Inhibition of Feline Collateral Vessel Development following Experimental Thrombic Occlusion


SUMMARY We compared development of feline hindlimb collateral circulation after acute occlusion of the terminal aorta by ligation, thrombus formation, and formation of a "closed" aortic loop containing thromboplastin. Collateral circulation development was assessed by aortograms, scintillation scans, neurological signs following occlusion, measurement of hindlimb muscle blood flow, and forelimb and hindlimb temperature. In cats in which aortic occlusion was the result of ligation or thromboplastin in the aortic loop, paralysis was not evident. Aortograms and scintillation scans indicated hindlimb blood flow. Both muscle temperature and blood flow data indicated that the return of blood flow was rapid. The 5th lumbar artery appears to be the origin of the collateral vessels. The midzone component is a dorsal and ventral vertebral route and an epaxial muscle route. The reentry components are the 6th or 7th lumbar arteries. The collateral vessels arise from preexisting collateral vessels. Of those cats in which aortic occlusion was the result of a thrombus, all exhibited paralysis. Aortograms, scintillation scans, muscle temperature, and hindlimb blood flow data indicated reduced hindlimb blood flow. The results suggest that the thrombus has an inhibitory effect on the development of collateral circulation.

OUR UNDERSTANDING of control mechanisms in the peripheral circulation has advanced significantly over the last half century. It has been well documented that following ligation of a peripheral artery, a significant collateral vasculature develops to restore tissue perfusion. The mechanisms involved in initiating, promoting, and modifying collateral development following acute arterial ligation have been investigated extensively. In distinct contrast, there have been few reports concerning collateral development following thrombic occlusion. In 1926 Lewis and Reichert reported that a human patient with severe pain and ulceration of the heel, as a result of a femoral thrombus, became asymptomatic following acute permanent ligation of the artery and removal of the thrombus. Their results suggest that collateral blood flow was reduced while the thrombus was present. The reduction of collateral vessel development by thrombic occlusion is further supported in a study by Imhoff on the feline aorta. Imhoff reported that acute ligation of the terminal feline aorta produced only transient abnormalities, whereas occlusion by an experimentally produced blood clot resulted in paralysis, lack of a femoral pulse, and cold rear extremities. Aortograms indicated significant collateral blood flow in the cats occluded by ligation, and poor hindlimb perfusion in the cats occluded by experimental thrombosis.

This feline model may provide an excellent source of information concerning events which influence development of collateral circulation after thrombic occlusion. There are, however, several problems associated with Imhoff's study which require clarification. Since the origin of the potential collateral vessels was not determined, it is not known whether the thrombus may have "grown" beyond its initial size and occluded potential collateral vessels, or whether small portions of the thrombus may have dislodged, thereby occluding potential collateral vessels. In addition, ligation of the 6th lumbar artery, which arises from the region of the aorta occluded both by the ligations and the clot, was not mentioned. It is possible that this artery was a part of the collateral system and could have been occluded by the methods used to produce the clot (i.e., injection of bovine thromboplastin). It is also possible that the collateral system may have been occluded by excess thrombin "leakage" during or after clot formation.

This study was undertaken to characterize further the events that follow both experimental thrombic occlusion and acute ligation of the feline aorta.

Methods

Thirty-two mixed breed cats weighing 3-5 kg were used in this study. To occlude the aorta, either by a thrombus or by ligation, the cats were anesthetized with thiamylal sodium (20 mg/kg, iv) and maintained in surgical anesthesia with 2% halothane. A laparotomy was performed and the caudal portion of the aorta was exposed from the mesenteric artery to the iliac trifurcation.

In a group of 16 cats the caudal aorta was acutely ligated. The aorta was ligated, using 3-0 silk, approximately 5 mm distal to the origin of the caudal mesenteric artery. The left deep circumflex iliaca and 6th lumbar arteries then were ligated. Ligatures also were placed around the aorta at the level of the trifurcation and around the right deep circumflex iliaca artery. These ligatures were tightened after blood had been removed from the aorta either by digital evacuation (three cats) or by flushing the aorta with saline solution, 37°C (13 cats). The aorta was flushed with saline by placing a 26-gauge needle in the aorta at the trifurcation and tightening the ligature. After the saline injection the right
deep circumflex iliac artery was ligated. The aortic ligature at the trifurcation was tightened as the needle was withdrawn. These ligations produced a 1.5-cm obstruction of the aorta that was free of blood (Fig. 1A).

Acute experimental thrombolic occlusion of the aorta was produced in a group of 12 cats. The aorta was temporarily occluded approximately 5 mm distal to the origin of the caudal mesenteric artery. The deep circumflex iliac and 6th lumbar arteries were acutely ligated, as was the aorta at the level of the trifurcation, after a 26-gauge needle had been positioned in the aorta (Fig. 1B). The temporary ligature then was slowly released and, after blood had entered the region, the aorta was again occluded. Between 10 and 20 units of thromboplastin (100 U/ml) were injected into the aorta to produce a clot. The temporary ligature and needle were removed 5 minutes after the thromboplastin injection (Fig. 1B).

In addition, three cats served as thromboplastin controls. Ligatures were placed in a manner similar to that used for the group with acute ligations. A 26-gauge needle was inserted into the aorta at the aortic trifurcation and the ligature was tightened around the needle. The aorta was flushed free of blood with saline, the saline digitally evacuated, and the right circumflex iliac artery ligated. Between 10 and 20 units of thromboplastin were injected into the aorta and the needle was removed from the aorta as the ligature was tightened. These procedures produced a "closed section" of the aorta approximately 1.5 cm in length which contained thromboplastin.

After surgery the cats were housed in individual cages for 3 days, at which time the experiment was terminated. Food and water were provided ad libitum. The ambient temperatures in the cages varied from 25°C to 27°C.

A neurological examination of all the cats was made immediately before surgery and at 24-hour intervals thereafter over the 3-day experimental period. The examination was based on the evaluation of the patellar tendon tap, flexor reflex (withdrawal), thigh adduction, digit extension, hindlimb weight bearing, locomotive ability, and cutaneous sensation. Each parameter was rated on a scale of 0 to 6. A 0 rating indicated absence of the parameter, while a 6 rating indicated a response equivalent to that obtained before the occlusion. The rating 3 days after occlusion is the mean rating of all evaluated parameters for both hindlimbs.

In seven of the cats with acute aortic ligation and in six of the cats with thrombolic occlusion, the temperatures of the hindlimb muscle and forelimb muscle were recorded immediately before occlusion and at 6, 12, 24, and 72 hours after occlusion. The temperature was measured with a Yellow Springs Instrument Co. recording telemeter (model 44-A) and thermistor probe (no. 520).

Hindlimb muscle blood flow was determined by the hydrogen electrode method in five of the cats with acute ligation, in five with thrombolic occlusion, and in three thromboplastin controls. In the first two groups blood flow was measured immediately before, immediately after, and 72 hours after occlusion.

Aortograms of each cat were made 3 days after occlusion of the aorta. The cat were anesthetized with pentobarbital sodium (25 mg/kg, iv) and aortograms were obtained by injection of 50% sodium diatrizoate (Hypaque) via a 20-gauge needle positioned into the left ventricle. In addition, in four cats with acute ligation and in two with thrombolic occlusion radiopaque latex was injected either into the aorta above the renal arteries or into the left femoral artery (retrograde injection) at 180 mm Hg pressure. After the aortograms were obtained a careful dissection was made to determine the origin, midzone, and reentry of the collateral vessels.

A whole body radioisotope scintillation scan was performed on one cat with acute aortic ligations, one cat with an aortic thrombus, and on one nonoperated cat. Each cat was anesthetized with pentobarbital sodium (25 mg/kg, iv), and technetium-labeled microspheres (5 mCi) were injected into the left ventricle via a 20-gauge needle. Following radioisotope injection scintillation scans were obtained with a gamma camera.

A careful gross postmortem examination of all cats not injected with latex was made. Special emphasis was placed on determining the location of the thrombus, the degree of embolization of the arteries arising from the aorta cranial or caudal to the thrombus, and whether or not the femoral artery or vein was thrombosed.

All statistical analyses was performed with Student's pool t, with significance taken at ≤0.05.

Results

On recovery from surgical anesthesia, cats with acute aortic ligation and thromboplastin injection withdrew and patellar tendon reflexes that were weaker than the presurgical reflexes. An ataxic hindlimb gait was evident in every cat in this group. There appeared to be muscular weakness and the hindlimbs tended to sag and, at times, collapse. All hindlimb reflexes steadily improved and, as shown in Figure 2, the cats exhibited minimal deficits 72 hours after occlusion. The major deficiencies at this time were dorsoflexion of the paws and weakness in weight bearing. In 15 of the 19 cats these deficiencies were minimal.

The neurological rating of the cats with thrombolic occlusion was severely depressed (Fig. 2). After the cats had recovered from anesthesia, all of the parameters were either severely depressed or absent. Over the 3-day observation period, the neurological rating either remained at the low level or became more depressed. Only seven cats exhibited...
some neurological function in the hindlimbs at the time the experiment was terminated. In these cats an extremely weak tendon tap and flexor reflex as well as some weight-bearing ability and cutaneous pain sensation above the patella were observed.

At 6 hours after acute aortic ligation the temperature of the hindlimb muscle was decreased from the preligation temperature of 36 ± 0.6°C to 34 ± 0.9°C while the forelimb muscle temperature increased from 36 ± 0.5°C to 38 ± 0.5°C (Fig. 3). The forelimb temperature remained relatively constant over the 72-hour period. At 24 hours after occlusion the hindlimb temperature had increased to a value which was not significantly different from that recorded presurgically, and it remained constant over the experimental period.

In the cats with thrombotic occlusion, hindlimb muscle temperature decreased from a preocclusion value of 35 ± 0.5°C to 31.9 ± 0.5°C at 6 hours after occlusion (Fig. 4). After a slight increase 24 hours after occlusion, temperature decreased further to 30.0 ± 2.0°C at 72 hours. This value was significantly less than the presurgical control temperature. The forelimb muscle temperature remained relatively constant over the 3-day experimental period (Fig. 4).

Immediately following acute aortic occlusion there was a significant decrease in blood flow to the hindlimb muscle in both the acutely ligated cats and those with the thrombotic occlusion (Fig. 5). This observation is in agreement with the data provided by measurements of hindlimb muscle temperature. At 72 hours after acute aortic ligation the hindlimb
muscle blood flow had returned to $90 \pm 18\%$ of preocclusion control values (Fig. 5). There was not a significant difference in blood flow between the cats in which the blood was removed from the aorta by digital expression and those in which the aorta was flushed with saline. Three days after occlusion in the thromboplastin control group, blood flow was found to be $102 \pm 17\%$ of preocclusion control values.

In distinct contrast, in the cats with thrombolic occlusion blood flow decreased from $31 \pm 6\%$ of preocclusion values immediately after occlusion to $23 \pm 11\%$ of preocclusion control flow 72 hours after occlusion (Fig. 5).

The blood distribution pattern of the hindlimbs of a nonoccluded, thrombus-occluded, and a ligation-occluded cat is shown in the scintillation scan (Fig. 6). The scan indicates a severely reduced blood supply to the hindlimbs following thrombolic occlusion. In the cat in which the aorta...
was occluded by ligation, there appears to be a reduced blood supply to the distal portion of the hindlimb while the more proximal portions appear to be normally perfused.

The direction of filling caudal to the acutely ligated aorta, as ascertained by serial aortograms, is shown in Figure 7. This aortogram also indicates dilation of the 5th and 7th lumbar arteries. The apparent enlargement of the caudal lumbar arteries and the direction of filling suggests that the lumbar arteries are important vessels in the collateral system. This suggestion is supported by postmortem aortograms with radiopaque latex as the contrast medium (Fig. 8). Small vessels which appear to originate from the 5th lumbar artery can be observed to span the junction of the 6th and 7th lumbar vertebrae within the epaxial musculature. Postmortem dissections confirmed the existence of the epaxial collateral vessels observed in the aortograms. Furthermore, filling of the aorta cranial to the occlusion following retrograde injection of latex was observed to occur via the 5th lumbar artery. In addition to the lumbar collateral system, on dissection anastomoses between the cranial superficial epigastric and caudal superficial epigastric arteries and between the urethral and umbilical artery were noted.

An aortogram of a cat with thrombolic occlusion is shown in Figure 9. The aorta was occluded by the thrombus from the aortic trifurcation to just distal to the caudal mesenteric artery. The thrombus apparently had not increased in size and the origins of the caudal mesenteric artery and 5th lumbar artery were patent. Although the 5th lumbar artery appeared to be increased in size, the 7th lumbar artery did not appear enlarged.

In thrombus-occluded cats injected with radiopaque latex

![Figure 8: Postmortem aortogram taken 3 days after acute aortic ligation. Arrow indicates the presence of small collateral vessels in the epaxial muscles which connect the 5th and 7th lumbar arteries.](http://circres.ahajournals.org/)}
either cranial or caudal to the occlusion, very little opaque material was found to cross the area of occlusion. Epaxial collateral vessels were not observed either radiographically or by dissection. The origin and major branches of the 5th and 7th lumbar arteries would fill, but anastomoses between the two were poorly developed.

Postmortem examination of the thrombus-occluded cats, not injected with latex, indicated that the thrombus had not significantly increased in size. The cranial end of the thrombus had a morphologically white appearance. The origin and branches of the 5th lumbar arteries and caudal mesenteric artery were patent, as were the femoral arteries and veins. Three cats died within 36 hours after the operation and were not included in the study. In the postmortem examination of these cats a thrombus was found to extend up and stop between the caudal mesenteric and the renal arteries. Also, severe necrosis of the hindlimbs, rectum, and portion of the colon and bladder was observed.

Discussion

The rapid restoration of lower limb function following acute ligation of the terminal aorta is comparable to the findings of other investigators following peripheral arterial ligation. Our studies demonstrate that there is substantial hindlimb perfusion 72 hours after acute ligation. The absence of chronic neurological deficits demonstrates that this blood supply is adequate to maintain hindlimb neuronal function. Aortographic and postmortem observations that the terminal aorta was occluded indicate that hindlimb muscle blood flow following aortic ligation originated from collateral vessels.
It is well documented that collateral vessels are functional almost immediately after acute ligation of a peripheral artery, suggesting that blood flow through preexisting collateral vessels is important if adequate perfusion distal to the occlusion is to occur. The restoration of muscle temperature within 24 hours and blood flow by 72 hours indicates that hindlimb perfusion approaches preocclusion values too rapidly to be accounted for by growth of new vessels. Although muscle temperature is altered by parameters other than blood flow, hindlimb muscle temperature may be an adequate reflection of blood flow. This is especially true in our present study, in which forelimb thrombus-occluded cats to develop an adequate collateral system appears to be a major collateral channel. Longland divided collateral vessels into stem, reentrant, and midzone components. In the lumbar collateral system, the primary stem or origin vessel appears to be the 5th lumbar arteries. The midzone component consists of a dorsal and ventral vertebral route and an epaxial muscle route. The reentry components are branches of either the 6th or 7th lumbar arteries.

Our results demonstrate that after acute thrombolic occlusion of the terminal feline aorta, collateral flow does not increase to ensure adequate tissue perfusion. If it is assumed that the cats with thrombolic occlusion have the same potential for collateral development as the acutely ligated cats, then it must be concluded that either the thrombus or the procedures used to produce the thrombus are responsible for the inadequate collateral flow. The ligation of the 6th lumbar arteries in our preparation and the restoration of proximal hindlimb blood flow following injection of thromboplastin into the closed aortic loop suggests that direct entry of thromboplastin into the 6th lumbar arteries, or thromboplastin leakage from the aorta, cannot account for the reduction in collateral development following thrombolic occlusion. The absence of thromboemboli near the origin of major branches of either the 5th lumbar arteries or caudal mesenteric artery, the lack of significant thrombus "growth," and the relatively small amount of thromboplastin used suggest that the possible release of excess thromboplastin when the anterior ligature was removed after 5 minutes cannot account for the effects observed. These data would suggest that the inability of the thrombus-occluded cats to develop an adequate collateral circulation is not a consequence of procedures used to produce the thrombus.

Miles et al. have provided experimental evidence for clot fragmentation. Fragmentation of a clot and transportation of the fragments into smaller arteries has been used to explain some cases of pseudoembolism in man. In experimental thrombolic occlusion, the ligation at the aortic trifurcation is occlusive. If there is clot fragmentation, for the emboli to alter collateral flow they must be transported cranially in the aorta to occlude the 5th lumbar arteries. The patenty of the 5th lumbar arteries and the absence of thromboemboli near the origin or branches of the 5th lumbar arteries suggest that the effects observed are not the result of clot embolization.

It also is unlikely that the thrombus increased in size in the 12 cats with thrombolic occlusion, because neither aortograms nor postmortem examinations indicated significant anterogrowth of the thrombus. In addition, if the 5th lumbar artery were occluded, the caudal mesenteric artery would also have been occluded, thereby causing intestinal ischemia. The intestine is extremely sensitive to ischemia, and necrosis quickly develops. Severe necrosis of the hindlimbs, rectum, and portions of the bladder and colon were observed only in the three cats that died 36 hours after experimental thrombolic occlusion. The experimental procedures, mechanical occlusion of the potential collaterals by thrombus "growth," or embolization do not appear to account for the inhibited collateralization. The marked differences in collateral flow between the cats with thrombolic occlusion and the acutely ligated cats provides strong support to the suggestion that the effects of an arterial thrombus may not be attributed entirely to mechanical vascular blockage.

There are several possible explanations to account for the reduced collateral flow. These include a decreased pressure gradient across the collateral systems and a reduction in the size of the lumen of collateral vessels. A reduction in lumen size could be accomplished by a neurogenic or humorally mediated increase in tone of the collateral vessel wall, by aggregation of formed blood elements, or by induced endothelial cell attenuation.

Neurogenic control of collateral vessels is poorly understood. Coffman has reported that collateral vessels show only a limited response to norepinephrine. A clinical study by Nielsen et al. of patients with occlusive arterial disease indicated that sympathetic tone was present primarily in the peripheral vascular bed rather than in collateral vessels. Although their studies indicate that there is a limited sympathetic control of collateral vessels, some alteration of neurogenic control cannot be excluded as a partial explanation of the our findings.

Platelets, a major constituent of natural arterial thrombi, can release or form many potent substances which could profoundly affect collateral flow. For example, platelets have been shown to release ADP and serotonin during clotting. Since these substances will aggregate feline platelets, it is possible that in the midzone region of collateral vessels the lumen is obstructed by platelet aggregates. Serotonin also has been shown to increase capillary permeability and to cause structural endothelial damage, as has prostaglandin E, which is produced by platelets in large amounts following thrombin treatment. These vasoactive agents may inhibit collateral flow by sufficiently damaging potential collateral vessels to render them nonfunctional. Platelets also can produce and release potent vasoconstrictor substances such as serotonin and prostaglandins during clotting. Although it is probable that vasoconstrictor substances are formed and that feline collateral vessels can react to these substances, any humorally mediated vasoconstrictor explanation must...
account for the prolonged reduction in hindlimb perfusion observed. Collateral flow may depend on the chemical environment in the collateral system or distal to it, or both.** It therefore is possible that a transient, humorally mediated vasoconstriction influences the delicate balance of local factors necessary for collateral vessel development. Collateral flow also has been shown to be governed by intravascular pressure differences throughout the circulation.** Lymph draining from limbs injured by several methods, including ischemia, contains significant kinin-forming activity.** It would be expected that such a situation would exist in the cats we have studied and that transient vasoconstriction would increase this activity. Since bradykinin is one of the most potent dilator substances known, formation of significant amounts within the circulation may lead to hypotension and reduced collateral flow.

If chemical substance(s) reduce collateral flow they must be present in effective amounts in the collateral systems. The lumbar collateral system is in close proximity to the clot. It therefore appears that in an important collateral system it is possible for a substance(s) to be in relatively high concentration as dilution and catabolism effects would be minimized. In addition, the morphology of the cranial end of the clot suggests that if the substance(s) was of platelet origin the concentration in the region of the clot, hence the lumbar collateral system, could be high.

The explanations that have been offered to account for the reduced collateral flow implicate platelets. Studies currently are in progress to ascertain whether platelets not only aggregate to obstruct the lumen of an artery but in certain circumstances initiate a mechanism(s) which inhibits collateral flow to the ischemic area.

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