C-Reactive Protein and Atherothrombosis: Cause or Effect?

Etty Grad, Rachel M. Pachino, Mordechai Golomb, Michal Levy & Haim Danenberg.

Cardiovascular Research Center, Hadassah-Hebrew University Medical Center, Jerusalem, Israel.
CRP and cardiovascular disease

First line host defense molecule
Rosuvastatin significantly reduced the incidence of major cardiovascular events.

**JUPITER study: Justification for the Use of Statins in Primary Prevention**

- 17,802 men and women with LDL <130 mg/dl and hsCRP >2 mg/L
- The trial was designed as a 4-year study but was stopped after a median follow-up of 1.9 years.
- Rosuvastatin reduced LDL cholesterol levels by 50% and high-sensitivity C-reactive protein levels by 37%.

### Table 3. Outcomes According to Study Group.

<table>
<thead>
<tr>
<th>End Point</th>
<th>Rosuvastatin (N=8901)</th>
<th>Placebo (N=8901)</th>
<th>Hazard Ratio (95% CI)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Primary endpoint</strong></td>
<td>142 0.77</td>
<td>251 1.36</td>
<td>0.56 (0.46–0.69)</td>
<td>&lt;0.00001</td>
</tr>
<tr>
<td>Nonfatal myocardial infarction</td>
<td>22 0.12</td>
<td>62 0.33</td>
<td>0.35 (0.22–0.58)</td>
<td>&lt;0.00001</td>
</tr>
<tr>
<td>Any myocardial infarction</td>
<td>31 0.17</td>
<td>68 0.37</td>
<td>0.46 (0.30–0.70)</td>
<td>0.0002</td>
</tr>
<tr>
<td>Nonfatal stroke</td>
<td>30 0.16</td>
<td>58 0.31</td>
<td>0.52 (0.33–0.80)</td>
<td>0.003</td>
</tr>
<tr>
<td>Any stroke</td>
<td>33 0.18</td>
<td>64 0.34</td>
<td>0.52 (0.34–0.79)</td>
<td>0.002</td>
</tr>
<tr>
<td>Arterial revascularization</td>
<td>71 0.38</td>
<td>131 0.71</td>
<td>0.54 (0.41–0.72)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Hospitalization for unstable angina</td>
<td>16 0.09</td>
<td>27 0.14</td>
<td>0.59 (0.32–1.10)</td>
<td>0.09</td>
</tr>
<tr>
<td>Arterial revascularization or hospitalization for unstable angina</td>
<td>76 0.41</td>
<td>143 0.77</td>
<td>0.53 (0.40–0.70)</td>
<td>&lt;0.00001</td>
</tr>
<tr>
<td>Myocardial infarction, stroke, or confirmed death from cardiovascular causes</td>
<td>83 0.45</td>
<td>157 0.85</td>
<td>0.53 (0.40–0.69)</td>
<td>&lt;0.00001</td>
</tr>
<tr>
<td>Death from any cause</td>
<td>190 0.96</td>
<td>235 1.19</td>
<td>0.81 (0.67–0.98)</td>
<td>0.03</td>
</tr>
<tr>
<td>Death on known date</td>
<td>198 1.00</td>
<td>247 1.25</td>
<td>0.80 (0.67–0.97)</td>
<td>0.02</td>
</tr>
</tbody>
</table>
CRP: a marker or a maker of atherothrombosis?

Does CRP affect atherogenesis, thrombosis or both components of the atherothrombotic process?
CRP and atherogenesis

• Histological staining of atherosclerotic lesions consistently demonstrates CRP within the lesion.
• *In vitro* experiments testing the addition of CRP to cultured Ecs, SMCs and monocytes identified several potential pro-inflammatory mechanisms by which CRP promote atherosclerosis.
• Studies in mice prone to atherosclerosis such as *APO-E KO* or *LDLR KO* have not come up with uniform conclusions.
• Accumulating data indicate that CRP is probably not pro-atherogenic in human.
How does it work?
CRP increases thrombosis

Pathways of CRP induced thrombosis

1. Down-regulates nitric oxide production and bioavailability
Transgenic expression of human C-reactive protein stimulates nitric oxide synthase expression and bioactivity after baseline injury.

Etty Grad,1 Mordechai Golomb,1 Irit Mor-Yosef,1 Nikolay Koroukidis,1 Chaim Lotan,1 Elazar R. Edelman,2 and Haim D. Danenberg1
1Cardiovascular Research Center, Hadassah Hebrew University Medical Center, Jerusalem, Israel
2Department of Health Sciences and Technology, Harvard-Massachusetts Institute of Technology

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* p < 0.05 n=5 per group
Pathways of CRP induced thrombosis

2. Modifies prostanoid balance and activity
CRP modifies prostanoid pathway

Phospholipids

Arachidonic acid

COX-2

COX-1

Prostaglandin H₂

Prostacyclin synthase

Prostacyclin

PGF₂, PGE₂, PGD₂

Thromboxane synthase

Thromboxane A₂

IP

TP
Aspirin reduces the prothrombotic activity of C-reactive protein


*Heart Research Centre, Hadassah Hebrew University Medical Centre, Jerusalem, Israel; and † The Institute for Translational Medicine and Therapeutics, The University of Pennsylvania, Philadelphia, PA, USA
Photochemical injury – realtime thrombus formation
Pathways of CRP induced thrombosis

3. Endothelial CRP increases platelet adhesion
MHEC derived from CRPtg mice express human CRP

Real time PCR analysis for human CRP show that MHEC derived from human CRP transgenic mice express mRNA for human CRP, while control wildtype MHEC do not.
Platelet adhesion to MHEC derived from CRPtg mice is significantly increased compared to wildtype. 

Number of platelet

Wildtype

CRPtg

N=6

* p<0.01, WT vs. CRPtg

** p<0.01 control vs. LPS
siRNA reduces hCRP expression in HUVEC by 60%

siRNA to hCRP significantly reduces platelet adhesion to endothelial cells

* p< 0.01 WT vs. CRPtg
# p<0.05 negative RNAi vs. siRNA/CRP

N=3-6
P-selectin mediates the pro-adhesive effect of CRP

P-selectin expression is significantly increased in MHEC derived of CRPtg mice

Protein expression

mRNA expression

sP-selectin secretion

n=3-4
* p<0.05 WT vs. CRPtg
** p< 0.05 control vs. LPS

* P< 0.05 P-selectin vs. IC

* p< 0.05 WT vs. CRPtg
** p< 0.05 control vs. LPS
Role of Thromboxane Receptor in C-Reactive Protein–Induced Thrombosis


CRP SiRNA reduced CRP & TP expression
SiRNA against TP in CRPtg MHECs reduced human CRP proadhesiveness
C reactive protein increases thrombosis:

1. CRP decreases nitric oxide production and activity
2. CRP modulates the COX-prostanoid pathway:
   suppresses PGI\(_2\) synthase and augments TXA\(_2\) activity by increasing TP. Aspirin treatment reverses the prothrombotic effect of CRP
3. Human CRP is locally secreted by endothelial cells: Local hCRP increases platelet adhesion to murine and human endothelial cells under normal shear flow conditions. The pro-adhesive effect of hCRP is mediated by P-selectin and TP
CRP increase thrombosis in a several interconnected pathways

- Reactive Oxygen Species
- TP
- Nitric Oxide
Thank You