Matrix extracellular phosphoglycoprotein causes phosphaturia in rats by inhibiting tubular phosphate reabsorption

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Abstract

Background. Matrix extracellular phosphoglycoprotein (MEPE), first isolated from tumour-derived tissue from a patient with oncogenic hypophosphataemia, is a putative phosphatonin that has received much less attention than fibroblast growth factor-23. To date, its effect on renal tubular phosphate reabsorption remains undefined.

Methods. A renal clearance study was performed in anaesthetized rats infused intravenously with a range of doses of MEPE.

Results. MEPE had no effect on glomerular filtration rate (inulin clearance) but caused rapid, dose-dependent increases in absolute and fractional phosphate excretion, wholly attributable to reduced phosphate reabsorption. At a maximal dose, MEPE increased fractional phosphate excretion more than 2-fold, whereas no change was observed in time controls.

Conclusion. The results lend support to the hypothesis that MEPE contributes to the phosphaturia of oncogenic hypophosphataemia and of hypophosphataemic rickets.

Keywords: kidney; phosphatonin; phosphaturia; renal clearance

Introduction

Matrix extracellular phosphoglycoprotein (MEPE) was first isolated from tumour-derived tissue obtained from a patient with oncogenic hypophosphataemic osteomalacia [1]. It was independently isolated from rat primary bone marrow cultures during osteoblast differentiation (where it was given the name osteoblast/osteocyte factor 45; OF45) [2]. MEPE, along with a number of other putative humoral regulators of phosphate balance (‘phosphatonins’), is up-regulated in oncogenic hypophosphataemia [3,4] and in the murine form of X-linked hypophosphataemic rickets [5], but the current and prevailing view is that MEPE is a regulator of bone mineralization rather than a contributor to phosphate wasting in these conditions [6–8]. Nevertheless, circulating levels of MEPE correlate with serum phosphate concentrations [9], and Rowe’s group has reported that repetitive high-dose intraperitoneal injections of MEPE into mice caused increased phosphate excretion, when compared with saline-injected controls [10]. However, since phosphate excretion was factored for creatinine excretion (an unreliable marker of glomerular filtration rate (GFR) in rodents [11]), it was not possible to determine whether the tubular reabsorption of phosphate was altered. Here, we report the results of a renal clearance study in rats, in which we have used a series of infusions of MEPE, delivered intravenously to control its bioavailability; GFR was determined using inulin.

Methods

Adult male Sprague-Dawley rats (weighing 220–260 g) were anaesthetized with sodium thiopentone (100 mg/kg; intraperitoneally; Link Pharmaceuticals, Horsham, Sussex) and prepared surgically for clearance experiments (jugular venous cannulae; tracheotomy; bladder catheter; femoral arterial catheter) as described previously [12]. Isotonic saline was infused intravenously throughout at 4 ml/h. Thirty minutes after the completion of surgery, [3H]inulin (Amersham Biosciences, Little Chalfont, Bucks) was included in the infusate (2 μCi primer; 2 μCi/h). After a further hour of equilibration, all rats underwent a 1 h clearance period, at the end of which the animals were divided into four groups: one group (n = 6; time controls) continued to receive the saline vehicle for a further 2 h; the second group (n = 6; low-dose MEPE) was infused with recombinant full-length human MEPE (raised in insect Spodoptera frugiperda cells; a gift from Acologix, Hayward, CA, USA) at a dose of 30 μg/h for 2 h; the third group (n = 6; medium-dose MEPE)
was infused with MEPE at 100 µg/h; and the final group (n = 6; high-dose MEPE) at 300 µg/h. During the final hour of the infusions, designated the experimental period, clearance measurements were repeated. Small arterial blood samples (~100 µl) were taken at the start and end of each clearance period.

**Analyses**

Urine and plasma samples were analysed for phosphate concentration and [³H]inulin activity by capillary electrophoresis [13] and β-emission spectroscopy (Packard Tricarb, model 2900TR), respectively.

**Calculations and statistics**

Glomerular filtration rate (GFR) was measured as the renal clearance of [³H]inulin (Cₜₐₛ). Renal clearances of phosphate (Cₚᵢ) and [³H]inulin were calculated using the standard formula. The fractional excretion of phosphate (FEPᵢ) was calculated as Cₚᵢ/Cₜₐₛ.

Values are presented as means ± SEM. Statistical assessment of the changes in renal variables between the control and experimental periods in the four groups of rats was made by analysis of variance (ANOVA) with repeated measures, followed, where appropriate, by Bonferroni’s multiple comparisons test. Comparison of the ΔFEPᵢ in each MEPE-treated group with that in the vehicle group was made by one-way ANOVA followed by Dunnett’s multiple comparisons test. A P-value of <0.05 was taken to be statistically significant.

**Results**

Figure 1 shows values for GFR, absolute phosphate excretion and FEPᵢ. GFR was similar in the four groups of rats during the baseline period and tended to fall slightly (NS) during the course of the experiment in all groups (Figure 1A). Phosphate excretion also did not differ significantly between the four groups during the baseline period. Whilst absolute phosphate excretion remained fairly stable in the time-control group, it tended to increase (though not significantly) in the low-dose MEPE group and increased markedly in both medium-dose and high-dose MEPE groups (Figure 1B). Because neither GFR nor plasma phosphate concentration differed significantly among the groups during either the control period or the experimental period, FEPᵢ followed a similar pattern to absolute phosphate excretion. Thus, FEPᵢ remained stable in the time controls, increased somewhat (NS) in the low-dose MEPE group and increased markedly in the medium- and high-dose MEPE groups (Figure 1C).

Figure 2 shows the dose–response curve for the effect of MEPE on FEPᵢ, showing the change in FEPᵢ between the control and experimental periods. MEPE had a dose-dependent effect on FEPᵢ, and the effect was already maximal (ΔFEPᵢ ~20% of the filtered load) at the medium dose.

**Discussion**

This study showed that intravenous infusion of MEPE into normal rats caused a rapid, dose-dependent increase in phosphate excretion that was wholly
attributable to a reduction in absolute and fractional phosphate reabsorption by the nephron. Unfortunately, since no reliable assay for MEPE is available to us, we were unable to relate this effect to a particular plasma concentration of MEPE. Nevertheless, the findings lend support to the hypothesis that MEPE (or a derivative) is at least partly responsible for the phosphaturia seen in oncogenic hypophosphataemia and in hypophosphataemic rickets. This action of MEPE does not diminish the claims of other candidate phosphatonin such as fibroblast growth factor-23 (FGF-23), which has been shown to inhibit phosphate transport in rabbit proximal tubules in vitro [14] and to increase fractional phosphate excretion in mice in vivo [15], and frizzled related protein-4 (FRP-4), which has been shown to increase fractional phosphate excretion in rats [16]. Indeed, a recent study of patients with oncogenic hypophosphataemia showed that most of the subjects had raised plasma concentrations of FGF-23 [17]. However, a significant proportion did not. There is some suggestion that both MEPE and FGF-23 are involved in the disordered phosphate balance of these two conditions [18], though the nature of any such interaction is complex and unclear [19].

The site of action of MEPE along the nephron could not be ascertained from the present study, but, given the magnitude of the response and the fact that the bulk of phosphate reabsorption occurs in the (early) proximal tubule, this nephron segment must be a likely candidate. In support of this, in vitro studies have indicated that phosphate uptake in cultured renal proximal tubule cells is reduced during incubation with MEPE [10], while a recent study has reported MEPE immunoreactivity and mRNA in the proximal tubule [20]. However, in vivo confirmation of the renal site(s) of action of MEPE awaits a systematic micropuncture investigation.

Fig. 2. Dose–response curve showing the change in fractional phosphate excretion ($\Delta$FEPi) between the control and experimental periods in rats infused with vehicle alone (zero dose) or with one of three doses of MEPE. Note the log scale on the x-axis. Values shown are means±SE; there were six rats in each group. *P<0.01 compared with the value in rats infused with vehicle alone.

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Conflict of interest statement. R. J. Unwin had previously acted as a medical advisor to Acologix and others have none.

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