FEATURED NEW INVESTIGATOR

Inhibition of the nucleotide-binding domain, leucine-rich containing family, pyrin-domain containing 3 inflammasome reduces the severity of experimentally induced acute pancreatitis in obese mice

JASON M. YORK, KARLA J. CASTELLANOS, ROBERT J. CABAY, and GIAMILA FANTUZZI

CHICAGO, ILL

Acute pancreatitis (AP), although most often a mild and self-limiting inflammatory disease, worsens to a characteristically necrotic severe acute pancreatitis (SAP) in about 20% of cases. Obesity, affecting more than one-third of American adults, is a risk factor for the development of SAP, but the exact mechanism of this association has not been identified. Coincidental with chronic low-grade inflammation, activation of the nucleotide-binding domain, leucine-rich containing family, pyrin-domain containing 3 (NLRP3) inflammasome increases with obesity. Lean mice genetically deficient in specific components of the NLRP3 inflammasome are protected from experimentally induced AP, indicating a direct involvement of this pathway in AP pathophysiology. We hypothesized that inhibition of the NLRP3 inflammasome with the sulfonylurea drug glyburide would reduce disease severity in obese mice with cerulein-induced SAP. Treatment with glyburide led to significantly reduced relative pancreatic mass and water content and less pancreatic damage and cell death in genetically obese *ob/ob* mice with SAP compared with vehicle-treated obese SAP mice. Glyburide administration in *ob/ob* mice with cerulein-induced SAP also resulted in significantly reduced serum levels of interleukin 6, lipase, and amylase and led to lower production of lipopolysaccharide-stimulated interleukin 1β release in cultured peritoneal cells, compared with vehicle-treated *ob/ob* mice with SAP. Together, these data indicate involvement of the NLRP3 inflammasome in obesity-associated SAP and expose the possible utility of its inhibition in prevention or treatment of SAP in obese individuals. (Translational Research 2014;164:259–269)

Abbreviations: AP = acute pancreatitis; ASC = apoptosis-associated speck-like protein containing a CARD; ATP = adenosine triphosphate; CASP1 = caspase 1; CER = cerulein; FFA = free fatty acid; GLY = glyburide; H&E = hematoxylin and eosin; IL-6 = interleukin 6; IL-12 = inter-

Jason M. York, PhD, *is a postdoctoral research fellow in the Department of Kinesiology and Nutrition at the University of Illinois, Chicago. His article is based on a presentation given at the Combined Annual Meeting of the Central Society for Clinical and Translational Research and the Midwestern Section American Federation for Medical Research, held in Chicago, Ill, on April 2014.*

From the Department of Kinesiology and Nutrition, University of Illinois at Chicago, Chicago, Ill; Department of Pathology, University of Illinois at Chicago, Chicago, Ill.

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Reprint requests: Jason M. York, 1919 West Taylor Street, MC 517, Chicago, IL 60612; Tel.: +1-312-413-5398; e-mail: jmyork@uic.edu.

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leukin 12; IL-18 = interleukin 18; IL-1β = interleukin 1β; K_{ATP} = ATP-sensitive potassium; LPS = lipopolysaccharide; NLRP3 = nucleotide-binding domain, leucine-rich containing family, pyrin-domain containing 3; PBS = phosphate-buffered saline; SAP = severe acute pancreatitis; TUNEL = terminal deoxynucleotidyl transferase dUTP nick end labeling; Veh = vehicle; WT = wild type

AT A GLANCE COMMENTARY

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Background

During acute pancreatitis (AP), obesity is a risk factor for developing severe acute pancreatitis (SAP). However, the mechanism(s) linking obesity and increased SAP risk are unknown. Concomitant with chronic inflammation, nucleotide-binding domain, leucine-rich containing family, pyrin-domain containing 3 (NLRP3) inflammasome hyperactivity also occurs during obesity. NLRP3 inflammasome functionality is required for the development of AP in lean mice, indicating its potential role in AP pathophysiology.

Translational Significance

We show that inhibition of the NLRP3 inflammasome with the sulfonylurea drug glyburide reduces the severity of experimental AP in genetically obese mice. As this drug is already approved for use in diabetic patients, our findings indicate glyburide's therapeutic potential in human patients with SAP.

INTRODUCTION

Although typically a mild and self-limiting disease, approximately 20% of acute pancreatitis (AP) cases worsen to severe acute pancreatitis (SAP), characterized by pancreatic tissue necrosis, high morbidity, and up to a 20% mortality rate.[1-3] Obesity is an established risk factor in the progression from AP to SAP and mortality because of SAP complications.[1,4,5] Despite the observed connection between obesity and SAP, the mechanisms of increased AP severity in the obese continue to remain unknown.

Both genetic and diet-induced obesity increase disease severity in multiple AP models in rodents, including coadministration of interleukin (IL)-12 + IL-18, pancreatic duct retrograde infusion of sodium taurocholate, and repeated injection of cerulein.[2,6-11] Former work by our group demonstrated that, despite differences in physiology between genetically obese and diet-induced obesity models, the presence of obesity rather than leptin and leptin signaling deficiency results in SAP.[7] Thus, although not exactly mimicking the pathophysiology of human obesity, genetically obese rodents provide relevant insight into the pathophysiological outcomes of SAP.

The nucleotide-binding domain, leucine-rich containing family, pyrin-domain containing 3 (NLRP3) inflammasome is an intracellular multimolecular pattern recognition receptor responsible for the caspase 1 (CASP1)-mediated activation and secretion of the proinflammatory cytokines IL-1β and IL-18.[12-14] Obesity is associated with a state of chronic low-grade inflammation and increased circulating inflammatory cytokines, which are linked to the pathogenesis of obesity-related diseases, such as cardiovascular disease and type 2 diabetes.[15-18] Coincidental with this chronic inflammation, NLRP3 inflammasome activity is increased in obese individuals as a result of adipocyte hypertrophy. Adipocyte and adipose tissue hypertrophy lead to localized tissue hypoxia due to poor vascularization of rapidly expanding adipose tissue in obesity, and subsequent necrotic cell death leads to reactive oxygen species production and free fatty acid (FFAs) and inflammatory cytokine release.[19-21]

NLRP3 inflammasome activation has also been implicated in rodent models of AP. Hoque et al have shown that *NLRP3*, *ASC* (apoptosis-associated speck-like protein containing a caspase recruitment domain (CARD); an adaptor molecule in the NLRP3 inflammasome structure), and *CASP1* genes are required for the development of pancreatic inflammation in AP in lean rodents.[22] Moreover, treatment with IL-1 receptor antagonist before or during the initiation of AP reduces pancreatic inflammation and tissue damage. Upstream of IL-1β, CASP1 inhibition in rats ameliorates disease severity and increases survival in sodium taurocholate–induced SAP.[23-25] Thus, blocking IL-1 maturation or activity effectively reduces AP severity in lean animals.

Glyburide (International nonproprietary name, glibenclamide), an antidiabetic sulfonylurea drug, inhibits NLRP3 inflammasome activation by several stimuli without affecting the nucleotide-binding domain, leucine-rich containing family, CARD domain containing 4 (NLRC4) or NLRP1 inflammasomes.[14] The mechanism of action of glyburide on the NLRP3 inflammasome is not completely understood, although the drug appears to interfere with signaling events upstream of the NLRP3 subunit. Specifically, glyburide inhibits the regulatory subunit of adenosine triphosphate (ATP)-sensitive potassium (K_{ATP}) channels on the cell