Intravascular Activation of the Clotting System with Phospholipids

Production of the Generalized Shwartzman Reaction with Platelet Factor 3

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The Sanarelli-Shwartzman phenomenon—generalized Shwartzman reaction (GSR)—is observed in rabbits after two properly spaced intravenous injections of endotoxin derived from gram-negative bacteria and is characterized by profound collapse and hemorrhagic diathesis. Marked changes in the blood clotting mechanism in animals undergoing GSR have been described and resemble those observed when blood clots in vitro. The platelets which remain in the circulation of these animals are altered both in number and in their specific procoagulant effects; the activities of both platelet factor 1 (adsorbed Ac-G) and of platelet factor 3 (phospholipid) are greatly impaired. Morphologically, the reaction is characterized by a generalized intravascular deposition of fibrin. Bilateral renal cortical necrosis with fibrin-thrombi in the glomerular capillaries is observed in animals that survive long enough to develop this lesion.

The present work deals with the morphologic and coagulative changes produced by infusing phospholipid having platelet factor 3 activity, or soya bean phospholipid (Inosithin), in rabbits previously given intravenous thorotrast with the intent of blocking the reticuloendothelial system. The alterations described were indistinguishable from those observed in the classical GSR produced in rabbits with two intravenous injections of endotoxin.

Materials and Methods

Materials

Material with platelet factor 3 activity was isolated from fresh pooled bovine platelets according to the method described by Alkjaersig, Abe and Seegers and was generously supplied to us by Dr. E. Mammen. Although a number of impurities are probably present, this material is largely phospholipid in nature. The soya bean phospholipid was the commercial Inosithin. Thorotrast (a 26 per cent thorium solution) was obtained commercially.
The infused activity of platelet factor 3 was estimated by the thromboplastin generation test of Biggs, Douglas and MacFarlane, as previously reported. It was calculated as being at least one-third of the total platelet factor 3 activity in the thrombocytes of the animal (assuming that the total amount of thrombocytes in the adult rabbit was approximately 375). The infused activity of platelet factor 3 was estimated by the thromboplastin generation test of Biggs, Douglas and MacFarlane, as previously reported. It was calculated as being at least one-third of the total platelet factor 3 activity in the thrombocytes of the animal (assuming that the total amount of thrombocytes in the adult rabbit was approximately 375).

Methods

Albino rabbits weighing an average of 3 Kg., fed Purina rabbit pellets ad libitum, received thorotrast intravenously in a dosage of 3 ml./Kg. of body weight. Twenty-four hours after injection, a catheter was inserted into the middle vein of the ear through which blood samples were taken and the phospholipids infused. Blood samples were taken for clotting determinations before the infusion of the procoagulant and at 10 minutes, 2, 4 and 6 hours thereafter.

Group A. Eight rabbits received the platelet factor 3 material: six within four hours and two within 10–15 minutes. The phospholipid was infused in 50 cc. of physiologic saline.

Group B. Four rabbits received Inosithin, all within 10–15 minutes.

Platelet counts were performed by the chamber method of Feissly and Lüdin. The activity of prothrombin and factor V (Ac-G) was assayed by the one-stage method with the reagents of Behringwerke AG (Marburg/Lahn). Fibrinogen was measured indirectly by determinations of tyrosin in the washed clot. In a few instances, thromboelastographic studies were performed.

The animals were autopsied immediately after death. The organs were fixed in 10 per cent neutral formalin and the histologic sections were stained with hematoxylin-eosin and Goldner-trichrom.

Results

No differences were found between the two experimental groups. The animals receiving the procoagulant infusion (either platelet factor 3 material or Inosithin) within four hours appeared very restless at the end of this period. Those receiving it within 10–15 minutes tolerated it well and became restless after 2–3 hours which, in most cases, ended in death about 10 hours after infusion.

Changes in Coagulation Factors

The platelets showed a marked drop from 100 per cent to 18 per cent in both groups of animals—i.e., from an initial average value of 273,000 platelets/cu. mm. only 51,000 remained in the circulation after 6 hours. In addition, there was a marked decrease in activity of prothrombin and factor V from 100 to 25 per cent and from 100 to 18 per cent, respectively, as well as in the plasma levels of fibrin which fell from 100 to 61 per cent within 6 hours (fig. 1).

Figure 2 depicts the thromboelastographic tracings from blood taken before and at various times after the infusion. There was a shortening of the r-values (reaction time) with later prolongation of the K-time (clotting time) and reduction of the M (maximal amplitude). All plasma samples withdrawn at 4 and 6 hours after the procoagulant injection showed a peculiar opalescent color, particularly when chilled, as described when cryoprofibrin is present.
Fig. 1.—Behavior of the plasma levels of fibrinogen, platelets prothrombin and factor V (Ac-G) in rabbits receiving the phospholipid infusion intravenously, 24 hours after RES blockage. 100 per cent fibrinogen = 252 gr. per cent. Platelet count, 100 per cent = 273,000 platelets cu. mm. Note the marked drop of the respective activities after the procoagulant infusion.

Fig. 2.—Hartert's thrombolastogram of blood samples taken from an animal receiving the procoagulant infusion within 10 minutes. A: initial value; B: during the infusion; C: ten minutes thereafter; D: two hours later. Note the reduction of r and Mε; also enlargement of K.
Fig. 3.—Transversal cuts of hearts of two rabbits of similar size treated with equiparable amounts of platelet factor 3, 24 hours after RES was blocked with thorotrast. Animal A died 20 hours thereafter; animal B died 2½ hours after the infusion. Note the severe dilatation of the right ventricle. Figure 7 shows intravascular deposition of fibrin in the lungs of this same animal.

Anatomical Changes

Macroscopic Findings: As a rule the lungs showed marked congestion and petechiae. The heart showed marked right ventricular dilatation in the animals dying shortly after infusion (fig. 3). The thymus showed diffuse petechiae; the liver was markedly congested in all animals, but was particularly so in the animals living a short period of time. The gross appearance of the kidneys of an animal that died 12 hours after initiation of the procoagulant infusion is shown in figure 4. In animals living a shorter length of time, only the cortex showed marked pallor and a well-defined congested area at the corticomedullary boundary. The spleen appeared congested in some areas and markedly ischemic in others. The adrenal cortex showed marked congestion in several instances. Petechiae were often observed in the peritoneum as well as ischemic areas in the intestines with some of the loops very distended.

Microscopic Findings. The most striking changes were seen in the kidneys. There were focal areas of necrosis limited to the cortex with tubular degeneration. The capillaries of the glomeruli were partially blocked with hyaline masses not adherent to the capillary wall and having the staining characteristics of fibrin (figs. 5 and 6). Other capillary loops were congested and the veins in the corticomedullary region showed stasis. In the majority of the animals, the lungs appeared markedly congested with subpleural hemorrhages. Fibrin thrombi were found in capillaries and arterioles (fig. 7). Localized bleeding was found in the thymus (fig. 8) and myocardium (fig. 9). The liver showed congestion of the central lobules (fig. 10). Mild congestion was found in the adrenals and spleen.
Fig. 4.—Macroscopic view of the kidney of a rabbit which expired 12 hours after the infusion of purified platelet factor 3. The RES was blocked 24 hours prior to the procoagulant infusion.

Fig. 5.—Microphotograph of a glomerulum of an animal treated with platelet factor 3, 24 hours after receiving 3 cc./Kg. of thorotrast. Note the intracapillary deposition of fibrin. Goldner-Trichom (900×).
Fig. 6.—Glomerulum of an animal whose RES was blocked 24 hours prior to the infusion of a procoagulant (Inosithin). Note the similarity with figure 5. Goldner-Trichrom (900 x).

Fig. 7.—Lung capillary showing deposition of hyaline material which has tinctorial properties of fibrin. Goldner-Trichrom (600 x).
Fig. 8.—Microphotograph of the thymus of an animal treated with phospholipid with procoagulant properties. Note hemorrhagic areas. Hematoxilin-eosin (375 x).

Fig. 9.—Myocardium showing focal hemorrhage. Hematoxilin-eosin (375 x).
Fig. 10.—Liver, centrolobular congestion. The experimental animal died 4 hours after the infusion of Inosithin. Hematoxilin-eosin (375 ×).

DISCUSSION

The results presented indicate that the infusion of phospholipids (platelet factor 3 or soya bean phospholipid) in animals previously given thorotrast in the attempt to block the reticuloendothelial system resulted in anatomic and coagulopathic pictures closely resembling those seen in animals undergoing the generalized Schwartzman reaction elicited with endotoxin. In previous experiments, we showed that gram-negative endotoxin had no thromboplastic effect on purified prothrombin but rather a double effect on the clotting mechanism—i.e., one activating the contact active factors and the other resulting in release of platelet factor 3 activity. Activation of the Hageman factor is currently considered to be the first step in blood clotting, although the exact mechanism by which the clotting process is triggered has thus far not been fully demonstrated. It is possible that the Hageman factor participates in the release of platelet factor 3, thus explaining the two apparently different points of action of endotoxin. This hypothesis is consistent with the fact that platelet factor 3 was not released from washed platelets incubated with endotoxin, while, on the other hand, the incubation of platelet-rich plasma with endotoxin resulted in the liberation of this factor.

Previous experiments have indicated that the platelets of animals undergoing the generalized Schwartzman reaction show reduced platelet factor 3 activity. Since the data presented here demonstrate that a condition resembling or identical with the GSR can be produced in rabbits pretreated with thorotrast by infusing phospholipids with high platelet factor 3 activity, it is thus conceivable that the activation of the clotting system by endotoxin is dependent upon...
Fig. 11.—Schematic representation of the clotting mechanism of the rabbit treated with endotoxin of gram-negative bacteria (generalized Shwartzman reaction). It is proposed that the Hageman factor (factor XII) acts either alone or concomitantly with endotoxin in releasing platelet factor 3 from the thrombocytes, which is able per se of activating the normal clotting system in vitro and in vivo (see text).

The release of platelet factor 3 from the thrombocytes. Whether or not release of this factor occurs directly or through the activation of Hageman factor (factor XII) is not clear. These studies indicate further that platelet factor 3 is sufficient to activate the so-called intrinsic mechanism of blood clotting.

Seegers and his co-workers have shown that purified prothrombin can be activated in vitro in the presence of platelet factor 3, Ac-G (factor V), one platelet cofactor—either I (factor VIII) or II (factor IX)—and calcium ions. All of these factors but the first one (and probably the platelet cofactor II) are present in circulating blood. Thus, the in vivo studies reported here tend to confirm, in accordance with Seeger’s studies in vitro, that the clotting mechanism is activated intravascularly when platelet factor 3 is released (fig. 11). These experiments also show that not only platelet factor 3, but another phospholipid (Inosithin) as well, sharing the procoagulant properties in vitro, is capable of activating the clotting system in vivo. It is to be expected that other methods for triggering the generalized Shwartzman reaction will activate the clotting mechanism by releasing (directly or indirectly) platelet factor 3 from the thrombocytes.

*In the extrinsic mechanism, prothrombin is activated by tissue thromboplastin to thrombin and autoprothrombin-C, the latter being a potent autocatalysing enzyme with potent toxic effects.

†Of special interest in this regard are the experiments of Gerber, who replaced the second endotoxin injection by an antigen-antibody reaction. Associations between "complement" and the clotting system have been discussed for many years.
Other authors have failed to observe activation of the clotting mechanism with "platelet extracts." This may be due to failure to block the reticuloendothelial system prior to the infusion of the platelet procoagulant. As shown recently, the reticuloendothelial system is capable of "clearing" clotting intermediates, fibrin and endotoxin, thus masking the triggering mechanism of the platelet procoagulant.

The intravascular activation of the clotting mechanism seen during the generalized Shwartzman reaction is reflected in the drop of the (plasma) clotting factors: fibrinogen, prothrombin, Ac-G (Factor V), antithrombin III, factor VIII, and also of the platelet count. All of these changes may be related to the intravascular presence of thrombin; that is, prothrombin decreases while generating thrombin and fibrinogen as immediate substrates of thrombin. Factors V (Ac-G) and VIII are inactivated by thrombin and antithrombin III activity is reduced in the presence of thrombin. Thrombin is also responsible for the drop of the platelet count, which is probably the result of viscous metamorphosis. The presence of thrombin activity intravascularly has been documented in animals undergoing the generalized Shwartzman reaction during the stage in which clotting factors decrease and at the time when the first fibrin thrombi can be seen microscopically.

The observed hemorrhagic diathesis of the experimental animal is apparently due to the development of a condition in which the (plasma) clotting factors are consumed, resulting in the circulation of "serum" rather than plasma, thus leading to a "consumption-coagulopathy." This condition may be of importance in possible human analogs of the generalized Shwartzman reaction.

The morphologic lesions observed in the generalized Shwartzman reaction are mainly of ischemic nature. The typical fibrin thrombi observed in this
lesion are the result of intravascular activation of the clotting mechanism and thus the cause of the morphologic pattern. Lesions similar to those reported herein were observed recently by infusing homologous fibrin in rabbits previously given thorotrast.

Summarizing the data shown above, it is apparent that in order to elicit a generalized Shwartzman reaction, the clotting system must be activated; fibrin deposition is the obligatory pathway for the morphologic pattern observed. In the pathogenesis of the generalized Shwartzman reaction the blood clotting system plays the main role, but concomitantly such factors as the activity of the reticuloendothelial system, a lack of endogenous activation of fibrinolysis, and the selective vasoconstrictor effects should not be disregarded. Figure 12 illustrates the possible mechanisms whereby this occurs.

**SUMMARY**

The infusion of phospholipids containing platelet factor 3 activity or of Inosithin (soya bean phospholipid) in animals previously given thorotrast in the attempt to block the reticuloendothelial system, resulted in histologic changes and in severe alterations of the clotting mechanism. These morphologic and analytical alterations of the clotting system could not be distinguished from the findings observed in animals undergoing the classical generalized Shwartzman reaction produced with two injections of endotoxin.

**SUMMARIO IN INTERLINGUA**

Le infusion de phospholipidos continente activitate de factor 3 plachettal o de Inosithina (phospholipido de soja) ad in animales previemente tractate con Thorotrast pro blocar le systema reticuloendothelial resultava in altera-
tiones histologic e in sever alterationes del mechanismo coagulatori. Iste altera-
tiones morphologic e analytic del systema coagulatori non poteva esser distinguite ab le phenomenos observe in animales subjicite al classic generalisate reaction de Shwartzman producite con duo injectiones de endotoxina.

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INTRAVASCULAR ACTIVATION


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