CONCISE REPORT

Sclerostin inhibition reverses systemic, periarticular and local bone loss in arthritis

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ABSTRACT

Objective To test whether inhibition of sclerostin by a targeted monoclonal antibody (Scl-Ab) protects from bone and cartilage damage in inflammatory arthritis. Sclerostin is a potent inhibitor of bone formation and may be responsible for the low level of bone repair in patients with rheumatoid arthritis.

Methods Human tumour necrosis factor transgenic mice (hTNFtg mice) developing inflammatory arthritis and local bone loss were administered either vehicle, anti-TNF antibody, ScI-Ab, or a combination of both agents. Inflammation, systemic and periarticular bone loss, bone erosion and cartilage damage were evaluated at baseline (week 8) and after 3 weeks of treatment by clinical assessment, micro-CT and histology.

Results Scl-Ab did not affect joint swelling or synovitis. Systemic bone loss in the spine and periarticular bone loss in the proximal tibia were completely blocked and partially reversed by inhibition of sclerostin but not by inhibition of TNF. Moreover, Scl-Ab completely arrested the progression of bone erosion in hTNFtg mice and in combination with TNF inhibition even led to significant regression of cortical bone erosions. Protective effects of Scl-Ab were also observed for the articular cartilage.

Conclusions These data suggest that sclerostin inhibition is a powerful tool to enhance bone repair in inflammatory arthritis.

INTRODUCTION

Rheumatoid arthritis (RA) leads to generalised bone loss and periarticular bone and cartilage damage.1 Bone and cartilage loss contribute to disease burden of RA by destroying joint architecture and increasing fracture risk.2–4 Current concepts suggest that inflammation creates an imbalance in bone homeostasis with high-level resorption but low-level bone formation. Whereas therapeutic interventions blocking increased bone resorption, like bisphosphonates and RANKL inhibitors, mitigate local and generalised bone loss in inflammatory arthritis, the effect of enhancing bone formation is poorly studied. This situation is surprising as patients with RA show only limited capacity to repair bone even when treatment with highly effective anti-inflammatory drugs, such as cytokine inhibitors, is commenced.5 6

Sclerostin, an osteocyte-specific protein and product of the sclerostin gene (SOST) is a potent suppressor of bone formation.7–9 Systemic administration of a targeted sclerostin antibody (Scl-Ab) increases bone mass in models of ovariectomy-induced osteoporosis, fracture repair and implant healing.10 11 Furthermore, a phase 1 clinical study has shown that Scl-Ab increases bone mass in postmenopausal women.12 In RA, variants of the SOST gene have been linked to structural progression of disease.13 These data support the concept that Scl-Ab can restore previously lost bone and suggest that such therapeutic approach could be beneficial to reverse the negative consequences of arthritis on bone.

To test this concept, we blocked sclerostin in human tumour necrosis factor transgenic (hTNFtg) mice which spontaneously develop arthritis associated with systemic bone loss, local bone destruction and cartilage damage. Sclerostin blockade was initiated when mice had already developed local and systemic bone loss to permit the assessment for bone repair.

METHODS

Mice and treatments

Forty-eight female 8-week-old mice were analysed in two consecutive independent experiments. Eight mice were non-arthritis wild-type littermates (controls). Forty were hTNFtg mice (C57Bl6 background, Tg197 strain): 8 were analysed at the age of 8 weeks (baseline); the other 32 mice were randomised into 4 treatment groups (each N=8 mice): IgG (10 mg/kg by intraperitoneal injection 3 times weekly; negative control), TNF-inhibiting antibody infliximab (10 mg/kg, 3 times weekly; TNFi) as positive control, Scl-Ab r15c7 (10 mg/kg, 3 times weekly, Scl-Ab) or combination of both antibodies for 3 weeks.

Clinical assessment

Clinical evaluation was performed weekly, starting at 4 weeks after birth. Arthritis was evaluated in a blinded manner as described previously.14

Micro-CT

The 2nd lumbar vertebral body (for analysis of systemic bone loss) and left proximal tibia metaphysis (for periarticular bone loss) were analysed by micro-CT (GE Healthcare). Images were reconstructed using a semiautomated algorithm, and the following parameters were assessed: trabecular bone volume per tissue volume (BV/TV), trabecular thickness (Tb. Th), number (Tb. N) and separation

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connectivity density, using a threshold of 585 mg/cm³. Additionally, bone mineral density (BMD) was generated without thresholding in the trabecular and cortical regions.

**Paw histology**

Both hind paws were fixed overnight in 4% paraformaldehyde, decalcified using EDTA and embedded in paraffin. Sections were stained with hematoxylin-eosin, tartrate-resistant acid phosphatase or toluidine blue for evaluation of synovitis, bone erosions and cartilage (surface area, thickness, proteoglycan content), respectively. Histomorphometric analysis was done by digital analysis system (OsteoMeasure; OsteoMetrics).

**Biochemical assays for serum**

Serum collected at the end of a study was used to quantify cytokines (IL-6, MCP1, keratinocyte chemoattractant (KC)) by using multiplex mouse-specific Luminex kits (EMD Millipore, Billerica, Massachusetts, USA)

**Statistical analysis**

Data are presented as the mean±SEM. For curve comparisons, non-parametric Mann–Whitney test was used. A p value of less than 0.05 was considered significant. For the group comparisons, Unpaired t test with p values two-tailed were used for the histological analyses.

**RESULTS**

**Scl-Ab does not affect clinical and biochemical signs of arthritis**

We first investigated whether Scl-Ab affects the signs and symptoms of arthritis. Vehicle-treated mice developed progressively increased joint swelling from week 8 (baseline) to week 11. TNFi arrested progression of arthritis either alone or in combination with Scl-Ab. Single blockade of sclerostin did not show significant reduction of signs and symptoms of arthritis as compared with vehicle treatment (figure 1A). When analysing grip strength, similar results were obtained (figure 1B). Furthermore, Scl-Ab did not significantly reduce or increase elevated serum levels of proinflammatory markers, such as interleukin-6, monocyte chemotactrant protein-1 and KC. Significant decreases of these markers were observed after TNFi. Body weight gradually decreased during the progression of arthritis in vehicle-treated mice (figure 1C). Surprisingly, Scl-Ab halted weight loss in hTNFtg mice, whereas TNFi permitted physiological increase in body weight between weeks 8 and 11.

**Scl-Ab reverses systemic bone loss in arthritis**

We next addressed whether Scl-Ab can influence systemic osteopenia in the lumbar spine. Micro-CT assessment showed a 35% decrease of bone volume per tissue volume (BV/TV) in vehicle-treated hTNFtg mice between week 8 and week 11 (figure 2A). As previously reported, TNFi monotherapy did not...
significantly affect systemic bone loss, although individual bone parameters showed slight improvement. By contrast, Scl-Ab either alone (±0% compared with baseline) or in combination with TNFi (+34% compared with baseline) completely abolished trabecular and cortical bone loss. Furthermore, the combination of TNFi and Scl-Ab restored vertebral bone to the level observed in non-arthritic wild-type mice (figure 2).

Sclerostin inhibition blocks periarticular bone loss in arthritis

We next assessed periarticular bone loss in the proximal tibia. BV/TV decreased by 38% between baseline and follow-up in vehicle-treated mice. TNFi did not inhibit periarticular bone loss (−31% compared with baseline), whereas Scl-Ab achieved complete protection either as monotherapy (+32% compared with baseline) or combined with TNFi (+11% compared with baseline). Both trabecular and cortical BMD increased in response to Scl-Ab.

Repair of arthritic bone erosions upon combined blockade of sclerostin and TNF

We then measured bone erosions in the paws of hTNFtg mice receiving the aforementioned treatments. Synovitis progressed from baseline (week 8) to follow-up (week 11). Only TNFi containing treatments did mitigate synovitis, whereas Scl-Ab did not show any anti-inflammatory effect. Bone erosions also progressed significantly from baseline to follow-up. TNFi and Scl-Ab did arrest their progression, whereas the combination of TNFi and Scl-Ab suppressed bone erosion to a level which was significantly lower than at baseline. Furthermore, TNFi and Scl-Ab halted the significant increase in osteoclast numbers from baseline to follow-up, and combination treatment even significantly suppressed them below baseline levels. Conversely, periarticular osteoblast number was generally low (vehicle: N=1.25±0.67/joint) in all groups and only increased significantly after TNFi/Scl-Ab combination treatment (N=7.25±2.46/joint) (figure 3).

Scl-Ab inhibition protects from cartilage damage in arthritis

Similar to bone erosions, cartilage damage progressed from baseline to follow-up showing significant decreases in cartilage area, cartilage thickness and proteoglycan-rich cartilage. TNFi arrested progression of cartilage damage to baseline level. Scl-Ab, either applied as monotherapy or in combination with TNFi showed even better results: cartilage area, thickness and proteoglycan
content were significantly higher than at baseline level suggesting Scl-Ab promoted the repair of articular cartilage (figure 3).

**DISCUSSION**

Our study shows that fostering bone formation by Scl-Ab reverses systemic, periarticular and local bone loss in inflammatory arthritis. Scl-Ab also improves articular cartilage damage suggesting it as powerful tool to protect bone and cartilage from the detrimental effect of inflammation and to induce repair of damaged bone and cartilage.

Treatment was initiated at a late stage of arthritis when significant bone and cartilage loss has already occurred. We thereby wanted to mimic the clinical situation of established RA. Bone and cartilage repair is considered an unmet need in patients with RA, since even the most potent anti-inflammatory therapies to date do not reverse bone loss and articular damage. Scl-Ab, but not TNFi, reversed systemic osteopenia in the spine, as well as periarticular osteopenia in the tibia, suggesting that fostering bone formation may be an effective treatment strategy for trabecular bone loss during inflammation. These findings are remarkable as protection of bone occurred despite no significant reduction of inflammation.

By contrast, TNFi alone was not able to reverse systemic and local trabecular bone loss despite its strong anti-inflammatory potential, a finding, which is consistent with observations in human RA. Given the high prevalence of osteoporosis and increased fracture risk in RA, reversal of trabecular bone loss is an important therapeutic task. The mere preservation of BMD in RA may not be sufficient, as bone loss starts early in the disease course and a significant proportion of patients with RA are osteopenic already at disease onset.

That repair of local bone and cartilage damage is feasible is reflected by the observation that Scl-Ab is highly effective in repairing cortical lesions, cartilage destruction and proteoglycan loss when combined with TNFi. Interestingly, a combination of Scl-Ab with TNFi was necessary to achieve repair, whereas inhibition of either Scl-Ab or TNFi alone only blocked progression of bone and cartilage damage but could not reverse it. The effect of TNFi is consistent with observations in human RA showing arrest of progression of bone erosions but only limited repair after TNFi treatment. These observations suggest that repair of existing bone/cartilage lesions can only be achieved when combining effective anti-inflammatory treatment, like TNFi, with strong bone anabolic agents, such as Scl-Ab. The observation that Scl-Ab effected cartilage repair was unanticipated. Restoration of articular cartilage structure may result from direct effects of Scl-Ab on cartilage. Although sclerostin is expressed on chondrocytes, its role in the cartilage is however poorly defined.

Another explanation is that cartilage regeneration essentially depends on effective repair of periarticular and subchondral bone which is facilitated by the anabolic effect of Scl-Ab. In summary, Scl-Ab treatment increases systemic and periarticular bone mass in arthritis. In combination with TNFi, Scl-Ab allowed repair of articular cartilage damage and bone erosion suggesting that such combination treatment could set new standards for joint protection.

**Contributors** CX-X, WB, MS, KS and DD collected the data. AB, MS, H-ZK and GS designed the study. MS and DD contributed to the data analysis and interpretation. AB and GS wrote the manuscript.

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**Figure 3** Effects of sclerostin inhibition on arthritic bone erosion and cartilage damage. Tartrate-resistant acid phosphatase staining (A) and toluidine blue staining (B) of hind paws of wild-type mice (WT) and human tumour necrosis factor transgenic mice at baseline (week 8) and after treatment with vehicle (VEH), tumour necrosis factor inhibitor (TNFi), sclerostin antibody (Scl-Ab) inhibitor or the combination of both agents (TNFi + Scl-Ab) at week 11. Purple colour: osteoclasts; dark blue: proteoglycan-rich cartilage; (C) Quantification by histomorphometry showing the area of synovitis in the tarsal joints, the area of bone erosions in the tarsal joints, the number of osteoclasts in the talonavicular joint and cartilage area, thickness and proteoglycan content (%×100 proteoglycan-rich cartilage) in the talonavicular joint; (*) indicates significant (p<0.05) difference to baseline; (#) indicates significant (p<0.05) difference to vehicle.
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**Competing interests** Denise Dwyer, Marina Stolina and Hua-Zhu Ke, are Amgen, Inc. employees, and own Amgen, Inc. stock and/or stock options.

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