

C-reactive protein (CRP) rise is associated with the development of acute events in a model of plaque rupture and thrombosis

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Abstract

Background. Elevated levels of C-reactive protein (CRP) have been associated with increased risk for development of cardiovascular events. **Objective.** To follow the trend of CRP over the course leading to an acute event, we evaluated CRP levels under three conditions: normal rabbits, atherosclerotic rabbits before and after pharmacological triggering of plaque rupture and thrombosis. Plaque rupture and thrombosis is induced using Russell viper venom (RVV) and histamine in an atherosclerotic rabbit model. **Methods.** Atherosclerosis was induced with balloon deendothelialization and feeding a high cholesterol diet for 9 months. Serum samples were obtained from control rabbits ($n = 3$), and atherosclerotic rabbits, before ($n = 6$) and 48 hours after RVV and histamine-induced thrombosis ($n = 8$). Rabbit specific high sensitivity ELISA was developed to detect the levels of serum CRP concentrations. **Results.** CRP levels were significantly lower in control normal rabbits compared with rabbits with atherosclerotic plaques. Our results further demonstrated that rabbits with RVV and histamine-triggered thrombosis had significantly higher levels of serum CRP than non-triggered atherosclerotic rabbits. **Conclusion.** The rise of serum CRP levels both after cholesterol feeding and the sudden rise after pharmacological triggering of thrombus may help using of CRP to evaluate not only the long-term risk but also a more short-term risk of events if CRP levels increase acutely. [Life Science Journal. 2008; 5(2): 21 – 24] (ISSN: 1097 – 8135).

Keywords: atherosclerosis; C-reactive protein (CRP); inflammation; plaque; thrombosis

1 Introduction

Atherosclerosis is associated with inflammation and acute coronary events (Li, 2004). Atherothrombosis is a complex disease which includes two different pathologies: atherosclerosis, the process of plaque formation in the arterial wall and thrombosis, the formation of a blood clot mostly at the site of a ruptured atherosclerotic lesion. Animal models for both pathologies have been useful to understand their aetiology and their evolution and they were used to evaluate the efficacy of new treatments. Numerous models to study venous and arterial thrombosis have been described, and Verbeuren *et al* (2006) used the

rat as the atherosclerotic model. In this study, we used rabbit as the atherosclerotic model.

A compelling amount of data using different technologies have demonstrated that often more than one plaque is ruptured both in the culprit artery with thrombosis and in adjacent non-occluded arteries at the time of an acute myocardial event (Fujimori, 2002). Rioufol *et al* (2002) demonstrated multiple atherosclerotic plaque ruptures in patients during acute coronary syndrome by conducting intravascular ultrasound scan of all three coronary arteries in 24 patients with acute coronary syndrome. Furthermore, patients with acute cardiovascular events seem to have plaques that have ruptured in other arterial beds such as the carotid arteries at the time of the event (Chang, 2002). Thus, it is almost inevitable to assume that the outcome at a specific arterial site is being influenced by a systemic process. Additional supportive data regarding

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the systemic nature and effects of atherosclerosis comes from the strong association between peripheral vascular disease and coronary artery disease (Aronow, 1994). In fact, carotid arterial wall thickness has been used as a strong index of coronary artery disease and predictor of future events (Salonen, 1991). Also, as with coronary artery disease, the elevation in C-reactive protein (CRP) is an indicator of future development of claudication and symptomatic peripheral vascular disease (Ridker, 2001). All these observations seem to support that coronary artery disease is part of a systemic condition reflected by the presence generalized inflammation.

CRP, a non-specific inflammatory marker may provide a link between systemic inflammation and the outcomes at a localized cardiovascular site. Studies have demonstrated that several arteries may be involved during an acute event (Buffon, 2003; Mukherjee, 2002). Carotid arterial wall thickness has been used as a strong index of coronary artery disease, which has been shown to be associated with increased CRP levels (Weiss, 2001). Further, as with coronary artery disease, the elevation in CRP is an indicator of future development of claudication and symptomatic peripheral vascular disease. Systemic inflammatory marker CRP also has recently been shown to play an important role in promotion of atherothrombosis by increasing recruitment of monocytes (Torzewski, 2000) and by increasing recruited monocyte synthesis of tissue factor (Cermak, 1993). Also, CRP is markedly higher in patients with metabolic syndrome as well as diabetes (Aranson, 2004). It has also been shown in monocytes that CRP induces the production of inflammatory cytokines and promotes monocyte chemotaxis and tissue factor (TF) expression (Cermak, 1993; Torzewski, 2000; Mackman, 2008). All this points in the direction that coronary artery disease is a part of systemic condition reflected by the presence generalized inflammation.

In this study we tried to determine if there was an association between the systemic inflammatory responses to an acute event. This was performed using an atherosclerotic model of plaque disruption and thrombosis that has been previously reported (Abela, 1995).

2 Materials and Methods

2.1 Atherosclerosis inducing and pharmacological triggering (Abela, 1995)

Atherosclerosis was induced in 14 New Zealand white rabbits using balloon deendothelialization and feeding a 1% cholesterol diet (Harlan-Sprague Dawley, Inc., Indianapolis, IN, USA) alternating with normal chow for

9 months. Under general anesthesia (ketamine 50 mg/kg and xylazine 20 mg/kg, *i.m.*) balloon-induced deendothelialization of the aorta was performed using a 4F Fogarty arterial embolectomy catheter (Baxter Healthcare Corporation, Irvine, CA, USA) introduced through the right femoral artery cutdown. The catheter was advanced in a retrograde fashion to the ascending aorta and pulled back three times. Plaque disruption and thrombus triggering were induced by histamine (0.02 mg/kg; *i.v.*, Sigma Chemical Co., St. Louis, MO, USA) and Russell viper venom (RVV) (0.15 mg/kg; *i.p.*, Sigma Chemical Co., St. Louis, MO, USA) given at 48 and 24 h prior to sacrifice (Abela, 1995). Rabbits were sacrificed with an overdose of pentobarbital (50 mg/ml, *i.v.*, Abbot Laboratories, North Chicago, IL, USA).

2.2 CRP serum levels were evaluated under three conditions

In order to follow the trend of CRP over the course leading to an acute event, we evaluated CRP levels under three conditions: normal rabbits ($n = 3$), atherosclerotic rabbits before ($n = 6$) and after ($n = 8$) pharmacological triggering of plaque rupture and thrombosis.

2.3 CRP measurements

Serum samples were obtained by venous puncture of the ear veins from control rabbits ($n = 3$), and atherosclerotic rabbits, before ($n = 6$) and 48 hours after Russell viper venom (RVV) and histamine-induced thrombosis ($n = 8$). Rabbit specific high sensitivity ELISA was used to detect the levels of serum CRP concentrations (Immunology Consultants Laboratory, Newberg, OR, USA).

3 Results

RVV and histamine triggering caused platelet rich thrombi to form over disrupted plaques in 70% of the atherosclerotic rabbits (Figure 1).

With the atherosclerotic/thrombotic rabbit model we evaluated the effect of CRP as an index of systemic inflammation when rabbits were on normal chow, after 9 months of cholesterol feeding and after thrombus triggering. Serum CRP levels increased significantly following each intervention. CRP levels in normal chow were very low (0.15 ± 0.05 mg/dl, $n = 3$) and rose by six fold after cholesterol feeding (0.92 ± 0.85 mg/dl, $n = 6$) and rose more than eighteen fold after thrombus triggering (2.71 ± 1.67 mg/dl, $n = 8$) (Figure 2). Furthermore, serum CRP was significantly higher in rabbits that had thrombi (3.40 ± 1.89 mg/dl) than those that did not develop thrombi (1.05 ± 0.66 mg/dl) after triggering (Figure 3).

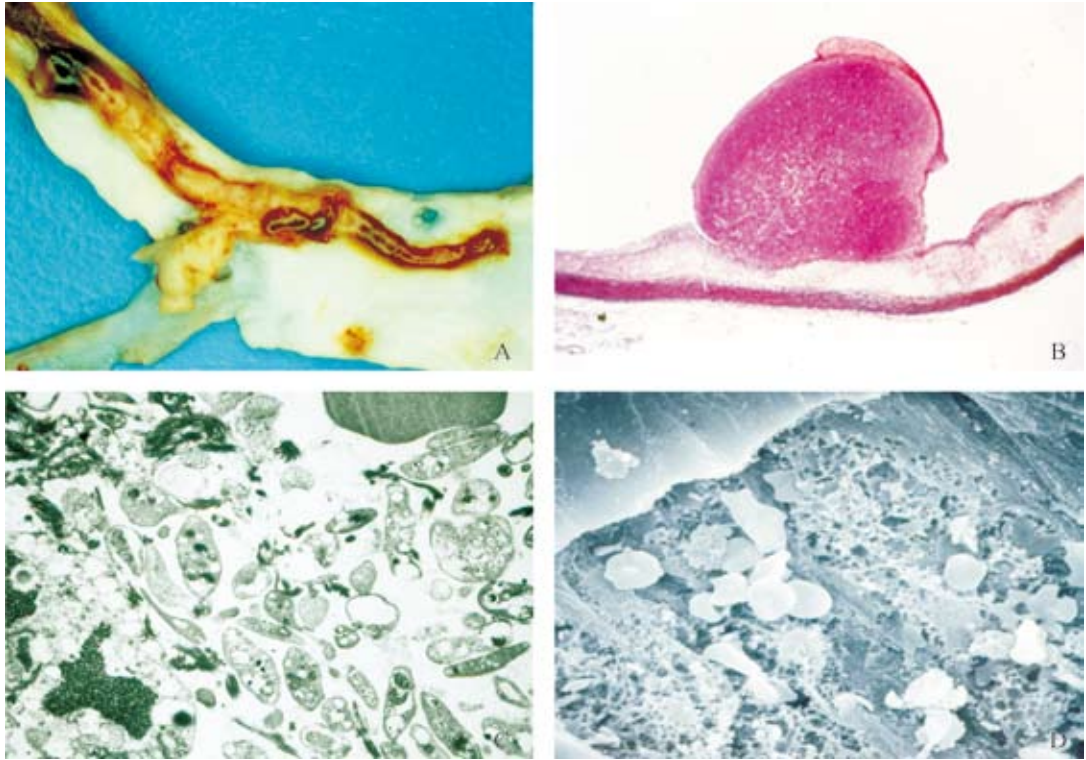


Figure 1. Histology of rabbit artery. Thrombus triggering was induced by histamine (0.02 mg/kg; *i.v.*) and Russell viper venom (0.15 mg/kg; *i.p.*) given at 48 h and 24 h prior to sacrifice. This caused platelet rich thrombi to form over disrupted plaques in 70% of rabbits. A: Gross examination. White thrombi with attached fibrin rich thrombi can be seen on the intimal surface of the aorta in more than half the triggered rabbits; B: Light microscopy. Platelet rich thrombi were noted overlying sites of eroded plaque. Other areas appeared to have had plaque disruption the thrombus site; C: Transmission electron microscopy. White thrombi were composed of a dense platelet rich matrix; D: Scanning electron microscopy. Fissures of various lengths could be seen at the site where thrombus was present.

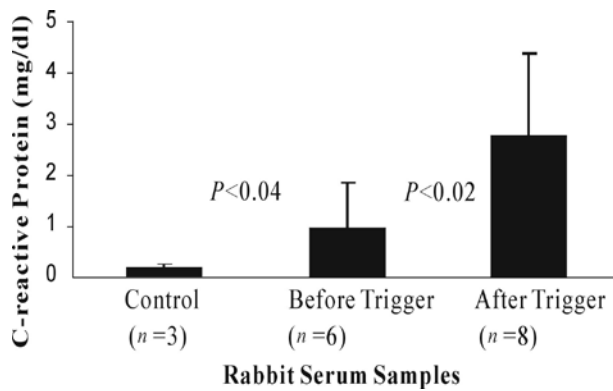


Figure 2. CRP levels were lowest at baseline (control) and rose following feeding a high cholesterol diet (before trigger) and jumping further after triggering.

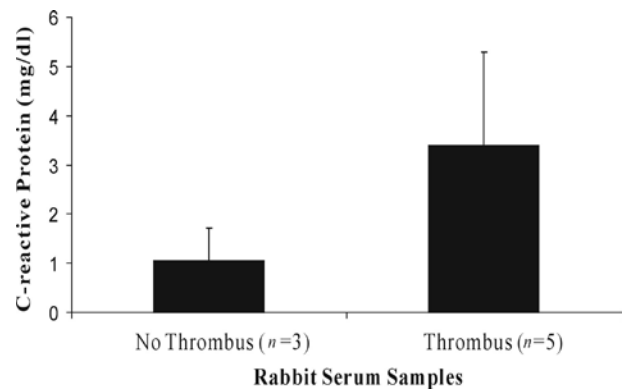


Figure 3. CRP levels in rabbits that developed thrombus were significantly greater than those without a thrombus ($P < 0.01$).

4 Discussion

Atherosclerosis and thrombosis are a kind of inflammation syndrome in this point (Choudhury, 2007). To di-

agnose atherosclerosis and thrombosis is important in clinical trial. CRP is one of the most important inflammation factors (Kampus *et al*, 2008). Even Pepys reported that CRP was neither a marker nor a mediator of atherosclerosis (Pepys, 2008), plenty experimental reports sug-

gest that CRP levels are a robust and independent predictor of future cardiovascular events (Verma, 2004). New research has identified signaling pathways that intertwine thrombotic and inflammatory pathways with the development and progression of atherosclerosis. These signaling pathways contain positive feedback loops that propagate atherogenesis. Targeting molecular regulators at the interface of thrombosis and inflammation simultaneously may reduce thrombosis and inflammation, thus breaking pathological cycles that promote atherosclerosis and associated thrombotic complications (Croce *et al*, 2007). Oxidative stress is a new risk factor for atherosclerosis. Increased oxidative stress in hemodialysis patients may arise from uremia-associated metabolic/humoral abnormalities and bioincompatibility of dialysis (Dursun *et al*, 2008).

5 Conclusion

Our study shows that CRP has direct relationship with atherosclerosis and thrombosis. With the atherosclerosis/thrombosis rabbit model, the elevation of CRP levels following cholesterol feeding and pharmacological triggering can provide a means to help monitor progression towards acute events. The results in this study gave information that measuring CRP can be a candidate of diagnosis on atherosclerosis and thrombosis. The fact that thrombosis is associated with higher levels of CRP may help predict severity of events and possibly recurrent events as well.

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