The myeloid mineralocorticoid receptor controls inflammatory and fibrotic responses after renal injury via macrophage interleukin-4 receptor signaling

Jonatan Barrera-Chimal[1,2], Gabriel R. Estrela[1,9], Sebastian M. Lechner[1,9], Sébastien Giraud[3,4,5], Soumaya El Moghrabi[1], Shiem Kaaki[3,6], Peter Kolkhof[7], Thierry Hauet[3,4,5] and Frédéric Jaisser[1,8]

[1]INSERM, UMRS 1138, Team 1, Centre de Recherche des Cordeliers, Pierre et Marie Curie University, Paris Descartes University, Paris, France; [2]Molecular Physiology Unit, Instituto de Investigaciones Biomedicas, UNAM and Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán, Mexico City, Mexico; [3]INSERM U1082 IRTOMIT, Poitiers, France; [4]Université de Poitiers, Faculté de Médecine et de Pharmacie, Poitiers, France; [5]CHU Poitiers, Service de Biochimie, Poitiers, France; [6]CHU Poitiers, Service d'Anatomopathologie, Poitiers, France; [7]Bayer AG, Cardiology Research, Wuppertal, Germany; and [8]INSERM, Clinical Investigation Center 1433, French-Clinical Research Infrastructure Network (F-CRIN) INI-CRCT, Nancy, France

Acute kidney injury induced by ischemia/reperfusion is an independent risk factor for chronic kidney disease. Macrophage recruitment plays an essential role during the injury and repair phases after an ischemic episode in the kidney. Here we show that the novel non-steroidal mineralocorticoid receptor antagonist finerenone or selective myeloid mineralocorticoid receptor ablation protects against subsequent chronic dysfunction and fibrosis induced by an episode of bilateral kidney ischemia/reperfusion in mice. This protection was associated with increased expression of M2-antiinflamatory markers in macrophages from finerenone-treated or myeloid mineralocorticoid receptor-deficient mice. Moreover, the inflammatory population of CD11b^{+}, F4/80^{+}, Ly6C^{high} macrophages was also reduced. Mineralocorticoid receptor inhibition promoted increased IL-4 receptor expression and activation in the whole kidney and in isolated macrophages, thereby facilitating macrophage polarization to an M2 phenotype. The long-term protection conferred by mineralocorticoid receptor antagonism was also translated to the Large White pig pre-clinical model. Thus, our studies support the rationale for using mineralocorticoid receptor antagonists in clinical practice to prevent transition of acute kidney injury to chronic kidney disease.

Kidney International (2018) **93,** 1344–1355; https://doi.org/10.1016/j.kint.2017.12.016

KEYWORDS: inflammation; ischemic injury; macrophage polarization

Correspondence: *Frédéric Jaisser, INSERM U1138, Centre de Recherche de Cordeliers, Team 1, 15 rue de l'Ecole de Médecine, 75006, Paris, France. E-mail: frederic.jaisser@inserm.fr*

[9]These authors contributed equally to this work.

Received 31 May 2017; revised 6 December 2017; accepted 15 December 2017; published online 13 March 2018

Chronic kidney disease (CKD) has become a global public health problem that threatens the health care system. According to a recent meta-analysis, it is estimated that the global prevalence of CKD is approximately 11% to 13%.[1] In recent years, it has been recognized that patients surviving an episode of acute kidney injury (AKI) are at increased risk of adverse outcomes, such as *de novo* CKD, worsening of pre-existing CKD, and end-stage kidney disease.[2] It has been demonstrated in experimental models that a single episode of renal ischemia-reperfusion (IR) may result in maladaptive repair and lead to chronic kidney fibrosis and dysfunction.[3,4] Intensive research in the field over the past few years has shed light on mechanisms involved in the AKI-to-CKD transition, such as capillary rarefaction, tubular epithelial cell G2/M arrest, sustained oxidative stress, and chronic inflammation.[5] The infiltration of inflammatory cells early after an AKI episode plays an important role in defining effective versus maladaptive repair.[6] Nevertheless, there are no therapeutic approaches in current clinical practice that have proven to be useful in preventing progression to CKD after an AKI episode.[7] There is thus a need to identify novel underlying mechanisms that facilitate CKD development to aid the development of more precise targeted therapy. We previously demonstrated that pharmacological mineralocorticoid receptor (MR) antagonism is an efficient strategy to prevent CKD after a single episode of AKI.[4,8] However, the detailed mechanisms of MR-mediated kidney fibrosis are poorly understood. MR is expressed in myeloid cells and has been shown to play a key role in aldosterone and angiotensin II–mediated heart fibrosis.[9,10] Thus, we hypothesized that myeloid MR may potentially be involved in the development of kidney fibrosis after an ischemic AKI episode.

RESULTS

Nonsteroidal MR antagonism is beneficial in AKI-induced CKD

We developed a model of bilateral IR-induced CKD in mice and demonstrated that pharmacological MR inhibition with

the nonsteroidal MR antagonist (MRA) finerenone can prevent CKD development after IR. Finerenone was administered at 48, 24, and 1 hour before the IR procedure, while no finerenone was given thereafter until killing (D30) (Supplementary Figure S1). In this model with important bilateral IR, the protection conferred by finerenone against the acute effects of IR (24 hours) was partial as shown by a limited reduction in plasma creatinine and urea levels (Supplementary Figure S2). Four weeks after transient ischemia, plasma creatinine (Figure 1a), urea (Figure 1b), and proteinuria levels (Figure 1c) were increased in the IR untreated mice. This was associated with increased expression of transforming growth factor beta as an indicator of kidney fibrosis (Figure 1d). These alterations were efficiently prevented by prophylactic finerenone administration. Histology analysis of Sirius Red–stained slides revealed substantially greater collagen deposition in the IR group than in sham- and finerenone-treated mice (Figure 1e–h). We tested whether finerenone was also protecting toward CKD progression in a more severe ischemic injury episode by using the 30-minute unilateral renal ischemia model and 4 weeks of follow-up. Unilateral IR induced severe tubulo-interstitial fibrosis as evidenced in the Sirius Red staining and fibrosis scoring (Supplementary Figure S2A–D). In contrast, in mice treated with finerenone before IR, the severity of kidney fibrosis was significantly reduced. Proteinuria was elevated in the untreated IR group compared with sham, and finerenone treatment prevented the increased proteinuria (Supplementary Figure S2E).

Role of myeloid MR in CKD progression and macrophage polarization

In previous studies, we have reported a role for the MR expressed in smooth muscle cells in acute kidney injury after IR.[11] Transient renal ischemia was induced in smooth muscle cell MR knockout mice (MR^{SMCKO}), and kidney injury was

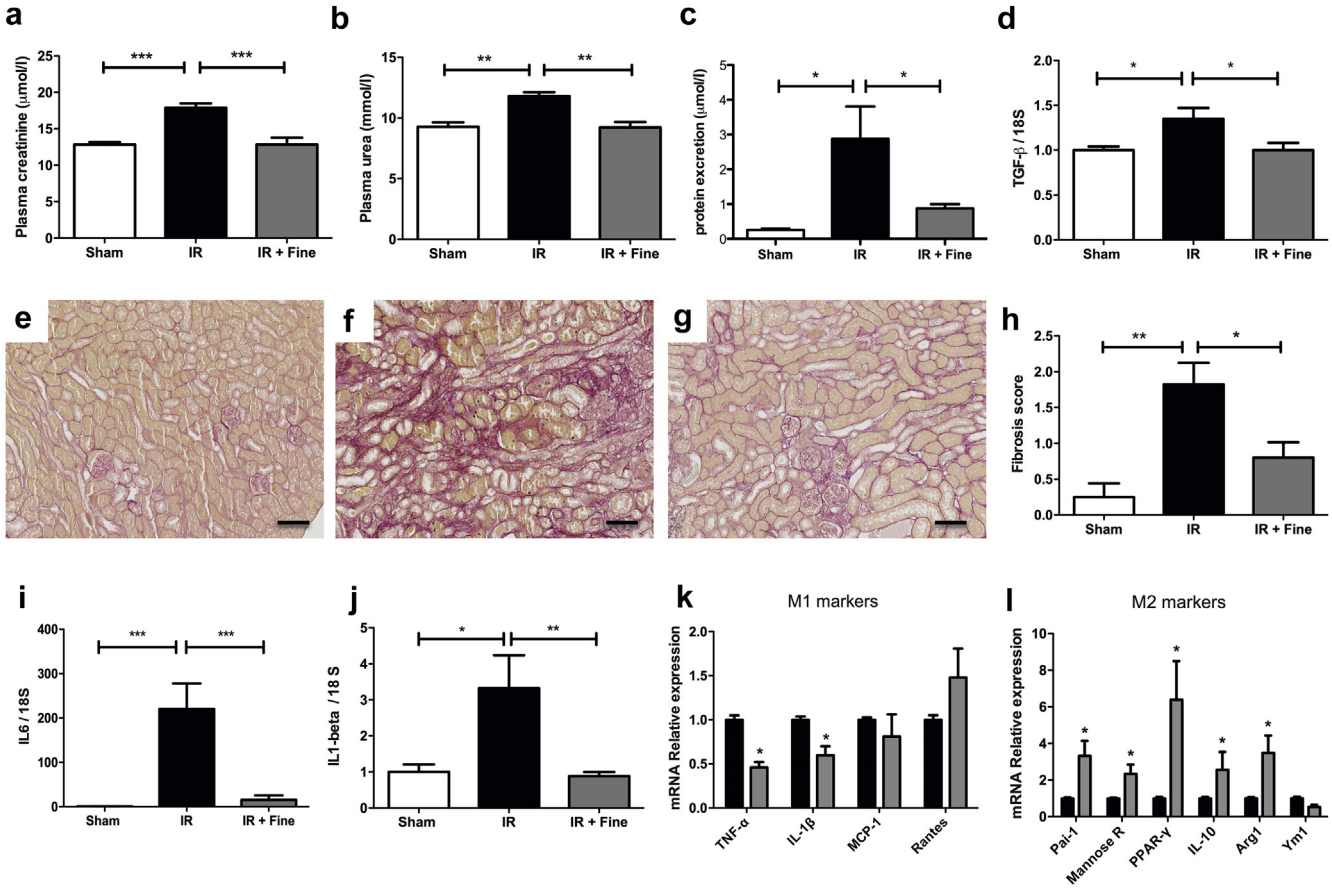

Figure 1 | Finerenone protects against the transition from acute kidney injury to chronic kidney disease. Renal function was determined by quantifying the plasma levels of (**a**) creatinine and (**b**) urea. (**c**) The urinary protein excretion was determined. (**d**) We measured kidney mRNA levels of transforming growth factor (TGF)-β1 by real-time polymerase chain reaction as a marker of kidney fibrosis. Representative Sirius Red–stained images for the (**e**) sham, (**f**) ischemia-reperfusion (IR), and (**g**) IR + finerenone (Fine) groups. (**h**) The fibrosis score was blindly quantified on 8 fields per mouse. The mRNA levels of the proinflammatory cytokines (**i**) interleukin (IL)-6 and (**j**) IL1-beta were quantified in whole kidney. Kidney macrophages ($CD45^+$, $F4/80^+$, $CD19^-$, $CD3^-$, and $Ly6g^-$ cells) were sorted and the RNA extracted. The mRNA levels of (**k**) M1 markers and (**l**) M2 markers were determined in the IR (black bars) and IR + Fine (gray bars) groups. $n = 6$ per group. Arg1, arginase 1; MCP, monocyte chemoattractant protein; Pai, plasminogen activator inhibitor; PPAR, peroxisome proliferator-activated receptor; TNF, tumor necrosis factor. One-way analysis of variance or Student *t*-test was performed. Bar = 100 µm. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$. To optimize viewing of this image, please see the online version of this article at www.kidney-international.org.