increase of excretion rates of LP was smaller (4–7 fold) than that of HP (8–16 fold). Thus, these longitudinal data suggest that about half of the amount of HP excreted in urine is derived from bone. The other half is likely to result from cartilage degeneration. This was confirmed by evaluation of the HP/LP ratio: in the nonresponder monkey the HP/LP ratio was about 5 throughout the experiment. In contrast, in the three responder monkeys, the initial HP/LP ratios of 5 doubled within three weeks to about 12.

Not unexpectedly, joint destruction (collagen crosslinks) occurred later than inflammation (ESR and CRP) in the three responder monkeys. Maximal elevation in crosslink excretion increased in the order: BB78 < BB67 < BB58. This is in contrast with clinical and inflammatory findings (BB58 < BB67 ≈ BB78), and suggests that a greater severity of inflammation does not necessarily lead to more destruction of the joint.

Conclusion

Longitudinal monitoring of urinary pyridinoline crosslinks in the collagen-induced arthritis model in rhesus monkeys allows monitoring of cartilage as well as bone degradation. About half of the amount of HP is derived from increased bone turnover; the other half may be accounted for by cartilage degradation. Therefore, collagen-induced arthritis in rhesus monkeys presents an interesting model to study efficacy of drug treatment on bone as well as cartilage degradation in arthritis.

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Determination of bone alkaline phosphatase isoforms in serum by a new high-performance liquid chromatography assay in patients with metabolic bone disease

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Four human gene loci are encoding for the alkaline phosphatase [orthophosphoric-monoester phosphohydrolase (alkaline optimum)]; EC 3.1.3.1, (ALP) isoenzymes; "tissue non-specific", placental, germ cell and small intestinal locus (Fishman 1990). ALP from the "tissue non-specific" locus is expressed in tissues such as bone, liver and kidney. Different carbohydrate side-chains or maybe remaining fragments of the in situ cell membrane glycosyl-phosphatidylinositol anchor, or both, yields "tissue specific" structures in the ALP isoforms from this gene locus (Moss 1992). In accordance with strict isoenzyme definition, bone and liver ALP should be referred to as isoforms of the same isoenzyme.

Patients and methods

We studied 70 healthy adults, 24 men and 46 women, mean age 37 (21–63) years, and 3 patients with metabolic bone disease. One man, age 71 years, with Paget's disease of the tibia; one man, age 21 years, with childhood onset hypophosphatasia; and one female race walker, age 29 years, with a pelvic stress fracture.

The bone and liver ALP isoforms were determined by a previously described high-performance liquid chromatography (HPLC) method (Magnusson et al. 1992, Magnusson et al. 1993). We used a new weak anion-exchange column, SynChropak AP300 (SynChrom Inc., Lafayette, IN, USA), instead of the referred SynChropak AX300. With SynChropak AP300 the resolution between the bone and liver ALP isoforms is increased. Serum total ALP activity was measured on a Hitachi 717 analyzer at 37 °C (Boehringer Mannheim GmbH, Mannheim, Germany). For adults, the upper reference limit for total ALP is 4.6 μkat/L in our laboratory.

Results and Discussion

With this HPLC method we were able to identify six ALP isoforms in serum samples from healthy adults:

one bone/intestinal (B/I), two bone (B1 and B2), and three liver ALP isoforms (L1, L2 and L3). A typical chromatographic ALP isoform pattern for a healthy adult has been presented previously (Figure 1, Magnusson et al. 1993). The ALP isoform reference intervals (mean ± 2SD) for adults were: B/I 0.04–0.17 μkat/L, B1 0.16–0.60 μkat/L, B2 0.20–1.50 μkat/L, B1/B2 ratio 0.21–0.66, and L1+L2+L3 0.71–1.75 μkat/L.

The patient with Paget's disease had increased bone ALP isoform activities, which is in agreement with the expected disease pathology; total ALP 9.7 μkat/L, B/I 0.22 μkat/L, B1 1.32 μkat/L, B2 7.17 μkat/L, B1/B2 ratio 0.18, and L1+L2+L3 0.99 μkat/L.

All ALP isoforms were decreased for the patient with hypophosphatasia, especially the bone isoforms, and the bone isoform B2 was not detectable at all (Figure 4, Magnusson et al. 1992); total ALP 0.5 μkat/L, B/I 0.02 μkat/L, B1 0.08 μkat/L, and L1 + L2 + L3 0.40 μkat/L.

The significance of the calculated ratio B1/B2 is not clear. However, we have found values of B1/B2 below and above the reference interval, in spite of activities within the reference intervals for the B1 and B2 isoforms, e.g., the patient presented here with a pelvic stress fracture; total ALP 1.5 μkat/L, B/I 0.04 μkat/L, B1 0.22 μkat/L, B2 0.26 μkat/L, B1/B2 ratio 0.85, and L1+L2+L3 0.98 μkat/L. For women, 15–16 years and in the pubertal stage groups IV–V, we have previously observed significantly higher levels for the calculated ratio B1/B2 than for men, due to a more rapid decline of B2 compared to B1 during puberty (Magnusson et al. 1995). This observation might indicate that B2, to a higher extent than the B1 isoform, represents a more mature expression of osteoblast differentiation during matrix mineralization in growing bone.

Conclusion

Measurements of these bone ALP isoforms, together with other biochemical markers of bone turnover, could provide new insights into bone and mineral metabolism. Nevertheless, morphological, structural and physiological aspects will need further investigation to clarify the clinical significance of the different bone ALP isoforms.

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