Road Map to Drug Discovery and Development – Inhibiting C-reactive protein for the Treatment of Cardiovascular Disease

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Abstract

Elevated plasma levels of high sensitivity C-reactive protein (hs-CRP), the prototype acute phase protein, are predictive for future cardiovascular events. Inclusion of information on hs-CRP values in patients with other cardiovascular risk factors assist physicians in medical decision making. Evidence also suggests that CRP plays a direct role in cardiovascular disease by activating the complement system and interacting with macrophages via Fcγ receptors. Thus, specific CRP inhibition may be a novel approach for reducing cardiovascular mortality. Here, we discuss the road map to the development of CRP inhibitors. As the topic is scientifically very controversial, the road map to drug development is severely influenced by diverse scientific views and also patent law considerations.

Keywords: C-reactive protein; Cardiovascular disease; Risk factor; Drug therapy, Risk marker

Abbreviations: CHD: Coronary Heart Disease; CRP (=hs-CRP): High Sensitivity C-reactive Protein; DCM: Dilated Cardiomyopathy; IL: Interleukin; LDL: Low Density Lipoprotein; TNF-α: Tumor Necrosis Factor Alpha

Background

For almost 15 years now, the role of C-reactive protein (CRP) in cardiovascular disease has been a very controversial topic of debate. The reasons for this international interest are summarized as follows: First, CRP has been identified as a powerful marker of cardiovascular risk. Secondly, evidence suggests that CRP may be causally involved in arteriosclerosis and its sequelae. If so, CRP may thirdly be a target for drug development. Whereas the role of CRP as a cardiovascular risk marker is almost generally accepted, both, its role as a risk factor and its role as potential drug target still lack international consensus. Here, we try to summarize the opposing arguments that were put forward by various groups and we also report on potential progress in pharmaceutical targeting of CRP.

C-reactive protein

C-reactive protein, the prototype acute phase protein in humans, is a pentameric molecule with a monomer molar mass of 25106 Da that has originally been identified by Tillett and Francis at the Rockefeller University in 1930 via its ability to bind to the C-fragment of streptococcus pneumoniae [1]. CRP is widely used in clinical medicine to monitor acute phase response, e.g. in intensive care medicine to control the effect of antibiotics in the treatment of pneumonia or sepsis [2]. CRP is synthesized almost exclusively in the liver in response to cytokines, mainly IL-1β and IL-6. The plasma half-life of the molecule is ~19 hrs. In acute phase response, CRP plasma levels rise up to 1000 times compared to normal [3].

Although CRP belongs to the most regularly quantified molecules in clinical medicine there is still substantial uncertainty about its biological function. CRP appeared very early in the evolution of the immune system as evidenced by the fact that ancient Limulus polyphemes, the horseshoe crab, expresses a CRP-analagon in its immune system [4]. This early appearance in evolution and its maintenance in the immune system of mammalians, suggests an important biological role of CRP. Contradictory, however, is the fact that CRP is not an acute phase protein in some higher organisms, especially in mice [3]. The latter has caused substantial difficulties in CRP research because the mouse is certainly the most broadly and easily available mammalian organism in experimental research. These species differences have led to some less reasonable experimental approaches to unravel CRP function, especially the overexpression of human CRP in mice or rabbits [5,6]. The latter, in fact, results in overexpression of a foreign antigen in the target organisms that causes unforeseeable and confounding effects to the experimental results [7].

The so far identified biological functions of CRP in humans include: 1. Activation of the classical complement pathway via C1q binding [8], 2. Opsonization of biological particles for macrophages [9], and 3. Binding to and signalling via Fcγ-receptors [10-12]. Interestingly, these major CRP functions are also typical antibody functions [13]. It is therefore not unlikely that CRP has been the first antibody-like molecule in the evolution of the immune system. Since adaptive immunity has developed in higher organisms and many of the original CRP functions may have been taken over and improved by antibodies, CRP may well be an atavism in the human immune system.

C-reactive protein as a cardiovascular risk marker

The first report on CRP as a cardiovascular risk marker was published by Frits Haverkate and colleagues in 1997 [14], and since then it was developed by several well controlled trials [15-17]. In 2007, the American Heart Association published a statement [18] claiming that “from all inflammatory biomarkers associated with CHD CRP is the only one that is employable in the clinical setting (commercially available, cheap, standardized assay with adequate precision).” It was

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suggested to quantify “hs-CRP, measured twice, either fasting or non-fasting, with the average expressed in mg/L, in metabolically stable patients”. Relative risk categories were defined: “Relative risk categories (low, average, high) correspond to approximate tertiles of value (<1.0, 1.0 to 3.0, >3.0 mg/L), based on an aggregation of population studies.”

Nonetheless, some studies have found less strong correlation of CRP plasma levels with cardiovascular risk, and thus, some authors doubt the usefulness of CRP as a cardiovascular risk marker [19]. Also, association of elevated CRP plasma levels with other diseases puts the specificity of the original observation into question [20]. A strong case for CRP as a helpful cardiovascular risk marker in decision making for patients was made by the JUPITER trial [21]. The JUPITER trial found that patients with elevated CRP levels without hyperlipidemia benefited from Statin (HMG-CoA-reductase-inhibitor)-treatment. Statins were selected because they have been proven to reduce levels of CRP. A subsequent trial however failed to find that CRP was useful for determining Statin benefit [22].

In summary, there is international consensus on the usefulness of measuring CRP plasma levels for the prediction of cardiovascular risk. Whether this will influence clinical decisions is mainly dependent on the question whether targeting CRP will reduce the incidence of cardiovascular events.

C-reactive protein as a cardiovascular risk factor

It may be important to reconsider some traditional rules in research before coming to a final conclusion on the topic “CRP as a cardiovascular risk factor”. These include the scientific commitment not to try to prove a hypothesis, the importance of clarifying the disease entity experimental research is performed on and, lastly, awareness of the fact that in order to investigate a molecule’s role in disease it may be helpful to reconsider its role in physiology.

Arteriosclerosis

Most research on the topic “CRP as a cardiovascular risk factor” has been performed on arteriosclerosis as the underlying cause for many cardiovascular disease entities.

Histopathology in humans: CRP deposition in all stages of human atherosclerosis has repetitively been demonstrated [23,24]. Interestingly, CRP in lesions deposits in association with activated complement fragments [24]. Thus, CRP may be the complement activating molecule in atherogenesis. CRP also colocalizes with macrophages [25]. As macrophages strongly express Fcy receptors [13], macrophages may be the target cells for CRP in atherosclerosis.

In vitro experiments: Numerous in vitro studies have been published that describe various effects of CRP on vascular cells [3]. Concerning these studies it is important to note that some of the rules mentioned above have not been considered appropriately. Especially the reconsideration of the biological roles of CRP has not regularly been the underlying basis for performing the in vitro experiments. Consequently, effects of CRP on vascular cells have been reported that were caused by contaminants of the CRP preparations used [26] and these artifacts have shed an unnecessarily dark light on the research topic in general [3].

CRP opsonizes biological particles for macrophages, CRP binds to and signals via Fcy-receptors and CRP activates the classical complement pathway via C1q binding. These biological functions should be the basis for any in vitro studies on CRP and atherosclerosis.

CRP and opsonization of LDL for macrophages via Fcy-receptors: The most prominent in vitro studies concerning CRP and opsonization of biological particles for macrophages report that (1) CRP binds to various unmodified and modified forms of LDL [27-29] (2) CRP binds to and signals via Fcy-receptors [10-12,30]. (3) LDL-bound CRP is taken up by macrophages via FcyR-dependent and FcyR-independent pathways [31] (4) LDL/CRP complexes induce intracellular Syk Kinase signaling [32].

In summary, colocalization of CRP with macrophages in atherosclerotic lesions, high expression levels of FcyRs on macrophages and CRP-mediated LDL uptake into macrophages suggest that CRP opsonizes LDL for macrophages and is thereby deeply involved in foam cell formation in atherogenesis.

CRP and complement activation: The most prominent in vitro studies concerning CRP and complement activation in atherogenesis report that (1) CRP-mediated complement activation is probably regulated by the conformation of CRP, either pentameric or monomeric [33], and (2) CRP may also have protective effects because it stops complement activation by modified lipoproteins before detrimental terminal sequence [34].

In summary, colocalisation of CRP with activated complement fragments in atherosclerotic lesions and complement activation by CRP/LDL complexes in vitro strongly suggest that lipoprotein-bound CRP is intimately involved in complement activation in atherogenesis.

Animal experiments: Animal models on CRP and atherosclerosis have provided very heterogenous results. The major problem is the fact that CRP is not an acute phase reactant in mice [3]. Thus, results form CRP knockout models are of unknown relevance [35] and overexpression of human CRP in mice results in immunological heterogeneity with unforeseeable effects on the immune system of the animals [5-7]. Rabbis seem to be the most promising animal model concerning CRP and atherosclerosis [36] and using inhibitors of CRP in this animal model may be the best way to receive answers on the question whether CRP is causal or not [7]. Overexpression of human CRP in rabbits causes similar problems as overexpression of human CRP in mice [6].

Myocardial infarction

Histopathology in humans: CRP plasma levels significantly rise after myocardial infarction indicating the human body’s acute phase response [37]. CRP also deposit in human myocardial scarcs following myocardial infarction [38]. Here, CRP again colocalizes with activated complement fragments suggesting that CRP mediated complement activation in necrotic tissue is a more general phenomenon.

Animal experiments: In rats, CRP also deposits in myocardial infarcts in the heart, and CRP-mediated complement activation seems to contribute to myocardial damage [39]. An inhibitor of CRP that cross-links the CRP subunits and thereby obviously prevents complement activation, seems to be protective in this animal model [40]. Whether inhibition of CRP-mediated complement activation after myocardial infarction is therapeutically useful, is at least questionable. The focus of therapy should certainly be reopening of the clotted coronary artery.

Dilated cardiomyopathy

The role of CRP in atherosclerosis and ischemic heart failure seems widely acknowledged. In contrast hardly any data is available on a potential involvement of CRP in non-ischemic heart failure, i.e. acute
myocarditis and its chronic course named dilated cardiomyopathy (DCM). In a retrospective study design, CRP was detected within the myocardium of patients suffering from DCM and, here again, co-localized with macrophages and the terminal complement complex C5b-9 [41]. It was hypothesized that CRP-mediated activation of complement could directly contribute to myocardial damage. Furthermore, colocalization of CRP with macrophages in the myocardium again suggested a contribution of CRP to opsonisation and chemotaxis of proinflammatory cells [41]. Nonetheless, neither a significant correlation of myocardial CRP with hsCRP plasma levels nor with clinical outcome could be found [41]. Satoh et al. [42] demonstrated that CRP colocalizes with TNF-α in DCM and that cardiomyocytes themselves are able to produce both molecules. This observation may explain the finding that CRP plasma levels are independent from myocardial CRP amounts. Furthermore, numerous clinical studies reported increased complication rates, aggravation of the clinical course and higher mortality in DCM when CRP levels were elevated [43-45]. In analogy to the JUPITER trial [21] patients with non-ischemic heart failure were treated with atorvastatin to reduce systemic inflammation [46]. After 12 weeks on treatment, CRP plasma levels were significantly lower compared to the placebo group. Additionally, clinical performance clearly improved.

In conclusion, there is accumulating evidence that CRP may be causally involved in the progression of DCM. Significantly more work will be necessary in order to explore the pathomechanistical impact of CRP in DCM and myocardial inflammation.

Genetics

1. The focus of genetic studies concerning CRP and cardiovascular risk is the so-called Mendelian randomization approach. Mendelian randomization is a method of using measured variation in genes of known function to examine the causal effect of a modifiable exposure on disease in non-experimental studies. Recent studies investigated the association between genetic polymorphisms that affect CRP gene expression and the risk of cardiovascular disease [47,48]. Both studies revealed an absence of increased risk attributable to genetic polymorphisms that affect CRP gene expression. It was thus concluded that CRP is unlikely to be a causal factor in cardiovascular disease. Although this is strong evidence contradicting the hypothesis that CRP contributes to atherogenesis and its sequelae it is important to note that these Mendelian randomization trials are fraught with difficulties: 1. The studies are based on the assumption that any causal relationship between CRP and CVD is linear, which is not necessarily the case [49].

2. Beside general problems in genetic studies such as population stratification and linkage disequilibrium, one important issue in these studies is the problem of "channelization", i.e. post-genetic adaptation for the genetic effects by other uncontrolled factors [50].

3. Genetic heterogeneity and complex regulation of CRP gene expression may confound the results of this approach.

C-reactive protein as a target for drug development

Although considerable effort has been made in order to design specific CRP inhibitors in the past decade only limited success was achieved. This is due to the fact that CRP is a very difficult target for drug design. Considering the structure and also the biological effects of CRP that have so far been identified, five strategies may be feasible:

1. Crosslinking of CRP subunits
2. Antisense strategies
3. Inhibition of CRP-mediated complement activation
4. Competitive inhibition of CRP/Fcy-receptor binding and
5. Inhibition of hepatic CRP synthesis

All these strategies have been examined. The idea to crosslink CRP subunits led to the identification of a low molecular weight inhibitor that, in animal models, reduced CRP-mediated complement damage in the rat myocardium [40]. As this molecule is a more or less ubiquitous cross-linker, in vivo utility of this inhibitor is at least questionable. Antisense strategies have led to the design of a CRP antisense molecule that was demonstrated to reduce CRP-mediated vascular injury in human CRP transgenic mice [51]. Inhibition of CRP-mediated complement activation via blockage of the C1q binding site was not possible due to sterical reasons. Competitive inhibition of CRP/Fcy-receptor binding was considered but found to be unreasonable in view of the identification of Fcy-receptors being the cellular receptors for CRP. Lastly, inhibition of hepatic CRP synthesis being one feasible strategy surprisingly led to the identification of cardiac glycosides as potent hepatic CRP synthesis inhibitors [52]. This observation published by our group in 2010 may finally turn out to be very helpful in coming to a conclusion on the question whether CRP is causal in cardiovascular disease or not. Cardiac glycosides, for the treatment of cardiac insufficiency, have been in clinical use since the late 18th century [53], and much is known about their toxicity and side effects. Clinical studies with these long known drugs are ethically much easier to justify, and reformulation of established substance classes has become one of the leading strategies for drug development. Maybe a reformulation of cardiac glycosides for CRP synthesis inhibition is possible.

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