EXPERIMENTAL ACUTE INFLAMMATORY REACTION AND HEMOSTATIC VARIABLES

Ioana Brudașcă*, M. Cucuianu*, Luminița Pleșca Manea**

*UMF Iuliu Hațieganu Cluj Napoca, Catedra de Biochimie, str. Pasteur nr.6
**UMF Iuliu Hațieganu Cluj Napoca, Catedra de Fiziopatologie
Correspondență: ioanabradasca@yahoo.com

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Abstract: Plasma fibrinogen levels and factor VIII:c activity were increased after intramuscular injections of turpentine in ten rabbits, which caused an acute inflammatory reaction, while plasma protein C activity (an anticoagulant mechanism) was slightly yet significantly decreased. The inflammatory reaction also caused an increase of plasma antithrombin III activity. These changes should be interpreted in the more complex context of hemostatic perturbations caused by the acute phase reaction and may be involved in the intravascular deposition of fibrin.

Clinical and experimental studies have suggested that inflammation leads to complex hemostatic disturbances, consisting of the increase of procoagulant variables such as fibrinogen, clotting factor VIII, von Willebrand factor, but also of the stimulation of antithrombin III, an anticoagulant mechanism. On the other hand, plasma protein C, another potent anticoagulant mechanism decreases during the acute phase reaction.

MATERIAL AND METHODS

The experiment was performed on 10 rabbits weighing 2 kg. Blood was harvested from the ear vein and 1,8 ml blood were immediately mixed with 0,2 ml of 3,8% sodium citrate. Platelet poor plasma (PPP) samples were obtained after a 20 min centrifugation of the citrated blood at 3000 rpm. Plasma samples were stored at – 20 °C in small capped plastic tubes. The frozen plasma samples were thawed at 37°C, 30 min prior the assay.

The rabbits were then submitted to an intramuscular injection with 0,75 ml turpentine into each thigh (total 1,5 ml turpentine). 48 hours after the injection blood was harvested again, in the conditions previously mentioned.

Plasma fibrinogen was measured according to the method described by Astrup (1). Protein C activity was measured using an end-point chromogenic method (Berichrom Protein C Behring). Factor VIII:c was measured using a coagulometric method consisting in the correction by the rabbit plasma samples of a factor VIII:c deficient plasma (Diagnostica Stago). Antithrombin III activity was measured using a kinetic chromogenic procedure (Berichrom Antithrombin III, Behring). The results were expressed as percentage of the activity of standard human plasma (Boehringer Mannheim).

Significance of changes of the investigated parameters was evaluated by paired difference analysis.

RESULTS

The acute inflammatory reaction induced by turpentine led to an important increase of plasma fibrinogen, and factor VIII:c, while antithrombin III displayed a less obvious, yet
statistically significant elevation. Protein C plasma levels showed a slight yet significant decrease (Table 1). Comparing to humans, plasma protein C levels are lower and plasma clotting factor VIII:c are higher in rabbits, even before the experimental inflammatory reaction.

Table 1 Effect of intramuscular injection of turpentine on plasma fibrinogen level, as on factor VIII:c, protein C and antithrombin III activities. Figures representing values ± SEM were obtained in 10 rabbits before (I) and 48 hours after turpentine injection (II).

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<tr>
<td>I</td>
<td>303 ± 26</td>
<td>80 ± 10,81</td>
<td>233 ± 19,3</td>
<td>52,7 ± 3,63</td>
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<td>II</td>
<td>650 ± 28</td>
<td>123 ± 6,56</td>
<td>528 ± 42</td>
<td>45,8 ± 2,8</td>
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<td>Statistical significance by paired difference analysis</td>
<td>p &lt; 0,001</td>
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**DISCUSSION**

Data in the literature and the authors’ own previous observations show that there is a relationship between the acute phase reaction and modification of the hemostatic variables, which may create the conditions for a thrombotic event. Trauma, sepsis or surgical procedures may lead to thrombosis, especially in subjects with other risk factors (2), and the acute phase reaction accompanying the above mentioned conditions may represent a triggering factor. Mechanisms linking inflammation to thrombosis are well documented, yet not fully elucidated. One may presume that the endothelial injury induced by the inflammatory reaction result in a down-regulation of some of the anticoagulant mechanisms, while the procoagulant factors and fibrinolysis inhibitors are up-regulated.

The presently reported changes of plasma fibrinogen and factor VIII:c levels are confirming previous observations (12, 14). These experimental data obtained in vivo are similar to those obtained by Hoffman et al (8) in rat hepatocyte cultures stimulated with macrophage stimulated cytokines.

Plasma fibrinogen is known as an acute phase reactant; its level is increased in inflammation, malignancy and pregnancy. High levels of plasma fibrinogen are associated with an important risk for coronary heart disease and stroke (7).

Concerning the behaviour of the anticoagulant mechanisms during the acute phase reaction, both up-regulation and down-regulation processes are reported. Proinflammatory cytokines induce an increased synthesis of antithrombin III, while impairing the hepatic synthesis protein C. The plasma level of antithrombin III was found to increase in several experimental and clinical conditions accompanied by an acute phase reaction (4,13). The actual plasma level of antithrombin III activity during an inflammatory reaction is the result of an increased synthesis and an enhanced consumption.

The presently reported decrease of plasma protein C suggests that this antithrombotic mechanism is down – regulated by inflammation (5). One may wonder what could be the pathophysiological relevance of this change. Protein C system may become down regulated by several other mechanisms associated with the acute phase reaction. Protein S, a cofactor of protein C is also decreased during the acute phase reaction (3). Furthermore, the increase in plasma level of c4b binding protein (an acute phase reactant that also binds protein S) results in a decrease of free protein S, and subsequently in a reduced cofactor activity for activated protein C.
The thrombin-thrombomodulin complex exerts an anticoagulant function through protein C system, and an antifibrinolytic effect by activating TAFI (10, 11). It seems that local concentration of thrombomodulin influences the balance between these two effects: at low concentration of thrombomodulin TAFI activation is predominant, while at higher concentrations, protein C activation is predominant, resulting in decrease of thrombin generation and subsequent TAFI activation.

Recent studies showed that activated protein C has a variety of antiinflammatory activities. It suppresses inflammatory cytokine elevation in animal models of severe sepsis, inhibits leukocyte adhesion, decreases leukocyte chemotaxis, reduces endothelial cell apoptosis, helps maintain endothelial cell barrier function through activation of the sphingosine-1 phosphate receptor, and minimizes the decrease in blood pressure associated with severe sepsis. Most of these functions are dependent on binding to endothelial protein C receptor (EPCR). It seems that this pathway is critical to both regulation of the blood coagulation process, and control of the innate inflammatory response and some of its associated downstream pathologies.(6,9).

An overall view of these inflammation-related mechanisms suggests that the enhanced procoagulant activities, represented by the increase of fibrinogen and factor VIII:C and the impaired function of the protein C system are favourable to the local deposition of fibrin, while the increase of the antithrombin III activity might represent an attempt to prevent or to reduce the intravascular dissemination of fibrin formation.

REFERENCES