see commentary on page 580

Effect of bisphosphonates on vascular calcification and bone metabolism in experimental renal failure

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Although it is known that bisphosphonates prevent medial vascular calcification *in vivo*, their mechanism of action remains unknown and, in particular, whether they act directly on the blood vessels or indirectly through inhibition of bone resorption. To determine this, we studied the effects of two bisphosphonates on calcification of rat aortas *in vitro* and on *in vivo* aortic calcification and bone metabolism in rats with renal failure. We produced vascular calcification in rats with adenine-induced renal failure fed a high-phosphate diet. Daily treatment with either etidronate or pamidronate prevented aortic calcification, with the latter being 100-fold more potent. Both aortic calcification and bone formation were reduced in parallel; however, bone resorption was not significantly affected. In all uremic rats, aortic calcium content correlated with bone formation but not with bone resorption. Bisphosphonates also inhibited calcification of rat aortas in culture and arrested further calcification of precalcified vessels but did not reverse their calcification. Expression of osteogenic factors or calcification inhibitors was not altered by etidronate *in vitro*. Hence, these studies show that bisphosphonates can directly inhibit uremic vascular calcification independent of bone resorption. The correlation between inhibition of aortic calcification and bone mineralization is consistent with a common mechanism such as the prevention of hydroxyapatite formation and suggests that bisphosphonates may not be able to prevent vascular calcification without inhibiting bone formation in uremic rats.

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Calcification of the medial layer of arteries is common in renal failure and is thought to contribute to the increased incidence of cardiovascular disease in this population. Recent studies have shown that medial calcification is a far more complex process than simple precipitation of calcium phosphate. It is clear that several endogenous inhibitors of hydroxyapatite formation are responsible for preventing vascular calcification under normal conditions[1] and must be deficient in pathologic calcification. Vascular calcification is also associated with osteogenic changes in smooth muscle[2] and with altered bone metabolism,[3] suggesting additional pathophysiologic mechanisms. We have shown that pyrophosphate (PPi), a potent inhibitor of hydroyapatite formation,[4–6] is produced by smooth muscle in quantities sufficient to prevent medial calcification *in vitro*.[7] Plasma PPi levels are reduced in hemodialysis patients[8] and PPi levels in the vascular wall may be reduced in uremia by virtue of increased alkaline phosphatase activity.[9] Humans and mice with low levels of PPi due to the absence of PC-1, a membrane-bound ecto-nucleotide pyrophosphatase/phosphodiesterase that produces PPi,[10–12], develop severe, fatal arterial calcification, indicating that PPi deficiency could contribute to vascular calcification in renal failure.

Systemic administration of PPi to vitamin D-toxic rats prevents vascular calcification[13] but this approach is limited by the susceptibility of PPi to hydrolysis. Bisphosphonates are nonhydrolyzable analogs of pyrophosphate that were originally developed to treat ectopic calcification. Although early compounds were successful at preventing vascular calcification in rats,[6,14,15], inhibition of bone formation limited this approach in humans.[15] It was assumed that the prevention of vascular calcification as well as the inhibition of bone formation were due to a physicochemical effect of the bisphosphonates related to their PPi-like structure. Subsequent chemical modifications imparted an additional biological action that yielded newer bisphosphonates that are far more potent at inhibiting bone resorption than bone formation. Recent studies with these compounds have shown that medial vascular calcification produced by vitamin D, warfarin, or renal failure in rats can be prevented at relative potencies that match those for inhibition of bone resorption rather than bone formation.[16–18]

Based on these results, it has been assumed that the prevention of vascular calcification is related to the antiresorptive activity of bisphosphonates in bone, thus supporting the putative link between bone resorption and vascular calcification. However, effects on bone were not examined in these studies and bisphosphonates can inhibit calcium deposition in vascular smooth muscle cells in culture.[19] Thus, the possibility that bisphosphonates directly inhibit vascular calcification in renal failure independent of effects on bone cannot be excluded. Whether calcification can be prevented without reducing bone mineralization is also unknown. Using two bisphosphonates with different relative potencies for inhibition of bone resorption and formation, we compared vascular calcification and bone metabolism *in vivo* in rats with renal failure. An *in vitro* model of vascular calcification in cultured rat aortas was used to examine direct effects of bisphosphonates independent of bone.

RESULTS

Rats were fed with adenine for 28 days together with a high-phosphate diet to induce renal failure and vascular calcification. One rat treated with the highest dose of etidronate was killed on day 22 due to distress. One rat receiving the lowest dose of pamidronate died on day 27 and was not included in the results. The resulting plasma concentrations of urea, calcium, and phosphate in rats receiving etidronate, pamidronate, or vehicle are shown in Figure 1. Plasma urea increased 2.5- to 4-fold in vehicle-treated, adenine-fed rats compared with pair-fed control rats. Pamidronate did not alter the plasma urea concentration at any dose but it was significantly increased at the two highest etidronate doses. Plasma urea did not differ between rats treated with etidronate or pamidronate at similar doses. The plasma phosphate concentration was substantially elevated in uremic rats and was slightly decreased and substantially increased at the highest and lowest doses of etidronate respectively. Plasma phosphate was not significantly altered by pamidronate although it was lower than in etidronate-treated rats. Plasma calcium was not altered by uremia or pamidronate but was significantly reduced at the highest etidronate dose. At similar doses, the plasma calcium concentration was lower in the pamidronate-treated rats. Plasma parathyroid hormone concentration was markedly elevated in uremic rats but not altered by bisphosphonates.

Extensive aortic calcification developed in half of the uremic rats treated with vehicle alone. As shown in Figure 2, both bisphosphonates reduced aortic calcification. Complete inhibition was obtained at 0.08 μmol/kg per day of pamidronate and at 8 μmol/kg per day of etidronate. Samples of bone were examined from the same rats. Bone volume, osteoid volume, and cortical thickness were not altered in uremic rats and were unaffected by bisphosphonate (Table 1). Parameters of bone resorption are shown in Figure 3. Erosion depth and osteoclast number both increased (but not significantly) in adenine-fed rats compared with pair-fed nonuremic rats and tended to be higher in etidronate-treated

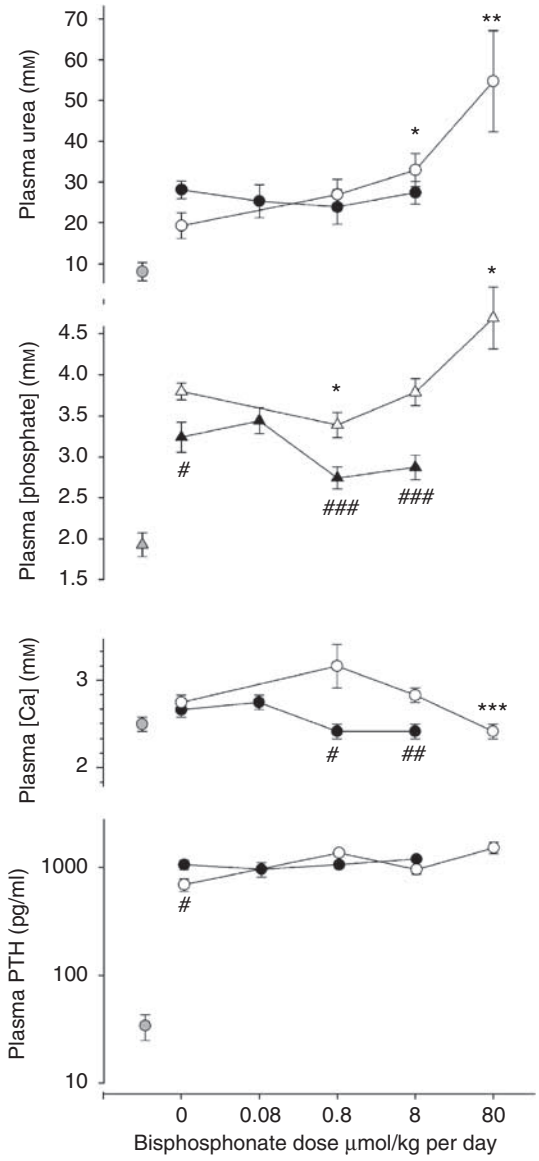

Figure 1 | Plasma chemistries in bisphosphonate-treated rats. Open symbols, etidronate-treated rats; solid symbols, pamidronate-treated rats; shaded symbols: nonuremic rats. There are separate untreated uremic controls for pamidronate and etidronate-treated rats. $^{*}P<0.05$, $^{**}P<0.02$, $^{***}P<0.005$ versus untreated adenine-fed rats; $^{\#}P<0.05$, $^{\#\#}P<0.02$, $^{\#\#\#}P<0.01$ versus etidronate-treated rats.

rats than in pamidronate-treated rats. Although there was a decreasing trend with increasing doses of bisphosphonate, none of the changes were significant. Parameters of bone formation are presented in Figure 4. Both bone formation rate (2.7-fold; $P=0.0009$) and osteoblast number (20-fold; $P=0.0013$) were increased in the adenine-fed rats. Bisphosphonates completely eliminated bone formation, with pamidronate being at least 10-fold more potent, but osteoblast number was reduced only at the highest dose of etidronate. Mineralizing surface (1.5-fold; $P=0.05$) was also increased in the adenine-fed rats and substantially reduced with bisphosphonates, again with pamidronate being at least 10-fold more potent (Table 1).