Evidence Suggesting the Regulation of Coagulation Factor Levels in Rabbits by a Transferable Plasma Agent

By Ellen W. Friedman, Margaret Karpatkin, and S. Karpatkin

New Zealand white rabbits were given 30 ml of goat serum intravenously. This procedure resulted in an immediate decrease in platelet count, fibrinogen, and levels of coagulation factors II, V, VII, and X, due to consumption coagulopathy. These factors returned toward baseline levels approximately 12 hr after the injection. Plasma from rabbits who had received goat serum 48 hr previously (donor rabbits) was injected into recipient rabbits. This procedure resulted in a slight rise in the level of coagulation factor II (range, 20%-30%) and a significant rise in factors V (35%-75%), VII (35%-235%), and X (35%-75%) in the recipients. When plasma from control donor rabbits who had not received goat serum was injected into recipients, there was no change in these coagulation factors. It is postulated that the reduction in coagulation factor levels in donor rabbits induces a "coagulopoietin" for each factor or one "coagulopoietin" for all factors which stimulates increased synthesis and/or release of these factors in recipient rabbits.

In a previous study from this laboratory, plasma from rabbits whose vitamin K-dependent coagulation factors had been depressed by coumadin was injected into normal recipient rabbits. Levels of the vitamin K-dependent factors rose in the recipients, whereas factor V did not rise, and normal donor plasma injected into normal recipient rabbits had no effect. It was postulated that depression of these coagulation factors caused production in the donor rabbits of a substance that stimulates synthesis of vitamin K-dependent coagulation factors. This substance, or "coagulopoietin," was transferred to the recipient rabbits. However, this observation was subject to another interpretation, since it was possible that animals receiving coumadin had biologically inert precursors of the vitamin K-dependent coagulation factors in their plasma, which closely resembled the active factors. The function of vitamin K appears to be the incorporation of a second carboxyl group into a number of glutamic acid residues of a preformed molecule. It was possible that precursors of the vitamin K-dependent factors had been transferred to the normal recipient rabbits and carboxylated to the active form in these animals. However, a positive response was obtained with as little as 0.25 ml of vitamin K-depleted plasma given twice daily for 3 days to a 3 kg rabbit. Furthermore, it was subsequently shown that inactive coagulation factors were not present in the plasma of rabbits receiving coumadin, and that of the species which have been studied, only man and cow have such precursors in their plasma. Nevertheless, an alternative method of lowering coagulation factor levels was sought.
A goat anti-rabbit factor II antibody was injected into rabbits in order to lower the level of factor II. Plasma from these animals was then injected into normal recipient rabbits, and factor II levels were measured in the recipients. Factor II did increase in the recipient animals, but levels of factors V, VII, and X also rose. The possibility was considered that injection of the goat serum was causing consumption coagulopathy in the donor rabbits. This proved to be the case. This communication reports evidence for the induction of a coagulopoietin for the coagulation factors consumed during consumption coagulopathy.

METHODS AND RESULTS

Measurement of coagulation factors. Factors II, V, VII, and X were assayed as described previously. Four dilutions were made of each plasma sample measured, ranging from 1:10 to 1:100. Clotting times obtained were plotted against dilutions on log-log graph paper, resulting in a straight line. Lines obtained were parallel with each other and a control rabbit plasma, irrespective of the level of coagulation factor. This finding indicated that alterations in clotting times were constant at all dilutions of each sample tested. Two standard deviation changes for the measurement of factors II, V, and X were ±18%, ±20%, and ±24%, respectively. A SD was not obtained for measurement of factor VII because of scarcity of VII-deficient substrate plasma. Because of the close similarity to the factor II assay, a SD of ±9% was assumed. Fibrinogen was measured by a heat precipitation method.

Platelet counts. These were performed under phase microscopy by a modification of the method of Brecher and Cronkite, employing 3% procaine hydrochloride as diluent.

Preparation of antibody against rabbit factor II. Antibody was raised by the injection of purified rabbit factor II into a goat. Blood collected from the goat was allowed to stand at 4°C overnight. The serum was separated and heated at 56°C for 30 min. Normal goat serum was obtained from Rockland Inc., Gilbertsville, Pa., and similarly heated at 56°C before use.

Effect of injection of goat antiserum (GAS) upon the coagulation factors. Two rabbits received 30 ml of goat serum containing antibody against factor II into a lateral ear vein, over a period of 15 min. This injection was preceded by the intravenous injection of 100 units/kg heparin, which did not affect the coagulation factor assays. Blood samples were drawn from the contralateral ear vein into 3.8% sodium citrate and 1 M e-aminocaproic acid (EACA) (9 parts blood in 1 part anticoagulant-EACA) at intervals for 46 hr. Plasma was separated immediately and frozen in aliquots. At the end of the experiment, all samples were assayed for factors II, V, VII, and X.

Platelet counts were also performed in one rabbit.

In the first rabbit, factors II, VII, and X fell below baseline levels during the first 30 min after injection of GAS (Fig. 1A). At 9½ hr, they were still below baseline. Thereafter, they rose to above baseline levels at varying time intervals.

In the second animal, more samples were taken in the 12 hr following the injection of GAS to examine the kinetics during this period (Fig. 1B). All four coagulation factors had fallen 30 min after injection of GAS. Factor VII was least affected and returned to baseline within 1 hr, remaining there for 5½ hr. The other coagulation factors remained low for a longer period of time and returned to normal by 11½ hr. The platelet count also fell immediately and remained low to the end of the experiment.

Effect of injection of normal goat serum (GS) upon the coagulation factors. One rabbit was injected with serum from a normal goat which had not been immunized against factor II, and similar assays were performed. Fibrinogen levels were also measured in this animal (Fig. 1C). Factor VII remained at baseline for 3½ hr and then rose to extremely high levels at 5 and 7 hr. It dropped slightly below baseline at 8 hr and remained at or near baseline for the remainder of the experiment. The other three factors were all depressed. The fall in factor II was not as profound as in the two animals who received antisera to factor II. Factors V and II had returned to baseline at 8 hr. Factor X had returned to baseline at 12 hr. Fibrinogen was undetectable at 30 min (< 40 mg/100 ml) and remained undetectable for 12 hr. At 24 hr, it had returned to slightly above baseline and remained so at 26 hr. The platelet count also fell immediately and remained low until the end of the experiment.

Preparation of donor rabbit plasma. Rabbits received an injection of GAS or GS at zero time.
Fig. 1. (A and B) Response of a normal rabbit to the intravenous injection of 30 ml of goat serum containing anti-rabbit factor II. The arrow refers to the time of injection. ○--○, factor II; ◯--◯, factor X; △--△, factor VII; ▲--▲, factor V; *=*, per cent platelet level change. The 100% value is the mean of three samples drawn on three separate days prior to the injection of goat antiserum. (C) Response of a normal rabbit to the intravenous injection of 30 ml of normal goat serum. The symbol □--□ refers to fibrinogen level expressed as per cent fibrinogen level prior to injection of goat serum; the arrow below the symbol indicates no detectable fibrinogen (< 40 mg/100 ml).
Table 1. Statistical Analysis of Elevation of Coagulation Factors in Recipient Animals Following Injection of Rabbit Plasma From Animals Subjected to Consumption Coagulopathy

<table>
<thead>
<tr>
<th>Day</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>7</th>
<th>(\chi^2) Analysis*</th>
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<tr>
<td>Factor II</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
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<td>NS</td>
<td>NS</td>
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<td>&lt; 0.02</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>Factor VII</td>
<td>NS</td>
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<td>&lt; 0.01</td>
<td>&lt; 0.02</td>
<td>&lt; 0.01</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>Factor X</td>
<td>&lt; 0.1</td>
<td>&lt; 0.05</td>
<td>NS</td>
<td>&lt; 0.05</td>
<td>&lt; 0.01</td>
<td>&lt; 0.01</td>
</tr>
</tbody>
</table>

*Performed by Student t test of unmatched pairs of seven experimental animals versus four control animals. NS refers to nonsignificant (\(p > 0.1\)). Other numbers refer to p values.

†Performed with a 2 x 2 \(\chi^2\) analysis with Yates correction comparing the number of days of elevation of experimental animal coagulation factors versus number of days of elevation of control coagulation factors.

(30 ml), 18 hr (15 ml), and 24 hr (25 ml). Each was given slowly into a lateral ear vein and preceded by 100 units/kg of heparin. The animals were exsanguinated 48 hr after the last injection, and their plasma was separated and stored as described previously. Control plasma was obtained by exsanguinating rabbits who had not received GAS or GS.

Effect of donor plasma from rabbits who had received GAS or GS. Five animals received plasma from rabbits who had been given GAS, and two from rabbits who had been given GS. Since no difference was found between the two groups, the data have been combined and averaged. Each received two daily intravenous injections of 1-4 ml for 4½ days (nine injections). Mean coagulation factor levels are shown in Fig. 2A. Factors V, VII, and X rose immediately and remained significantly (Table I) above baseline for at least 6 days for factors VII and X, and 9 days for factor V. Factor II fell initially and then rose and remained above baseline for 8 days. However, this change was not significant, possibly because of the small number of samples or possibly due to the presence of residual antibody against factor II in five of the rabbit donor samples.

Effect of donor plasma from rabbits who had received no injections. Four rabbits received normal rabbit plasma with the same injection schedule as above. Two received 1 ml per injection, and two received 4 ml per injection. Mean factor levels for these animals are shown in Fig. 2B. Factor VII remained below baseline throughout the experiment. Factors II and X did not rise more than 8% above baseline. Factor V rose to 120% on day 3, but remained below baseline on days 4 through 9.

DISCUSSION

The changes in coagulation factor levels and platelet count following injection of GAS or GS into rabbits were similar to those seen during intravascular coagulation despite the presence of heparin. Factor II fell to levels that were lower in the two animals who received GAS than in the one who received GS, presumably due to the additional effect of the antibody. In each animal, there was some degree of overshoot above baseline after recovery from the intravascular coagulation. This pattern has been observed in other circumstances following intravascular coagulation. The data strongly suggested that reduction in levels of circulating coagulation factors stimulated increased synthesis and/or release of these factors. It is postulated that the low levels of coagulation factors induce the formation of a coagulopoeitin, which stimulates the hepatic cell to synthesize and/or release coagulation factors. An alternative mechanism is direct feedback upon the hepatic synthesizing cells.

Our previous1 as well as present data suggest the presence of a humoral substance, which is elaborated in animals with depleted coagulation factors, and which is capable of elevating coagulation factors in recipient rabbits. An alternative explanation for the rise in coagulation factors in the recipient animals is
Fig. 2. (A) Response of seven normal recipient rabbits to the intravenous injection of plasma from rabbits given goat serum containing anti-rabbit factor II (five recipients) or normal goat serum (two recipients). The combined mean of seven experiments is given. The arrows refer to the intravenous injection of 1-4 ml plasma. ○—○, factor II; ■—■, factor V; ▲—▲, factor VII; △—△, factor X. The 100% value refers to the mean of three samples drawn on each animal on three separate days prior to starting the injection schedule. (B) Response of four normal recipient rabbits to the intravenous injection of plasma from normal rabbits. Protocol and symbols are the same as in (A).

that the donor rabbit plasma contained activated coagulation factors due to the injection of GAS or GS. Four lines of evidence are against this: (1) While it is well recognized that injection of serum causes activation of clotting factors, there is evidence that, in the intact healthy animal, activated factors are very rapidly removed from the circulation by the liver and reticuloendothelial system, probably within minutes. It is therefore unlikely that any circulating activated factors remained when the donor animal was exsanguinated 48 hr after the last injection of serum. (2) Even if activated factors had been present in the donor plasma, the volumes injected into a recipient rabbit were extremely small (i.e., between 1 ml and 4 ml per injection into a calculated blood volume of approximately 210 ml). (3) Samples from the recipient animals were drawn at least 12 hr after an injection of donor plasma. (4) The clotting factors in the recipients were still elevated 3 days after the last injection of donor plasma (Fig. 2). It is therefore unlikely that activated factors induced by the injection of donor plasma could still be present at this point in time.

Many questions remain to be answered. Is the material in the donor plasma a specific coagulopoi etin, or is it a nonspecific acute phase reactant formed in the donor animals and capable of stimulating the synthesis and/or release of coagulation factors in the recipients? If it is a coagulopoi etin, is there one for each coagulation factor? Our previous data on vitamin K-dependent coagulation factors suggest that this may be the case, since factors II, VII, IX, and X rise in recipient animals following the injection of plasma from coumadin-treated animals, whereas factor V levels are no different in control and experi-
mental recipient animals. In the case of the vitamin K–dependent factors, is the rise observed due to de novo protein synthesis, or to rapid carboxylation of precursor protein present in the hepatic cells? Further studies are needed to elucidate these points.

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