Hydroxyethyl Starch Reduces Leukocyte Adherence and Vascular Injury in the Newborn Pig Cerebral Circulation After Asphyxia

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Background and Purpose—Hydroxyethyl starch (HES) has beneficial effects on ischemic brain injury; however, its mechanism of action remains unclear. The present study was undertaken to test the hypothesis that HES can attenuate increases in leukocyte adherence and vascular permeability in the cerebral vasculature after global cerebral ischemia induced by asphyxia.

Methods—Pial venular leukocyte adherence and permeability to sodium fluorescein were quantified in anesthetized newborn piglets by in situ fluorescence videomicroscopy through closed cranial windows during basal conditions and during 2 hours of reperfusion after global ischemia induced by 9 minutes of asphyxia. Experimental animals received HES after the asphyxial insult (10% HES 257/0.47, 600 mg/kg IV bolus 5 minutes after asphyxia, followed by 600 mg/kg per hour IV drip during reperfusion; n=9).

Results—A progressive and significant (P<0.05) increase in adherent leukocytes was observed during the initial 2 hours of reperfusion after asphyxia compared with nonasphyxial controls. In this model, vascular injury, as determined by significant (P<0.05) increases in fluorescein permeability at 2 hours of reperfusion, is largely dependent on adherent leukocytes. HES significantly reduced (P<0.05) leukocyte adherence at 1 hour and 2 hours of reperfusion and reduced fluorescein permeability at 2 hours. HES did not change hematocrit or alter pial arteriolar diameter.

Conclusions—These findings indicate that a vascular anti-inflammatory action may underlie the beneficial effects of HES in global cerebral ischemia secondary to asphyxia. Since this compound is well tolerated by patients, future preclinical and clinical studies may reveal improvements in functional outcome with the early introduction of this or similar agents after perinatal asphyxia or global ischemia. (Stroke. 2000;31:2218-2223.)

Key Words: cerebral ischemia, global inflammation leukocytes reperfusion injury

Hydroxyethyl starch (HES) is a clinically well-tolerated complex polysaccharide that has recently been used in the therapeutic treatment of stroke and vasospasm after subarachnoid hemorrhage. It is available in multiple preparations, each with different pharmacological characteristics based on concentration, molecular weight, degree of substitution, and C2/C6 hydroxyethylation ratio. While HES has shown a protective effect with reductions in infarct size and/or improvement in outcome in experimental models of ischemic injury in both central nervous system (CNS) and peripheral tissues, results of its use in clinical stroke trials have been less encouraging. This may relate to the time window of administration of the compound relative to stroke onset, as well as the pharmacological characteristics of the particular HES preparation; nevertheless, clinical interest still remains, particularly in situations of elevated intracranial pressure.

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The mechanistic basis of the beneficial effects of HES remains unclear. Postulated actions include improvements in cerebral blood flow, tissue oxygen delivery, and rheology. Recent in vitro and in vivo studies in peripheral tissues have demonstrated that HES can significantly reduce leukocyte-endothelial adherence. It is now recognized that cerebral ischemia/reperfusion leads to activation of leukocytes and cerebrovascular endothelium, and the resulting adherence of polymorphonuclear leukocytes to the microvascular wall contributes to reperfusion injury through the release of cytotoxic enzymes and the generation of oxygen free radicals. Whether or not HES affects leukocyte-endothelial interactions in the CNS microvasculature in the setting of cerebral ischemia, with potential implications for blood-brain barrier breakdown, is not known.
We have previously shown in newborn piglets that reperfusion after global cerebral ischemia or asphyxia is associated with significant and progressive increases in the number of leukocytes adherent to cerebral venules and a leukocyte-dependent increase in vascular permeability. We used the asphyxial model in the present study to test the hypothesis that HES would attenuate ischemia-induced increases in leukocyte adherence and vascular permeability and that the previously reported neuroprotective effects of this agent may be partially mediated by its anti-inflammatory actions.

Materials and Methods

Animal Preparation
Thirty-nine newborn piglets (age, 1 to 4 days) weighing between 1.5 and 3.5 kg were used in experimental protocols that were consistent with Public Health Service guidelines and approved by our institutional animal studies committee. The preparation for in vivo monitoring of leukocyte dynamics has been detailed previously. In brief, animals were premedicated with ketamine (20 mg/kg IM), tracheotomized, and mechanically ventilated with a mix of room air and oxygen with anesthesia maintained for the remainder of the experiment with isoflurane (1.0% to 1.5%). End-tidal CO₂ and transcutaneous oxygen saturation were continuously monitored by a capnometer and forepaw sensor, respectively. Both femoral arteries were cannulated for continuous recording of arterial blood pressure and intermittent withdrawal of arterial blood samples for analyses of blood gases, glucose, and hematocrit (before ischemia and before the animals were killed). A femoral vein was cannulated for body fluid maintenance (6 mL/kg per hour of 5% dextrose in 0.45% normal saline) and paralysis (0.25 mg/kg per hour pancuronium). A thermoregulated heating pad and overhead heating lamp were used to maintain core body temperature at 38°C to 39°C, the normal temperature for newborn piglets.

The animal was placed in a stereotaxic frame, and the head was held securely by ear bars placed against the mandibles. An 18-mm craniotomy was performed, the dura was removed, and a closed cranial window (12 mm) made of Plexiglas was mounted over the craniotomy. The window was fashioned to create a 2-mm-deep chamber with significant and progressive increases in the number of leukocytes that remained stationary anywhere within each venular network under observation for >10 consecutive seconds. The adherence values reported indicate the mean number of leukocytes per square millimeter of total endothelial vessel surface examined in the 2 networks, as determined by image analysis software (2-dimensional surface area times π [3.142]).

Quantification of Vascular Permeability

Leukocyte adherence to the endothelium of the pial venular wall was quantified in 2 preselected venular networks that included several secondary and tertiary (20 to 45 µm diameter) postcapillary branches and 1 or 2 larger venules (60 to 90 µm diameter) into which they drained. Adherence was quantified manually by counting the number of leukocytes that remained stationary anywhere within each venular network under observation for >10 consecutive seconds. The adherence values reported indicate the mean number of leukocytes per square millimeter of total endothelial vessel surface examined in the 2 networks, as determined by image analysis software (2-dimensional surface area times π [3.142]).

Statistical Analyses

Differences in the physiological parameters within and between groups were assessed by repeated-measures ANOVA or nonparametric Kruskal-Wallis with Dunn’s or Dunnett’s multiple range test applied when appropriate. Differences in the hematological parameters within and between groups, both before and after asphyxia, were assessed by unpaired Student’s t tests. Determinations of significant differences in arteriolar diameter, leukocyte adherence, and vascular permeability within and between groups were by paired
or unpaired Student’s t tests, respectively, with signed rank or Mann-Whitney rank sum tests as needed. P, 0.05 was considered significant.

Results

Physiological and Hematological Parameters
There were no significant differences in the monitored physiological variables (blood pressure, blood glucose, arterial pH, PaCO₂, and PaO₂) among the 3 animal groups during baseline conditions or at 1 and 2 hours of reperfusion, and all values were within normal ranges for newborn piglets (data not shown). None of these parameters changed significantly over time in the control group. Asphyxial animals became severely hypoxic (PaO₂ = 14 ± 2 mm Hg), hypotensive (mean arterial blood pressure = 22 ± 5 mm Hg), acidicotic (pH = 7.10 ± 0.04), bradycardic (21 ± 8 bpm), and hypercapnic (PaCO₂ = 76 ± 5 mm Hg) by the end of the 9-minute asphyxial insult; however, these parameters recovered to preasphyxial levels by 30 minutes of reperfusion. There were no significant differences in hematocrit between the level measured at baseline and that measured at 2 hours of reperfusion, within and between groups.

Pial Arteriolar Diameter
No significant change in pial arteriolar diameter occurred over time in the nonasphyxial control group. In animals rendered asphyxial, arteriolar diameters remained at preasphyxial baseline levels throughout the 2-hour reperfusion period. Posttreatment with HES also did not affect pial arteriolar diameter at any time.

Leukocyte-Endothelial Adherence
Under baseline conditions, no significant differences were noted among groups with respect to the number of leukocytes adherent to cerebral venules. In nonasphyxial control animals, a slight but significant increase in leukocyte adherence occurred over the 2-hour observation period relative to that measured during baseline conditions. However, as shown in Figures 1 and 2, asphyxia resulted in a much more robust and significantly greater increase in the number of leukocytes adherent to the venular endothelium during the initial 2 hours of reperfusion relative to time-matched controls. Animals treated postischemically with HES exhibited significantly less leukocyte-endothelial adherence at both 1 hour and 2 hours of reperfusion compared with asphyxial, time-matched controls.

Vascular Permeability
Figures 2 and 3 show that the pial venular permeability to sodium fluorescein was significantly elevated after 2 hours of reperfusion in the asphyxial animals relative to controls. In animals treated with HES, this asphyxia-induced vascular leak was significantly attenuated to levels equivalent to nonasphyxial controls. As shown qualitatively in Figure 2, in nonasphyxial animals, fluorescein lightly stained the venular endothelium and defined the vascular network against a darker background. In asphyxial animals, fluorescein appeared extravascularly, spreading away from the venules; this typical result caused the vascular network to appear dark against a lighter background. The fluorescence pattern in HES-treated asphyxial animals looked like that seen in nonasphyxial controls. Fluorescein leakage was not observed in the perivascular space adjacent to arterioles.
did similarly stimulated cells treated with pentafraction,18 endothelial cells in vitro bound 229% more neutrophils than integrins. Indeed, thrombin-stimulated human umbilical vein sion of adhesion molecules, including the selectins and/or and may include the reduction or modulation of the expres-

...completely in asphyxial animals treated with HES (n=6). *P<0.05 vs control group at same time; †P<0.05 vs asphyxia group at same time.

Figure 3. Vascular fluorescein permeability, measured at 2 hours of reperfusion and expressed as optical density units nor-

Discussion
Accumulating evidence in peripheral vascular beds supports an inhibitory effect of a variety of complex polysaccharides on leukocyte-endothelial interactions. Ongoing studies in our laboratory21,23 indicate that, in this model of asphyxia-induced global ischemia, microvascular leakage of low-molecular-weight fluorescein is largely dependent on the magnitude of leukocyte adherence to venular endothelium. Consistent with these observations is our present finding that postischemic treatment with HES concomitantly attenuates increases in leukocyte adherence and vascular permeability during early reperfusion after global cerebral ischemia. This early improvement in microvascular integrity secondary to these anti-inflammatory effects of HES gives further impetus to continuing examinations of this agent in clinical stroke.14,15 particularly if study designs dictate that patients receive the agent early after stroke onset, which was not the case in previous trials.11-13

Studies in the peripheral vasculature indicate that HES can reduce leukocyte-endothelial cell interactions promoted by ischemia/reperfusion. For example, isovolemic hemodilution with 6% HES 200/0.62 to a hematocrit of 30% resulted in a 40% decrease in number of postischemic neutrophils adherent to skeletal muscle postcapillary venules at 30 minutes and 2 hours of reperfusion, but this effect was lost by 24 hours.16 In a porcine model of fecal peritonitis, resuscitation with pentafracton decreased the accumulation of neutrophils in the pulmonary capillary and hepatic sinusoids.17 The mechanistic basis of these antiadherent actions of HES is unclear and may include the reduction or modulation of the expres-

...in microvascular permeability after focal brain ischemia2-4 or spinal cord ischemia1 were also reduced by medium-molecular-

...osmotic mannitol.27 HES may also reduce the transcytosis of serum proteins.4

The effect of HES on the postischemic CNS microvascu-

...day and animal species used; different anesthetics, methods, and tracers used to measure vascular permeability; the time at which postischemic edema assessments were made; the dose administered as well as other intrinsic property differences between the various HES compounds30; and the extent to which HES and HES-like compounds affected hematocrit, blood viscosity, and autoregulatory ca-

...the type and severity of the cerebral ischemic insult and the associated degree of blood-brain barrier compromise, as well as the resultant physiological state of the brain, could also modulate the ability of HES to affect postischemic cerebrovascular integrity. The physiological and hemodynamic changes induced by asphyxia in our model are distinctly different from those induced by global ischemia by cardiac arrest, for example, and these changes may not only affect the integrated response of the tissue but the efficacy of therapeutic measures as well. Clearly, mech-

...and other indirect effects may contribute to the acute anti-inflammatory effects of HES that we
observed in the present study, such as a lowering of blood viscosity. However, hematocrit was unaffected at the concentration of HES used herein; thus, it appears unlikely that hemodilution played a significant role in reducing the post-ischemic inflammatory response in our study. We also cannot rule out the possibility that the HES-mediated reduction in postischemic leukocyte-endothelial adherence is secondary to an increase in cerebral blood flow, particularly an increase in venular blood velocity. However, the lack of change in the caliber of the resistance arterioles both during reperfusion after global ischemia and in response to HES administration argues against this mechanism, although absolute measures of cerebral blood flow are needed to conclusively address this possibility.

The important contribution of leukocytes to ischemic brain injury has become increasingly clear over the last few years. The present study supports the hypothesis that HES is capable of attenuating elevations in leukocyte adherence and leukocyte-dependent increases in vascular permeability in the newborn brain after an asphyxial insult. Although our previous study with a CD18 monoclonal antibody indicated some degree of dependence of fluorescein leakage on leukocyte adherence, it is likely that additional mechanisms, independent of an effect on adherent leukocytes, could contribute to the reduction in microvascular permeability resulting from HES administration. Moreover, our observations were made during the initial 2 hours of reperfusion; additional studies will be required using other models of ischemia/reperfusion to determine whether this anti-inflammatory effect is maintained over longer postischemic time periods and, ultimately, whether improvements in neuronal viability and functional outcome are realized. It is possible that the lack of significant efficacy in clinical stroke trials of HES is related primarily to the protracted time (48 hours) between ischemia and the initiation of therapy, although one recent study still found no treatment effect when HES was given within 6 hours of stroke onset. Other factors that might contribute to the demonstrated efficacy of HES in our and other preclinical studies relative to human stroke trials showing little or no benefit may also include differential alterations in volume status and differential effects of HES on coagulation and hemorheology, depending on the degree of substitution and the resultant in vivo molecular weight of the particular HES preparation.

In any event, the present in vivo documentation of an anti-inflammatory action of HES reveals one mechanism whereby beneficial effects of this agent might be realized in the setting of global cerebral ischemia.

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