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A Pilot Study of Peripheral Blood Neutrophil Aldose Reductase Expression in Pediatric Sepsis

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Abstract

Background: The role played by Aldose Reductase (AR) in chronic diabetic complications is well documented, though its role in acute inflammation is under investigation. AR expression in rodents can be associated with an exacerbated, acute inflammatory response. However, it is not known if AR has a role in acute inflammation in children e.g. Acute Lung Injury (ALI) and/or Acute Respiratory Distress Syndrome (ARDS) associated with sepsis. Objective: Our study was carried out in order to determine whether AR protein expression in peripheral blood neutrophils of children with acute respiratory failure would be increased in those with sepsis or ALI compared to other children. Methods: Patients <18 years of age with acute respiratory failure were enrolled in an observational cohort study. We measured AR protein expression in peripheral blood neutrophils by Western blot and correlated with clinical markers for critical illness, sepsis, and lung injury. Results: Of 16 subjects (median age: 6, range: 0.7-18 years), 4 had ALI (2 with acute respiratory distress syndrome) and 7 had sepsis (3 with septic shock). AR protein expression was detected in 4 subjects, and each of these subjects had sepsis (1 with septic shock) while 1 also had ALI. Subjects with AR protein expression were similar to all other subjects with respect to PRISM III score, peak oxygenation index, lowest PaO2/FiO2, length of mechanical ventilation and oxygen therapy, duration of PICU stay, peak glucose at 24 hours and mortality. Conclusion: AR protein expression was associated with sepsis (p=0.02); not enough patients with ALI were recruited to permit analysis.

Key words: Aldose Reductase, Acute Lung Injury, Acute Respiratory Distress Syndrome, Oxygenation Index, Pediatric Intensive Care Unit

INTRODUCTION

Aldose reductase (AR) catalyzes the first and rate-limiting step of the polyol pathway. AR is a member of the aldo-keto reductase family and is a NADPH-dependent enzyme that catalyzes the reduction of aldose sugars and saturated and unsaturated aldehydes. Increased flux through the polyol pathway associated with increased AR expression has been implicated in chronic inflammation related to diabetes mellitus.[1-5] Recent studies have suggested a role for increased AR expression in mouse models of acute inflammation such as sepsis,[6,7] and myocardial ischemia,[8] in which inhibition of AR protects ischemic hearts. Transgenic mice overexpressing human AR had greater inflammatory response and increased accumulation of neutrophils in the lungs compared to wild type mice in early phase following induction of sepsis.[9] AR expression was markedly elevated in the neutrophils and pulmonary endothelial cells of these transgenic mice compared with wild type mice, and AR inhibition attenuated inflammatory changes including IL-6 and TNF-α expression, neutrophil infiltration into the lungs, and activation of lung endothelial cells.[9]

We hypothesized that AR protein expression in peripheral blood neutrophils is increased early after the onset of respiratory failure in children with sepsis and/or acute lung injury (ALI) compared to other children, and designed this prospective, observational, cohort, pilot study to test this hypothesis in children with acute respiratory failure. We decided to assess clinical markers of critical illness, sepsis, and lung injury, and look for any correlation between these markers and AR protein expression.

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MATERIALS AND METHODS

This prospective study at Morgan Stanley Children's Hospital–Columbia University College of Physicians and Surgeons was approved by the Institutional Review Board (Columbia University Medical Center IRB 2: IRB-AAAD4859), and recruitment occurred from October, 2008 to April, 2010. Patients<18 years of age and weighing more than 5 kg with acute respiratory failure (<24 hours of mechanical ventilation- either invasive or noninvasive) in the Pediatric Intensive Care Unit (PICU) were eligible for recruitment following informed verbal consent. The requirement to obtain written documentation of informed consent was waived, as the study was judged to involve minimal risk, based on a total blood requirement for the study of less than 1% of estimated blood volume for each patient.

ALI was defined as the acute onset of lung disease characterized by bilateral infiltrates on chest radiograph and PaO2/FiO2 200 to 300 without evidence of left-sided heart failure, while acute respiratory distress syndrome (ARDS) was defined similarly except for PaO2/FiO2<200.[10] Sepsis was defined as a clinical syndrome characterized by infection (either suspected or documented) with a systemic inflammatory response and at least one of the following indicators of altered organ function: altered mental status, hypoxemia, increased serum lactate level, or bounding pulses.[11] Septic shock was defined as the presence of sepsis with tachycardia (unless hypothermic) and evidence of decreased perfusion (either decreased peripheral pulses, altered alertness, flash capillary refill, mottled or cool extremities, or decreased urine output).[11]

Neutrophil isolation and measurement of AR expression

A whole blood specimen was taken at study enrollment from an existing arterial or venous catheter, as follows: 1. Patients weighing 5 kg to 10 kg: a single 5 mL specimen 2. Patients weighing>10 kg: a single 10 mL specimen.

Neutrophils were isolated immediately from each specimen by density gradient separation using Histopaque solutions (Sigma-Aldrich) as previously described.[9] Residual red blood cells were lysed using ammonium chloride and the resultant purity of isolated neutrophils was >95%. AR protein expression in neutrophils was examined using immunoblot as previously described.[9] Briefly, neutrophils were lysed in 50 µL of cell lysis buffer containing protease inhibitors. This neutrophil lysate was stored at -80°C until there were enough specimens for processing. The total protein concentration of the neutrophil extract was measured by spectrophotometry (absorbance was measured at a wavelength of 595 nm) after a small amount of the extract was added to Bio-Rad Protein Assay Dye Reagent (Bio-Rad Laboratories). The proteins of the neutrophil extract were separated using NuPAGE 4-12% BisTris gel in MES-SDS buffer (Invitrogen) after equal protein loading per lane. The proteins were transferred to a PVDF membrane (Whatman) and nonspecific binding of the membrane was blocked with 5% nonfat dry milk. The membrane was then probed with a rabbit antibody against AR, followed by an antibody against rabbit IgG coupled with horseradish peroxidase. Catalase was used as a housekeeping protein to verify equal protein loading.

Clinical markers

Clinical markers of critical illness and sepsis included PRISM III score,[12] length of PICU stay, incidence of bacteremia, and the mortality rate. Clinical markers of lung injury included the peak oxygenation index (OI),[13] the lowest PaO2/FiO2, and the length of mechanical ventilation and oxygen therapy. Data from non-survivors was not included in either length of mechanical ventilation, length of oxygen therapy, or length of PICU stay, but was included in all other analyses. The OI was defined as mean airway pressure multiplied by the fractional concentration of inspired oxygen multiplied by 100 divided by the arterial partial pressure of oxygen; the peak value from the entire course of respiratory failure for each patient was reviewed. The ratio of the arterial partial pressure of oxygen divided by the fractional concentration of inspired oxygen (PaO2/FiO2) was also determined, and the lowest value for the entire course of respiratory failure for each patient was reviewed. The peak OI and lowest PaO2/FiO2 were not calculated for patients with hypoxemia secondary to a cardiac shunt.

Statistical analysis

Data are presented as medians with ranges. Continuous variables were compared using Mann-Whitney tests, while dichotomous variables were compared using Fisher's exact tests, and correlations were performed using Spearman nonparametric correlations. All statistical analyses were performed using InStat version 3.1a (GraphPad Software, Inc; San Diego, CA, USA).

RESULTS

We recruited 26 subjects, all of whom were intubated and treated with invasive mechanical ventilation at the time of sample collection. We were unable to isolate sufficient neutrophil protein in specimens from 10 subjects due to either neutropenia or a technical error in specimen handling. As a result, we were left with 16 subjects in our cohort: median age was 6 years (range 0.7-18). Table 1 provides demographic data for this cohort. A primary respiratory problem was documented in 5 patients (#4, 5, 7, 8, 12; Table 1). One subject had an intracardiac shunt (#16) and thus did not contribute a peak OI or lowest PaO2/FiO2; this subject did not have infiltrates on chest radiograph, and thus did not have evidence of ALI or ARDS. At the time of study entry, 2 subjects had ALI, 2 had ARDS, and 7 subjects had sepsis (including 1 with ALI and both subjects with ARDS; Table 2). Bacteremia was documented in 2 subjects: an infant with fever, pneumonia and respiratory failure requiring invasive mechanical ventilation, and a femoral central venous catheter had Staphylococcus epidermidis (patient 8), while another child with pneumonia and empyema had Streptococcus pneumoniae (patient 12).

AR protein expression was detected in 4 subjects (#5, 8, 14, and 15; Table 2, Figure 1). All 4 subjects (3 males and 1 female) with detectable AR protein expression had sepsis (1 had septic shock), and 1 also had lung injury (ARDS); all 4 of these subjects survived. Not enough patients with ALI were recruited to permit analysis of any potential association with AR protein expression. AR protein expression was associated with sepsis (p=0.02). Band intensity for AR protein expression was greatest in 2 subjects (Figure 1): one of these subjects (#5) had pneumonia and empyema with lung necrosis on computed tomography scan, while the other (#8) had pneumonia and bacteremia. Subjects with detectable AR protein expression were similar to all other subjects with respect to PRISM III score (median: 6 vs 7, range: 0-66; p=0.85), peak OI (median: 5 vs 4, range: 2-49; p=1), lowest PaO2/FiO2 (median: 212 vs 217, range 51-419; p=0.57), length of mechanical ventilation (median: 5 vs 3, range 1-14 days; p=0.75) and oxygen therapy (median: 6 vs 7, range 2-23 days; p=0.57), peak glucose at 24 hours (p=0.4), PICU length of stay (median: 8 vs 9, range 1-28 days; p=0.74), bacteremia (1 of 4 vs 1 of 12; p=0.45), and mortality (0 of 4 vs 1 of 12; p=1). One subject died during this PICU admission.

DISCUSSION

This translational, pilot study was performed to investigate whether AR protein expression by peripheral blood neutrophils was associated with sepsis and/or lung injury in children with acute respiratory failure. AR has been implicated in the generation of acute inflammation via its role in oxidative stress.[14] The metabolism of glucose and aldehyde substrates by AR depletes NADPH, which is essential for the maintenance of antioxidant glutathione reductase. The increased flux via AR and its link to oxidative stress promotes inflammation by modulating signaling pathways that depend on reactive oxygen species. AR may also help generate acute inflammation via regulation of the arachidonic acid pathway following endotoxin stimulation,[7] as well as via alteration in glucose uptake by endotoxin-stimulated macrophages.[15] Animal models suggest a role for AR in sepsis] and ALI associated with sepsis.[9] The severity of ALI and ARDS in adults is associated with levels of the soluble form of the receptor for advanced glycation endproducts;[16] this receptor binds to various products of the polyol pathway.

We found that 4 out of 7 patients with clinical sepsis and acute respiratory failure had detectable peripheral blood neutrophil AR expression, while only 1 of 4 patients with ALI/ARDS had detectable peripheral blood neutrophil AR expression. To our knowledge, this is the first report showing an

Table 1: Subject characteristics

Patient	Age Years)	Gender	Underlying diagnosis	Reason for acute respiratory failure
1	8	Male	Medulloblastoma	Impending cerebral herniation
2	13	Male	Dilated cardiomyopathy	Cardiac surgery (heart transplantation)
3	2	Male	Liver transplantation for hepatoblastoma	Diaphragmatic hernia repair
4	11	Male	None	Multiple trauma (subdural hematoma, pulmonary contusion)
5	5	Male	None	Pneumococcal pneumonia, empyema
6	6	Male	Seizure disorder	Status epilepticus, sagittal sinus thrombosis
7	0.7	Male	None	Retropharyngeal abscess*
8	1	Male	Asthma	Status asthmaticus, pneumonia
9	18	Male	Scoliosis, trisomy 21	Scoliosis surgery
10	12	Female	Brainstem tumor	Brainstem tumor resection
11	1	Female	None	Progressive shock with multiorgan system failure after cardiac arrest
12	2	Female	None	Pneumococcal pneumonia, empyema, bacteremia; 2009 influenza A(H1N1)
13	1	Female	Heart transplantation for hypoplastic left heart syndrome	Viral sepsis
14	8	Male	Autism	Viral encephalitis, intracranial hypertension
15	15	Female	Autoimmune hepatitis	Septic shock
16	3	Female	Pulmonary atresia	Cardiac surgery (Fontan procedure)

*This patient developed sepsis and 2009 influenza A (H1N1) several days following participation in this study.

Table 2 Clinical presentation and AR expression

	Patient	ALI	ARDS	Septic shock	AR expression
No sepsis	1	-	-	-	-
	2	-	-	-	-
	3	-	-	-	-
	4	-	-	-	-
	6	+	-	-	-
	7	+	-	-	-
	9	-	-	-	-
	10	-	-	-	-
	16	-	-	-	-
Sepsis	5	-	-	-	+
	8**	-	+	-	+
	11*	-	-	+	-
	12**	-	+	+	-
	13	-	-	-	-
	14	-	-	-	+
	15	-	-	+	+

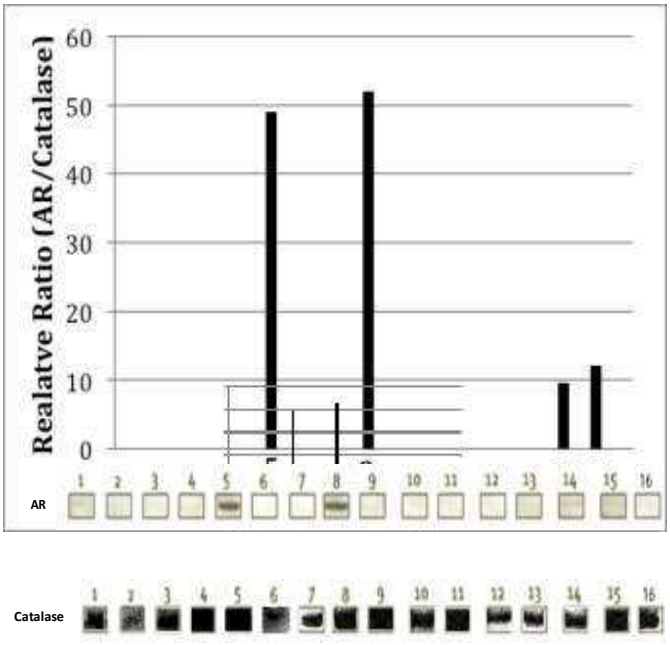

Figure 1: Shows AR expression in peripheral blood neutrophils of pediatric subjects. Neutrophils were isolated by density density gradient using Histopaque solutions. AR protein expression was examined by immunoblot.

association between clinical sepsis in children and increased AR protein expression. We were unable to recruit enough children with ALI or ARDS, and cannot comment on any potential role of AR in that setting.

Corticosteroids have been implicated in the suppression of AR,[17] and it is possible that elevated endogenous cortisol levels during shock were responsible for suppressing AR expression in several patients (#11, 12). Of note, patients with and without increased peripheral blood neutrophil AR expression had similar blood glucose, suggesting that AR expression in sepsis may be unrelated to glycemia.

Previous reports on AR documents the role played by the polyol pathway in diabetic complications, such as neuropathy and cataract formation in both children and adults. AR reduces many glutathione conjugates and lipid peroxidation-derived aldehydes which help regulate the inflammatory cascade initiated by cytokines, growth factors, and lipopolysaccharide. It is notable that therapies to inhibit AR are available and have been used successfully to help inhibit progression of some types of inflammatory injury in animal models; there may be a role for inhibitors of AR in sepsis and acute inflammation.[6,7,18] However, AR inhibitors have not been used in the clinical arena to modulate this acute inflammatory response.

As a pilot study designed to provide an initial test of the hypothesis, the small sample size, the occasional presence of neutropenia, as well as the presence of endogenous proteases (which may interfere with the expression of neutrophil proteins) all limit our ability to provide definitive answers; however, we believe that our experience in an animal model helped minimize some of these problems.[9] Additional drawbacks not yet mentioned include the heterogeneity of disease processes such as ALI, ARDS, and sepsis, the undefined relationship between sepsis and lung injury, and the arbitrary nature of several of our clinical markers. A larger study population may be required to account for underlying disease, genetic predisposition, as well as other acute illness in children with lung injury and/or sepsis. The association between sepsis and lung injury is also likely to be heterogeneous, and may reflect multiple pathophysiologic mechanisms. Several of the clinical markers we employed are arbitrary (PICU length of stay, length of mechanical ventilation and oxygen therapy) and may reflect principally our practice patterns. An effective strategy to study AR in a larger patient population would involve collection of an adequate blood sample (our current methodology suggests at least 10 mL is necessary) in patients without neutropenia. Demonstration of the soluble receptor for advanced glycation endproducts and AR expression in aspirates from the airways concurrent with blood sampling might help reveal both the systemic and local roles played by the polyol pathway in sepsis and lung injury.

CONCLUSION

Our pilot study is, to our knowledge, the first to investigate AR protein expression in peripheral neutrophils in children, and we suggest that AR may play a role in pediatric sepsis. Further investigation of the possible role of AR and the polyol pathway in early pediatric sepsis and lung injury is important.

REFERENCES

1. Dunlop M. Aldose reductase and the role of the polyol pathway in diabetic nephropathy. Kidney Int Suppl. 2000;77:3-10.
2. Larkins RG, Dunlop ME. The link between hyperglycaemia and diabetic nephropathy. Diabetologia. 1992;35:499-504.
3. Srivastava SK, Ramana KV, Bhatnagar A. Role of aldose reductase and oxidative damage in diabetes and the consequent potential for therapeutic options. Endocr Rev. 2005;26:380-92.
4. Kaneko M, Bucciarelli L, Hwang YC, Lee L, Yan SF, Schmidt AM, et al. Aldose reductase and AGE-RAGE pathways: key players in myocardial ischemic injury. Ann N Y Acad Sci. 2005;1043:702-9.
5. Kronenberg. Williams Textbook of Endocriology. 11th ed. WB Saunders, Philadelphia: 2008.
6. Ramana KV, Fadl AA, Tammali R, Reddy AB, Chopra AK, Srivastava SK. Aldose reductase mediates the lipopolysaccharide-induced release of inflammatory mediators in RAW264.7 murine macrophages. J Biol Chem. 2006;281:33019-29.
7. Shoeb M, Yadav UC, Srivastava SK, Ramana KV. Inhibition of aldose reductase prevents endotoxin-induced inflammation by regulating the arachidonic acid pathway in murine macrophages. Free Radic Biol Med. 2011;51:1686-96.
8. Hwang YC, Sato S, Tsai JY, Yan S, Bakr S, Zhang H et.al. Aldose reductase activation is a key component of myocardial response to ischemia. FASEB Journal. 2002;16:243-5.
9. Ravindranath TM, Mong PY, Ananthakrishnan R, Li Q, Quadri N, Schmidt AM et al. Novel role for aldose reductase in mediating acute inflammatory responses in the lung. J Immunol. 2009;183:8128-8137.
10. Bernard GR, Artigas A, Brigham KL, Carlet J, Falke K, Hudson L et al. The American-European Consensus Conference on ARDS. Definitions, mechanisms, relevant outcomes, and clinical trial coordination. Am J Respir Crit Care Med. 1994;149:818-24.
11. Levy MM, Fink MP, Marshall JC, Abraham E, Angus D, Cook D et al. 2001 SCCM/ESICM/ACCP/ATS/SIS International Sepsis Definitions Conference. Crit Care Med. 2003;31:1250-6.
12. Pollack MM, Patel KM, Ruttimann UE. PRISM III: an updated Pediatric Risk of Mortality score. Crit Care Med. 1996;24:743-52.
13. Trachsel D, McCrindle BW, Nakagawa S, Bohn D. Oxygenation index predicts outcome in children with acute hypoxemic respiratory failure. Am J Respir Crit Care Med. 2005;172:206-11.
14. Ramana KV. ALDOSE REDUCTASE: New Insights for an Old Enzyme. Biomol Concepts. 2011;2:103-14.
15. Reddy AB, Srivastava SK, Ramana KV. Aldose reductase inhibition prevents lipopolysaccharide-induced glucose uptake and glucose transporter 3 expression in RAW264.7 macrophages. International J Biochem Cell Biol. 2010;42:1039-45.
16. Jabaudon M, Futier E, Roszyk L, Chalus E, Guerin R, Petit A et al. Soluble form of the receptor for advanced

glycation end products is a marker of acute lung injury but not of severe sepsis in critically ill patients. Crit Care Med. 2011;39:480-8.

17. Endo S, Matsunaga T, Mamiya H, Ohta C, Soda M, Kitade Y et al. Kinetic studies of AKR1B10, human aldose reductase-like protein: endogenous substrates and inhibition by steroids. Arch Biochem Biophys. 2009;487:1-9.

18. Pandey S, Srivastava SK, Ramana KV. A potential therapeutic role for aldose reductase inhibitors in the treatment of endotoxin-related inflammatory diseases. Expert Opin Investig Drugs. 2012;21:329-39.