Epiphyseal growth plate growth hormone receptor signaling is decreased in chronic kidney disease–related growth retardation

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Linear growth retardation in children with chronic kidney disease (CKD) has been ascribed to insensitivity to growth hormone. This resistance state has been attributed to impaired growth hormone signaling through the JAK2/STAT5 pathway in liver and skeletal muscle leading to reduced insulin-like growth factor-I (IGF-I). Here we determine whether systemic and growth plate alterations in growth hormone signaling contribute to CKD-induced linear growth retardation using partially nephrectomized and pair-fed control 20-day-old rats. Serum growth hormone did not change in rats with CKD, yet serum IGF-I levels were decreased and growth retarded. The tibial growth plate hypertrophic zone was wider and vascularization at the primary ossification center was reduced in CKD. This was associated with a decrease in growth plate vascular endothelial growth factor (VEGF) mRNA and immunostainable VEGF and IGF-I levels. Growth plate growth hormone receptor and STAT5 protein levels were unchanged, while JAK2 was reduced. Despite comparable growth hormone and growth hormone receptor levels in CKD and control rats, relative STAT5 phosphorylation was significantly depressed in CKD. Of note, the mRNA of SOCS2, an inhibitor of growth hormone signaling, was increased. Thus, linear growth impairment in CKD can in part be explained by impaired long bone growth plate growth hormone receptor signaling through the JAK2/STAT5 pathway, an abnormality that may be caused by an increase in SOCS2 expression.

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Linear growth retardation is a major problem in children with chronic kidney disease (CKD). Nearly 40% of North American children with CKD suffer from short stature. Even though growth failure correlates with the degree of renal impairment, children with mild reduction of glomerular filtration rate may also exhibit short stature. In addition, growth failure in CKD is associated with comorbidity.[1] Several factors contribute to this growth retardation, including the age at onset and severity of CKD, the existence of concomitant acidosis, hypovolemia and hyponatremia, secondary hyperparathyroidism, decreased appetite, and resistance to anabolic hormones such as growth hormone (GH) and insulin-like growth factor-I (IGF-I).[2] GH stimulates linear growth, and increases muscle strength and bone density.[3] After binding to its receptor (growth hormone receptor (GHR)), several signal transduction pathways can be activated, including the Janus kinase 2 (JAK2) signal transducer and activator of transcription (STAT) pathway in which GH-induced JAK2 activation phosphorylates STAT5b, which dimerizes and translocates to the nucleus, to bind response elements that regulate transcription of GH target genes, among them IGF-I.[4] Although STAT1, -3, -5a, and -5b can all be activated by GH, STAT5b is the major target for growth promotion because it regulates IGF-1 expression.[4] In children with CKD and growth retardation, serum GH levels are normal or even elevated, reflecting a state of acquired GH resistance.[5]

Linear growth is the product of an elaborate cascade of events that takes place in the cartilaginous growth center of the long bones, the epiphyseal growth plate. Longitudinal growth is mediated by endochondral ossification in the growth plate area. It begins with the proliferation of early chondrocytes, followed by their alignment in columns, and finally their maturation into hypertrophic chondrocytes.[6] The hypertrophic chondrocytes then die, and as they do so, the transverse septa of cartilage matrix surrounding them are broken down, allowing entry of the invading cells of the ossification front: blood vessels, osteoclasts, and precursors of osteoblasts and bone marrow cells.[6] Vascular endothelial

growth factor (VEGF) is expressed in hypertrophic chondrocytes and is required for vascular invasion to the primary ossification center. The absence of VEGF results in delay of vascular invasion and ossification.[7] GH is an important regulator of longitudinal bone growth.[8] Impaired growth is seen in children with GH deficiency or a genetic inability to respond to GH owing to mutations in its receptor or components of its signaling pathway, including STAT5b.[7] GH affects bone growth directly, by binding to its receptors in the growth plate, and also indirectly, via IGF-I. GH stimulates many body tissues, especially the liver, but also the growth plate, to produce IGF-I, thereby increasing circulating and local IGF-I levels. IGF-I serves as both the main mediator of GH action and as a GH-independent growth factor. The main effect of GH on growth plate chondrocytes is to stimulate their proliferation[7,9] and differentiation (in a spatial-dependent manner[6]) and to protect cells from apoptosis. Few studies have previously specifically investigated the bone GHR signaling pathway in CKD. Rabkin *et al.* examined liver and muscle tissue of mature CKD rats and showed that CKD caused a postreceptor defect in GH signal transduction, which is responsible for GH resistance, without affecting GHR protein levels.[10,11] Our purpose was to test the hypothesis that there are changes in the GH/IGF-I axis, both in the growth plate and systemically, that could contribute to the retardation of linear body growth that occurs in CKD. To that end, we have studied young growing rats with surgically induced CKD and pair-fed sham-operated controls (C).

RESULTS

Kidney function was moderately decreased in this subtotal nephrectomy model, as reflected by a 3-fold increase in serum urea levels (Table 1). Creatinine and Urea clearance was around 8-fold lower in the CKD group compared with the C group (Table 1). No changes in serum phosphate or bicarbonate levels were seen. Serum parathyroid hormone (PTH) was mildly elevated and plasma hemoglobin was mildly decreased in CKD (Table 1). Serum GH did not change in CKD compared with C, whereas serum IGF-I decreased significantly in CKD vs. C (Table 1). Similar endocrine changes were previously described,[5] including reports in some human studies. These results confirm a model of early stage of CKD.

Table 1 | Blood and urine analysis

Parameter	Control	CKD	*P*-value
Serum urea (mg/dl)	45.8 ± 3.1	132.8 ± 7	< 0.0001
Serum creatinine (mg/dl)	0.36 ± 0.02	0.81 ± 0.04	< 0.0001
Creatinine clearance (μl/min)	1658 ± 120	190 ± 23	< 0.001
Urea clearance (μl/min)	838 ± 44	115 ± 10	< 0.001
24-h urine volume (ml)	15.5 ± 0.7	20.5 ± 0.7	< 0.001
Plasma bicarbonate (mmol/l)	24.7 ± 0.7	23.7 ± 1.5	NS
Serum phosphate (mg/dl)	12.3 ± 0.8	11.9 ± 0.6	NS
TRP (%)	99.9 ± 0.03	99.5 ± 0.14	< 0.05
Serum PTH (pg/ml)	195 ± 35	385 ± 38	< 0.005
Hemoglobin (g/dl)	14.3 ± 0.2	11.7 ± 0.4	< 0.0001
Serum GH (ng/ml)	128 ± 15	127 ± 12	NS
Serum IGF-I (ng/ml)	1138 ± 83	662 ± 57	< 0.001

Abbreviations: CKD, chronic kidney disease; GH, growth hormone; IGF-I, insulin-like growth factor-I; NS, not significant; PTH, parathyroid hormone; TRP, total reabsorption of phosphate.
N = 16 for each group.

Growth retardation in CKD

Growth was followed for 2 weeks since the full induction of CKD. Body weight and length, tail length gain, and tibial length all decreased significantly in the CKD group compared with the C group (by 25 ± 3, 42 ± 4, 29 ± 2 and 15 ± 1%, respectively). These changes in growth parameters were not the result of differences in food consumption, because the animals were pair-fed (Table 2). Epiphyseal growth plate width was larger in the CKD group compared with the C group, but there was no change in the number of cells per column between groups (Table 2). There were no changes in proliferative zone width between the groups, whereas a significant increase in the width of the hypertrophic zone was seen (Table 2). Accordingly, the ratios between the widths of each zone to the total width of the growth plate showed a decrease in the fraction of the proliferative zone and an increase in the fraction of the hypertrophic zone in CKD (Table 2). In addition, type X collagen mRNA, which is abundant in the hypertrophic zone, was increased in CKD (Table 2).

Defects in growth plate GHR signal transduction

Bovine GH (100 μg/kg) or buffer was injected into the vena cava 15 min before killing the animal, creating four groups: control + buffer (C), control + GH (Cgh), CKD + buffer (CKD), and CKD + GH (CKDgh). Tibial epiphyseal growth plate GHR mRNA levels were unchanged between groups (1.2 ± 0.1-, 1.1 ± 0.3-, and 1.3 ± 0.05-fold of C, in Cgh, CKD, and CKDgh groups, respectively, p: NS) (Figure 1a). Growth plate GHR protein levels were also unchanged (88 ± 11,

Table 2 | Somatic linear growth and epiphyseal growth plate parameters

	C	CKD	*P*-value
Somatic growth			
Body weight gain (g)	80.9 ± 1.8	60.3 ± 2.3	< 0.0001
Body length gain (cm)	8.2 ± 0.3	4.8 ± 0.3	< 0.0001
Tail length gain (cm)	5.1 ± 0.13	3.6 ± 0.1	< 0.0001
Tibial length (mm)	31.5 ± 0.2	26.9 ± 0.4	< 0.0001
Food consumption/day (g)	14.5 ± 0.2	13.8 ± 0.4	NS
EGP parameters			
EGP width (% of C)	100 ± 1.9	123 ± 3.4	< 0.0001
Proliferative zone width (% of C)	100 ± 3.5	105 ± 4.2	NS
Hypertrophic zone width (% of C)	100 ± 2.2	140 ± 6.2	< 0.0001
Proliferative/total width ratio (%)	47.7 ± 1.3	40.7 ± 0.9	< 0.0001
Hypertrophic/total width ratio (%)	45.5 ± 1.1	54.2 ± 1.1	< 0.0001
Proliferative/hypertrophic ratio (%)	104.8 ± 6.3	54.2 ± 1.1	< 0.0001
Number of cells/row	30.7 ± 1.5	32.1 ± 1.2	NS
EGP type X collagen mRNA (fold of C)	1.0 ± 0.1	3.7 ± 0.4	< 0.0001

Abbreviations: C, control; CKD, chronic kidney disease; EGP, epiphyseal growth plate; NS, not significant.
N = 16 for each group.