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Dietary advanced glycation end-products, its pulmonary receptor, and high mobility group box 1 in aspiration lung injury

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ABSTRACT

Background: Gastric aspiration is a significant cause of acute lung injury and acute respiratory distress syndrome. Environmental risk factors, such as a diet high in proinflammatory advanced glycation end-products (AGEs), may render some patients more susceptible to lung injury after aspiration. We hypothesized that high dietary AGEs increase its pulmonary receptor, RAGE, producing an amplified pulmonary inflammatory response in the presence of high mobility group box 1 (HMGB1), a RAGE ligand and an endogenous signal of epithelial cell injury after aspiration.

Materials and methods: CD-1 mice were fed either a low AGE or high AGE diet for 4 wk. After aspiration injury with acidified small gastric particles, bronchoalveolar lavage and whole-lung tissue samples were collected at 5 min, 1 h, 5 h, and 24 h after injury. RAGE, soluble RAGE (sRAGE), HMGB1, cytokine and chemokine concentrations, albumin levels, neutrophil influx, and lung myeloperoxidase activity were measured.

Results: We observed that high AGE-fed mice exhibited greater pulmonary RAGE levels before aspiration and increased bronchoalveolar lavage sRAGE levels after aspiration compared with low AGE-fed mice. Lavage HMGB1 levels rose immediately after aspiration, peaking at 1 h, and strongly correlated with sRAGE levels in both dietary groups. High AGE-fed mice demonstrated higher cytokine and chemokine levels with increased pulmonary myeloperoxidase activity over 24 h *versus* low AGE-fed mice.

Conclusions: This study indicates that high dietary AGEs can increase pulmonary RAGE, augmenting the inflammatory response to aspiration in the presence of endogenous damage signals such as HMGB1.

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1. Introduction

Aspiration is an important cause of direct acute lung injury (ALI) and acute respiratory distress syndrome (ARDS), affecting thousands of patients each year [1–4]. In a recent multicenter study to accurately identify patients at high risk for ALI, aspiration was one of the strongest predictors of ALI development [5]. Gastric aspiration is of particular concern in the trauma and critical care setting. These patients frequently suffer from altered levels of consciousness and impaired airway protection reflexes, two major risk factors for aspiration. As current therapeutic strategies for ALI and ARDS remain limited to mostly supportive care, focus has turned toward preventative practices [6]. From recent work, we identified a potentially modifiable risk factor, dietary advanced glycation end-products (AGEs). We demonstrated that the preinjury accumulation of high dietary AGEs led to a more pronounced inflammatory lung injury after gastric aspiration [7].

AGEs are proteins and lipids that are nonenzymatically glycated and oxidized in the presence of reducing sugars. These glycated structures accumulate in the body with age and are abundant in processed foods. AGEs can bind and cross-link extracellular structures, altering tissue function and activating proinflammatory pathways through engagement of the AGE receptor, RAGE [8,9]. RAGE is a multi-ligand, pattern recognition receptor (PRR) that is abundantly expressed in the lung on type 1 alveolar epithelial cells and is upregulated in the presence of its ligands [10].

Based on our previous study, we proposed that the AGE-RAGE axis is important to moderate the pulmonary inflammatory response and subsequent lung injury after aspiration [7]. Schmidt *et al.* [10] hypothesized a "two-hit model" of tissue inflammation and injury mediated by RAGE and its ligands. In our aspiration model, increased expression of RAGE in the lung, upregulated by high dietary AGEs, constitutes the "first-hit." The "second-hit" comes from the low pH cellular injury of gastric aspiration, releasing a variety of endogenous danger signals known as damage associated molecular patterns (DAMPs) [11].

DAMPs are endogenous molecules that are released on cell death, stimulating a number of PRRs to activate the innate immune system. We recently reported on the early release of mitochondrial DNA (a DAMP) in a murine model of acid aspiration [12]. High mobility group box 1 (HMGB1) is another DAMP released from its normally intranuclear location on cell necrosis. HMGB1 is a prototypical RAGE ligand, acting as a proinflammatory cytokine through RAGE engagement. Although HMGB1 can directly stimulate pulmonary inflammation, our understanding of its interaction with RAGE in ALI is limited and it has not been studied in aspiration-induced lung injury [11,13].

The purpose of this study was to investigate the mechanism by which high dietary AGEs amplify the acute inflammatory response to gastric aspiration. We hypothesized that high dietary AGEs increase pulmonary RAGE, and in the presence of HMGB1 released from injured respiratory epithelial cells after aspiration, is associated with an enhanced proinflammatory state. We primarily focused on defining the kinetics of RAGE, its soluble receptor (sRAGE), and HMGB1 in the early phase of ALI, for which a paucity of information is available. Using our unique *in vivo* murine model of acute aspiration pneumonitis which combines small gastric particles and acid to best simulate clinical aspiration events, we hope these results provide new insight into the complex mechanics of this highly morbid lung injury.

2. Materials and methods

2.1. Experimental animal model

Male CD-1 mice (4 wk old) were purchased and housed according to protocols approved by the Institutional Animal Care and Use Committees of the University at Buffalo and the Veterans Administration Western New York Healthcare System. Mice were randomized to a group receiving either a low AGE (LAGEs) or high AGE (HAGEs) diet *ad libitum* for 4 wk. The details of the diet preparation are described in our previous publication [7]. After 4 wk, the animals in each dietary designation were randomly assigned to one of five groups ($n = 9–15$ per group): uninjured control animals or gastric aspiration injury with samples collected at 5 min, 1, 5, or 24 h after injury. Injury and sample collection were performed under general anesthesia using isoflurane in 100% oxygen, delivered via nose cone.

2.2. Gastric aspiration-induced lung injury

The aspirate injury solution was a combination of 20 mg/mL of small gastric food particles and hydrochloric acid, adjusted to a pH of 1.25. The small, non-acidified gastric particles were obtained from the stomachs of necropsied CD-1 mice and prepared according to previously published protocol [7]. After anesthesia induction, a midline tracheotomy was performed and a 22-gauge needle containing the aspirate solution (volume of 3.6 mL/kg of body weight + 0.2 mL air bolus) was inserted into the trachea. The chest wall was then compressed and rapidly released following aspirate instillation, maximizing pulmonary distribution. Once the incision was closed and anesthesia ceased, the mice received 100% oxygen and were monitored for recovery of regular, spontaneous breathing patterns. Mice were then returned to their respective cages, breathing room air only, until their prespecified postinjury time point when they were sacrificed under anesthesia for bronchoalveolar lavage (BAL) sampling and lung tissue harvesting.

2.3. BAL sampling and processing procedures

After puncturing the diaphragm and performing a sternotomy, the pulmonary vasculature was flushed via the right ventricle with 5 mL of Hanks balanced salt solution. BAL was performed by instilling five 1 mL aliquots of Hanks balanced salt solution through a tracheal cannula and recovering the pooled aliquots with a syringe. The BAL was kept on ice until processing, beginning with centrifugation at 1,500 × *g* for 5 min at 4°C to pellet cells. The resulting supernatant was stored at −80°C for analysis of sRAGE, HMGB1, albumin, and cytokines, and chemokines. The cell pellet was then