The surfactant poloxamer-188 protects against glutamate toxicity in the rat brain

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Membrane repair of damaged neurons by surfactant poloxamers has been noted in experimental spinal cord injury and *in vitro* excitotoxicity. We examined poloxamer-188 (P-188)-mediated neuroprotection in a rat model of glutamate toxicity. Quinolinate was infused into the striatum followed 10 min and 4 h later by P-188 administered either i.v. or intracisternally (i.c.), or by vehicle. Mean neuronal loss examined volumetrically 7 days later in control animals was 50% greater ($P < 0.01$) than after i.c. P-188 treatment; control lesion volumes were 38% greater than lesion volumes after i.v. P-188 treatment; however, that comparison did not reach significance. This robust protection against glutamate toxicity may predict P-188-mediated neuroprotection against a broad range of clinically relevant neural insults. *NeuroReport* 15:171–174 © 2004 Lippincott Williams & Wilkins.

Key words: Intracisternal; NeuN; Neuroprotection; Poloxamer; P-188; Quinolinic acid; Rat striatum

INTRODUCTION

The poloxamers are a family of multi-block co-polymers which are non-ionic surface-active agents. The primary structure of the biocompatible polymers is $H(OCH_2CH_2)_a(OCHCH_3CH_2)_b(OCH_2CH_2)_aOH$ [1,2]. The poloxamer family includes liquids, pastes, gels, and solids with mol. wts from 1000 to 14 000 and polyethylene oxide/polypropylene oxide weight ratios from about 1:9 to 8:2. Poloxamer 188 (P-188) has an average mol. wt of 8400 and its hydrophile comprises about 80% of the total molecular weight ($H(OCH_2CH_2)_{38}(OCHCH_3CH_2)_{29}(OCH_2CH_2)_{38}OH$).

As a modulator of viscosity, P-188 reduces the discomfort associated with sickle-cell crisis [3]. P-188 reduces platelet aggregation [4] and modulates the construction of fibrin clots [5], properties which may also allow decreased sludging and thrombosis in the setting of extracorporeal oxygenators. Consistent with these effects, P-188 has shown efficacy in a dog model of cardiac ischemia [6]. Poloxamer 188 has been also shown to re-seal permeabilized muscle cell membranes without affecting the membrane transport properties of uninjured cells [2]. A poloxamer molecule seals permeabilized erythrocyte membranes after radiopermeabilization [7]. In an *in vitro* model of glutamate toxicity in dispersed hippocampal neuronal culture, potent neuroprotective effects of P-188 have been observed [8] concurrent to evidence that P-188 directly intercalates into the neuronal membranes and prevents lipid peroxidation. These observations argue for a primary molecular membrane repair property of the molecule in addition to any possible effects on blood viscosity or anti-oxidation. However, previous studies of the effect of P-188 on ischemic injury have not shown any beneficial effects of improved perfusion or improved functional outcome from spinal cord injury after aortic cross-clamping [9] and no direct protective effect in reducing focal stroke size in the brain [10]. P-188 did not improve outcome and caused renal toxicity at a high rate when used as an adjunct to angioplasty for acute myocardial infarction in humans [11]. The limitations of these studies are the use of systemic delivery of P-188, which may not be able to penetrate into the CNS, as well as possibly excessive systemic doses that may have caused the reported side effects of treatment. To address these issues, we sought to determine if P-188 was neuroprotective in an *in vivo* model of glutamate toxicity. We also investigated whether intracisternal (i.c.) dosing has a greater neuroprotective effect than i.v. dosing.

MATERIALS AND METHODS

Thirty Sprague–Dawley rats underwent stereotactic lesioning in the right striatum under ketamine cocktail anesthesia (ketamine, xylazine, acepromazine, saline). A Kopf stereotactic head frame with nose bar set at −3 from the interaural line was used to calculate the target from bregma as AP 1.6, L −2.5, and V −4.5 from the dura. Quinolinic acid (120 nmol in 1 ml) was hand-injected through a 5 μl Hamilton syringe and 28G cannula over 1 min. Animals were then treated i.v. with poloxamer at 400 mg/kg, i.c. with poloxamer at 40 mg/kg (injected into the cisterna magna) or with vehicle (injected through either route). There was no observed

lesion size difference between animals injected i.v. or i.c. with vehicle and these animals were combined into a single control group. The i.c. dose of poloxamer was determined by the maximal soluble concentration of the surfactant and the maximal volume that could be injected into the cisterna magna safely. Twenty-six animals survived until they were sacrificed under deep anesthesia 1 week after lesioning. Each animal underwent cardiac perfusion with PBS followed by 4% paraformaldehyde. Brains were post-fixed in 4% paraformaldehyde for 12 h after sacrifice and allowed to equilibrate in 30% sucrose prior to sectioning. Tissue was cut at 40 μm and immunocytochemically stained for the presence of NeuN, a neuronal nucleus marker, by standard peroxidase reaction. Adjacent sections were stained with cresyl violet to assess lesion morphology (Fig. 1).

Image analysis: Brain sections containing the lesion were photographed with a digital microscope at ×10. The perimeter of the lesion was drawn for each section and included lesion areas outside the striatum (cortex and neocortex) in animals where lesions were large. Lesion area was calculated for each section perimeter using NIH Image. Each lesion area was multiplied by the thickness of the section (40 μm) and summed to calculate the volume of the lesion for each animal. The lesion volumes within each group were then compared by ANOVA with *post hoc* comparison (Dunnett) to detect significant differences in lesion size between groups.

RESULTS

Quinolinate infusions produced neuronal loss in the striatum in all animals and outside the striatum in several animals where lesions where large. The lesions were centered on the infusion site. Lesion volume analysis showed a significant main effect between groups ($F=3.4$; $df=2$; $P<0.04$). The animals that received intrathecal poloxamer were found to have significantly smaller lesion volumes (15.3 ± 2.1, $n=10$) than those that received vehicle (23.5 ± 2.2, $n=9$; $P<0.01$). Animals that received i.v. polox-

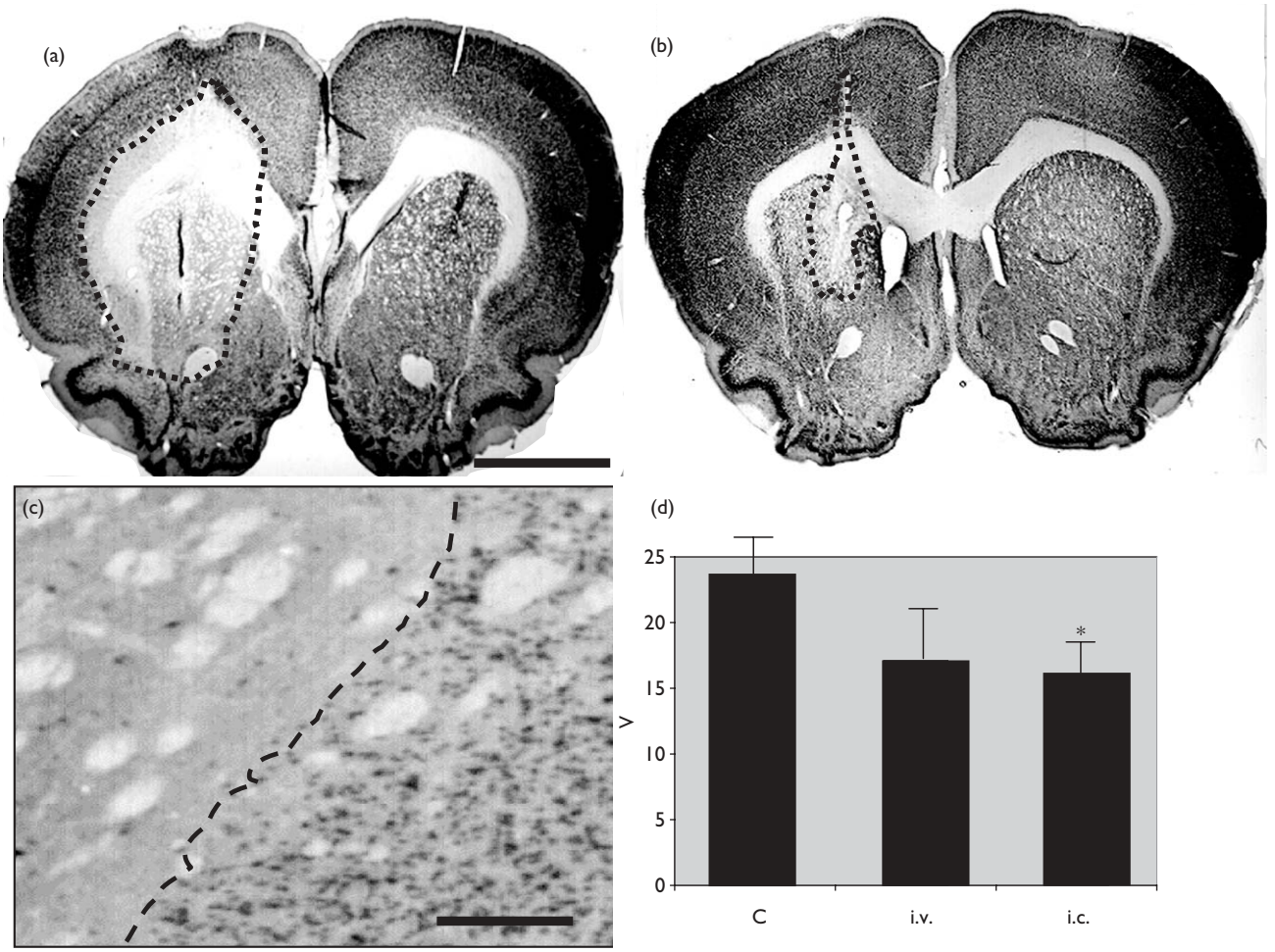

Fig. 1. Effects of P-188 on striatal quinolinic acid lesions. (**a**) Coronal section through a control rat brain at the level of the quinolinic acid injection needle track immunostained for NeuN, a neuronal marker. Dashed line marks outline of area of neuronal loss within the section; solid line=4 mm. (**b**) Coronal brain section at level of quinolinic acid injection needle track immunostained for NeuN in a rat treated with P-188 delivered intracisternally; note decreased area of neuronal loss (dashed line) seen; magnification identical to (**a**). (**c**) Photomicrograph showing higher power view of NeuN immunostaining at boundary of striatal quinolinic acid lesion. Area to left of dashed line shows neuronal loss within the lesion; area to right shows near normal neuronal density; solid line=0.4 mm. (**d**) Graphical representation of effects of P-188 delivered either i.v. or i.c. on striatal lesion volume after quinolinic acid delivery into the striatum. V, lesion volume in mm^3; C, control; $^*P<0.01$ *vs* control; error bars, s.e.m.

amer had somewhat smaller lesion volumes than controls (17.1 ± 3.1, $n=7$), but this difference failed to reach statistical significance in *post hoc* analysis, perhaps due to group size. No significant difference was found between the two treatment groups. Incidentally noticed after cresyl violet staining was that the lesions of the animals that received intrathecal poloxamer had less inflammatory infiltrate along the needle track than the lesions of the control animals.

DISCUSSION

We sought to determine whether the surfactant poloxamer P-188 has neuroprotective effects in an *in vivo* excitotoxic model of brain injury. After infusion of quinolinic acid into the rat striatum, P-188 was delivered i.v. or i.c. Significant reduction in the volume of subsequent neuronal loss in and around the striatal quinolinate infusion site was seen after i.c. delivery of P-188 when compared to control animals. There was a reduction in mean lesion sizes after i.v. P-188 delivery; however, this effect did not reach statistically significance.

Neuronal injury, whether from trauma, vascular insult, or a neurodegenerative state, contains a region of potential cell death, separate from any acute direct injury, where as yet undefined factors determine neuronal survival. The determinants of survival may include neuronal resistance to glutamate toxicity, cellular ability to detoxify free radical metabolites [12], or neuronal ability to repair membrane damage due, perhaps, to lipid peroxidation [13–15]. We have previously modeled this penumbral area of at-risk neurons by a striatal excitotoxic lesion caused by quinolinic acid in order to investigate potential neuroprotectants such as NGF [16,17], the glucocorticoid steroid methylprednisolone, and the non-glucocorticoid steroid antioxidant U78517F [17]. In the present study, we noted the neuroprotective effects of P-188 in this well-defined and reproducible model. Previous studies of surfactant- or poloxamer-mediated neuroprotection have been conducted *in vitro* [8] or centered on spinal cord injury [18] except for use of P-188 in a rat model of focal brain ischemia where no reduction in infarct size was noted with P-188 treatment [10]. Delivery of the P-188 in the rabbit model of focal ischemia was i.v. We observed significant neuroprotection after delivery of P-188 directly into the CSF, whereas i.v. infusion of P-188 produced less neuroprotection that did not reach significance. These observations, coupled with lack of protection in the reported rabbit ischemia model after i.v. delivery, imply that penetration of the P-188 across the blood–brain barrier may be impaired and reduces its ability to modulate neuronal injury. This may be due to the size or some other physical property of the P-188. Further studies are in progress to demonstrate the levels of P-188 in the CNS after various modes of administration. Modification of the chemical properties of the poloxamer molecule may eventually overcome this delivery requirement. Incidentally noted in our tissue sections was a tendency towards reduced inflammatory infiltrate around the striatal lesions in animals treated with poloxamer. This may possibly be an effect of the reduction in lesion size, although it was also seen in the intravenously treated animals. P-188 is a known immunomodulator [19] and the decreased inflammation may be a direct effect of the P-188. If this is true, it could impact on the mechanism of our observed P-188-mediated neuroprotection. P-188-mediated protection of dispersed hippocampal neurons after glutamate injury appears to be related to direct intercalation of the P-188 into the neuronal membrane to potentially seal membrane injury [8]. This may also occur in the *in vivo* situation. However, an alternative or additional neuroprotective mechanism involving P-188 effects on immune cell infiltration into an injury or on the immunologic response to neuronal stress may also play a role in P-188-mediated neuroprotection. Our initial observations of significant P-188 neuroprotective effects suggest a role for surfactant poloxamers in the treatment of a variety neural injuries. Further studies into the mechanism of poloxamer-mediated neuroprotection will help to define the extent of that role. Evaluation of P-188 neuroprotection in more clinically relevant models of neural injury, such as intraparenchymal hemorrhage or hydrocephalus, will define its applicability.

REFERENCES

1. Borgens RB. Cellular engineering: molecular repair of membranes to rescue cells of the damaged nervous system. *Neurosurgery* **49**, 370–378 (2001); discussion 378–379.
2. Lee RC, River LP, Pan FS, Ji L and Wollmann RL. Surfactant-induced sealing of electropermeabilized skeletal muscle membranes *in vivo*. *Proc Natl Acad Sci USA* **89**, 4524–4528 (1992).
3. Adams-Graves P, Kedar A, Koshy M, Steinberg M, Veith R, Ward D *et al.* RheothRx (poloxamer 188) injection for the acute painful episode of sickle cell disease: a pilot study. *Blood* **90**, 2041–2046 (1997).
4. Armstrong JK, Meiselman HJ and Fisher TC. Inhibition of red blood cell-induced platelet aggregation in whole blood by a nonionic surfactant, poloxamer 188 (RheothRx injection). *Thromb Res* **79**, 437–450 (1995).
5. Carr ME Jr, Powers PL and Jones MR. Effects of poloxamer 188 on the assembly, structure and dissolution of fibrin clots. *Thromb Haem* **66**, 565–568 (1991).
6. Mezrow CK, Mazzoni M, Wolfe D, Shiang HH, Litwak RS and Griepp RB. Poloxamer 188 improves neurologic outcome after hypothermic circulatory arrest. *J Thorac Cardiovasc Surg* **103**, 1143–1146 (1992).
7. Hannig J, Yu J, Beckett M, Weichselbaum R and Lee RC. Poloxamine 1107 sealing of radiopermeabilized erythrocyte membranes. *Int J Radiat Biol* **75**, 379–385 (1999).
8. Marks JD, Pan C-y, Bushell T, Cromie W and Lee RC. Amphiphilic, tri-block copolymers provide potent, membrane-targeted neuroprotection. *FASEB J* **10**, 1096 (2001).
9. Follis F, Jenson B, Blisard K, Hall E, Wong R, Kessler R *et al.* Role of poloxamer 188 during recovery from ischemic spinal cord injury: a preliminary study. *J Invest Surg* **9**, 149–156 (1996).
10. Colbassani HJ, Barrow DL, Sweeney KM, Bakay RA, Check IJ and Hunter RL. Modification of acute focal ischemia in rabbits by poloxamer 188. *Stroke* **20**, 1241–1246 (1989).
11. O'Keefe JH, Grines CL, DeWood MA, Schaer GL, Browne K, Magorien RD *et al.* Poloxamer-188 as an adjunct to primary percutaneous transluminal coronary angioplasty for acute myocardial infarction. *Am J Cardiol* **78**, 747–750 (1996).
12. Frim DM, Wullner U, Beal MF and Isacson O. Implanted NGF-producing fibroblasts induce catalase and modify ATP levels but do not affect glutamate receptor binding or NMDA receptor expression in the rat striatum. *Exp Neurol* **128**, 172–180 (1994).
13. Hall ED and Braughler JM. Central nervous system trauma and stroke. II: Physiological and pharmacological evidence for involvement of oxygen radicals and lipid peroxidation. *Free Rad Biol Med* **6**, 303–311 (1989).
14. Hall ED. Inhibition of lipid peroxidation in CNS trauma. *J Neurotrauma* **8A**, 831–840 (1991).
15. Hall ED, Braughler JM, Yonkers PA, Smith SL, Linseman KL, Means ED *et al.* U-78517F: a potent inhibitor of lipid peroxidation with activity in experimental brain injury and ischemia. *J Pharm Exp Ther* **255**, 688–694 (1991).
16. Frim DM, Short MP, Rosenberg WS, Simpson J, Breakefield XO and Isacson O. Local protective effects of nerve growth factor-secreting

fibroblasts against excitotoxic lesions in the rat striatum. *J Neurosurg* **78**, 267–273 (1993).
17. Uhler TA, Frim DM, Pakzaban P and Isacson O. The effects of megadose methylprednisolone and U-78517F on toxicity mediated by glutamate receptors in the rat neostriatum. *Neurosurgery* **34**, 122–127 (1994); discussion 127–128.
18. Borgens RB and Shi R. Immediate recovery from spinal cord injury through molecular repair of nerve membranes with polyethylene glycol. *FASEB J* **14**, 27–35 (2000).
19. Moghimi SM and Murray JC. Poloxamer-188 revisited: a potentially valuable immune modulator. *J Natl Cancer Inst* **88**, 766–768 (1996).