Modification of Acute Focal Ischemia in Rabbits by Poloxamer 188

Harold J. Colbassani, MD, Daniel L. Barrow, MD, Kevin M. Sweeney, MD, Roy A.E. Bakay, MD, Irene J. Check, PhD, and Robert L. Hunter, MD, PhD

We studied the effect of a synthetic copolymer surfactant, poloxamer 188, on cerebral blood flow in a rabbit model of focal cerebral ischemia. Following retro-orbital craniectomy, the parietal branch of the middle cerebral artery was occluded with bipolar current. Cerebral blood flow was measured by the hydrogen clearance technique using platinum-iridium electrodes placed within the parietal cortex. Ten rabbits were infused with 50 mg/kg poloxamer 188 in saline beginning 30 minutes after occlusion; 12 control rabbits received an equal volume of saline. Poloxamer 188 increased blood flow significantly in areas of severe or moderate ischemia but had little effect in areas with mild or no ischemia. The improvement in blood flow could not be accounted for by hemodilution, and the copolymer did not affect blood viscosity at any shear rate from 1 to 100 sec⁻¹. We hypothesize that poloxamer 188 increases circulation in ischemic tissue by inhibiting adhesive interactions among proteins (fibrin and fibrinogen) and cells in the microcirculation. (Stroke 1989;20:1241-1246)

Arterial stenosis, thrombosis, embolization, and vasospasm often reduce regional brain perfusion, resulting in focal cerebral ischemia. This ischemia may produce neurologic deficits that are reversible or irreversible, depending on the depth and duration of ischemia. Numerous studies have suggested that the progression to irreversible damage can be influenced by therapeutic intervention. Therefore, agents that influence cerebral blood flow in pathologic states have potential as therapeutic agents in cerebral vascular disease.

Hemorheologic studies have identified hematocrit and whole-blood viscosity as factors that influence cerebral blood flow. Several clinical reports including the Framingham Study found a correlation between hematocrit and the risk of cerebral infarction. Harrison et al studied patients with occlusive vascular disease and found that the size of the infarct is directly proportional to the hematocrit. Several therapeutic modalities that influence hematocrit and blood viscosity have been evaluated for the treatment of focal cerebral ischemia. Sundt et al demonstrated that lowering the hematocrit by hemodilution before an ischemic event was protective and reduced infarct size. Wood et al found a significant increase in cerebral blood flow in the presence of focal ischemia after hemodilution with low-molecular-weight dextran. Hint pointed out that diluting plasma proteins, notably fibrinogen, by administering low-molecular-weight dextran decreases plasma viscosity at the level of the microcirculation. The increased blood flow following hemodilution results in an increased oxygen carrying capacity of the blood; this effect is maximal at a hematocrit of approximately 30%. Fein measured oxygen availability and found oxygen extraction to be maximal at a hematocrit of 35%.

Strand et al reported a randomized controlled trial of hemodilution in patients with acute ischemic stroke, which revealed significant short- and long-term improvement in the neurologic recovery of patients treated with hemodilution. In an effort to increase the oxygen carrying capacity of blood by means other than hemodilution, perfluorocarbon emulsions prepared as blood substitutes have been used for the treatment of focal cerebral ischemia. Sundt et al demonstrated that lowering the hematocrit by hemodilution before an ischemic event was protective and reduced infarct size. Wood et al found a significant increase in cerebral blood flow in the presence of focal ischemia after hemodilution with low-molecular-weight dextran. Hint pointed out that diluting plasma proteins, notably fibrinogen, by administering low-molecular-weight dextran decreases plasma viscosity at the level of the microcirculation. The increased blood flow following hemodilution results in an increased oxygen carrying capacity of the blood; this effect is maximal at a hematocrit of approximately 30%. Fein measured oxygen availability and found oxygen extraction to be maximal at a hematocrit of 35%.

Poloxamer 188 is currently undergoing Phase I clinical studies as a hemorheologic agent. Poloxamer 188 is a nonionic block polymer surfactant...
composed of a single chain of hydrophobic polyoxypropylene flanked by two chains of hydrophilic polyoxyethylene. In earlier studies, poloxamer 188 by itself was found to produce beneficial effects in several situations in which blood flow was impaired. The surfactant reduced tissue damage in a rabbit model of frostbite, increased survival in dogs with hemorrhagic shock, and maintained electroencephalographic activity in an isolated perfused ischemic brain preparation. Poloxamer 188 was reported to reduce viscosity without hemodilution.

In rabbits, we evaluated the effects of poloxamer 188 on cerebral blood flow in the distribution of the middle cerebral artery, which had been surgically occluded. Our results demonstrate an increase in cerebral blood flow without changes in either hematocrit or viscosity, suggesting a new mechanism of action.

Materials and Methods

We chose rabbits because the internal carotid artery is the predominant supplier of blood to the cerebral hemispheres and because there are few external-to-internal anastomoses. In addition, Meyer et al. have used a rabbit model of cerebral ischemia with excellent reproducibility of ischemic zones following middle cerebral artery occlusion.

Twenty-two New Zealand White rabbits weighing 3.5-4.5 kg were sedated with a mixture of ketamine and xylazine. A peripheral intravenous cannula was placed in the marginal ear vein. A tracheostomy was performed, and the rabbits were anesthetized with 0.5% halothane in 70% N\textsubscript{2}O/30% O\textsubscript{2}. Paralysis was achieved using 1-2 mg/hr pancuronium bromide. The rabbits were placed on a warming blanket to maintain normothermia (38.5-39.0°C). A cutdown was performed in the right groin, and catheters were placed in the femoral artery and vein. Ventilation was adjusted to maintain PO\textsubscript{2} at ≥100 torr and PCO\textsubscript{2} at 35-45 torr. Serum glucose concentration was measured hourly and was corrected with insulin if >200 mg/dl.

Each rabbit’s head was fixed in a stereotactic head frame, and the left hemisphere was exposed via a retro-orbital craniectomy involving the parietal bone just lateral to the superior sagittal sinus, the temporal bone to just below the zygomatic arch, and the frontal bone down to the optic canal. The craniectomy was done using a high-speed drill to prevent compression of the underlying brain.

A dural incision was made, and the middle cerebral artery was identified at the anterior inferior margin of the craniectomy between the frontal and temporal lobes. The main parietal branch of the middle cerebral artery was identified, and two to four platinum-iridium electrodes 25 μm thick were inserted into the parietal cortex in the distribution of this parietal branch as well as into the vascular distribution between the frontal and parietal branches. A silver-silver chloride reference electrode was placed into the temporalis muscle. The exposed cortex was covered with a plastic film to prevent dehydration and surface oxygenation. The electrodes were allowed to stabilize for 1 hour before cerebral blood flow was measured.

Cerebral blood flow was determined by the hydrogen clearance technique following administration of 5% H\textsubscript{2} administered intratracheally. Before starting and after concluding these experiments, aliquots of blood from 10 rabbits were obtained in which to measure hematocrit and whole-blood viscosity; the latter was determined using the Litt-Kron capillary step-response whole-blood viscometer (Philadelphia, Pennsylvania). Cerebral blood flow was determined until values were stable for two consecutive measurements (baseline). At this point the main parietal branch of the middle cerebral artery was occluded using a bipolar current, and cerebral blood flow (initial extent of ischemia) was determined 20 minutes after occlusion. Initial extent of ischemia demonstrated at each electrode was classified as minimal (>40 ml/100 g/min), mild (26-40 ml/100 g/min), moderate (16-25 ml/100 g/min), or severe (0-15 ml/100 g/min).

The first five rabbits were allocated to the control group. Thirty minutes after occlusion, 10 minutes after blood flow determination, the second five rabbits were given a bolus injection of a formulation of poloxamer 188 for intravenous administration (RheothRx, CytRx Corp., Norcross, Georgia) at 0.6 mg/ml blood volume followed by an infusion of 0.6 mg/ml blood volume/hr until the end of the experiment. Poloxamer 188 has also been referred to as Pluronic F68, a trademark of BASF Corp. (Wyandotte, Michigan). Thereafter, rabbits were alternately allocated to control and treatment groups. The 12 control rabbits were given a volume of saline equal to that of poloxamer 188 given to the 10 treated rabbits. Cerebral blood flow was determined 1, 2, 3, and 4 hours after occlusion. The effect of treatment was assessed by calculating the percentage change in blood flow detected by each electrode at various times relative to the initial extent of ischemia. At approximately 6 hours, the rabbits were killed by intravenous injection of euthanasia agent.

Data from each electrode were analyzed separately. Data for groups of rabbits are expressed as mean±SEM. Differences between groups were analyzed using Student’s paired (for hematocrit and viscosity) and unpaired t tests and Fisher’s exact test.

Results

The surgical occlusion of the parietal branch of the middle cerebral artery was complete in all rabbits. Consequently, the extent of ischemia recorded by each electrode depended on its anatomic placement in the brain relative to the vascular supply and the collateral circulation. Three electrodes were placed in the cortex of 17 rabbits, four in three rabbits, and two in two rabbits, for a total of 67 electrodes in 22 rabbits.
FIGURE 1. Graph of effect of poloxamer 188 on cerebral blood flow determined by hydrogen washout technique in 10 rabbits (30 electrodes) with poloxamer 188 (●) and in 12 controls (37 electrodes) treated with saline (○). Baseline blood flow was determined, and parietal branch of middle cerebral artery was occluded with bipolar current at time 0. Initial extent of ischemia was measured at 20 minutes. Treatment was started at 30 minutes, and blood flow was determined over 4 hours. Results are shown as mean±SEM for all electrodes in each group. Differences between control and treated groups are significant at 1, 3, and 4 hours (p=0.03, 0.001, and 0.001, respectively, by Student’s t test).

Baseline blood flow was 75±6 ml/100 g brain tissue/min in the control group and 76±3 ml/100 g brain tissue/min in the treated group. Twenty minutes after occlusion, both groups showed similar initial extents of ischemia (28±2% vs. 27±3% of baseline, control vs. treatment). However, by 30 minutes after the start of treatment (1 hour after occlusion), the groups differed significantly (Figure 1). After 3.5 hours of treatment (4 hours after occlusion), blood flow in the control group remained essentially unchanged (27±2%). In treated rabbits, brain perfusion improved after treatment; by 3.5 hours, blood flow had increased to 39±3 ml/100 g/min, a 69±13% improvement over the initial extent of ischemia. The difference in cerebral blood flow between groups at 4 hours after occlusion was significant (p=0.001, t test).

The extent of ischemia detected by each electrode ranged widely, from a blood flow as low as 4 to one as high as 78 ml/100 g min, as expected from the differences in electrode placement. To examine the relation between the initial extent of ischemia and the degree of improvement with treatment, the percentage change in blood flow at 4 hours was plotted as a function of the initial extent of ischemia (Figure 2). Only 11 of 37 (30%) electrodes in control rabbits (Figure 2, top) compared with 21 of 30 (70%) in treated rabbits (Figure 2, bottom) showed >20% improvement in blood flow (p=0.001, Fisher’s exact test). All instances of improved perfusion occurred in areas of the brain where the initial extent of ischemia was <45 ml/100 g/min. In 10 electrodes in control rabbits, perfusion actually decreased by >20%; such a decrease was seen in only two electrodes from treated rabbits (p=0.005), and these two were in areas showing the least initial extent of ischemia.

Subgroups of electrodes were defined based on the initial extent of ischemia. Blood flow as a function of time after occlusion is shown for the two subgroups of electrodes with the greatest initial extents of ischemia (Figure 3). The most compromised areas showed the greatest improvement in blood flow with treatment. Percentage improvement in blood flow 4 hours after occlusion as a function of the initial extent of ischemia is summarized in Figure 4. Whereas overall, poloxamer 188 increased blood flow by 69%, it improved blood flow by >120% in tissue with severe initial ischemia but not at all in areas with minimal initial ischemia. Areas with intermediate initial extents of ischemia showed intermediate percentage improvement. The control rabbits showed no significant improvement of blood flow in any subgroup of electrodes.

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FIGURE 3. Graph of effect of poloxamer 188 on cerebral blood flow in subgroups of electrodes demonstrating regions of moderate (left) or severe (right) initial extents of ischemia in rabbits treated with poloxamer 188 (•; nine electrodes for moderate, nine electrodes for severe) and in controls treated with saline (○; nine electrodes for moderate, 10 electrodes for severe). Data from Figure 1 were subgrouped according to initial extent of ischemia (moderate ischemia, 16–25 ml/100 g/min; severe ischemia, 0–15 ml/100 g/min). Results are shown as mean±SEM for all electrodes in each subgroup. Differences between control and treated groups are significant at all times (p<0.01 by Student’s t test).

Improved perfusion in the treated rabbits was not explained by hemodilution or by changes in viscosity. Over the course of the experiment, hematocrits of five treated and five control rabbits increased by 0.6±0.4% and 0.4±0.6%, respectively. Viscosity measured at shear rates of 1 to 100 sec⁻¹ (Table 1) increased with decreasing shear rate in both groups, but there were no differences between groups.

Discussion

Poloxamer 188 increased blood flow by an average of 121% in areas of rabbit brain with severe ischemia produced by surgical occlusion of the middle cerebral artery; the surfactant had lesser effects in areas with moderate or mild ischemia and no significant effect in adjacent areas with minimal or no ischemia. This apparently selective effect of poloxamer 188 to enhance blood flow in ischemic tissue was not accompanied by changes in hematocrit or bulk viscosity of blood measured at either high or low shear rates. Ours were acute studies, and we did not evaluate preservation of brain function. However, studies in several other systems in which comparable doses of poloxamer 188 were used demonstrate preservation of function in addition to increasing blood flow in ischemic or damaged tissues. These other systems include isolated cat brain,27 rabbit frostbite,23,24 dog hemorrhagic shock,25,26 and isolated perfused rat heart32 models.

TABLE 1. Effect of Poloxamer-188 on Viscosity of Blood From Rabbits

<table>
<thead>
<tr>
<th>Shear rate (sec⁻¹)</th>
<th>Time</th>
<th>Control (n=5)</th>
<th>Treated (n=5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>100</td>
<td>Before</td>
<td>2.6±0.6</td>
<td>2.4±0.2</td>
</tr>
<tr>
<td></td>
<td>After</td>
<td>2.0±0.4</td>
<td>2.5±0.3</td>
</tr>
<tr>
<td>10</td>
<td>Before</td>
<td>3.9±1.2</td>
<td>3.2±0.5</td>
</tr>
<tr>
<td></td>
<td>After</td>
<td>4.1±0.9</td>
<td>3.4±0.5</td>
</tr>
<tr>
<td>1</td>
<td>Before</td>
<td>6.0±3.0</td>
<td>6.5±2.6</td>
</tr>
<tr>
<td></td>
<td>After</td>
<td>4.1±1.1</td>
<td>4.4±1.3</td>
</tr>
</tbody>
</table>
Poloxamer 188 is a nonionic block polymer surfactant that is not metabolized and is rapidly excreted by the kidneys. The material has been infused into humans on many occasions as a pump prime for cardiac bypass surgery and as an inactive component of an intravenous hyperalimentation preparation and of perfluorocarbon blood substitutes. The report of adverse pulmonary reactions due to activation of complement by perfluorocarbon artificial blood is troublesome. It is unclear, however, whether this reaction is due to poloxamer 188, especially considering that the reaction has not been seen in patients receiving the agent with other substances. At the time of writing, a new formulation of poloxamer 188 (RheothRx) is undergoing Phase I clinical trials. Consequently, questions regarding its safety for use in humans will be resolved in due course.

Recent studies indicate that poloxamer 188 has a number of unusual biologic effects. In an in vitro model, poloxamer 188 increased the flow of blood through tortuous capillary-sized fibrin-lined channels by as much as 20-fold without affecting bulk viscosity. This agent also reduced pathologic elevations in whole-blood viscosity that were associated with high-molecular-weight polymers of soluble fibrin. Progressive microcirculatory obstruction, which occurs primarily at the capillary level and is compounded by compression from swollen parenchyma, has been demonstrated in areas of focal cerebral ischemia. The loss of fluids from the capillaries produces hemoconcentration, with increasing viscosity, sludging, and obstruction. Under normal conditions, the capillary diameter is approximately equal to that of erythrocytes. In ischemic tissue with swelling and damaged endothelial cells, the capillaries are narrowed and intravascular fibrin forms. In this situation, the friction of erythrocytes passing through the narrowed capillaries probably becomes important. We have proposed that poloxamer 188 enhances blood flow in the damaged microvascularature by reducing friction due to adhesive reactions between the blood cells and the vessel walls. There are fundamental relations between hydrophobicity, adhesion, and friction. Damaged cells and certain macromolecules, especially fibrin, frequently have hydrophobic domains exposed on their surfaces; contact of such domains produces adhesion and friction. There is no direct evidence that this mechanism operates in ischemic cerebral tissue. However, the observation that poloxamer 188 increases cerebral blood flow through damaged tissue without hemodilution makes it an interesting reagent for further studies of the pathophysiology of microvascular obstruction in ischemic tissue.

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