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SHORT COMMUNICATION

Influence of Rituximab on markers of bone remodeling in patients with rheumatoid arthritis: a prospective open-label pilot study

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Abstract Immune system and bone are interacting in a complex way. Rheumatoid arthritis is characterized not only by joint destruction, but also by development of systemic osteopenia and osteoporosis. The CD20-depleting antibody Rituximab (Rtx) is a novel therapeutic option able significantly to slow the destructive joint process of rheumatoid arthritis. However, there are little data whether Rtx influences systemic bone remodeling. In the present prospective study, we evaluated the influence of Rtx on markers of bone metabolism with a follow-up of 3–15 months after Rtx therapy (2 dose of each 1,000 mg) in 13 patients with rheumatoid arthritis. There was no significant change of the bone formation markers bone alkaline phosphatase and c-terminal propeptide of collagen I. However, a non-significant tendency of decrease of RANKL (with no chance of osteoprotegerin) and a significant decrease of the bone degradation marker desoxypyridinolin crosslinked collagen I was observed 15 months after Rtx application. These initial results provide no evidence of a negative systemic influence of Rtx on bone remodeling. In contrast, it appears that Rtx lowered osteoclast activity often found increased in active rheumatoid arthritis contributing to osteoporosis in this disease.

Keywords Rituximab · Rheumatoid arthritis · Bone remodeling · Inflammation · B-cells

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Introduction

Rheumatoid arthritis (RA) is a chronic inflammatory disease driven by autoimmune processes that consequently results in progressive degradation and destruction of the affected joint cartilage and juxtaarticular bone [1]. A common finding of RA is a systemic osteopenia/osteoporosis caused by multiple factors including chronic inflammation [2–4]. The interaction between various inflammatory cells and bone is mediated by cytokines, chemokines but also by direct cell–cell contact [5]. Two cell types are critically involved in bone remodeling, the bone matrix generating osteoblasts and the bone resorbing osteoclasts. The development and function of osteoblasts and osteoclasts is fundamentally influenced by inflammatory and immunological processes [6–8].

In recent years, new principles were introduced into RA therapy with more specific mode of action concerning the influence on the autoimmune processes, the so-called biologicals.

TNF-α blocking substances have been shown to reduce the progressive joint destruction of RA. Furthermore, there is increasing evidence that TNF-α blockers are also able to stop the systemic bone loss and may even improve the bone mineral density and systemic bone mass [9–11]. Rituximab (Rtx) is a chimeric B-cell depleting anti-CD20-antibody, inducing depletion of different B-cell populations with the exception of the pro-B cells and plasma cells. This novel therapy can substantially improve signs and symptoms as

well as physical function and inhibit radiological progression in RA [12, 13]. However, a potential influence of Rtx on systemic bone remodeling in RA has not been so far investigated. The crosstalk between the immune system (including the B cells) and the cells participating in bone remodeling (osteoblasts and osteoclasts) is highly complex, and few experimental data indicate even possible unfavorable effects of B-cell depletion on bone remodeling [14–16]. Therefore, the aim of our study was to evaluate the influence of Rtx therapy on markers of bone remodeling in patients with RA in a pilot study.

Patients and methods

The influence of Rtx on markers of bone markers was studied in 13 patients suffering from active RA (9 females, 4 males), mean age 54 years (32–72 years) before the first Rtx infusion, and 3, 6, 9 and 12 months after Rtx application. Rtx was given in two doses of 1,000 mg with an interval of 14 days. Before treatment with Rtx, all patients failed to respond to therapy with TNF-α blocking substances. The study was approved by the ethic committee of the university.

Blood samples were taken between in the morning after overnight fasting. Serum was immediately separated, transferred into aliquots, frozen, and stored at −70°C until measurement of parameters. All parameters were determined by ELISA using the following commercial kits: Type I C-terminal collagen propeptide (CICP) and tartrate-resistant acid phosphatase isoform 5b (TRAcP 5b) enzyme immunoassay were from Quidel Corporation (San Diego, CA, USA), Osteoprotegerin and total sRANKL ELISA kits were obtained from Immundiagnostik AG (Bensheim, Germany). The ELSIA for desoxypyridinolin crosslinked collagen I was from Wampole Laboratories (Princeton, NJ, USA). Bone-specific alkaline phosphatase (ostase) was measured with the LIAISON®BAP OSTASE® Assay (DiaSorin Inc., Stillwater, MN, USA).

Statistical analysis

All data are presented as the means ± standard deviation (SD). Statistical significance between different groups was first tested with the non-parametric Kruskal–Wallis test. Individual groups were subsequently tested using the Mann–Whitney *U*-test.

Results

Rtx therapy had no significant effect on the measured markers of bone formation (bone-specific alkaline phosphatase (ostase) and the c-terminal propeptide of collagen I (Fig. 1a, b) up to 15 months after RTX application. In addition, RTX therapy had no significant effect on the bone resorption parameter tartrate-resistant acid phosphatase but significantly reduced serum desoxypyridinolin crosslinked collagen I concentrations after 15 months (Fig. 2a, b). Serum RANKL and osteoprotegerin concentrations were not significantly influenced after Rtx therapy (Fig. 3a, b).

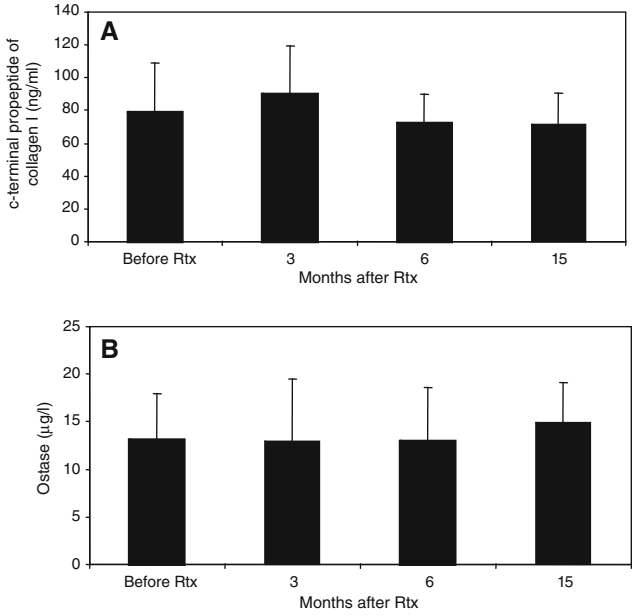

Fig. 1 Bone formation markers

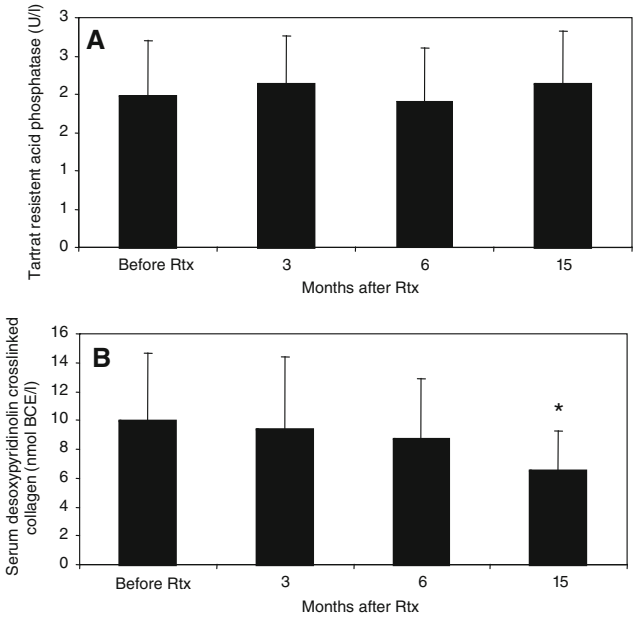

Fig. 2 Bone resorption markers (* $P < 0.001$ vs. patients before Rtx)

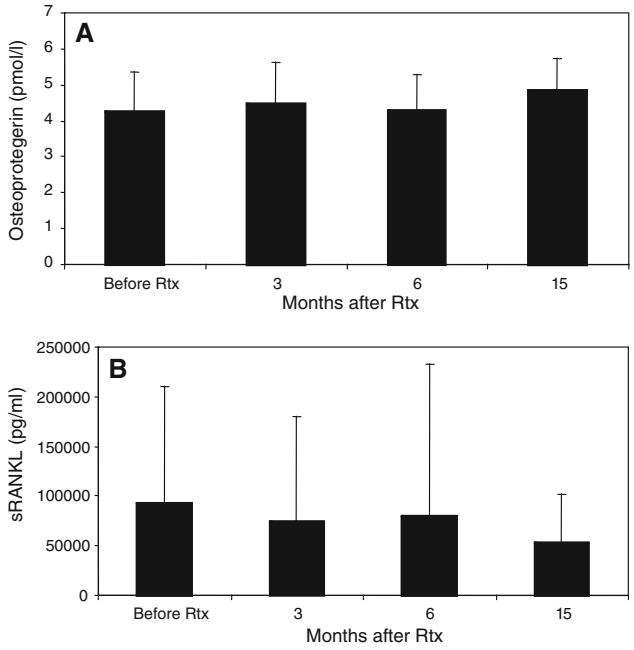

Fig. 3 RANKL and osteoprotegerin

Discussion

The interaction between the immune system and the bone is characterized by intensive crosstalk. Activated T-cells are able to produce RANKL, the down stream activator molecule of osteoclasts [17, 18], and different immunocompetent cells also produce osteoprotegerin, the decoy receptor of RANKL [14]. In addition, immune cells and their mediators influence the expression of SOST and the release of sclerostin, thereby influencing Wnt-signaling with effects on generation and function of osteoblasts [19, 20]. Different immunomodulating therapies such as methotrexate, azathioprine, cyclosporine, leflunomide but also biologicals (TNF-α blocking substances, CTLA4- antagonists or IL6 antagonists) inhibit juxtaarticulare bone resorption and joint destruction in RA. Especially for TNF-α blocking substances, an increase in systemic bone density and bone mass has been previously described [9–11]. Furthermore, the first report on improvement in bone turnover after application of the IL-6 receptor inhibitor tocilizumab has been published [21]. On the other hand, methotrexate or cyclosporine may have in part unfavorable effects on bone density and quality indicating divergent effects on juxtaarticulare and systemic bone [22, 23].

The B-cell depleting therapy with Rtx for patients with progressive RA revealed good results in the decrease in inflammatory activity and destructive potency (for review see [13]), but potential effects on bone remodeling have not been studied.

Experimental investigations have provided evidence that the same network of transcription factors (e.g. Ebf1 and Pax5) which regulate the B-cell development is also important in the regulation of bone cells [24]. For example, Pax5 is needed for the development of B cells [25], but the loss of Pax5 resulted in significant decrease in trabecular bone in a mouse model [26]. Ebf1 is an essential transcription factor for B-cell specification and function but also regulates the genesis of osteoblasts. Bruton's tyrosine kinase (Btk) is not only a factor essential for B-cell development, but also is involved in the regulation of terminal differentiation of osteoclasts together with Tec kinase [27]. Using isolated bone marrow cells, Li et al. [14] showed that cells of the B lineage in mice were responsible for 64% of total bone marrow osteoprotegerin production, and mice depleted of B cells are osteoporotic.

Our study provided no evidence that Rtx therapy negatively influences bone remodeling. This therapy had no significant effect on the measured parameter of bone formation. In contrast, the serum concentrations of the bone resorption marker desoxypyridinolin crosslinked collagen I was significantly reduced 15 months after treatment.

However, our study has several limitations. The patient number of this initial study was low, and studies with more patients are needed. Furthermore, the measured markers are the result of ongoing or decreased juxtaarticulare bone destruction/resorption and the systemic bone remodeling. We can also not further separate whether the observed Rtx effects are due to the efficient treatment of RA resulting in a decrease in inflammation or are due to direct effects of the antibody on the bone. However, there are currently no markers that are able to differentiate between local and systemic bone loss. In addition, bone markers alone are not sufficient to describe a decrease, a maintenance or an increase in bone density and bone mass. Imaging methods, at least the measuring of bone mineral density is needed, but because of the time limitation of 12 months, a significant change in the bone mass is unlikely to occur.

Conclusion

In contrast to some experimental studies, our results give no support that a depletion of B cells by therapy with Rtx negatively influences bone remodeling.

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