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European Journal of Pharmacology 577 (2007) 156–161
www.elsevier.com/locate/ejphar

Haemostatic imbalance following carrageenan-induced rat paw oedema

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Received 2 March 2007; received in revised form 30 July 2007; accepted 6 August 2007
Available online 14 August 2007

Abstract

Carrageenan-induced rat paw oedema is a widely used model to investigate the physiopathology of an acute local inflammation. Recently, much attention has been focused on the link between haemostasis and inflammation, and on the impact that inflammation might have on thrombotic events. It is known that the systemic response to inflammation is the "*acute phase reaction*" that represents a highly complex reaction of the organism to a variety of injuries, aimed to restore homeostasis; one important feature of the acute phase reaction is the hepatic synthesis of proteins involved in the coagulation cascade. Much attention has been focused on the role that systemic inflammation might have on thrombotic events, while there is not much information on the role played by an acute local inflammation on haemostasis, that can lead toward a pro-thrombotic state. The present study was conducted to evaluate the haemostatic balance in the early and the late phase of carrageenan-induced rat paw oedema; i.e. at 3 h, when paw inflammation is maximally expressed, and 24 h following carrageenan injection, when there is an almost complete absence of local inflammatory symptoms. We found that in inflamed animals, 24 h following oedema induction, there was an increase in plasma fibrinogen levels, antithrombin III activity and serum interleukin-6 levels, concomitant to a shortened prothrombin time and to an increased platelet responsiveness to ADP. Furthermore, in inflamed tissues at 3 h there was an increase in antithrombin III proteic expression. Our results demonstrate that a haemostatic imbalance occurs following carrageenan-induced rat paw oedema.

Keywords: Carrageenan; Haemostasis; Fibrinogen; Platelet aggregation; Antithrombin III

1. Introduction

Both experimental and clinical evidence have shown that thrombosis and atherosclerosis might be associated to an inflammatory reaction, suggesting an important link between inflammation and haemostasis (Jurado and Ribeiro, 1999; Khatun et al., 1999; Esmon, 2003; Strukova, 2006). Indeed, inflammation initiates clotting, decreases the activity of natural anticoagulant mechanisms and impairs the fibrinolytic system. On the other hand, proteases involved in coagulation system contribute to inflammation not only by promoting fibrin formation at the site of injury, but also by stimulating several cell functions (Cicala and Cirino, 1998; Cirino et al., 2000; Esmon, 2005). Anticoagulants may be beneficial in limiting inflammation triggered by coagulation, not only by inhibiting coagulation proteases, but also by limiting inflammatory cell activation (Roemisch et al., 2002; Wiedermann and Romisch, 2002; Strukova, 2006).

An important contribution to haemostatic changes observed following inflammation is given by the "acute phase reaction", that represents a highly complex reaction of the organism to a variety of injuries, aimed to restore homeostasis. One important feature of the "acute phase reaction" is the hepatic synthesis of proteins involved in the coagulation cascade (Baumann and Gauldie, 1994; Cucuianu et al., 1996; Jones et al., 1996; Ceciliani et al., 2002). However, changes in the haemostatic balance caused by the "acute phase reaction" may lead to an increased risk of thrombosis, both in human and in experimental animals (Plesca et al., 1995; Cucuianu et al., 1996; Robson et al., 1996). There is evidence that fibrinogen, an acute phase reactant that is increased under inflammatory conditions, is an important link between inflammation and haemostasis; high fibrinogen levels have been associated with an increased risk of thrombotic diseases (Ernst, 1993; Hernandez et al., 2000; Esmon, 2005).

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0014-2999/$ - see front matter © 2007 Elsevier B.V. All rights reserved.
doi:10.1016/j.ejphar.2007.08.007

Carrageenan-induced oedema in the rat paw is a widely used model, developing in about 6 h, to investigate the physiopathology of an acute local inflammation, and to test the potential anti-inflammatory of new molecules (Morris, 2003). Up to now, identified mediators involved in this model of acute inflammation are biogenic amines; bradykinin; prostaglandins (Di Rosa et al., 1971; Di Rosa and Willoughby, 1971); nitric oxide (Salvemini et al., 1996) and, more recently, hydrogen sulphide has been shown to be involved (Bhatia et al., 2005). We have demonstrated that carrageenan-induced rat paw oedema is inhibited by systemic administration of the thrombin inhibitor, hirulog, outlining a possible involvement of thrombin in the development of oedema (Cirino et al., 1996).

Carrageenan-induced rat paw oedema remains localized in the injection area. Little is known about the systemic reaction to the local injection of carrageenan in the rat. In the past, studies carried out in order to evaluate the systemic response to carrageenan oedema induction in rats have only analyzed platelet function, without taking in account any coagulation parameter (Srivastava and Srimal, 1986; 1990); thus, there is no information on the impact that this local acute inflammation may have on the haemostatic balance.

In order to investigate whether even a local acute inflammation might represent a factor risk for pro-thrombotic events, in the present study we evaluated the haemostatic balance in the early and the late phase of carrageenan-induced rat paw oedema; i.e. at 3 h, when paw inflammation is maximally expressed, and 24 h following carrageenan injection, when there is an almost complete absence of local inflammatory symptoms.

2. Materials and methods

2.1. Materials

ADP, aprotinin, bovine serum albumin (BSA), carrageenan, trisodium citrate were purchased from SIGMA (Italy). Dithiothreitol (DTT), phenyl-methylsulphonyl fluoride (PMSF), leupeptin were purchased from ICN Pharmaceutical, S.r.l. (Milan, Italy). Bradford reagent was purchased from Bio-Rad Laboratories (Segrate, MI). Polyclonal antibody to antithrombin III was purchased from DPC, Biermann (Germany). Anti-rabbit IgG conjugated to horseradish peroxidase (HRP-anti-rabbit IgG) was purchased from Dako (Denmark). Enhanced chemiluminescence's system (ECL) was from Amersham Pharmacia Biotech (Milan, Italy).

2.2. Carrageenan oedema

Male Wistar rats (200–250 g; Harlan Nossan, Italy) were slightly anaesthetized with enflurane and oedema was induced by injecting in the left hind paw carrageenan 1% w/v (100 μl). Paw volume was measured at the time zero and each hour for 6 h; and then was measured at 24 h, by a hydropletismometer (Ugo Basile, Comerio, VA). In control animals, vehicle (distilled water, 100 μl) was injected in the paw. All animal experiments complied with the Italian D.L. no. 116 of 27 January 1992 and associates guidelines in the European Communities Council Directive of 24 November 1986 (86/609/ECC).

2.3. Blood cell count

At 3 and 24 h after induction of oedema, or vehicle injection, blood was withdrawn by cardiac puncture, anticoagulated with trisodium citrate 3.8% (ratio 1:9) and utilized for cell count, by using a cell counter CELL DYN 610 (SEQUOIA TURNER).

2.4. Coagulation parameters

An aliquot of blood withdrawn 3 and 24 h following oedema induction, was centrifuged at 650×*g* for 15 min and the plasma obtained was utilized to evaluate prothrombin time, activated partial thromboplastin time and fibrinogen content by a coagulometer (KoaguLab MJ Ortho Diagnostic Systems, Raritan, NJ). Quantitative determination of antithrombin III activity was performed by a chromogenic assay Unitest™ (Unicorn Diagnostics Ltd, London UK). Briefly, thrombin is added to a plasma dilution containing antithrombin III in the presence of heparin. After an initial incubation period, residual thrombin is determined with a thrombin specific chromogenic substrate. The residual thrombin activity is inversely proportional to the antithrombin III concentration. Reference antithrombin III activity was 0.90 UI/ml.

2.5. Interleukin-6 assay

Serum samples obtained from rat blood 3 and 24 h following oedema-induction were subjected to interleukin-6 (IL-6) levels determination using the rat specific DuoSet ELISA kit (ELISA; DuoSet R&D Systems, UK), according to the manufacturer's protocol.

2.6. Platelet preparation and aggregation

Blood was withdrawn by cardiac puncture after 3 and 24 h following the injection of carrageenan or of vehicle, as described above. Platelet-rich plasma and platelet-poor plasma were prepared as previously described (Cicala et al., 1996). Platelet count in platelet-rich plasma was adjusted to 3×10^5 platelets/μl with autologous platelet-poor plasma. Platelet aggregation to ADP (3, 10 and 30 μM) was monitored in an Elvi 840 light transmission aggregometer by measuring changes in turbidity of 0.25 ml platelet-rich plasma warmed at 37 °C and under continuous stirring.

2.7. Determination of antithrombin III protein by Western blot analysis

3 and 24 h after oedema induction, from each rat soft tissue was removed from individual inflamed paws. The same tissue samples were also removed from non-inflamed animals. Tissues were homogenised with the Polytron homogenizer (3 cycles of 10 s at the maximum speed) in a lysis buffer containing: $MgCl_2$, 2 mM; sodium ortovanadate, 100 μM; β glycerophosphate, 50 mM; EGTA, 1 mM; DTT, 1 mM; PMSF, 1 mM; leupeptin, 10 μg/ml and aprotinin, 10 μg/ml. After centrifugation at

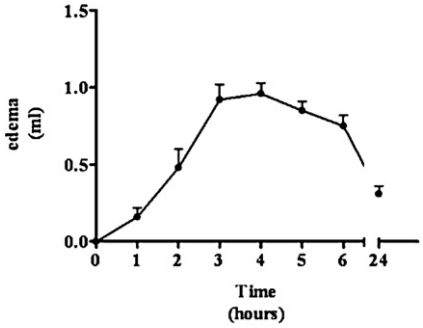

Fig. 1. Time course of carrageenan-induced oedema in the rat paw. Carrageenan (1% w/v; 100 µl) was injected in the rat paw at time zero. Paw volume was evaluated at the time zero and each hour for 6 h following oedema injection and at 24 h. Oedema was evaluated by the difference of paw volume at each hour and basal value ($n=8$).

650 ×g at 4 °C for 10 min, the supernatants were collected and centrifuged at 10,000 ×g for 30 min at 4 °C. The protein concentration was measured by Bradford assay using BSA as a standard. Membrane proteins (30 µg) were briefly boiled and loaded on 12% sodium dodecyl sulphate (SDS)-polyacrilamide gel electrophoresis (PAGE) and transferred onto nitrocellulose membranes for 45 min at 250 mA.

Non-specific antibody binding to the membrane was blocked with 5% non-fat milk and 0.1% BSA in PBS-Tween 20 (0.1%, v/v) for 1 h at room temperature. Membranes were washed with PBS-Tween 20 (0.1%, v/v) at 5 min intervals for 30 min and then incubated with a polyclonal antibody to antithrombin III (1:2000 in PBS-Tween 20 0.1%, v/v, containing 5% non-fat milk and 0.1% BSA) overnight at 4 °C. Detection blots were washed with PBS-Tween 20 (0.1%, v/v) at 5 min intervals for 30 min and incubated with HRP-anti-rabbit IgG (1:10,000) for 2 h at 4 °C. The immunoreactive bands were visualised using an enhanced chemiluminescence's system (ECL).

2.8. Statistical analysis

All data are expressed as mean ± S.E.M. and analyzed with one way analysis of variance (ANOVA) followed by Dunnett's test. A value of $P<0.05$ was considered significant.

3. Results

3.1. Blood cell count and coagulation parameters

Injection of carrageenan in the rat paw induced an oedema reaching its maximum after 3 h and steadily decreasing over 6 h. At 24 h, the oedema was almost absent. Blood cell analysis showed that in animals treated with carrageenan, either after 3 h or 24 h before, total white blood cell number was unchanged compared to control animals; however the percentage of granulocytes was significantly increased at 3 h returning to control value 24 h thereafter. There was no difference in red blood cell and platelet count between control and inflamed animals (Fig. 1; Table 1A).

24 h following carrageenan-induced oedema, plasma fibrinogen content was significantly increased compared to the value obtained from non-inflamed animals. At the same time point, prothrombin time was significantly reduced compared to control value, while activated partial thromboplastin time was unchanged. Conversely, prothrombin time, activated partial thromboplastin time and fibrinogen content were unchanged 3 h after carrageenan injection. Plasma antithrombin III activity was significantly increased on samples obtained 24 h following carrageenan injection, while it was unchanged on samples at 3 h (Table 1B).

3.2. Platelet aggregation assay

Rat platelets obtained 24 h following carrageenan-induced oedema showed a response to ADP increased compared to platelets obtained from non-inflamed animals (Fig. 2).

3.3. IL-6 levels

IL-6 serum levels were significantly increased at 24 h following oedema induction, but not at 3 h (Fig. 3).

3.4. Western blot analysis

In carrageenan injected paws obtained 3 h following oedema induction there was an increased antithrombin III proteic expression compared to the non-injected paws and to paws obtained 24 h following oedema induction (Fig. 4).

4. Discussion

The present study was conducted in order to evaluate the haemostatic balance in the early and the late phase of carrageenan-induced rat paw oedema.

Differences in the haemostatic balance between 3 and 24 h following oedema induction were clearly observed, demonstrating that a systemic reaction occurs in response to the local inflammation, that seems to be consistent with an "acute phase response". Indeed, 24 h following injection of carrageenan in

Table 1A
Rat blood cell count following carrageenan-induced paw oedema

Treatment	WBC 10^3/µl	Gran %	Lymph %	RBC 10^6/µl	PLT 10^3/µl
Control	12.57±0.87	12.17±0.23	87.83±0.23	5.75±0.13	744±41.52
3 h	11.53±0.93	30.37±1.49 [a]	69.63±1.49 [a]	6.20±0.27	759±56.40
24 h	8.93±1.58	16.83±7.8	83.17±7.80	5.88±0.60	740±106

Cell count were evaluated on blood obtained from rats at 3 and 24 h following carrageenan-induced oedema. Control rats received only vehicle injection in the paw. WBC, white blood cells; Gran, granulocytes; Lymph, lymphocytes, RBC, red blood cells; PLT, platelets. Data represents means±S.E.M. [a]$P<0.001$ vs. control. (ANOVA followed by Dunnett's test; $n=6$–8).

Table 1B
Rat coagulation parameters evaluated following carrageenan-induced paw oedema

Treatment	PT sec	APTT sec	Fibrinogen mg/dl	AT III %
Control	9.95±0.20	21.83±0.91	204±13	100.30±5.65
3 h	10.27±0.09	22.55±0.93	207±10	87.45±2.93
24 h	9.27±0.13 [b]	22.00±0.23	450±33 [c]	128.00±8.17 [a, d]

Coagulation parameters were evaluated on plasma obtained from rats 3 and 24 h following carrageenan-induced oedema. Control rats received only vehicle injection in the paw. PT, prothrombin time; APTT, activated partial thromboplastin time; AT III, antithrombin III. All data represent means±S.E.M (n=6–8). AT III activity was evaluated as % activity normalized to control activity value (0.586 UI/ml). [a]P<0.05; [b]P<0.01 and [c]P<0.001 vs. control. [d]P<0.01 vs. 3 h.

the rat paw, there was an increase in fibrinogen plasma levels, that was not observed at 3 h. Fibrinogen is an acute phase reactant whose plasma levels are increased under inflammatory situations and also a clotting factor (Glenn, 1969; Degen, 1999; Ceciliani et al., 2002; Gruys et al., 2005). Here, we demonstrate an increase in plasma fibrinogen only evident when there was no more sign of local inflammation; this suggests that following carrageenan-induced oedema in rat paw fibrinogen might be produced as a an acute phase protein.

High fibrinogen levels have been associated with an increased risk of thrombotic diseases, with a multifactorial mechanism involving also an increased platelet aggregability, an important factor for the genesis of vascular lesion (Ross et al., 1974; Meade et al., 1985; Ernst, 1993; Hernandez et al., 2000; Steinhubl and Moliterno, 2005). Based on these observations, we also evaluated platelet aggregation. We found an increased platelet aggregability in response to ADP at 24 h following oedema induction but not at 3 h. Furthermore, we observed that the increase in fibrinogen plasma levels was also paralleled by an increase in IL-6 serum levels, a cytokine known to act as an inducer of acute phase reactants and as a mediator of the crosstalk between inflammation and coagulation (Baumann and Gauldie, 1994; Kerr et al., 2001).

The activated partial thromboplastin time and prothrombin time assays were performed, as non-specific tests of the haemostatic system, on plasma samples obtained 3 and 24 h following oedema induction. Activated partial thromboplastin time reflects activation of the intrinsic and common coagulation pathways, while prothrombin time reflects activation of extrinsic and common coagulation pathways. We found that 24 h following oedema induction, prothrombin time but not activated partial thromboplastin time was significantly shortened compared to control values and to values obtained at 3 h, the peak time of inflammation. The shortening of prothrombin time value observed, without any change of activated partial thromboplastin time value, could only reflect the increase in coagulation factors involved in the extrinsic pathway (factors VII and tissue factor). Although it is known that inflammation causes tissue factor generation, and hence increased activation of factor VII (Rapaport and Rao, 1995; Esmon 2003; Chu, 2005), we did not measure plasma tissue factor levels in our model, thus whether the reduced prothrombin time value is due to the increased tissue factor generation needs further investigation.

Antithrombin III is a natural thrombin inhibitor; a reduction of its activity in systemic inflammatory disorders, such as sepsis has been demonstrated, concomitant to a prolonged prothrombin time and coagulation factors consumption (Esmon, 2003, 2005). We found that antithrombin III plasma activity was unchanged at 3 h but increased 24 h following induction of oedema. An increase in antithrombin III activity has already been described to take place during inflammation, paralleled by a down-regulation of protein C system (Plesca et al., 1995; Cucuianu et al., 1996), and also during hepatitis A (De Jonge et al., 1995). The increased antithrombin III activity concomitant to a reduced prothrombin time value suggest that even though there is an increased production of coagulation factors, likely tissue factor, due to the systemic response to the local inflammation, the extrinsic coagulation pathway is not activated and there is no coagulation factor consumption. Thus, the increased antithrombin III activity might represent a defence mechanism able to prevent intravascular fibrin deposition. Thus, we hypothesise that following carrageenan-induced rat paw oedema, an increased antithrombin III liver synthesis might be a homeostatic defence to limit thrombin activity and the consequent fibrin

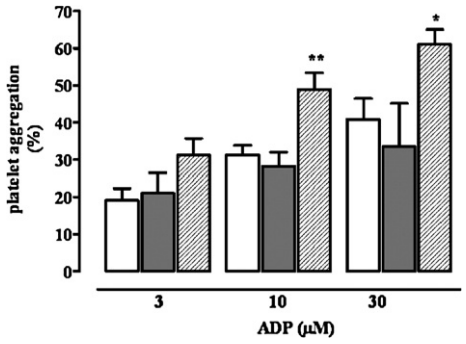

Fig. 2. Aggregation to ADP of platelets obtained from inflamed animals. Aggregation to ADP (3, 10 and 30 μM) was evaluated on rat platelets obtained 3 h (grey bar) and 24 h (crosshatched bar) following carrageenan-induced oedema. Control animals (open bar) received only vehicle (distilled water, 100 μl) injection in the paw. *P<0.05 and **P<0.01 (ANOVA followed by Dunnett's test) vs. control (open bars; n=15–20).

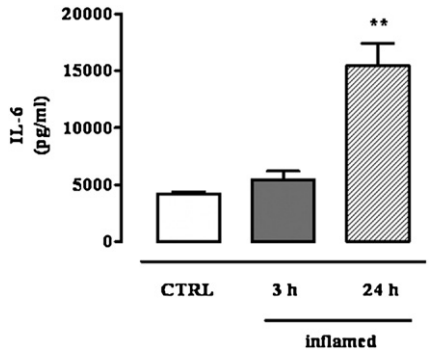

Fig. 3. Serum IL-6 levels from inflamed rats. IL-6 levels were measured by an ELISA kit in serum obtained from rat blood 3 h (grey bar) and 24 h (crosshatched bar) following carrageenan oedema induction. **P<0.01 vs. CTRL (ANOVA followed by Dunnett's test; n=8).

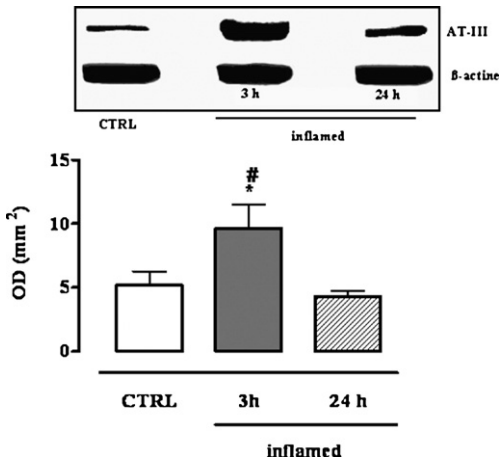

Fig. 4. Panel A: Detection of antithrombin III (AT III) by Western blot analysis in inflamed paws excised 3 h and 24 h following carrageenan-induced oedema, and in control (vehicle injected) paws. Panel B: Densitometric analysis (OD = optical density) showing that there is a remarkable increase of AT III proteic expression in inflamed paws 3 h (grey bar) following carrageenan-induced oedema compared to paws at 24 h (crosshatched bar) and to control paws. $^{*}P<0.05$ vs. CTRL and $^{\#}P<0.05$ vs. 24 h (ANOVA followed by Bonferroni's test; $n=8$).

formation. In this respect, it is known that thrombin plays a role in the development of carrageenan-induced rat paw oedema. Systemic administration of the thrombin inhibitor, hirulog, inhibits oedema development (Cirino et al., 1996). Furthermore, it is known that in the inflamed paw there is local platelet and fibrin deposition (Vincent et al., 1978). Interestingly, we found that antithrombin III proteic expression was increased locally, in the inflamed tissues at 3 h but not at 24 h, when inflammation was dampened out. It is possible that this antithrombin III over-expression at the inflamed site might participate to modulate local fibrin deposition.

It is worth considering that our findings are inconsistent with a late systemic effect of carrageenan due to absorption from the paw. Firstly, because it is known that the inflammatory effect of intraplantar injection of carrageenan in the rat is related to its poor absorption rate (Di Rosa, 1972). Furthermore, because systemic exposure to carrageenan causes disseminated intravascular coagulation (Fowler et al., 1977; Thomson and Fowler, 1981). It is known that disseminated intravascular coagulation is characterised by consumption of coagulation factors, thus reduction of fibrinogen levels and prolongation of both prothrombin time and activated partial thromboplastin time, and reduced platelet number (Esmon, 2003, 2005); all features very different from the haemostatic changes that we have observed.

In conclusion, taken together our results show for the first time that following carrageenan-induced rat paw oedema there are changes in blood parameters that are suggestive of an acute phase reaction leading to a haemostatic imbalance. Thus, we suggest that even a local acute inflammation that is resolved in about 6 h triggers a systemic reaction, mainly characterized by high fibrinogen levels, increased platelet reactivity, a shortened prothrombin time value, that might lead toward a pro-thrombotic state and further highlight the important link between inflammation and haemostasis.

References

Baumann, H., Gauldie, J., 1994. The acute phase response. Immunol. Today. 15, 74–80.

Bhatia, M., Sidhapuriwala, J., Moochhala, S.M., Moore, P.K., 2005. Hydrogen sulphide is a mediator of carrageenan-induced hindpaw oedema in the rat. Br. J. Pharmacol. 145, 141–144.

Ceciliani, F., Giordano, A., Spagnolo, V., 2002. The systemic reaction during inflammation: the acute-phase proteins. Protein Pept. Lett. 9, 211–223.

Chu, A.J., 2005. Tissue factor mediates inflammation. Arch. Biochem. Biophys. 440, 123–132.

Cicala, C., Cirino, G., 1998. Linkage between inflammation and coagulation: an update on the molecular basis of the crosstalk. Life Sci. 20, 1817–1824.

Cicala, C., Santacroce, C., Itoh, H., Douglas, G.J., Page, C.P., 1996. A study on rat platelet responsiveness following intravenous endotoxin administration. Life Sci. 60, PL 31–PL 38.

Cirino, G., Cicala, C., Bucci, M.R., Sorrentino, L., Maraganore, J.M., Stones, S.R., 1996. Thrombin functions as an inflammatory mediator through activation of its receptor. J. Exp. Med. 183, 821–827.

Cirino, G., Napoli, C., Bucci, M., Cicala, C., 2000. Inflammation-coagulation network: are serine protease receptors the knot? Trends Pharmacol. Sci. 21, 170–172.

Cucuianu, M., Plesca, L., Bodizs, G., Colhon, D., Brudasca, I., 1996. Acute phase reaction and the hemostatic balance. Rom. J. Intern. Med. 34, 13–18.

Degen, J.L., 1999. Hemostatic factors and inflammatory disease. Thromb. Haemost. 82, 858–864.

De Jonge, N., Woudstra, M., Maartense, E., Ponjee, G.A., 1995. High plasma antithrombin levels in acute hepatitis A. Scand. J. Gastroenterol. 30, 95–96.

Di Rosa, M., Willoughby, D.A., 1971. Screens for anti-inflammatory drugs. J. Pharm. Pharmacol. 23, 297–298.

Di Rosa, M., 1972. Biological properties of carrageenan. J. Pharm. Pharmacol. 24, 89–102.

Di Rosa, M., Giroud, J.P., Willoughby, D.A., 1971. Studies on the mediators of the acute inflammatory response induced in rats in different sites by carrageenan and turpentine. J. Pathol. 104, 15–29.

Ernst, E., 1993. Fibrinogen as a cardiovascular risk factor-interrelationship with infections and inflammation. Eur. Heart. J. 14, 82–87.

Esmon, C.T., 2003. Inflammation and thrombosis. J. Thromb. Haemost. 1, 1343–1348.

Esmon, C.T., 2005. The interactions between inflammation and coagulation. Br. J. Haematol. 131, 417–430.

Fowler, E.F., Thomson, A.W., Horne, C.H.W., 1977. Alleviation of carrageenan-induced hepatotoxicity and acronecrosis by aprotinin. Br. J. Exp. Pathol. 58, 260–267.

Glenn, E.M., 1969. Fibrinogen and experimental inflammation. Biochem. Pharmacol. 18, 317–326.

Gruys, E., Toussaint, M.J.M., Niewold, T.A., Koopmans, S.J., 2005. Acute phase reaction and acute phase proteins. J. Zhejiang Univ. Sci. 6B, 1045–1056.

Hernandez, L.R., Lundberg, U., Arocha-Pinango, C.L., 2000. Experimental thrombosis I: relation with fibrinogen and other haemostatic parameters. Thromb. Res. 99, 295–305.

Jones, R., Braam, M.J., Cooley, B.C., 1996. Arterial thrombosis enhanced by an acute phase response. Ann. Plast. Surg. 36, 14–17.

Jurado, R., Ribeiro, M., 1999. Possible role of systemic inflammatory reaction in vascular access to thrombosis. South. Med J. 92, 877–881.

Kerr, R., Stirling, D., Ludlam, C.A., 2001. Interleukin 6 and haemostasis Br. J. Haematol. 115, 3–12.

Khatun, S., Kanayama, N., Belayet, H.M., Tokunaga, N., Sumimoto, K., Kobayashi, T., Terao, T., 1999. Induction of hypercoagulability condition by chronic localized cold stress in rabbits. Thromb. Haemost. 81, 449–455.

Meade, T.W., Vickers, M.V., Thompson, S.G., Seghatchian, M.J., 1985. The effect of physiological levels of fibrinogen on platelet aggregation. Thromb Res. 38, 527–534.

Morris, C.J., 2003. Carrageenan-induced paw edema in the rat and mouse. Methods Mol. Biol. 225, 115–121.

Plesca, L.A., Bodizs, G., Cucuianu, M., Colhon, D., 1995. Hemostatic balance during the acute inflammatory reaction; with special reference to antithrombin III. Rom. J. Physiol. 32, 71–76.

Rapaport, S.I., Rao, L.V.J., 1995. The tissue factor pathway: how it has become a 'Prima Ballerina'. Thromb. Haemost. 74, 7–17.

Robson, S.C., White, N.W., Aronson, I., Woollgar, R., Goodman, H., Jacobs, P., 1996. Acute phase response and the hypercoagulable state in pulmonary tuberculosis. Br. J. Haematol. 93, 943–949.

Roemisch, J., Gray, E., Hoffmann, J.N., Wiedermann, C.J., 2002. Antithrombin: a new look at the actions of a serine protease inhibitor. Blood Coagul. Fibrinolysis 13, 657–670.

Ross, R., Glomset, J., Kariya, B., Harker, L., 1974. A platelet-dependent serum factor that stimulates the proliferation of arterial smooth muscle cells in vitro. Proc. Natl. Acad. Sci. U. S. A. 71, 1207–1210.

Salvemini, D., Wang, Z.Q., Wyatt, P.S., Bourdon, M.D., Marino, M.H., Manning, P.T., Currie, M.G., 1996. Nitric oxide: a key mediator in the early and late phase of carrageenan-induced rat paw inflammation. Br. J. Pharmacol. 118, 829–838.

Srivastava, R., Srimal, R.C., 1986. Inflammation induced changes in platelets. Indian. J. Pharmacol. (suppl.1), 0–2.

Srivastava, R., Srimal, R.C., 1990. Amplification of platelet response during acute inflammation in rats. Biochem. Pharmacol. 40, 357–363.

Steinhubl, S.R., Moliterno, D.J., 2005. The role of the platelet in the pathogenesis of atherothrombosis. Am. J. Cardiovasc. Drugs 5, 399–408.

Strukova, S., 2006. Blood coagulation-dependent inflammation. Coagulation-dependent inflammation and inflammation-dependent thrombosis. Front. Biosci. 11, 59–80.

Thomson, A.W., Fowler, E.F., 1981. Carrageenan: a review of its effects on the immune system. Agents and Actions 11, 265–273.

Vincent, J.E., Bonta, I.L., Zijlstra, 1978. Accumulation of blood platelets in carrageenin rat paw oedema. Possible role in the inflammatory process. Agents Actions 8, 291–295.

Wiedermann, Ch.J., Romisch, J., 2002. The anti-inflammatory actions of antithrombin — a review. Acta Med. Austriaca 29, 89–92.