α₁-Acid glycoprotein reduces local and remote injuries after intestinal ischemia in the rat

JULIAN P. WILLIAMS, MARTIN R. WEISER, TAINÉ T. V. PECHET, LES KOBZIK, FRANCIS D. MOORE, J R., AND HERBERT B. HECHTMAN. α₁-Acid glycoprotein reduces local and remote injuries after intestinal ischemia in the rat. Am. J. Physiol. 273 (Gastrointest. Liver Physiol. 36): G1031–G1035, 1997.—The aim of this study was to look at the role of α₁-acid glycoprotein as a natural anti-inflammatory agent with particular respect to its antineutrophil and anticomplement activity. A recombinantly engineered form of sialyl Lewisα (sLeα)-bearing α₁-acid glycoprotein (sAGP) was administered intravenously to pentobarbital-anesthetized rats after 50 min of intestinal ischemia just before 4 h of reperfusion. A non-sLeα-bearing form of AGP (nsAGP) was used as control. sAGP-treated animals had a 62% reduction (P < 0.05) in remote lung injury, assessed by 125I-albumin permeability, compared with those treated with nsAGP (permeability index of 3.61 ± 0.15 × 10⁻³ and 5.18 ± 0.67 × 10⁻³, respectively). There was a reduction in pulmonary myeloperoxidase levels in sAGP-treated rats compared with nsAGP-treated rats. Complement-dependent intestinal injury, assessed by 129I-albumin permeability was reduced by 28% (P < 0.05) in animals treated with sAGP (7.58 ± 0.63) compared with those treated with nsAGP (10.4 ± 0.54). We conclude that sAGP ameliorates both complement- and neutrophil-mediated injuries.

METHODS

Materials. sAGP, non-sLeα-bearing AGP (nsAGP), and 125I-labeled SAGP were kind gifts from Dr. Dale Cummings and Ravindra Kumar (Genetics Institute, Cambridge, MA). Partially sLeα-bearing AGP (psAGP), human albumin, and ³⁵S-labeled ovalbumin were purchased from Sigma Chemical (St. Louis, MO). ¹²⁵I-labeled albumin was purchased from ICN Biomedicals (Costa Mesa, CA).

Production of AGP. A mammalian cell expression vector pED (12) containing a cDNA encoding human AGP (17) was constructed. This plasmid and a second plasmid, pED.FT3, containing a cDNA encoding human FT3 (13) were cotransfected and amplified via methotrexate selection in DHFR-CHO cells. In the case of nsAGP, the AGP expression vector was amplified in DHFR-CHO cells lacking the transfected FT3 gene. The secreted SAGP and nsAGP were purified from serum-free CHO cell-conditioned medium by conventional chromatography methods, and the presence of sLeα on AGP N-linked glycans was confirmed by in vitro E-selectin binding assays and nuclear magnetic resonance analysis.

Animal model. Adult male Sprague-Dawley rats were anesthetized with intraperitoneal pentobarbital sodium (50 mg/kg), and a cannula was inserted into the tail vein. A midline laparotomy was performed. Collateral vessels from the caudal mesenteric artery were ligated with silk ties, and the superior mesenteric artery was occluded with a microvascular clip. The laparotomy incision was closed and then reopened at 50 min. The microvascular clip was removed, and reperfusion of the mesenteric vasculature was confirmed by the return of pulsation to the vascular arcade. The incision was then closed, and all animals were maintained supine and kept warm with radiant lamps for an additional 4 h of monitoring during reperfusion. Sham-operated animals un-

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underwent an identical procedure except for the omission of superior mesenteric artery occlusion.

sAGP was administered at a dose of 10 mg/kg in 2 ml/kg saline intravenously, 15 min before reperfusion (n = 12). This gave an initial serum concentration of ~3 µmol/l. A control group (n = 16) received nsAGP at the same dose. A further control group (n = 12) underwent ischemia-reperfusion and was treated with saline. The sham animals (n = 12) received saline.

All animals were infused with 125I-labeled albumin 15 min before reperfusion. After 4 h of reperfusion, the animals were killed by overdose of pentobarbital sodium followed by sternotomy and exsanguination by cardiac puncture. Blood samples were taken for gamma counting and assay of serum alanine aminotransferase (ALT) as a measure of remote liver injury (24). Lungs were harvested, and the right lung was used for myeloperoxidase (MPO) assay as a measure of pulmonary neutrophil sequestration (24). The left lung was used for assessment of permeability from the ratio of 125I-labeled albumin in bronchialalveolar lavage fluid to that in blood (24). Intestine was harvested for MPO assay. Intestinal contents were collected for assessment of dry stool-to-blood contents were collected for assessment of dry stool-to-blood contents were collected for assessment of dry stool-to-blood permeability (7, 11, 27).

Systemic complement activity. To demonstrate that AGP on complement activity we devised a modified in vitro alternative pathway 50% hemolysis (CH50) analysis (APCH50) (31). In brief, 200-µl aliquots containing 6.6 × 10^7 washed guinea pig erythrocytes (Rockland, Gilbertsville, PA), test rat serum in varying concentrations, and Mg-EGTA were incubated for 1 h at 37°C. Reactions were quenched with excess 0.9% sodium chloride solution, and supernatants were assessed for free hemoglobin by measuring OD414. Samples were assessed in duplicate and the APCH50 for each animal’s serum was calculated using the von Krogd plot (31).

Localization of AGP to sites of injury. AGP that had been labeled with 125I was used to demonstrate localization of the AGP to sites of injury. A group of five rats underwent intestinal ischemia-reperfusion as described above but were given the 125I-labeled sAGP 15 min before reperfusion (0.5 µCi/animal in 0.25 ml saline). To control for localization caused by extravasation of AGP because of increases in permeability, the rats also received ovalbumin labeled with 11C (0.5 µCi/animal in 0.25 ml saline). This albumin has the same molecular weight as the sAGP (45,000) and would therefore be expected to extravasate to the same degree as the sAGP if localization were the result of permeability changes alone. The thesis that sAGP was concentrated at sites of endothelial selectin expression means that one would expect AGP levels to be in excess of albumin levels, giving a ratio of sAGP to albumin of >1. After 4 h of reperfusion, blood and tissue samples (small bowel mucosa, small bowel submucosa, liver, lung, large bowel, stool, liver, and kidney) were counted for 125I using a gamma counter (Cobra II Auto-Gamma, Packard Instruments, Downers Grove, IL). The samples were then minced, solubilized, decolorized, and mixed with scintillation fluid (Biosol/Bioscient, National Diagnostics, Atlanta, GA). These were then counted on a beta counter (Delta 300 Liquid Scintillation Counting System, Searle Analytic, Des Plaines, IL). The counting windows were set such that there was maximal detection of 14C-β-particles with minimal detection of the lower energy Compton electrons and β-particles released by the 125I. For each sample, the permeability index for both albumin and sAGP were calculated by the ratio of radioactivity per gram of tissue to that per gram of blood. The ratio of these “permeabilities” was used to estimate the degree of localization of sAGP to each tissue.

Results are expressed as means ± SE. Where percentages are used to describe the reduction in injury, these are estimated after subtraction of the sham values. Significance was tested using analysis of variance for multiple comparisons, and when a difference was found Student’s t-test was performed.

RESULTS

sAGP reduces remote organ injury. Pulmonary injury was estimated by the ratio of radioactivity in bronchialalveolar lavage to that in blood. sAGP reduced the pulmonary permeability index from a mean of 5.18 ± 0.67 × 10^-3 in nsAGP-treated animals to a mean of 3.61 ± 0.15 × 10^-3, a reduction of 62% (P < 0.05; Fig. 1). There was a similar reduction in pulmonary neutrophil sequestration, as assessed by MPO levels, from 0.12 ± 0.01 U/g in nsAGP-treated rats to 0.08 ± 0.01 U/g in sAGP-treated animals (P < 0.05; Fig. 2). MPO levels in the sham group were 0.07 ± 0.01 U/g. Liver injury was also reduced by sAGP as assessed by the level of serum ALT. Animals treated with sAGP had reduced serum levels of ALT (50.7 ± 2.5 U/l) compared with those treated with nsAGP (148.8 ± 35.2 U/l) (P < 0.05). ALT levels were 33.23 ± 4.14 U/l in sham animals and 126.7 ± 48.2 U/l in saline-treated animals.

sAGP reduces ischemia-reperfusion-induced changes in intestinal permeability. Intestinal permeability was reduced by 28% (P < 0.05) in those animals treated with sAGP (7.58 ± 0.63) compared with those treated with nsAGP (10.4 ± 0.54) (Fig. 3). Despite reduced intestinal injury as measured by the permeability index in animals treated with sAGP, a significantly higher number of neutrophils was sequestered, indicated by MPO levels (0.65 ± 0.04 U/g vs. 0.45 ± 0.04
AGP inhibits complement deposition. To demonstrate an effect of sAGP on complement activity, we used the modified in vitro APCH50 assay (31). The results show a significant inhibition of the alternative pathway by sAGP and psAGP at physiological concentrations when compared with albumin. The effect of the fully sLex-bearing molecule was significantly greater than that of the only partially sLex-bearing molecule (Fig. 4).

sAGP does not impair systemic complement activity. There was no decrease in systemic complement activity in serum from animals treated with saline (APCH50 44.7 ± 7.99 U/ml) compared with those treated with sAGP (APCH50 47.02 ± 5.40 U/ml). These APCH50 results are similar to those of other studies in normal rats (45 U/ml; Ref. 26). This is important because generalized inhibition of complement could result in an increased susceptibility to infection. Animals genetically deficient in complement C3 or C4 show a marked sensitivity to bacteremia (30).

sAGP is localized to sites of injury. The ratio of labeled AGP to labeled albumin detected in small bowel submucosa was 1.6 compared with 1 in all other tissues. This is most likely caused by the fact that this is the site of the most severe injury by far, and therefore detection of localization to the lung, which has a more limited degree of injury, would require a test of more sensitivity. Thus there is a localization of sAGP in excess of what would be expected because of increased permeability, which indicates that the sLex-bearing molecule localizes to the site of greatest injury.

DISCUSSION

We have demonstrated that sAGP reduces remote and local injuries after intestinal ischemia-reperfusion.
injury. However, the absence of neutrophils in a tissue either activated or involved in producing a particular neutrophils in a tissue does not imply that they are for leukosequestration. Furthermore, the presence of other adhesion molecules or neutrophil cytoskeletal sAGP blocks neutrophil-selectin interaction, because this finding does not conflict with the hypothesis that they are not instrumental in producing the local injury. These data are consistent with our thesis that this injury is relatively neutrophil independent. The converse is true in the lung, in which a relatively minor degree of injury (compared with that in the intestine) is dependent on neutrophil-selectin interactions to a much greater degree (7), and a molecule that blocks this interaction (sAGP) would be expected to have a much greater effect in reducing injury, as is indeed demonstrated in this study. As such, the reduction in the intestinal injury would suggest that there is an inhibitory action on local complement activation. This is supported by the in vitro demonstration of inhibition of the alternative pathway of complement activation by sAGP. In studies that have demonstrated attenuation of gut injury as well as a reduction in intestinal neutrophil accumulation with sCR1 (10), the dose of sCR1 given was sufficient to abolish all systemic complement activity. When lower doses of sCR1 were given in the same study, there was no reduction in intestinal neutrophil sequestration but still a significant reduction in injury results from a less severe local injury in sAGP-treated rats. Sequestration of neutrophils can be CD18 and selectin independent, and in a local injury as severe as inflicted in these experiments one might expect these other mechanisms to be fully activated (6, 18, 23). Our primary thesis based on prior studies with regard to reduction of local injury is that this injury is almost entirely initiated by deposition of complement, as demonstrated by the fact that neutropenia does not modify the local injury (24), whereas complement inhibition with soluble complement receptor type 1 (sCR1) does (10). The fact that sAGP leads to a moderate reduction in local injury in sAGP-treated animals implies that the reduction is a result of reduced neutrophil sequestration and reduced injury, as seen in the intestine. Sequestration of neutrophils can be CD18 and selectin independent, and in a local injury as severe as inflicted in these experiments one might expect these other mechanisms to be fully activated (6, 18, 23). The acute-phase response in mucosal injury. It is likely that the sAGP in our model behaves more like the lower dose of sCR1 and is less “potent” inhibitor of complement.

The difference in alternative pathway inhibition between the sLe^g-bearing sAGP and the psAGP would suggest that the degree of sialylation of the molecule is important in this action. Meri and Pangburn (16) showed that sialic acid-bearing polyanions inhibit the alternative pathway by enhancing binding of complement C3b to factor H, which would support this thesis. It could be argued that the reduction in pulmonary injury results from a less severe local injury in sAGP-treated animals. However, the difference in magnitude of reduction in injury, with intestinal injury less than pulmonary, would make this explanation unlikely.

Levels of circulating AGP can rise up to fourfold in acute inflammatory conditions, and the degree of sialylation and structure of the supporting glycan moiety can change in a characteristic fashion dependent on the pathological stress (9, 19). The acute-phase response has been shown not only to result in an increase in circulating levels of AGP but also to increase the relative amount of sLe^g-substituted AGP molecules (4).

Fig. 4. Dose response of AGP in inhibiting alternative pathway of complement. There was up to 80% inhibition of the alternative pathway by sAGP (○) at equimolar concentrations compared with human albumin (■) at equimolar concentration. Partially sLe^g-bearing AGP (psAGP; △) obtained from pooled human sera demonstrated inhibition that was intermediate between sAGP and control. Values represent means ± SE of assay performed in 3 replicates.
Thus it may be that AGP has a moderating role as an anti-inflammatory agent, being produced by the liver in response to interleukin 1, interleukin 6, or other cytokines (7), and as such may modify not only neutrophil interactions via selectin binding but also limit the effects of complement activation. Indeed, it may well be that by virtue of selectin binding, the molecule can adhere to inflamed endothelium in areas of injury, thus inhibiting complement deposition without causing generalized depression of complement activity. In these respects, we believe AGP to be unique and therefore valuable as a possible agent in treating many types of inflammatory condition that have both neutrophil- and complement-mediated components.

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