JACC: HEART FAILURE VOL. ■, NO. ■, 2014

ISSN 2213-1779/$36.00
PUBLISHED BY ELSEVIER INC. http://dx.doi.org/10.1016/j.jchf.2014.08.002

The Impact of Galectin-3 Inhibition on Aldosterone-Induced Cardiac and Renal Injuries

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ABSTRACT

OBJECTIVES This study investigated whether galectin (Gal)-3 inhibition could block aldosterone-induced cardiac and renal fibrosis and improve cardiorenal dysfunction.

BACKGROUND Aldosterone is involved in cardiac and renal fibrosis that is associated with the development of cardiorenal injury. However, the mechanisms of these interactions remain unclear. Gal-3, a β-galactoside–binding lectin, is increased in heart failure and kidney injury.

METHODS Rats were treated with aldosterone-salt combined with spironolactone (a mineralocorticoid receptor antagonist) or modified citrus pectin (a Gal-3 inhibitor), for 3 weeks. Wild-type and Gal-3 knockout mice were treated with aldosterone for 3 weeks. Hemodynamic, cardiac, and renal parameters were analyzed.

RESULTS Hypertensive aldosterone-salt-treated rats presented cardiac and renal hypertrophy (at morphometric, cellular, and molecular levels) and dysfunction. Cardiac and renal expressions of Gal-3 as well as levels of molecular markers attesting fibrosis were also augmented by aldosterone-salt treatment. Spironolactone or modified citrus pectin treatment reversed all of these effects. In wild-type mice, aldosterone did not alter blood pressure levels but increased cardiac and renal Gal-3 expression, fibrosis, and renal epithelial-mesenchymal transition. Gal-3 knockout mice were resistant to aldosterone effects.

CONCLUSIONS In experimental hyperaldosteronism, the increase in Gal-3 expression was associated with cardiac and renal fibrosis and dysfunction but was prevented by pharmacological inhibition (modified citrus pectin) or genetic disruption of Gal-3. These data suggest a key role for Gal-3 in cardiorenal remodeling and dysfunction induced by aldosterone. Gal-3 could be used as a new biotarget for specific pharmacological interventions. (J Am Coll Cardiol HF 2014;■:■–■) © 2014 by the American College of Cardiology Foundation.

Aldosterone (Aldo) is a well-known key regulator of blood pressure (BP) and electrolytic balance that acts classically via an intracellular mineralocorticoid receptor (MR) (1). A growing body of evidence suggests that Aldo plays an important pathophysiological role in cardiac, vascular, and renal remodeling via its MR by promoting oxidative stress, inflammation, fibrosis, and hypertrophy

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Manuscript received June 9, 2014; revised manuscript received August 14, 2014, accepted August 22, 2014.

ABBREVIATIONS AND ACRONYMS

Aldo = aldosterone

BP = blood pressure

Gal = galectin

HF = heart failure

KO = knockout

LV = left ventricular

MCP = modified citrus pectin

MR = mineralocorticoid receptor

NGAL = neutrophil gelatinase associated lipocalin

SMA = smooth muscle actin

WT = wild-type

(2–4). The pivotal role of Aldo in cardiac and renal fibrosis is reinforced at the cellular level in tubule epithelial cells and in cardiac fibroblasts where Aldo increases collagen synthesis (5,6). However, the precise mechanism responsible for Aldo-induced cardiac and renal fibrosis remains to be determined, and despite advances in treatment of both cardiovascular and kidney diseases, cardiorenal injury remains a major clinical problem (7).

Galectin-3 (Gal-3) is a protein member of a β-galactoside binding lectin family, localized in the nucleus, cytoplasm, cell surface, and extracellular space (8). Several inhibitors of Gal-3 have been described, such as the modified citrus pectin (MCP) (9), a complex water-soluble indigestible polysaccharide rich in β-galactose. The expression of this lectin has been reported in many tissues (10) (including heart and kidney) and cells (in fibroblasts [11], endothelial cells [12], epithelial cells of tubules or collecting ducts [13], and inflammatory cells [14]). This lectin induces cardiac and renal fibrosis that leads to cardiac dysfunction (11) or acute kidney injury (13) in different experimental models of heart failure (HF) and kidney injury. Our group recently showed that Gal-3 is up-regulated by Aldo and that it mediates the inflammatory and fibrotic response to Aldo in vascular smooth muscle cells both in vitro and in vivo, indicating a key role for this lectin in Aldo-induced vascular fibrosis (15). In humans, the serum Gal-3 level has been correlated with serum markers of cardiac extracellular matrix turnover and therefore Gal-3 emerges as a biomarker associated with HF onset, morbidity, and mortality (16). In addition to this cardiac association, it has been shown that plasma Gal-3 is associated with renal impairment in patients with or without HF (17). The predictive value of Gal-3 appeared to be stronger in patients with HF with preserved ejection fraction, postulating that Gal-3 might be a particularly useful biomarker in HF with preserved ejection fraction (18). However, although previous studies have investigated separately the effects of Aldo and Gal-3 on cardiac remodeling and function, the interaction between these 2 factors and the pharmacological blockade or gene inactivation of Gal-3 have never been explored in the heart and the kidney in the context of high Aldo levels.

The hypothesis that we explore here is that Gal-3 is involved in Aldo-induced cardiac and renal fibrosis and dysfunction, and, therefore, Gal-3 could be a new key factor in the development of the cardiac and renal injuries. The present study was designed to examine the protective effect of the Gal-3 inhibition or absence in the progression of the cardiac and renal injuries using 2 animal models: 1) hypertensive hyperaldosteronism model of rats (treated with Aldo-salt) in combination with the Gal-3 inhibitor MCP; and 2) normotensive model of wild-type (WT) and Gal-3 knockout (KO) mice infused with Aldo with a normal salt diet.

METHODS

Detailed materials and methods are available in the Appendix.

ANIMALS. The investigation was performed in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

For the Gal-3 inhibition model, adult male Wistar rats were treated for 3 weeks with vehicle (n = 10), Aldo-salt (1 mg/kg/day diluted in sunflower oil and administered by subcutaneous injection and 1% NaCl as drinking water, n = 10), Aldo-salt plus spironolactone (200 mg/kg/day, n = 10), Aldo-salt plus MCP (100 mg/kg/day, n = 9), spironolactone (n = 7), or MCP (n = 5) alone. After 2 weeks of treatment, urine was collected in metabolic cages. At the end of the treatment, hemodynamic parameters were evaluated.

For the Gal-3 ablation model, adult male C57BJ6 WT mice and Gal-3 KO mice (19) were infused for 3 weeks with Aldo (1 mg/kg/day, osmotic minipump) or vehicle (n = 7, each group). Tail cuff blood pressure was monitored throughout the treatment.

ANALYSIS. For histology, sections were stained with Sirius red. For immunohistochemistry, sections were incubated with primary antibody, then with peroxidase-labeled secondary antibody and revealed using a 3,3′-diaminobenzidine substrate kit (Vector Laboratories, Burlingame, California). Classical electrophoresis was performed on sodium dodecyl sulfate polyacrylamide gels and transferred to a nitrocellulose membrane. Reverse-transcription polymerase chain reaction was performed with SYBR green polymerase chain reaction technology. Protein concentrations were measured by an enzyme-linked immunosorbent assay according to the manufacturer's instructions. Urinary Na^+ and K^+ concentrations were measured by flame photometry (IL943, Instrumentation Laboratory, Bedford, Massachusetts).

STATISTICS. Because means were found very close from the medians, results are presented as mean ± SEM, computed from the average measurements obtained from each group of animals. Normal distribution of data was checked by means of the Shapiro-Wilk test, and a Levene statistic test was performed to