the plasma membrane of *Oenococcus oeni*: A response to ethanol stress? *Microb. Ecol.* 2002; 43:146–153. [PubMed: 11984636]