Intravascular Coagulation and Fibrinolysis within Primate Extremities During Tourniquet Ischemia

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A common although infrequently recognized complication associated with the use of a pneumatic tourniquet is profuse bleeding from the wound after deflation of the tourniquet. The purpose of this study was to determine whether intravascular coagulation and fibrinolysis could be induced in subhuman primates by tourniquet ischemia, and whether this phenomenon could be altered by pretreatment of the animal with heparin. It was shown that, after 2½ hours of tourniquet ischemia, (400 mmHg) to one lower limb, fibrinogen levels were significantly lower ($p < .005$), antithrombin III levels were significantly lower ($p < .05$), plasminogen levels were significantly lower ($p < .05$), fibrin split products significantly higher ($p < .025$) and fibrinopeptide A levels were significantly higher ($p < .02$) than values measured simultaneously in the control limbs. After pre-treatment with sodium heparin, 30 units/kg, there was no change in antithrombin III levels or fibrinogen levels, but fibrin split products in the experimental limbs were significantly elevated ($p < .05$) when compared to control limbs. In both groups the abnormal levels returned to control levels 5–30 minutes after tourniquet deflation. We conclude that intravascular coagulation and fibrinolysis develop within ischemic subhuman primate limbs during tourniquet ischemia. Pretreatment with heparin prevents the consumption of fibrinogen and antithrombin III but does not prevent the increase in fibrin split products which was observed. It is possible that intravascular coagulation and fibrinolysis contribute to post tourniquet bleeding.

A COMMON ALTHOUGH INFREQUENTLY recognized complication associated with the use of a pneumatic tourniquet is profuse bleeding from the wound after deflation of the tourniquet. This phenomenon has been attributed to the reactive hyperemia which develops after tourniquet ischemia, but its true etiology is obscure.[5]

The purpose of this study was to determine whether intravascular coagulation and fibrinolysis could be detected within subhuman primate limbs during tourniquet ischemia, and whether this phenomenon could be altered by pre-treatment of the animal with heparin.

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Methods

Part I

Ivy bleeding times were performed and venous blood samples were drawn from groups of ten stump tailed monkeys. Specimens were taken from awake animals and monkeys anesthetized with intravenous sodium pentobarbital alone or in combination with intravenous infusion of sodium heparin, 30 units per kg.

Three layers of three inch wide cotton bandage were wrapped circumferentially around the mid-thigh of one lower extremity, and a pneumatic tourniquet was inflated to 400 mm of mercury for 2.5 hours. Tourniquets were calibrated daily with a mercury manometer. Bleeding times were repeated and venous blood samples were collected from each lower extremity during tourniquet ischemia and at varying intervals following tourniquet deflation. Because no more than 5 ml of free flowing blood could be obtained from the edematous ischemic limb, a series of separate experiments was carried out. In each series, blood samples from ten monkeys were aliquoted into polystyrene tubes containing the appropriate anticoagulant. For routine coagulation studies, nine parts blood plus one part 3.8% sodium citrate were placed on ice for no more than two hours until the platelet-poor plasma was separated in a refrigerated centrifuge. Paired specimens of plasma prepared in this manner from ischemic and control limbs were assayed within two hours of collection or stored frozen at −80°. Hematocrit determinations and platelet counts were performed immediately after each series of experiments. Ivy bleeding times were performed using a BD long-point microlance (Becton-Dickinson, Rutherford, N.J.). Fibrinogen determinations were performed on citrated plasma by

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TABLE 1. *Control Values in Experimental Animals Prior to Tourniquet Ischemia*

	Awake	Anesthetized	Anesthetized Heparinized
Hematocrit (%)	37 ± 1.3 (6)	36 ± 0.8 (10)	37 ± 1 (8)
Platelet Count (× 10^3/cu mm)	423 ± 36 (10)	437 ± 35 (11)	422 ± 33 (9)
Bleeding Time (min)	3.1 ± 0.2 (10)	3.6 ± 0.4 (8)	3.5 ± 0.7 (8)
Fibrinogen (mg/dl)	246 ± 30 (9)	216 ± 11 (10)	236 ± 30 (8)
Fibrin Split Products (μg/ml)	6.4 ± 0.8 (10)	7.0 ± 0 (11)	8.0 ± 1 (9)
Antithrombin III (%)	122 ± 5.2 (8)	116 ± 6 (10)	118 ± 5.5 (8)

Values expressed as mean ± 1 SEM.

Numbers in parentheses represent the number of specimens used for each determination.

a modification of the turbidimetric clotting technique of Ellis and Stransky, according to the method of Saleem et al.[16] Antithrombin III was measured in plasma by the two stage method of Ødegard which measures thrombin neutralization in the presence of a chromogenic substrate.[12] Fibrin split products were measured in serum from blood clotted in the presence of small amounts of enzyme inhibitor, thrombin and reptilase, using the Thrombo-Wellcotest® rapid latex test kit (Burroughs Wellcome Co., Research Triangle Park, N.C.).

Part II

Fibrinopeptide A levels were measured in platelet-poor plasma which was flash frozen and shipped on dry ice to Dr. Hymie Nossel at the Columbia Presbyterian Medical Center. For this test, 5 ml of blood was mixed with 0.5 cc of anticoagulant containing 100 mg heparin and 1,000 units Trasylol (aprotinin buffer).[9] Plasminogen determinations were performed on acidified neutralized plasma by the caseinolytic method using oxalated plasma.[1] Results from ischemic limbs and control limbs were compared using the Student's paired t-test.

Results

Part I (Tables 1–3)

Normal control values prior to tourniquet ischemia are shown in Table 1. No significant alterations occurred in the hematocrits, platelet counts, bleeding times, fibrinogen levels, levels of fibrin split products or antithrombin III levels as a result of the administration of sodium pentobarbital alone or in combination with heparin.

However, after 2½ hours of tourniquet ischemia, fibrinogen levels fell to 168 mg/dl compared to 235 mg/dl in the control limbs ($p < .005$), antithrombin III levels dropped to 95% compared to 119% ($p < .05$), and fibrin split products increased to 19.4 μg/ml compared to 7.7 μg/ml ($p < .025$) in the control limbs. (Table 2, Group A). Mean platelet counts decreased by 20% in the ischemic limbs, but they were not significantly lower than those measured prior to tourniquet ischemia in either leg, or to those measured in the control limbs after tourniquet ischemia. Within five minutes after tourniquet deflation, all parameters had returned to normal. Hematocrits and bleeding times remained stable throughout the course of the experiment.

In animals receiving heparin, no significant changes occurred in fibrinogen or antithrombin III levels when compared to the control limbs (Table 2, Group B). However, fibrin split products remained elevated during tourniquet ischemia. Five minutes following tourniquet deflation, fibrin split products returned to control levels.

Comparison of the coagulation studies from the two sets of experimental limbs revealed that blood from

TABLE 2. *Coagulation Studies Following Tourniquet Ischemia*

	Control Limb	Experimental Limb After 2½ Hours of Ischemia	p Value	Experimental Limb 5 Minutes After Tourniquet Deflation
		Nonheparinized Group A		
Fibrinogen (mg/dl)	235 ± 14 (10)	168 ± 13.8 (8)	<.005	234 ± 13.1 (8)
Fibrin Split Products (μg/ml)	7.7 ± 0.7 (10)	19.4 ± 4.9 (10)	<.025	7.5 ± 0.9 (10)
Antithrombin III (%)	119 ± 4.7 (10)	95.5 ± 8.9 (8)	<.05	122 ± 4.6 (8)
		Heparinized Group B		
Fibrinogen (mg/dl)	282 ± 38 (6)	236 ± 28 (8)	NS	226 ± 24.8 (8)
Fibrin Split Products (μg/ml)	7.9 ± 1.0 (6)	19.5 ± 5.4 (8)	<.05	8.1 ± 1.1 (8)
Antithrombin III (%)	128 ± 3.6 (6)	119 ± 5.6 (8)	NS	131 ± 5 (8)

TABLE 3. *Comparison of Experimental Limbs in Nonheparinized Versus Heparinized Animals*

	Nonheparinized Group A	Heparinized Group B	p Value
Fibrinogen (mg/dl)	168 ± 13.8 (8)	236 ± 27.8 (8)	<.02
Fibrin Split Products (μg/ml)	19.5 ± 4.9 (10)	19.5 ± 5.4 (8)	NS
Antithrombin III (%)	95.5 ± 8.9 (8)	119 ± 5.6 (8)	<.01

the heparinized ischemic limbs had a significantly higher fibrinogen level and antithrombin III concentration than blood from nonheparinized limbs (Table 3). No significant differences were noted in the fibrin split products from the two sets of experimental limbs.

Part II (Tables 4 and 5)

To investigate whether these changes were due to intravascular coagulation, fibrinopeptide A levels were measured in a group of nine animals. As can be seen in Table 4, after 2½ hours of tourniquet ischemia, fibrinopeptide A levels were 158 pmoles/ml in the ischemic limbs compared to 14.9 pmoles/ml in the control limb ($p < .02$). Values returned to normal within 30 minutes after release of the tourniquet.

Finally, to confirm that the elevated fibrin split products were due to the activation of plasminogen, plasminogen levels were measured in a group of six animals under similar experimental conditions, except that samples were drawn from the femoral vein proximal to the tourniquet immediately after release of the tourniquet. This modification in technique was necessary because of difficulty obtaining access to superficial veins which become fibrotic with repeated use. As can be seen in Table 5, plasminogen levels were 9.1 nkat units in the experimental limbs compared to 24 nkat units in the control limb ($p < .05$).

Discussion

Potential hazards associated with the use of a pneumatic tourniquet include direct injury to local tissues by compression and injury to tissues distal to the tourniquet because of ischemia, hypoxia and acidosis.[6,14,18–20] Tissue thromboplastin which is released after injury causes activation of the extrinsic system of blood coagulation.[13] Ischemia, hypoxia and acidosis also produce injury to the vascular endothelium and activate the intrinsic system of coagulation as well as the fibrinolytic system.[3,11]

Some authors have demonstrated that a bleeding tendency secondary to tourniquet use develops in rats and dogs after prolonged ischemia.[8,15] Paletta et al. noted that the limbs of dogs treated with heparin prior

TABLE 4. *Fibrinopeptide A Levels in Nonheparinized Animals*

	Control Limb	Experimental Limb	p Value
Preischemia	22 ± 0.2 pmoles/ml		
During ischemia 2½ hours duration	14.9 ± 6.03 (9)	158 ± 52 (9)	<.02
30 minutes after ischemia	7.9 ± 1.7 (8)	15.7 ± 45 (9)	NS
24 hours after ischemia	3.8 ± 2.4 (6)	3.0 ± 1.1 (6)	NS

to and during tourniquet ischemia (600–650 mmHg for five hours) had more rapid "normalization" of skin and muscle temperature, less local edema and did not develop foot drop as did the nonheparinized group.[14] Strock and Majno were unable to find evidence of intravascular coagulation within rat limbs subjected to tourniquet ischemia for hours.[17,18] Eriksson et al. have observed vascular thrombosis within the vasculature of the tenuissimus muscle after six hours of tourniquet ischemia to the hind limb of a cat.[2] Wilgis reported that, after 1½ hours of tourniquet ischemia in human beings, prothrombin times in blood samples drawn from ischemic limbs were 70–75% normal and partial thromboplastin times were prolonged.[19,20]

Our studies clearly indicate that during tourniquet ischemia (400 mgHg for 2½ hours) intravascular coagulation develops within the ischemic limbs of subhuman primates. Significant decreases in fibrinogen and antithrombin III levels prevented by heparin support this conclusion, despite the fact that the platelet counts, while lower, were not significantly less than in control limbs.

Further credence for the diagnosis of consumptive coagulopathy is lent by the markedly elevated fibrinopeptide A levels measured in the experimental limbs after 2½ hours of tourniquet ischemia. Fibrinopeptide A is released when thrombin cleaves the Aα chain of fibrinogen.[10] Thus as a direct index of thrombin action, it is a highly specific and extremely sensitive test for intravascular coagulation.

The significant decrease in plasminogen and the rise in fibrin split products is evidence that plasminogen activation was accompanying intravascular coagulation, as expected. However, intravascular coagulation

TABLE 5. *Plasminogen Levels after 2.5 Hours Tourniquet Ischemia*

	Control Limb	Experimental Limb†	p Value
Plasminogen*	24 ± 5 (6)	9.1 ± 1.8 (6)	<.05

* Normal values 18.6 ± 1.7 nkat units, n = 8.

† Specimens drawn from femoral vein proximal to tourniquet immediately after release of tourniquet in 5 of 6 animals.

with secondary fibrinolysis does not explain the increase in fibrin split products which was observed in the heparinized animals. It is possible that, in response to severe anoxic stimuli, plasminogen activator was released from damaged endothelial cells in sufficient quantities to overwhelm the normal inhibitory mechanisms, causing hyperplasminemia and proteolysis of circulating fibrinogen (*i.e.* primary fibrinolysis). Since plasmin levels were not measured, we cannot exclude this possibility. However, the insignificant change in fibrinogen suggests that primary fibrinolysis was not the cause of the elevated split products in the heparinized animals. A more likely explanation is that nonplasmin fibrinolytic activies were also operative. These may include proteinases released by polymorphonuclear leukocytes, as well as other nonplasmin proteinases.[7]

The rapid return of fibrinogen, antithrombin III and fibrin split products to normal levels five minutes after tourniquet deflation is most likely due to the ingress of clotting factors from the systemic circulation, dilution of the fibrin split products, and removal of these products by the now-patent and functional venous system.

An alternative explanation for the decrease in platelet counts, fibrinogen, and antithrombin III levels, which must be considered is whether hemorrhage or hemodilution occurred during the course of the experiment.[4] These possibilities are not likely because the hematocrit remained stable, no bleeding occurred other than during removal of blood samples (total volume was 30 cc), and no intravenous fluids which might have produced hemodilution were administered. Moreover, as stated previously, thrombin activity as measured by FPA generation was specifically increased.

Caution should be exercised in equating the coagulation abnormalities which developed after tourniquet ischemia to clinical bleeding since the observed changes in fibrinogen and platelet counts were not of a magnitude which would be expected to impair hemostasis. However, in association with ischemic changes and vasodilutation, bleeding might well be potentiated by consumption and fibrinolysis of this magnitude.

We conclude that intravascular coagulation and fibrinolysis manifested by a significant fall in fibrinogen, antithrombin III, and plasminogen levels, accompanied by a significant increase in fibrin split products and fibrinopeptide A levels develops within ischemic subhuman primate limbs during tourniquet ischemia. Pretreatment with heparin prevents the consumption of fibrinogen and antithrombin III but does not prevent the elevation of fibrin split products which was observed. It is possible that these changes in association with local ischemic changes may contribute to post tourniquet bleeding.

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