Surfactant poloxamer 188–related decreases in inflammation and tissue damage after experimental brain injury in rats

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Object. The surfactant, poloxamer 188 (P-188), has been found to protect against tissue injury in various experimental models. Its protective mechanism may involve the effects of the surfactant against oxidative stress and inflammation. The authors investigated the role of P-188 in the reduction of tissue injury and macrophage response in a model of excitotoxic brain injury in the rat striatum.

Methods. Fifteen Sprague–Dawley rats underwent stereotactic injection of 120 nmol of quinolinic acid into the striatum and received intracisternal injection of vehicle or P-188 (40 mg/kg) at 10 minutes and 4 hours postinjury. Rats were killed after 1 week, and the histological score was determined based on the degree of overall tissue injury (Grades 1–4) at the lesion site. The number of macrophages within the lesioned striatum was compared with that found within the striatum on the nonoperated contralateral side. The scores related to tissue damage and the macrophage ratios of each group were then compared using t-tests.

Striatal injection of the toxin produced a lesion characterized by necrosis and inflammation surrounding the injection site in all six control animals. In rats in which intracisternal P-188 was administered, significantly less tissue injury was demonstrated (mean score 2.45 ± 0.74) than in controls (mean score 3.14 ± 0.75) (p = 0.045). The rats that received intracisternal surfactant also had significantly less macrophage infiltrate (mean ratio 1.06 ± 0.18) than control animals (mean ratio 2.00 ± 0.48) (p = 0.004).

Conclusions. The surfactant P-188 reduces tissue loss and macrophage infiltrate after excitotoxic brain injury in the rat. Possible mechanisms of this effect may include direct surfactant modulation of inflammatory cell membrane fluidity.

KEY WORDS • surfactant • poloxamer • traumatic brain injury • chemotaxis • membrane fluidity • cytokine • pediatric neurosurgery

A significant portion of the neuronal loss after TBI occurs by secondary brain injury. Secondary brain injury is caused by neurochemical changes in the injured brain that affect ion homeostasis and energy metabolism and are in part mediated by the immune response. Glutamate, and glutamate receptor agonists such as quinolinic acid, cause toxicity, in part, by generating reactive oxygen species and thereby depleting energy in neurons. As such, quinolinate can serve as a model for certain aspects of neural trauma, particularly those related to delayed reaction to the initial mechanical trauma which includes release of glutamate and delayed inflammation. In this capacity, we have undertaken extensive studies involving infusion of quinolinic acid into the rat striatum to develop a model neural injury in the assessment of neurotrophic agent–mediated neuroprotection.

When neuronal death is caused by necrosis, the immune response is thought to be initiated by the secretion of cytokines such as IL-1, IL-6, and TNF from microglia, which lead to the recruitment of neutrophils and macrophages. This inflammatory infiltrate corresponds directly to the formation of cerebral edema. Cytokine production has also been associated with the excitotoxicity of experimental brain injury in the rat. Interruption of this inflammatory process would be of potential therapeutic benefit in the management of TBI.

Surfactants are amphipathic molecules that have the ability to self-aggregate in an aqueous medium and create ionic compartmentalization. Poloxamer 188 is a synthetic surfactant that is a multiblock copolymer. A P-188 molecule is composed of two blocks, 38 moieties each, of hydrophilic polyoxyethylene, flanking one block of 29 moieties of hydrophobic polyoxypropylene. It has a molar weight of 8.4 kD, and, being 80% hydrophilic, it is water soluble. Despite its large size, P-188 can cross the blood–brain barrier when injected into the peritoneum, but the efficiency of this transport is unknown. Intrathecal administration of P-188 has not been reported in the literature. It is excreted by the kidney unmetabolized. Poloxamers can intercalate into the lipid bilayer of the...
plasma membrane, affecting the membrane fluidity, making the membrane more rigid, and giving intramembranous molecules less freedom of movement. This study was conducted to investigate the ability of surfactant P-188 to reduce tissue loss and inflammation in an experimental model of excitotoxic TBI in the rat.

Materials and Methods

Fifteen Sprague–Dawley rats underwent stereotactic lesioning of the right striatum after administration of a ketamine anesthetic cocktail (ketamine, xylazine, acepromazine, and saline). A Kopf stereotactic frame was used to calculate striatal target coordinates from bregma as anteroposterior 1.6, lateral −2.5, and ventral −4.5 from dura; 120 nmol of quinolinic acid in 1 μl was infused though a Hamilton syringe for a 1-minute period. The animals subsequently received either 50 μl of P-188 or vehicle (artificial CSF) by direct infusion into the CSF at the cisterna magna. Two groups of rats were studied: Group 1 (six animals) served as a control, receiving vehicle only. Group 2 (nine rats) received surfactant injection at 10 minutes and 4 hours after lesioning. The poloxamer dose was determined by limitations in the soluble concentration of the surfactant, viscosity of injection, and the maximum volume that could be safely delivered via the intracisternal route. The animals were killed 7 days postinjury after induction of deep anesthesia and cardiac perfusion with phosphate-buffered saline followed by 4% paraformaldehyde. The rat brains were postfixed in 4% paraformaldehyde for 12 hours and allowed to equilibrate in 30% sucrose for sectioning. Serial specimens were cut in 40-μm sections, stained with cresyl violet, and stained immunocytochemically by a standard peroxidase reaction for the presence of macrophages with HIS36, an antibody to a rat ED2-like antigen found on tissue macrophages. Serial specimens were cut in 40-μm sections, stained with cresyl violet, and stained immunocytochemically by a standard peroxidase reaction for the presence of macrophages with HIS36, an antibody to a rat ED2-like antigen found on tissue macrophages.

In an additional experiment to assess P-188 penetration into the ventricles and the brain after cisterna magna injection, two deeply stained histological section of brain containing the needle track for each rat was mounted and scored to determine the degree of tissue loss and inflammation surrounding the track. There were five separate grades of tissue injury (Fig 1). Lesion Grade 0 was defined as no discernable tissue loss and no evidence of inflammation outside the needle track; Grade 1 as a mild inflammatory infiltrate along the needle track only, not exceeding 2 mm in width, and no tissue loss; Grade 2 as inflammation within 5 mm of the needle track with tissue loss observed along or adjacent to the needle track; Grade 3 as a moderate inflammatory infiltrate of greater than 5 mm in width and tissue loss at or remote from the needle track not exceeding 50% of the striatal width; and Grade 4 as massive inflammation and tissue loss at and distant from the needle track exceeding 50% of the width of the striatum visible on the section. The tissue injury grade was determined in a blinded fashion by two independent examiners who performed light microscopy. The two grades of the individual section that contained the needle track were averaged for each rat. The injury grades for the treated group were compared with those for the control group by Student t-test, with the level of significance at a probability value of 0.05.

Macrophage Infiltrate

The degree of macrophage infiltrate was again determined by analysis of the individual anti-HIS36 immunocytochemically stained section obtained in each rat that contained the needle track. All immunopositive cells within the boundaries of the striata (Fig 2) were counted on that section—both on the lesioned and the nonlesioned sides. A ratio was determined for each section, which was the total number of immunocytochemically positive cells within the striatum on the lesioned side divided by the number of immunocytochemically positive cells on the nonlesioned side. Both intravascular and intraparenchymal cells were counted. The ratios of cell counts, as determined by two independent observers for each animal, were averaged. The ratios for the treated and nontreated groups were then compared using Student t-test, with the level of significance at a probability value of 0.05. Mean values are presented with SDs.

Results

In rats receiving P-188, significantly less striatal tissue damage and gross inflammatory infiltrate were observed (Fig. 1A and B; Grade 2.5 ± 0.7) than controls (Fig. 1C and D; Grade 3.1 ± 0.8) (p = 0.045) 1 week after lesioning (Fig. 3 left). The number of macrophages in the perilesional striatum was also decreased in the animals receiving poloxamer (Fig. 3 right). In those that received poloxamer there were approximately equal numbers of macrophages on the lesioned and nonlesioned hemissections (ratio 1.1 ± 0.2) compared with the control animals in which there were approximately twice as many macrophages on the treated hemisection than on the nonlesioned hemisection (ratio 2.0 ± 0.5). This comparison was statistically significant (p = 0.004).

Discussion

We describe the use of the surfactant, P-188 as a potential protective molecule after striatal injury with the glutamate agonist quinolinic acid. In this model, the P-188–treated rats appear to exhibit less tissue loss and inflammatory infiltrate. There was also a striking reduction in macrophage infiltration around the injury in the P-188–treated animals. There are many possible explanations for the observed activity of the P-188 molecule, although most are related to the specific biochemical properties of the molecule relative to membrane intercalation.

The role of surfactants has been investigated as membrane-ordering agents. Membrane-ordering agents, such as cholesterol and polyethylene glycol, increase cell rigidity and decrease membrane fluidity. In normal cells, an increase in plasma membrane order can alter the cell’s transmembrane signaling by decreasing the ability of the membrane bound receptors to modulate their shape in the lipid matrix.7 Taken to extremes, this process is known as molecular freezing and may be part of the disease process in the vascular degeneration of atherosclerosis and diabetes.8,9 In cases involving pathological membrane fluidity or permeability, however, surfactants can restore and maintain ionic gradients by intercalating into the plasma membrane.6,21-25,26

There have been many proposed and attempted uses of poloxamer molecules during the last 30 years. The molecules have been used as a food additive, stool softener, topical wound cleanser, emulsifying agent in intravenous fat emulsions, and an organ perfusate. Poloxamer 188 specifically has been evaluated as an agent to reduce discomfort in sickle cell crisis, to enhance drug delivery of antimycobacterials, as an antiparasite, and as a chemotherapy agent in multidrug-resistant cancer cells.5 It
has also been used as antiplatelet therapy in coronary angioplasty,\(^2,44\) an antisludging agent in extracorporeal membrane oxygenators,\(^2,28,49\) a soluble bone wax for hemostasis,\(^47\) and as a suppressor of carcinogenesis in a rodent model of colon cancer.\(^33\)

The effects of poloxamer on excitable tissues have been mostly protective. Poloxamer 188 has been found to reduce neurological injury in a dog model of hypothermic circulatory arrest,\(^27\) and to reduce tissue injury in a rabbit model of frostbite.\(^20,48\) It has also been shown to increase blood flow in the rabbit after middle cerebral artery ligation, although it did not decrease the extent of stroke when delivered intravenously.\(^8\) It has been demonstrated to seal electropermeabilized muscle tissue when injected intravenously, as well as to reduce inflammation.\(^22\)

Poloxamers have also been reported to have antiinflammatory effects in certain model systems.\(^30,43\) Their antiinflammatory effect can derive from modulation of any of the following stages in the inflammatory process: 1) interference with intercellular adhesion molecule–mediated leukocyte margination; 2) modulation of the cytokine response; 3) modulation of chemotaxis; 4) blocking of opsonization; and 5) modulation of phagocytosis. The majority of studies involving the immunological effects of poloxamers have been focused on their effects on cell migration and phagocytosis.

Poloxamer’s mediation of leukocyte chemotaxis is based mainly on its capacity to act as a membrane-ordering agent, decreasing plasma membrane fluidity and increasing membrane rigidity. It has been shown that polymorphonuclear leukocyte plasma membrane fusion with esterified polyethyl glycerol (a membrane-ordering agent) decreases both cell locomotion and superoxide activation to 20% of those demonstrated in controls at high concentration (1 g/l).\(^10\) This effect was only observed when the ester was greater than C14. Wolach, et al.,\(^50\) reported that polymorphonuclear membrane rigidification produced by cholesteryl hemisuccinate (a membrane-ordering agent) initially increased mobility in chemotaxis-deficient neonatal polymorphonuclear membrane cells, with mobility returning to baseline after 5 minutes of incubation. Rigidification of normal adult polymorphonuclear membrane cells decreased chemotaxis, which implies that there is an optimal membrane fluidity for chemotaxis. A known antiinflammatory fatty acid, eicosapentaenoic acid, was found to increase membrane fluidity in resting polymorphonuclear membrane cells, but decreased membrane fluidity in activated polymorphonuclear membrane cells, and thus decreased their chemotaxis and phagocytosis.\(^42\) In a canine myocardial reperfusion injury model, treatment with Fluosol, a P-188–containing agent, decreased neutrophil infiltration and chemotaxis, but it only decreased lysozyme production 1 hour after reperfusion.\(^3\) Indirect correlation of decreased chemotaxis to increased membrane rigidity has been shown in an examination of lazy leukocyte syndrome.\(^36\) This syndrome is remarkably similar to vinblastine exposure on electron microscopy, a known defect in leukocyte mobility due to increased rigidity.

Poloxamers may also have an antiinflammatory effect by inhibiting aggregation and adherence. Tan and Saltzman\(^43\) reported that P-188 decreased neutrophil migration on a collagen matrix, as well as random cellular motion. The poloxamer, however, did not inhibit neutrophil migration in the absence of collagen. These authors interpreted
this as a reflection of the cell’s decreased adherence to “coated” collagen. This view is shared by others who have shown its inhibitory effects on cell aggregation.32 This coating capacity could also modulate the inflammatory process by decreasing opsonization. Jackson, et al.,17 demonstrated that a high-weight molecular poloxamer, Pluronic F127, inhibits the plasma opsonization of polymeric microspheres. Additionally, Perskin and Cronstein35 found that in the polymorphonuclear membrane cells obtained in the elderly in whom decreased viscosity (increased rigidity) was present, there was an age-related decline in adherence to a component of the extracellular matrix (denatured collagen). Although poloxamers have been used as potent surface-active adjuvants to the immune response, this property has been shown to decrease with hydrophilic surfactants, measured as a hydrophillic–lipophile balance of two or greater.16 The hydrophillic–lipophile balance of P-188 is two.

Whether poloxamers modulate the cytokine response of inflammation is unclear. Poloxamer 188 was found not to affect the levels of IL-6, -10, -12, or TNF in murine peritoneal macrophage.41 On the contrary, CRL 1072, a small 3.5-kD poloxamer, was found to increase the secretion of IL-8 from human macrophages, increasing the killing response of polymorphonuclear cells to mycobacteria and increasing their secretion of IL-2 and TNF.18 The CRL 1072—stimulated macrophages were also shown to secrete TNF and granulocyte–macrophage colony—stimulating factor, but not to polymorphonuclear cell—inducing levels.18

Analysis of our data indicates that P-188 reduces tissue damage and macrophage infiltrate after excitotoxic TBI in the rat. As discussed, its antiinflammatory effect in this model could be based on inhibiting chemotaxis, modulating phagocytosis, inhibiting opsonization, or decreasing cell adherence to the extracellular matrix. On the other hand, P-188 has been shown to reduce cell loss in an in vitro model of excitotoxicity by resealing plasma membranes and preventing necrosis, indicating that it affects lipid peroxidation.23 This phenomenon could contribute to an antiinflammatory effect by preventing liberation of
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intracellular contents, thereby reducing the chemoattractants during the inflammatory process. Although further experimentation is required to delineate the mechanism of the P-188 antiinflammatory effect, our results indicate that it provides a novel and potentially beneficial therapeutic approach to a various brain injuries.

References


*J. Neurosurg: Pediatrics* / Volume 101 / August, 2004

Manuscript received October 13, 2003.
Accepted in final form February 24, 2004.

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