Influence of Factor VIII/von Willebrand Factor Protein (F VIII/vWF) and F VIII/vWF-Poor Cryoprecipitate on Post-Ischemic Microvascular Reperfusion in the Central Nervous System

J. M. Hallenbeck, M.D., T. W. Furlow, Jr., M.D., and H. R. Gralnick, M.D.

SUMMARY To test the hypothesis that plasma contains native constituents capable of impairing microcirculatory flow in zones of acute ischemic tissue damage, we performed 14C-antipyrene autoradiographic blood flow studies in splenectomized dogs subjected to 35 min of cerebrospinal fluid compression ischemia followed by 30 min of recirculation to the neuraxis. The animals were anticoagulated with heparin and were divided into 4 groups by exposure to various measures before induction of ischemia. Groups 1 and 2 served for comparison with the other groups and underwent, respectively, no glass-wool filtration and glass-wool filtration via an arteriovenous shunt. Post-ischemic brain blood flows in Group 1 were low and focal zones of greatly impaired reperfusion were present. In Group 2, post-ischemic brain blood flows were high and focal perfusion impairment did not occur. Group 3 received homologous purified factor VIII/von Willebrand factor protein (F VIII/vWF) after glass-wool filtration but before induction of ischemia; Group 4 received F VIII/vWF-poor cryoprecipitate at the same time point. The purpose of administering the plasma preparations was to check for the presence of activity that nullified the enhancement of post-ischemic reperfusion expected after exposure to glass-wool. The results indicate that activity deleterious to post-ischemic reperfusion primarily resides in the F VIII/vWF fraction of cryoprecipitate. The F VIII/vWF-poor cryoprecipitate infusate, containing 250 to 800-fold more protein than the F VIII/vWF fraction, produced an intermediate reduction of blood flow.

Methods

Nineteen male mongrel dogs that had been splenectomized 2–3 weeks previously to prevent autotransfusion of sequestered blood were divided into 4 groups as outlined in table 1. The groups were entered into a previously described protocol1,2 that may be conveniently divided into 6 stages: 1) preparation of the animal; 2) observation of animals in group 1 and glass-wool filtration of the blood in animals in groups 2–4; 3) infusion of a plasma fraction into dogs in groups 3 and 4; 4) compression ischemia of the CNS for 35 min; 5) post-ischemic recirculation of the CNS for 30 min; and 6) an autoradiographic blood flow study in the final minute. Arterial blood pressure was measured at 2-min intervals in the femoral artery by an arterial line to a pressure transducer and an oscillograph. Arterial blood pressure was selected to maintain mean arterial pressure equal to mean arterial pressure during the ischemic interval.2 Both mean arterial pressure and central venous pressure were recorded at the time of reperfusion. Arterial blood pressure was measured by blood pressure catheters placed in the femoral artery and draining into a Travenol pressure transducer. Arterial blood pressure was selected to maintain mean arterial pressure equal to mean arterial pressure during the ischemic interval.2

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Naval Medical Research and Development Command, Work Unit No. M0099.PN001.1159. The opinions and assertions contained herein are the private ones of the writers and are not to be construed as official or reflecting the views of the Navy Department or the Naval Service at large. The experiments reported herein were conducted according to the principles set forth in the "Guide for the Care and Use of Laboratory Animals," Institute of Laboratory Animal Resources, National Research Council, DHEW, Pub. No. (NIH) 78-23.

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TABLE 1. Summary of Experimental Groups of Dogs

<table>
<thead>
<tr>
<th>Group</th>
<th>No. animals</th>
<th>Glass-wool filtration</th>
<th>Stage 3 infusate</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>6</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>2</td>
<td>6</td>
<td>+</td>
<td>None</td>
</tr>
<tr>
<td>3</td>
<td>4</td>
<td>+</td>
<td>F VIII/vWF-rich fraction of cryoprecipitate*</td>
</tr>
<tr>
<td>4</td>
<td>3</td>
<td>+</td>
<td>V VIII/vWF-poor fraction of cryoprecipitate*</td>
</tr>
</tbody>
</table>

*The fractions were obtained by gel chromatography of cryoprecipitate. The words rich and poor are used quantitatively because virtually no F VIII/vWF protein was detected in the "poor" fraction and the F VIII/vWF protein was the only protein detected in the "rich" fraction.

continuously monitored and blood gases were periodically measured during each experiment.

Stage 1 (Animal Preparation)

Each animal was anesthetized with sodium pentobarbital 25-30 mg/kg and mechanically ventilated through an endotracheal tube. End-tidal PCO₂ was continuously monitored, and core temperature was maintained at 38°C with heating pads and infrared light. The femoral arteries and veins were catheterized bilaterally to permit 1) monitoring of hematocrit, platelets, arterial blood gases, and arterial blood pressure; 2) infusing saline, drugs, and plasma fractions; and 3) arteriovenous shunting of blood through glass-wool.

Stage 2 (Observation or Glass-Wool Filtration for 1 Hour)

All animals were anticoagulated with heparin 300 units/kg intravenously. Those in Group 1 were observed for 60 min with careful monitoring of aortic blood pressure, temperature, blood gases, and blood pH. Animals in Groups 2-4 underwent glass-wool filtration.

Glass-wool filters were fashioned from the filter housing of a Fenwal® blood recipient set that was packed loosely with washed Pyrex® glass-wool after removal of the original Dacron mesh. After heparinization, the animal's blood was shunted for 20 min at a time through each of 3 glass-wool filters successively inserted between femoral arterial and venous catheters (total filtration time, 60 min).

Stage 3 (Infusion of a Plasma Fraction)

A plasma fraction prepared as described below was infused intravenously over 5 min into animals in Groups 3 and 4 after glass-wool filtration. The volume of this infusate varied between 50 and 90 ml. Groups 1 and 2 received no infusion.

Stage 4 (Compression Ischemia)

Stages 4, 5, and 6 were identical for all groups. A spinal needle was placed percutaneously into the cisterna magna and connected to a line containing Elliott's solution B tween warmed to 38°C. Global ischemia of the CNS which may be regarded as complete was induced by raising the bottle of artificial CSF until hydrostatic pressure in the cisternal line equalled the mean arterial blood pressure. Details of the CSF compression ischemia procedure have been published previously.

Stage 5 (Post-ischemic Recirculation)

After 35 minutes of compression ischemia, the CSF pressure was lowered to 10 mm Hg (136 mm H₂O). Systolic blood pressure was kept at 110 to 120 mm Hg during 30-min recirculation to the neuraxis.

Stage 6 (Blood Flow Study)

During the final 60 sec of each experiment, an autoradiographic blood flow study was carried out. This assay required constant intravenous infusion of a total dose of 100 μCi/Kg of ¹⁴C-antipyrine for 1 min with serial sampling of arterial blood repeated at 4- to 6-sec intervals. The procedure was terminated by inducing cardiac arrest with a bolus injection of 20 cc of saturated potassium chloride solution through the right femoral vein catheter into the right ventricle. The brain, spinal cord, and heart were removed, frozen in liquid Freon® maintained at −50 to −60 °C by suspension over liquid nitrogen, and later cut into 20-micrometer sections. The tissue concentration of the isotope was determined autoradiographically. Local blood flow was calculated from the formula:

\[ C_b(T) = \frac{\lambda k_i C_a e^{-k_i(T-t)}}{1 + \lambda} dt \]

where \( C_b(T) \) is the concentration of tracer substance in the tissue at time T; \( \lambda \) is the tissue-blood partition coefficient for the tracer material; \( k_i \) is the rate of blood flow per unit weight of tissue multiplied by the reciprocal of the partition coefficient for that tissue; and \( C_a \) is the concentration of tracer substance in the arterial blood.

Preparation of Plasma Fractions

Two plasma fractions were prepared with sterile technique for infusion into Groups 3 and 4 during Stage 3 of the protocol. The 2 fractions were isolated from homologous plasma cryoprecipitate and characterized as a F VIII/vWF-rich fraction and a F VIII/vWF-poor fraction. For preparation of homologous cryoprecipitate, 1,000 ml of whole blood, the average estimated blood volume of the dogs, was collected from 4 donors in 250 ml Fenwal® blood-packs containing 35 ml of citrate-phosphate-dextrose solution (114 mg citric acid hydrous USP, 921 mg sodium citrate hydrous USP, 77.7 mg sodium biphosphate USP, 893 mg dextrose hydrous USP). The whole blood was spun at 1600 X G for 15 min at 25°C. The supernatant plasma was recovered and spun again at 4000 X G for 15 min at 25°C. The supernatant was recovered and spun again at 18,000 X G for 60 min at 25°C. The cell-free superna-
The factor VIII/von Willebrand factor protein was purified from cryoprecipitate by precipitation with polyethylene glycol and chromatographic separation on Sepharose 4B as previously described. The column fractions were monitored for protein and procoagulant VIII activity. The initial peak of protein appearing at the void volume was pooled and concentrated either with ammonium sulfate or by Ficoll as previously described. In some experiments the void volume fractions were not concentrated and were used as they came from the column. All void volume fractions were handled in sterile plastic and sterile pipettes. No additives were used to insure sterility. In some instances the void volume and other fractions (see below) were frozen and then used later. The other portions of the column were also assayed for activity deleterious to reperfusion. These portions included the chromatographic fractions rich in fibrinogen and the area of the column rich in immunoglobulins. These were detected by appropriate immunologic procedures. These fractions were collected and pooled as described from the void volume fraction; however, in comparing studies of these fractions with void volume fractions, no correction was made for the protein concentration, i.e., approximately 250- to 800-fold increase of protein in the later fractions compared to the void volume fraction. All chromatographic fractions prepared for these animal experiments were analyzed by polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate with and without reducing agents. In addition, immunologic studies including immunodiffusion and immunoelectrophoresis of the concentrated and nonconcentrated fractions were performed for the detection of the factor VIII-and-related antigen, fibrinogen, and cold insoluble globulin.

Results

Physiologic data determined before the blood flow study are displayed in table 2. The number of samples in each group, n, is equal to the number of animals in each group.

Table 3 compares the 4 groups with regard to local blood flow in 16 neuroanatomic areas and heart. Heterogeneous flows within a single neuroanatomic structure were analyzed by averaging the highest and lowest local flows such that blood flow within each structure was represented by a single mean value. There were focal zones of extremely low post-ischemic flow (i.e., 0-20 ml/100 g/min in gray matter; 0-10 ml/100 g/min in white matter) in the non-filtered animals of Group 1. These low-flow areas are believed to correspond to zones of impaired microvascular perfusion noted in other models. In Group 2 animals the rates of reperfusion were much higher and foci of deviant flow were in the direction of reactive hyperemia. Zones of extremely low flow did not occur in this group. The mean local blood flows in Groups 1 and 2 serve as reference standards representing, respectively, states in which the processes leading to post-ischemic microvascular perfusion impairment have progressed unimpeded and states in which such processes have been attenuated or interrupted.

Table 2. Systolic Arterial Blood Pressure and Blood Gas Values Before Blood Flow Study (mean ± SEM)

<table>
<thead>
<tr>
<th></th>
<th>Group 1</th>
<th>Group 2</th>
<th>Group 3</th>
<th>Group 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Systolic BP*</td>
<td>115 ± 2</td>
<td>118 ± 2</td>
<td>117 ± 3</td>
<td>114 ± 1</td>
</tr>
<tr>
<td>pH</td>
<td>7.40 ± 0.03</td>
<td>7.36 ± 0.03</td>
<td>7.34 ± 0.02</td>
<td>7.31 ± 0.02</td>
</tr>
<tr>
<td>Po2*</td>
<td>88 ± 5</td>
<td>101 ± 9</td>
<td>90 ± 3</td>
<td>84 ± 4</td>
</tr>
<tr>
<td>Po2o</td>
<td>30 ± 2</td>
<td>29 ± 2</td>
<td>31 ± 3</td>
<td>33 ± 2</td>
</tr>
</tbody>
</table>

*mm Hg

Table 3. Local Blood Flows in Various Neuroanatomic Areas and Heart in ml per 100 g/min (mean ± SEM)

<table>
<thead>
<tr>
<th></th>
<th>No Infarct</th>
<th>Infarct</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1</td>
<td>Group 2</td>
<td>Group 3</td>
</tr>
<tr>
<td>Anterior centrum ovale</td>
<td>14 ± 3</td>
<td>28 ± 1</td>
</tr>
<tr>
<td>Auditory cortex</td>
<td>46 ± 11</td>
<td>110 ± 13</td>
</tr>
<tr>
<td>Caudate nucleus</td>
<td>54 ± 15</td>
<td>97 ± 9</td>
</tr>
<tr>
<td>Corpus callosum</td>
<td>17 ± 5</td>
<td>32 ± 2</td>
</tr>
<tr>
<td>Hippocampus</td>
<td>34 ± 9</td>
<td>76 ± 12</td>
</tr>
<tr>
<td>Internal capsule</td>
<td>20 ± 5</td>
<td>36 ± 2</td>
</tr>
<tr>
<td>Middle centrum ovale</td>
<td>11 ± 4</td>
<td>31 ± 1</td>
</tr>
<tr>
<td>Optic radiations</td>
<td>14 ± 3</td>
<td>31 ± 1</td>
</tr>
<tr>
<td>Sensorimotor cortex</td>
<td>42 ± 6</td>
<td>115 ± 15</td>
</tr>
<tr>
<td>Thalamus</td>
<td>55 ± 12</td>
<td>108 ± 9</td>
</tr>
<tr>
<td>Visual cortex</td>
<td>43 ± 9</td>
<td>101 ± 15</td>
</tr>
<tr>
<td>Posterior-middle cerebral watershed cortex</td>
<td>18 ± 6</td>
<td>109 ± 16</td>
</tr>
<tr>
<td>Anterior-middle cerebral watershed cortex</td>
<td>32 ± 6</td>
<td>96 ± 12</td>
</tr>
<tr>
<td>Anterior association cortex</td>
<td>38 ± 6</td>
<td>98 ± 18</td>
</tr>
<tr>
<td>Heart</td>
<td>248 ± 80</td>
<td>211 ± 32</td>
</tr>
<tr>
<td>Spinal cord gray</td>
<td>53 ± 13</td>
<td>29 ± 4</td>
</tr>
<tr>
<td>Spinal cord white</td>
<td>19 ± 2</td>
<td>16 ± 3</td>
</tr>
</tbody>
</table>

*Significance of difference between Groups 2 and 3 by Student's t-test.
†The p-value less than 0.05 for the difference between Groups 3 and 4 by Student's t-test.
parison of flow values in Group 3 with those in Group 2 reveals that the infusion of the F VIII/vWF-rich fraction of homologous cryoprecipitate after glass-wool filtration nullifies the reflow-enhancing effect of the glass-wool and reactivates the process leading to impaired microvascular reperfusion. Autoradiograms from Group 3 animals showed areas of extremely low flow (fig.). The mean flow value for each measured brain structure in this group fell far below its counterpart in Group 2.

Infusion into Group 4 animals of column fractions designated F VIII/vWF-poor did not interfere with the glass-wool effect to the same extent as infusion of F VIII/vWF-rich cryoprecipitate into Group 3 animals. Group 4 mean local blood flows in such structures as auditory cortex, sensorimotor cortex, visual cortex, hippocampus, thalamus, posterior-middle cerebral watershed cortex, and anterior-middle cerebral watershed cortex exceeded those of corresponding structures in Group 3 by a margin that attained significance despite the small sample size. Nevertheless, structure-by-structure comparison of mean local blood flow below levels expected after glass-wool filtration did occur in the brains of animals subjected to F VIII/vWF-poor cryoprecipitate infusion.

No focal zones of impaired reperfusion occurred in the spinal cord gray or white matter, and in all animals the hearts were uniformly perfused without evidence of focal flow disruption.

The void volume fraction of the dog cryoprecipitate on Sepharose 4B® could not be distinguished from that of the human material that has already been described. In brief, on polyacrylamide gel electrophoresis the protein in the void volume did not enter the gel. After reduction, there was one band with an approximate molecular weight of 230,000. This band stained positive with PAS as well as with Coomassie. The procoagulant activity of the canine factor VIII/von Willebrand factor protein was approximately 21/2 to 3 times that of the human material; the antigen content measured with the human antifactor VIII/von Willebrand factor antibody was approximately 50% that of normal human material. Neither cold insoluble globulin, fibrinogen or immunoglobulins could be detected in the void volume fraction used for these studies by immunoelctrophoresis or immunodiffusion against appropriate antibodies, although these proteins were detectable in the other chromatographic fractions used in these studies.

Discussion

Some assumptions that underlie the present experiments should be clearly stated. Ischemia of the brain of a duration sufficient to cause tissue damage is viewed as setting into motion a process that leads to progressive impairment of microvascular perfusion. Development of this state of perfusion disruption is regarded as depending upon an interaction between components present in blood and elements in the zone of damaged tissue. Pre-ischemic modification of whole blood by exposure to glass-wool inserted into an arteriovenous shunt somehow reduced the blood's capacity to participate in this interaction. Consequently, the process of microcirculatory shut-down is aborted or attenuated leaving microvascular reperfusion largely unimpaired.

The plasma infusates administered after the glass-wool filtration step contain constituents native to blood in a quantity that is less than the total amount normally present in the recipient animal's plasma. Constituents in a plasma fraction are viewed as having activity deleterious to reperfusion when addition of that fraction nullifies the beneficial effect on reperfusion of pre-ischemic exposure of blood to glass-wool and reactivates the process leading to impaired microvascular perfusion.

To the extent that this theoretical framework is valid, the present results indicate that the void volume from Sepharose 4B® gel chromatography of pooled canine cryoprecipitate contains activity deleterious to
post-ischemic reperfusion. Previous work localized activity deleterious to reperfusion to the cryoprecipitate fraction of whole plasma. The present work further delimits the activity to a mixture of proteins copurifying with factor VIII. Because this fraction does not contain immunoglobulins, the results cannot be ascribed to immunologic incompatibility between pooled homologous donor plasma and the recipient. The same fraction from autologous plasma would be predicted to produce an identical effect. Studies in progress appear to support this.

However, in the case of the F VIII/vWF-poor infusion, the homologous preparation could exert an effect in a recipient that would not be observed with autologous material. This fraction does contain immunoglobulins as well as fibrinogen and cold-insoluble globulin and a 250 to 800 times greater protein concentration than the F VIII/vWF-rich fraction. The question as to whether the interference with reperfusion associated with F VIII/vWF-rich fraction infusion is mediated through platelet aggregation as suggested previously or some other mechanism cannot be answered from the design of the present studies.

Acknowledgment

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References

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