Postersession

Urinary collagen crosslinks to monitor bone and cartilage degradation in collagen-induced arthritis in rhesus monkeys

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Pyridinoline crosslinks are present in collagen proteins in mature bone and cartilage. Articular cartilage contains exclusively the crosslink hydroxylysylpyridinoline (HP). In bone, HP as well as lysylpyridinoline (LP) are present in a characteristic three to one ratio. Degradation of mature bone matrix results in the excretion of pyridinoline crosslinks in urine. Typically, HP and LP are excreted in urine in a ratio of about three. As such, these crosslinks (and in particular LP) present the best available biochemical markers of bone resorption.

Since type II collagen in cartilage contains 8-fold higher levels of HP than type I in bone, one might speculate that urinary excretion rates of HP are (partly) indicative of cartilage degeneration. Excretion rates of HP (and also LP) are known to be increased in patients with joint diseases such as rheumatoid arthritis and osteoarthritis. However, the HP/LP ratio does not deviate from the normal value of three. Thus, excretion rates of collagen crosslinks in joint diseases are considered to be indicative of bone turnover and not of cartilage degeneration. This consensus is based on cross-sectional studies. Inasmuch as longitudinal investigations usually facilitate data interpretation, we wondered whether the relative abundance of HP in articular cartilage could be used in longitudinally designed studies to monitor cartilage degradation.

Therefore, the present study was designed to evaluate whether continuos monitoring of HP and LP allows assessment of cartilage degeneration as well as bone turnover in arthritic disease. The collagen-induced arthritis model in rhesus monkeys was selected for this purpose since it reflects characteristic features of joint-inflammation and -erosion in human arthritides and since it allows determination of baseline values before onset of arthritis. Another interesting feature of this well-characterized animal model is the availability of genetically-determined responder and nonresponder animals (i.e. both types of animals undergo identical experimental procedures: responder monkeys develop collagen-induced polyarthritis whereas such response is lacking in non-responder animals; Bakker et al. 1992).

Methods

Rhesus monkeys were immunized with bovine type II collagen according to Bakker et al. (1992). Three responder animals (codes BB58, BB67, and BB78) and one nonresponder (code BB 77) were included. Blood and overnight-urine were collected weekly to determine inflammatory parameters (CRP and ESR) and crosslink excretion, respectively. Clinical assessment of arthritis was performed weekly. Collagen crosslinks HP and LP were quantitated in unhydrolyzed urine samples by HPLC after sample purification with CF1 cellulose according to Black et al. (1988), and were expressed per mol of creatinine.

Results

In responder monkeys clinical symptoms of arthritis became manifest around three weeks after immunization. The inflammatory parameters (CRP and ESR) reached their highest levels at around three weeks. Subsequently, the inflammatory response subsided. Severity of inflammation (ESR and CRP) and clinical assessment were comparable: all three parameters increased in the order: BB58 (mild) < BB67 ≈ BB78 (severe). Clinical and inflammatory parameters in the nonresponder monkey indicated the absence of arthritis.

Urinary excretion rates of the collagen crosslink HP increased in responder animals from the third week onward. Maximal excretion rates were observed at 6–8 weeks after immunization: excretion rates were 8–16 fold higher than baseline values or values from the nonresponder animal. After 8 weeks, excretion rates of HP declined.

Excretion rates of LP followed a similar time course. LP levels started to increase at 3–4 weeks; 6–8 weeks after immunization the levels of this established marker of bone turnover declined. Maximal

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.

References

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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: