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Inhibition of Endothelial Nitric Oxide Synthase by C-Reactive Protein: Clinical Relevance

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In 2002, 2 groups independently reported that C-reactive protein (CRP) inhibits endothelial nitric oxide synthase (eNOS) activity and bioactivity (1,2). There was much reported debate as to whether these effects were artifacts attributable to CRP contamination with azide and endotoxin. In cells in which the endotoxin receptor Toll-like receptor 4 was knocked down by use of the small-interfering RNA technique, investigators convincingly showed that extensively dialyzed solutions of CRP inhibited eNOS, confirming that this inhibition was not an effect of endotoxin or azide (3). In a subsequent study, the molecular mechanism by which CRP inhibits eNOS was elucidated (4). This study demonstrated that CRP inhibited GTP cyclohydrolase 1 and stimulated NADPH oxidase, causing a decrease in tetrahydrobiopterin and an increase in reactive oxygen species, resulting in uncoupling of eNOS, which led to decreased eNOS activity, decreased phosphorylation of Ser1177 of eNOS, and decreased eNOS binding to Hsp90. Other investigators had shown that CRP also impairs vasoreactivity in vivo. In particular, Mineo and coworkers demonstrated in C57BL mice that intraperitoneal administration of CRP (250 μ g) compared to administration of a vehicle control impaired acetylcholine-induced carotid artery vascular conductance by 50%, but no mechanistic insights were provided (5). Until the results of these 2 studies were reported, it had not been appreciated that endotoxin stimulates eNOS whereas CRP inhibits eNOS (4,5).

In 2007, Schwedler and coworkers (6) showed that subcutaneous administration of CRP for 8 weeks induced endothelial dysfunction in apolipoprotein E^{-/-} mice, and that this effect was reversed with an inducible NOS inhibitor (6). However, these authors found no effect of CRP delivery on eNOS or inducible NOS in aortic tissue. Importantly, the authors found anti-CRP antibodies following subcutaneous treatment of the apolipoprotein E^{-/-} mice with CRP. These findings led these investigators to speculate that the potential pathogenic effect of CRP was due to antigen–antibody complexes inducing endothelial dysfunction. Most recently, Teoh and coworkers showed that the CRP transgene resulted in decreased vasoreactivity following administration of turpentine (7). These investigators also demonstrated decreased eNOS phosphorylation and nitrite/nitrate levels in CRP transgenic mice. However, no effect was seen in the basal state but only after turpentine administration, which produced mean CRP concentrations of 276 mg/L, a concentration range that is usually seen with severe inflammation such as infection.

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The validity of using CRP transgenic mice as a model to study the role of CRP in atherosclerosis has been questioned (8). However, the use of a rat model to study the vascular effects of CRP has proved very rewarding. As reviewed elsewhere (8), human CRP administration in rat models has been shown to induce myocardial infarction following coronary ligation to increase cerebral infarct size following middle cerebral artery occlusion, and most recently, to promote oxidatively modified LDL uptake, cholesterol ester accumulation, and matrix metalloproteinase activity and to stimulate NADPH oxidase and tissue-factor activity in macrophages (9,10). A most relevant finding in the rat model was reported by the Pepys group, who demonstrated that with the use of a small molecule inhibitor to CRP they could prevent an increase in infarct size following coronary ligation (11). Thus, the rat appears to be a more valid model than CRP transgenic mice to study the vascular effects of CRP.

In the current issue of *Clinical Chemistry*, Guan and coworkers (12) report their finding that a single intravenous injection of adeno-associated virus vector with CRP into male rats resulted in efficient and sustained expression of CRP in the liver and other tissues and an increase in serum CRP to 15 mg/L at 2 and 4 months. This effect was associated with an increase in systolic and mean arterial pressure. In addition, these authors demonstrated impaired endothelium-dependent vasoreactivity in rats administered adeno-associated virus vector with CRP compared with control rats administered adeno-associated virus vector–green fluorescent protein. Guan and coworkers documented that the impaired vasoreactivity was associated with increased expression of angiotensin type 1 (AT1) receptor, endothelin (ET)-1, and ET type A receptors and decreased expression of eNOS and AT2 receptors. Furthermore, these investigators found a decrease in serum nitrate/nitrites and in guanosine-3',5'-cyclic-monophosphate, confirming an observed decrease in eNOS mRNA and protein. On the basis of their data, Guan and coworkers argue that the effects on AT1, ET type A, ET-1, and AT2 are secondary to eNOS inhibition, a plausible argument given the results of their experiments and of other reported studies. Thus, the underpinning of impaired vasoreactivity and hypertension is inhibition of eNOS by CRP.

Although the study of Guan and coworkers is interesting and clearly advances understanding in this area by supplying in vivo confirmation that CRP can induce endothelial dysfunction and hypertension, their investigation also has certain deficiencies. Previously, CRP has been shown to inhibit prostacyclin synthase (13), and prostacyclin is well known to be a potent vasodilator, but this aspect was not reported in the Guan study. Furthermore, because Singh and coworkers (4) convincingly showed that the molecular mechanism for the impaired vasoreactivity was due to uncoupling of eNOS, some measurement of reactive oxygen species would have strengthened this study. An additional deficiency of the reported study was the fact that human CRP was available in the circulation of these rats for 4 months, but the authors did not provide data regarding the formation of anti-CRP antibodies and whether the effects were due to antigen–antibody complexes, as reported by Schwedler et al. (6), who administered CRP subcutaneously. Despite these deficiencies, the study of Guan and coworkers advances the field by convincingly showing impaired vasoreactivity in vivo. Prospective epidemiological study results suggest that higher quantiles of CRP concentration predict hypertension. Vongpatanasin and coworkers have previously reported the effect of CRP in inducing hypertension in CF1-transgenic mice expressing rabbit CRP (14). It should be emphasized, however, that unlike the current study of Guan and coworkers they suggest that this effect is not attributable to the effect of AT1 but to a decrease in AT2. Vongpatanasin and coworkers' study provided little information regarding molecular mechanisms.

The in vivo implications of the inhibition of eNOS by CRP offer abundant evidence that CRP has a clear role in atherothrombosis, and the majority of effects reported for CRP (azide-free and without endotoxin contamination) appear to be related to endothelial dysfunction and activation. Thus, the initial reports regarding inhibition of eNOS activity and bioactivity by

CRP now appear to have clinical implications and suggest that CRP, by inducing endothelial dysfunction, could put patients at risk for hypertension. If human studies confirm that CRP induces hypertension or that antisense therapy to CRP lowers blood pressure, then it will be imperative to institute more aggressive management, initially with therapeutic lifestyle changes, of patients who present with high CRP. The recently reported results of JUPITER (Justification for the Use of Statins in Primary Prevention: An Intervention Trial Evaluating Rosuvastatin) (15) further endorse the important effect of CRP in atherothrombosis, because reductions in LDL cholesterol and high-sensitivity CRP from rosuvastatin therapy were associated with a significantly reduced incidence of cardiovascular events in treated individuals who initially had LDL cholesterol within the reference interval [3.37 mmol/L (130 mg/dL)], but increased high-sensitivity CRP (>2 mg/L).

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