Molecular targets of hyperphosphataemia in chronic renal failure

Ken-Ichi Miyamoto, Mikiko Ito, Hiriko Segawa and Masashi Kuwahata

Nutritional Science, Department of Nutrition, School of Medicine, Tokushima University, Japan

Abstract
Dietary phosphate restriction can prevent or retard the progress of chronic renal failure (CRF) and secondary hyperparathyroidism. The klotho gene is involved in the development of a syndrome resembling human ageing, and klotho mutant mice show abnormal calcium/vitamin D metabolism, developing hyperphosphataemia and vascular calcification. Phosphate retention rescues the phenotype of klotho mice. The level of expression of klotho RNA was greatly reduced in the kidneys of all CRF patients. Dietary Pi restriction induced klotho expression, which enhances the beneficial effect of Pi restriction in patients with CRF and/or on haemodialysis.

Keywords: chronic renal failure; dietary phosphate; hyperphosphataemia; klotho; secondary hyperparathyroidism

Introduction
Phosphate (Pi) retention, or hyperphosphataemia, has been identified as playing a major role in the progression of renal failure and in the generation of secondary hyperparathyroidism and uraemic bone disease [1]. Hyperphosphataemia, independent of calcium and calcitriol, enhances uraemia-induced hyperplasia of the parathyroid glands, as well as parathyroid hormone (PTH) synthesis and secretion [2]. As shown in Figure 1, there is now considerable evidence that hyperphosphataemia regulates several signalling pathways of cell functions [1–3]. Of great interest is the recent identification of a novel Pi-regulating gene, klotho [4,5], which in mice is involved in the development of a syndrome resembling human ageing. The klotho mutant mice show abnormal calcium/phosphate/vitamin D metabolism and develop hyperphosphataemia and vascular calcification [4,5].

Phenotypes of klotho mice
The new mouse mutant known as klotho exhibits a syndrome resembling human ageing, including a reduced life span, decreased activity, infertility, osteoporosis, arteriosclerosis, atrophy of the skin, etc. [4]. All these phenotypes were caused by the disruption of a single gene, klotho [4]. Klotho protein functions through a signalling pathway involving a circulating humoral factor [4]. Ectopic calcification is evident in various organs of klotho mice, as well as in arterial walls, including vessels in the stomach, bronchial mucosa, alveolar cells, choroid plexuses, skin, testes and cardiac muscle [4]. It appears ~4 weeks after birth and progresses according to age. The distribution of ectopic calcification in klotho mice resembles that in natural human ageing [4]. Plasma Pi and calcium

Cardiac disease
Klotho
Ursolic bone disease

Novel phosphate-regulating genes

Soft-tissue calcification

Osteocalcin Cbfα-1

Hyperphosphataemia

Kidney

Calcium and phosphate deposition

Vitamin D
1α-OHase

Figure 1. Hyperphosphataemia accelerates the progression of renal failure to secondary hyperparathyroidism and uraemic bone disease. A diet high in inorganic phosphate (Pi) increases parathyroid expression of transforming growth factor-α (TGF-α), which promotes growth of PTG cells. Dietary Pi restriction induces the cyclin-dependent kinase inhibitor p21, which arrests growth. Hypophosphataemia also leads to less stable PTH transcription and a decrease in PTH mRNA levels. Hyperphosphataemia induces osteocalcin and Cbfα-1 in vascular smooth muscle cells and promotes vascular calcification. Hyperphosphataemia also down-regulates klotho gene expression.
concentrations are significantly increased. The serum concentrations of calcitonin and PTH of klotho mice (5 weeks) are normally up- and down-regulated, respectively, in response to high concentrations of calcium. Despite the high concentration of calcium, serum concentrations of 1,25-dihydroxyvitamin D in klotho mice are significantly higher than those of wild-type [5].

**Phosphate retention rescues the phenotypes of klotho mice**

Reduction in klotho expression causes accelerated senescence in klotho mutant mice. Morishita et al. demonstrated that a low Pi diet (0.4%) modulated the phenotypes of klotho mice [6]. Analysis of the structure of klotho shows that the insertional mutation in the klotho mice is not located in the gene, but in the 5’ flanking region ~6 kb upstream from the transcription start site. This may be the reason why klotho is only slightly transcribed in the mutants. A mutation in the promoter region of klotho made it hypersensitive to a low Pi diet compared with normal mice, and dietary Pi restriction induced klotho expression in the kidney. We found a dietary Pi-responsive element in the promoter region of klotho that is also present in the promoter of the type IIa Na/Pi co-transporter gene in the kidney, and therefore explained why a phosphate-restricted diet rescues the phenotypes of klotho mice [7].

**Mechanisms of hyperphosphataemia in klotho mice**

Renal proximal tubular reabsorption of Pi is a key element in overall Pi homeostasis, and type IIa Na/Pi co-transporters are the key players [8]. We analysed the concentrations of type IIa protein in klotho mutant mice and found that it was significantly increased in the brush border membrane of the proximal tubular cells. Elevation of renal type IIa Na/Pi co-transporter activity causes hyperphosphataemia in klotho mice; thus, reduction in klotho expression increases the renal type IIa Na/Pi co-transporter.

**klotho expression in chronic renal failure (CRF) patients**

Patients with CRF develop multiple complications that are reminiscent of the phenotypes observed in klotho mice. The klotho gene is expressed mainly in kidney and brain, and the evidence presented here suggests that there might be involvement of klotho function in the complications arising in CRF patients. Indeed, Kho et al. demonstrated that the levels of klotho RNA expression were greatly reduced in the kidneys of all CRF patients [9].

**Hyperphosphataemia represses the expression of klotho**

A reduction in klotho concentration causes up-regulation of calcitriol synthesis and hyperphosphataemia. In renal failure models, hyperphosphataemia promotes secondary hyperparathyroidism whereas hypophosphataemia prevents the development of this condition [1]. Hyperphosphataemia also suppresses the expression of klotho in the aorta, colon and thyroid gland of CRF models. Suppression of klotho may modulate the function of the type III Na/Pi co-transporter in the aorta and then promote vascular calcification in haemodialysis patients (H. Segawa et al., personal communication).

**Conclusion**

There is substantial evidence from studies in experimental animals and patients with CRF that dietary phosphate restriction can prevent or retard the progression of this condition to secondary hyperparathyroidism with associated vascular calcification. A gene, klotho, is involved in the development of a syndrome resembling human ageing in mice. Klotho mice develop hyperphosphataemia and vascular calcification, but dietary Pi restriction induces expression of klotho and rescues the phenotypes of the mice. Up-regulation of klotho may enhance the beneficial effect of Pi restriction in CRF and haemodialysis patients.

**Acknowledgements.** Thanks to D. Nabeshima for providing klotho mice and klotho cDNA, and to Dr Segawa for helpful discussion about the manuscript.

**References**