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Pharmacokinetics of Heparin VII: Effect of Pregnancy on the Relationship Between Concentration and Anticoagulant Action of Heparin in Rats

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Abstract □ The effect of pregnancy on the anticoagulant action of heparin was determined by comparing the slope of the relationship between the natural logarithm of the activated partial thromboplastin time (APTT) and heparin concentration (the heparin slope) in the plasma of pregnant and nonpregnant female inbred Lewis rats. Also determined were the prothrombin time, hematocrit, and the activities of coagulation factors II, VII, VIII, X, XI, and XII. The heparin slope was significantly decreased in pregnant rats at the 20th day of gestation but not in rats at the 10th day of gestation, indicative of a decreased anticoagulant action of heparin in late pregnancy. The hematocrit and prothrombin time were decreased, and the baseline APTT (*i.e.*, the APTT without added heparin) as well as the activities of factors II, VII, and X were increased in pregnant rats at the 20th day of gestation. Both pregnant and nonpregnant animals showed a significant negative correlation between prothrombin time and factor II activity and a significant positive correlation between the activities of factors II and X. The effects of pregnancy in rats on heparin slope, prothrombin time, hematocrit, and factors VII, VIII, X, and XII are qualitatively the same as those in pregnant women in the third trimester. The increases in factor II activity and baseline APTT found in the rats were not observed in humans. Pregnant rats, like pregnant women, are relatively resistant to the anticoagulant action of heparin.

Keyphrases □ Heparin—anticoagulant action, effect of pregnancy, rats □ Anticoagulants—heparin, effect of pregnancy on the anticoagulant action, rats □ Pregnancy—effect on the anticoagulant action of heparin in rats

Rats and humans exhibit grossly similar characteristics with respect to the pharmacokinetics and pharmacodynamics of heparin: both species eliminate heparin by dose-dependent kinetics that are not of the Michaelis–Menten type and both show an essentially linear relationship between an index of the anticoagulant response, the logarithm of the activated partial thromboplastin time of plasma (APTT), and the concentration of heparin added to plasma (1). Human pregnancy is associated with a state of progressive blood hypercoagulability due, in part, to increased concentrations or activities of certain components of the coagulation system (2). The heparin dose requirements for treatment or prevention of thromboembolic disorders are increased in human pregnancy (3–6). Recent studies in this laboratory have shown this to be due, at least in part, to a decreased anticoagulant effect of heparin (7). Changes in the distribution (7) and clearance (3) of heparin may also occur, but this is presently uncertain.

A previous investigation of blood coagulation characteristics and response to heparin in rats (8) has revealed a number of important differences relative to humans, despite the gross similarity in the pharmacokinetics and pharmacodynamics of heparin in the two species (1). To further explore the relative characteristics of the two species with respect to heparin, we have determined the effect of pregnancy on the anticoagulant action of heparin and on a number of related physiological variables in rats.

EXPERIMENTAL

Groups of two female inbred Lewis rats[1], 200–250 g, were placed in plastic cages with sawdust bedding taken from cages previously occupied by adult male rats. They had free access to food[2] and water at all times. After 2 d an adult male Sprague–Dawley rat was placed in each cage with the two females for an overnight period. The male rat was removed in the morning, and that day was designated as the first day of gestation. The female rats were then housed in groups of four per cage for the duration of the study; nonimpregnated females from the same colony were kept similarly. This procedure was repeated on three occasions. For the first study, groups of eight pregnant and eight control rats each were tested on the 10th and 20th days of gestation; for the second study, nine pregnant and nine control rats were tested on the 20th day of gestation; for the third study, seven pregnant and seven control rats were tested on the 20th day of gestation.

Blood samples were obtained from the abdominal aorta while the animals were under light ether anesthesia. The hematocrit was first determined from a small sample of blood (<0.1 mL) taken from the tail vein. Sufficient acid citrate anticoagulant (9) was then added to a 5-mL plastic syringe such as to yield a 6:1 plasma–citrate solution volume ratio when 4.5 mL of blood was drawn from the abdominal aorta into the syringe. The citrated blood was transferred to plastic tubes which were centrifuged to separate the plasma as previously described (8). The plasma samples were stored at −80°C for no longer than 3 d. Plasma from two of the control rats (10th day) in the first study contained small clots on thawing. To prevent this in subsequent experiments, the plasma–citrate solution volume ratio was changed from 6:1 to 5:1 in the second and third studies. All animals in the pregnant groups were dissected after blood sampling to confirm pregnancy.

The APTT was determined with a coagulation timer[3] after a 15-min plasma incubation time (unless stated otherwise), using automated APTT[4] as the principal reagent. Heparin of beef lung origin[5] was added to the plasma to yield concentrations of 0.1–0.7 U/mL in six steps; an equivalent volume of heparin-free solvent was added for baseline APTT determination. Details of these procedures have been described previ-

[1] Charles River Farms, Wilmington, Mass.
[2] Charles River Formula RMH 1000, Syracuse, N.Y.
[3] Fibrometer, Baltimore Biological Laboratories, Cockeysville, Md.
[4] General Diagnostics, Morris Plains, N.J.
[5] The Upjohn Co., Kalamazoo, Mich., (lot no. 955FW).

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Table I—Effect of Pregnancy on Baseline APTT, *In Vitro* Anticoagulant Action of Heparin, Prothrombin Time, Hematocrit, and Body Weight of Rats (First Study)

Study Group[a]	Baseline APTT, s	Slope[b], mL/U	Intercept[b], s	Prothrombin Time, s	Hematocrit, %	Body Weight, g
10th Day of gestation						
Control	15.1 ± 1.0	2.72 ± 0.50	12.9 ± 1.3	21.0 ± 1.2	43.5 ± 2.2	254 ± 16
Pregnant	17.4 ± 2.0	2.06 ± 0.53	15.9 ± 1.8[c]	20.9 ± 0.8	41.5 ± 0.9[c]	275 ± 12[c]
20th Day of gestation						
Control	16.7 ± 1.2	2.29 ± 0.25	15.1 ± 0.7	18.5 ± 1.4	45.0 ± 1.1	243 ± 9
Pregnant	25.6 ± 2.8[c]	1.32 ± 0.32[c]	24.6 ± 2.1[c]	16.0 ± 0.8[c]	36.1 ± 2.8[c]	366 ± 26[c]

[a] Lewis inbred rats, eight per group. Results are expressed as mean ± *SD*. [b] Slope and intercept of the least squares regression line of ln APTT *versus* concentration of heparin added to plasma. APTT was measured after a 15-min incubation of the plasma–APTT reagent mixture. [c] Significantly different from the appropriate control group, $p < 0.01$.

Table II—Effect of Pregnancy on Baseline APTT, *In Vitro* Anticoagulant Action of Heparin, Prothrombin Time, Coagulation Factors Activity, Hematocrit, and Body Weight of Rats (Second Study)[a]

Variable	Control Rats	Pregnant Rats[b]
Baseline APTT, s	20.1 ± 4.5	26.2 ± 3.5[e]
Slope, mL/U[c]	2.45 ± 0.36	1.70 ± 0.08[e]
Intercept, s[c]	17.9 ± 6.3	23.6 ± 3.3[f]
Prothrombin time, s	19.0 ± 0.6	16.3 ± 0.3[e]
Factor II, %[d]	100 ± 8	133 ± 8[e]
Factor VII, %[d]	100 ± 19	203 ± 13[e]
Factor VIII, %[d]	100 ± 59	140 ± 69
Factor X, %[d]	100 ± 9	135 ± 13[e]
Factor XI, %[d]	100 ± 36	121 ± 34
Factor XII, %[d]	100 ± 33	85 ± 21
Hematocrit, %	45.7 ± 0.9	38.8 ± 1.4[e]
Body weight, g	254 ± 19	337 ± 12[e]

[a] Lewis inbred rats, nine per group. Results are expressed as mean ± *SD*. [b] At the 20th day of gestation. [c] See footnote b in Table I. [d] Determined by assay procedure for human coagulation factors; mean of control group taken as 100%. [e] Significantly different from the control group, $p < 0.01$. [f] Significantly different from the control group, $p < 0.05$.

ously (1, 8). Prothrombin time was determined as described previously (10). The activity of coagulation factors was determined by one-stage clotting assays using commercially available plasma deficient in the appropriate coagulation factor[6] as substrate, either actin-activated cephaloplastin reagent[6] or activated thromboplastin reagent[6], and the coagulation timer. The assay procedures were essentially as specified by the manufacturer.

The slope of the essentially linear portion of the relationship between ln APTT and heparin concentration (Fig. 1) and the intercept at zero heparin concentration were determined by linear regression analysis after transforming the APTT values to their natural logarithmic values. APTT values for the plasma samples without added heparin were not used in the regression analysis because these values were consistently higher than the extrapolated values based on the regression line for the other data (8). Results of the first study were analyzed statistically by two-way analysis of variance. If significant ($p < 0.05$) differences were found, the Student–Newman–Keuls test was used to determine, by multiple comparisons, which groups were different from one another. Differences between groups in the second and third studies were determined by unpaired t tests. Associations among variables were assessed by correlation analysis.

RESULTS

There was an essentially linear relationship between ln APTT and heparin concentration up to 0.7 U/mL ($r^2 > 0.96$) in plasma of both pregnant and nonpregnant rats, except that the APTT at zero heparin concentration was consistently higher than the extrapolated value (Fig. 1). Consequently, the intercept at zero heparin concentration of the least-squares regression line was always less than the actual baseline APTT.

The results of the first study are summarized in Table I. Pregnant rats at the 10th day of gestation had a lower hematocrit than the nonpregnant controls, but did not differ significantly from the controls with respect to baseline APTT and heparin slope. At the 20th day of gestation, the baseline APTT was significantly greater than and the slope was significantly lower than the corresponding control values. The prothrombin time and the hematocrit were decreased in late pregnancy.

The changes in baseline APTT, heparin slope, prothrombin time, and hematocrit in late pregnancy observed in the first study were confirmed in the second study which was performed ~6 months later. In addition, the activity of a number of coagulation factors was determined in the second study. This revealed a significant increase in the activities of factors II, VII, and X in late pregnancy (Table II).

A number of statistically significant correlations were found between pairs of some of the variables measured in this investigation (Table III). Of these, only the negative correlation between prothrombin time and factor II activity and between the activities of factors II and X are common to both the control and pregnant rats at the 20th day of gestation.

The APTT of rat plasma undergoes considerable change during incubation for activation of the intrinsic coagulation pathway (8). The APTT decreases rapidly in the first few minutes and then, unlike the APTT of human plasma under our experimental conditions (11), slowly increases (8). To minimize the effect of potential differences between pregnant and control rats with respect to the rate of this apparent *in vitro* degradation of some component(s) of the clotting process, a third study was performed in which the plasma incubation time was reduced from 15 to 3 min. The results of that study, summarized in Table IV, also show an increase in the baseline APTT and a decrease in heparin slope at the 20th gestational day, in agreement with the results of the first and second studies.

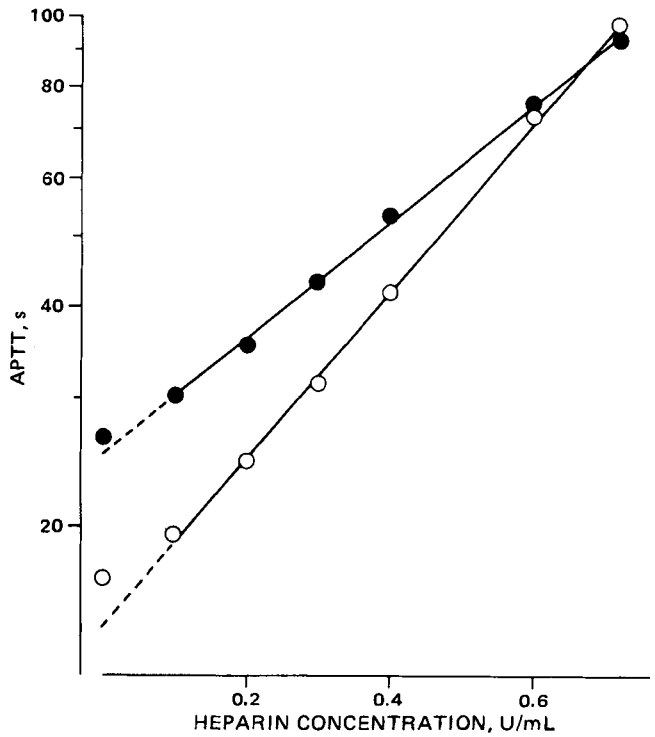

Figure 1—*Relationship between activated partial thromboplastin time (APTT) and heparin concentration in plasma of a pregnant rat (●) at the 20th day of gestation and a nonpregnant female control rat (○).*

[6] American Dade, Miami, Fla.

Table III—Correlations Among Variables in Control and Pregnant Rats of the Second Study [a]

Correlates	Control Rats		Pregnant Rats[b]	
	Correlation Coefficient	Statistical Significance (p)	Correlation Coefficient	Statistical Significance (p)
Prothrombin time–baseline APTT	0.76	<0.05	0.63	<0.1
Prothrombin time–hematocrit	0.85	<0.01	0.05	N.S.[c]
Prothrombin time–factor II	−0.68	<0.05	−0.76	<0.05
Prothrombin time–factor VII	−0.87	<0.01	−0.14	N.S.
Prothrombin time–factor X	−0.80	<0.05	−0.62	<0.1
Hematocrit–factor X	−0.73	<0.05	0.21	N.S.
Hematocrit–factor VII	−0.87	<0.01	−0.37	N.S.
Factor II–factor VII	0.69	<0.05	0.35	N.S.
Factor II–factor X	0.70	<0.05	0.76	<0.05
Factor VII–factor X	0.71	<0.05	0.15	N.S.
Factor XII–factor X	0.26	N.S.	−0.69	<0.05
Baseline APTT–factor II	−0.38	N.S.	−0.85	<0.01
Baseline APTT–factor X	−0.53	N.S.	−0.79	<0.05
Body weight–factor XI	0.06	N.S.	−0.72	<0.05

[a] Based on data summarized in Table II. [b] At the 20th day of gestation. [c] N.S. = not significant.

Table IV—Effect of Pregnancy on Baseline APTT and *In Vitro* Anticoagulant Action of Heparin Determined After a 3-min Incubation of the Plasma–APTT Reagent Mixture (Third Study) [a]

	Control Rats		Pregnant Rats[b]
Baseline APTT, s	12.9 ± 2.6	$p < 0.05$	16.0 ± 0.8
Slope, mL/U	1.24 ± 0.12	$p < 0.001$	0.95 ± 0.09
Intercept, s	12.8 ± 2.2	$p < 0.01$	15.4 ± 0.7

[a] Results are mean ± SD, $n = 7$. [b] At the 20th day of gestation.

DISCUSSION

This investigation has shown that rats, like humans, become relatively resistant to the anticoagulant effect of heparin in late pregnancy. The decrease of prothrombin time and hematocrit and the increase in the activities of clotting factors II, VII, and X in pregnant rats at the 20th day of gestation also occur in human pregnancy (7). One notable difference between rats and humans is that the baseline APTT increases in pregnant rats, but remains in the normal range during the third trimester of human pregnancy. The coagulation factor determinations in the second study were made to explore the possible reasons for this species difference.

The prothrombin time test is affected by alterations in the activity of clotting factors in the extrinsic coagulation pathway (factor VII) and in the common coagulation pathway (factors II, V, X, XIII, and fibrinogen). A deficiency in any of these components of the coagulation system or the presence of endogenous inhibitor(s) would be reflected by an increase in prothrombin time. In fact, the pregnant rats had a decreased prothrombin time and, consistent with this alteration, an increase in the activity of factors II, VII, and X. The decreased prothrombin time suggests that pregnancy is not associated with a deficiency of the components of the common coagulation pathway.

The APTT is affected by alterations in the activity of coagulation factors in the intrinsic coagulation pathway (factors VIII, IX, XI, XII, high molecular weight kininogen, and prekallikrein) and, like the prothrombin time, by factors in the common coagulation pathway. In view of the decreased prothrombin time, the increased baseline APTT in pregnancy is not likely to be due to a deficiency in any of the factors in the common coagulation pathway. Prolongation of the baseline APTT in pregnancy by an endogenous inhibitor was ruled out (12) because this prolongation was reversed on mixing equal volumes of plasma from pregnant and nonpregnant rats. This reversal, together with the other evidence, points to a deficiency of one or more components of the intrinsic coagulation pathway. Of these, factors VIII, XI, and XII were determined directly and were found not to be deficient. Factor IX activity was not measured, but since the other vitamin K-dependent factors (II, VII, and X) were actually above normal, it is probable that factor IX activity is not deficient. Thus, it appears that the increased baseline APTT in pregnant rats may be due to a deficiency of high molecular weight kininogen and/or prekallikrein, or to a deficiency of as yet unidentified factor(s) (13).

One more possibility was considered, namely that the prolonged baseline APTT in pregnant rats may have been due to more rapid *in vitro* degradation of one or several components of the coagulation system. That possibility is virtually excluded by the results of the third study, in which APTT was determined after 3 min rather than 15 min of plasma incubation for contact activation. The difference between pregnant and nonpregnant rats with respect to baseline APTT remained when the samples were incubated for only 3 min, although the absolute APTT values were different, as observed previously (8).

In summary, rats demonstrate many of the effects of pregnancy observed in humans with respect to blood coagulation: resistance to the anticoagulant effect of heparin relative to the response in nonpregnant individuals, increased activity of certain clotting factors, and decreased prothrombin time. However, there are also subtle differences, as reflected by the increased baseline APTT in pregnant rats which apparently does not occur in humans (7). The gestational age of the pregnant women (7) ranged from 33 to 41 weeks (mean, 36.8 weeks), and the correlation between their baseline APTT and gestational age was not statistically significant ($r = 0.63$, $n = 7$, $p > 0.1$). The rats were in their 20th day of gestation, *i.e.*, 1 d before parturition. Thus, their relative duration of gestation was similar.

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