Metformin prevents the development of severe chronic kidney disease and its associated mineral and bone disorder

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Chronic kidney disease (CKD) causes dysregulation of mineral metabolism, vascular calcification and renal osteodystrophy, an entity called 'CKD–Mineral and Bone Disorder' (CKD-MBD). Here we determine whether metformin, an anti-diabetic drug, exerts favorable effects on progressive, severe CKD and concomitant mineral metabolism disturbances. Rats with CKD-MBD, induced by a 0.25% adenine diet for eight weeks, were treated with 200 mg/kg/day metformin or vehicle from one week after CKD induction onward. Severe, stable CKD along with marked hyperphosphatemia and hypocalcemia developed in these rats which led to arterial calcification and high bone turnover disease. Metformin protected from development toward severe CKD. Metformin-treated rats did not develop hyperphosphatemia or hypocalcemia and this prevented the development of vascular calcification and inhibited the progression toward high bone turnover disease. Kidneys of the metformin group showed significantly less cellular infiltration, fibrosis and inflammation. To study a possible direct effect of metformin on the development of vascular calcification, independent of its effect on renal function, metformin (200 mg/kg/day) or vehicle was dosed for ten weeks to rats with warfarin-induced vascular calcification. The drug did not reduce aorta or small vessel calcification in this animal model. Thus, metformin protected against the development of severe CKD and preserved calcium phosphorus homeostasis. As a result of its beneficial impact on renal function, associated comorbidities such as vascular calcification and high bone turnover disease were also prevented.

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Over the past decades, metformin has been used as a first-line treatment for type 2 diabetes because of its effect on glucose metabolism. This therapeutic improves insulin sensitivity and thus stimulates glucose uptake, increases glycolysis, and decreases gluconeogenesis in the liver. However, it is becoming clear that metformin exerts multiple actions beyond its prescribed use as an antihyperglycemic agent, including beneficial effects on the kidney.[1] Because metformin is cleared by the kidney and thus, for the time being, is contraindicated in patients with advanced stages of renal disease due to the fear of lactic acidosis, studies of the effect of metformin on the kidney are few and limited to preclinical experiments. In a rat model of gentamicin-induced nephropathy, metformin treatment fully blocked the induction of acute renal failure, prevented the histological changes in the kidney, and normalized oxidative stress.[2] Furthermore, this antidiabetic agent was able to ameliorate renal fibrosis in 5/6 nephrectomized rats[3] and mice with unilateral ureteral obstruction.[4] Also, with regard to the cardiovascular system, benefits of metformin are reported in diabetic patients,[3,5–7] although the mechanism underlying its action is not yet fully understood. Vasculoprotective effects by metformin may indirectly be ascribed to improvements in glucose and lipid metabolism, but direct actions on the heart and vasculature also are increasingly being reported.[8] Metformin has been shown to attenuate atherosclerosis and vascular senescence,[9] improve diabetic cardiomyopathy by stimulation of cardiac autophagy and inhibition of apoptosis of cardiomyocytes,[10,11] and to protect against myocardial ischemia reperfusion injury.[12–14]

Patients with chronic kidney disease (CKD) suffer from disturbances in mineral homeostasis including dysregulation of calcium, phosphorus, parathyroid hormone, and fibroblast growth factor 23, which ultimately entails ectopic calcification in the arteries and bone abnormalities. These complications of chronic renal impairment cover a broader syndrome and are defined as CKD-mineral and bone disorder (CKD-MBD). In the current study, we aimed to investigate whether metformin exerts protective effects on the kidney, blood vessels, and bone in a rat model of CKD-MBD, recently optimized by our group[15] and characterized by the induction of severe, stable CKD along with the development of moderate vascular

calcification and high bone turnover disease. To evaluate the isolated effect of metformin on arterial calcification, independently of its effect on renal function, an additional study was executed in a rat model of warfarin-induced vascular calcification.

RESULTS

Adenine-induced CKD rat model: effect of metformin on CKD-MBD

Mortality was limited because only 1 animal of the metformin group died before the planned sacrifice, which was not related to the metformin treatment. Food intake was slightly higher in the vehicle-treated group in weeks 2, 3, and 4 and slightly higher in the metformin-treated group in weeks 6, 7, and 8 (Supplementary Figure S1). No significant differences in body weight were noted between vehicle- and metformin-treated CKD rats throughout the study period (Supplementary Figure S1).

Renal function. Incessant adenine dosing resulted in severe, stable CKD in vehicle-treated rats. Serum creatinine levels dramatically rose to approximately 6 mg/dl, which was 10-fold higher than control values at the start of the experiment (Figure 1a). In line with this result, creatinine clearance significantly decreased over time in vehicle-treated CKD rats (Figure 1b). Interestingly, metformin dosing protected adenine-exposed rats from the evolution toward severe CKD. Serum creatinine concentrations in the metformin-treated CKD rats remained relatively low and were only 2.5-fold increased at the end of the study compared to baseline values. From week 4 onward, serum creatinine levels were significantly lower, and creatinine clearance was significantly higher in the metformin-treated CKD rats than in the vehicle-treated animals.

Renal histology, fibrosis, and inflammation. Histological examination of periodic acid–Schiff-stained renal sections revealed that induction of CKD by adenine exposure caused dilation of proximal and distal tubules, as well as epithelial flattening, proximal brush border loss, basement membrane thickening, and tubulointerstitial cellular infiltration, whereas glomeruli remained relatively intact (Figure 2c). Masson staining clearly confirmed the presence of excess extracellular matrix in the expanded tubulointerstitium (Figure 2d). The tubulointerstitial area percentage, consisting of both extracellular matrix and infiltrating cells, was 35% lower in metformin-treated animals ($P < 0.05$) (Figure 2a).

The 2,8-dihydroxyadenine crystals were observed both in the tubules and in the interstitium. To investigate whether the beneficial impact of metformin on renal function was not due to interference with the adenine metabolism and thus with the formation of 2,8 dihydroxyadenine crystals in the kidney, the percentage of surface covered by these crystals was measured. Results indicated that the percentage of surface covered by 2,8 dihydroxyadenine crystals in the metformin-exposed CKD rats did not differ significantly from that of the animals exposed to vehicle (Figure 2b).

The mRNA expression of the proinflammatory cell adhesion molecule-1 (VCAM1) was markedly decreased in CKD rats exposed to metformin (Figure 3). Metformin treatment also led to a significantly decreased expression of the inflammatory cytokines TNF-α and IL-1β in the kidney. In addition, TGF-β and collagen type 1a1 expression levels were significantly reduced in CKD rats treated with metformin compared with those treated with vehicle. No differences were observed between the bax-to-bcl2 ratios of either study group, suggesting no effect of metformin on apoptosis.

Mineral homeostasis. In line with the increase in serum creatinine and, thus, with the decrease in renal function, serum phosphorus levels significantly rose with the duration of CKD (Figure 1c), whereas serum total calcium (Figure 1d) was significantly decreased after 8 weeks of CKD in vehicle-treated animals compared with baseline values, which is inherent to chronic renal impairment. Metformin treatment prevented development of hyperphosphatemia and hypocalcemia in CKD rats. Serum ionized calcium levels also were significantly higher in CKD rats treated with metformin than those treated with vehicle (1.23 $\pm$ 0.03 mmol/l in the metformin group versus 0.77 $\pm$ 0.07 mmol/l in the vehicle group). Hypocalcemia and hyperphosphatemia led to a rise in serum parathyroid hormone (PTH) (Figure 1e) and FGF-23 (Figure 1f) levels, respectively, in CKD rats exposed to vehicle treatment. Metformin treatment was also able to significantly attenuate the increase in both of the hormones.

Acid/base balance and anemia. To evaluate whether daily metformin doses caused acidosis, serum pH and bicarbonate were measured at the end of the study. Levels of both pH (7.40 $\pm$ 0.02 in the vehicle group vs. 7.36 $\pm$ 0.03 in the metformin group) and bicarbonate (31.75 $\pm$ 0.77 mmol/l in the vehicle group vs. 27.78 $\pm$ 1.93 mmol/l in the metformin group) in serum did not significantly differ between vehicle- and metformin-treated CKD animals. Due to the beneficial effect of metformin on renal function, metformin-treated rats displayed significantly higher hematocrit (34.08% $\pm$ 1.47% in the metformin group versus 26.18% $\pm$ 1.73% in the vehicle group; $P = 0.001$) and serum hemoglobin concentration (11.60 $\pm$ 0.50 g/dl in the metformin group vs. 8.89 $\pm$ 0.59 g/dl in the vehicle group; $P = 0.001$) compared with vehicle-treated CKD animals.

Vascular calcification. Adenine-induced CKD rats treated with vehicle developed calcification in the aorta and peripheral arteries as shown by the increased calcium content of each vessel type (Figure 4a–c). The calcium content of the aorta, carotid, and femoral arteries in the metformin-treated CKD rats was low and comparable with values of rats with normal renal function, measured in the frame of previous studies conducted by our group,[15] indicating that no vascular calcification had developed in this study group. Although total arterial calcium content was clearly higher in the vehicle-treated CKD group than in the metformin-exposed study group, significant differences between both study groups were reached only in the femoral artery. Figure 4d shows a von Kossa-stained section of a calcified aorta of a CKD rat treated with vehicle versus an intact aorta of a metformin-treated CKD rat. Calcification had developed in the medial layer of the vessel wall.

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