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Preliminary report

Inhibition of tumor necrosis factor-alpha (TNF-alpha) in patients with early rheumatoid arthritis results in acute changes of bone modulators

A. Fassio*, G. Adami, D. Gatti, G. Orsolini, A. Giollo, L. Idolazzi, C. Benini, E. Vantaggiato, M. Rossini, O. Viapiana

Rheumatology Unit, University of Verona, Ospedale Civile Maggiore, Piazzale A. Scuro, 37134 Verona, Italy

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ABSTRACT

Objective: Dicckopf-1 (Dkk-1) is a potent inhibitor of the Wnt canonical pathway. In rheumatoid arthritis (RA), Dkk-1 is upregulated by tumor necrosis factor-α (TNF). Certolizumab pegol (CMZ) is a biologic TNF-inhibitor (TNFi) effective in RA and slows radiographic progression. Data on the immediate effects (≤1–8 weeks) of TNFi on Wnt modulators are lacking. This study investigated the acute influence of TNFi treatment on Wnt modulators (Dkk-1 and sclerostin) and bone turnover markers (BTM), including intact N-terminal propeptide of collagen type I (PINP) and C-terminal telopeptide of type I collagen (CTX-I).

Methods: This longitudinal, uncontrolled study involved female RA patients with inadequate response to conventional methotrexate who underwent treatment with CMZ. ESR, Dkk-1, sclerostin, BTM, parathyroid hormone (PTH), and 25OH-vitamin D levels were evaluated at baseline, week 1, week 4, and week 8. Radiographs of the hands and feet were obtained at baseline and the total and erosion scores were assessed using the Simple Erosion Narrowing Score method (SENS).

Results: Seventeen patients were enrolled. Dkk-1 and CTX-I significantly decreased after one week of treatment with CMZ ($-49.1 \pm 17.1\%$ and $-25.0 \pm 20.6\%$, respectively, $p < 0.01$), whereas PINP increased ($+43.2 \pm 31.5\%$, $p < 0.01$). These changes persisted at week 4 and 8.

Conclusions: Our study showed that TNF-alpha inhibition with CMZ promptly results in a rapid decline of serum Dkk-1 levels, alongside decreased bone resorption and increased bone formation.

1. Introduction

Dicckopf-1 (Dkk-1) is a potent inhibitor of the Wnt canonical pathway and a master regulator of bone metabolism [1]. In rheumatoid arthritis (RA), Dkk-1 is mainly expressed by synovial fibroblasts and it is known to be upregulated by pro-inflammatory cytokines such as tumor necrosis factor-α (TNF) [1]. Presently, there is strong evidence of elevated serum Dkk-1 levels in patients with RA [2]. Moreover, some studies have suggested a direct correlation between serum Dkk-1 levels and the risk of developing typical bone erosions [3–5]. Consistent with these data, Dkk-1 blockade in TNF-transgenic mice could prevent the development of bone erosions [6]. Sclerostin is another inhibitor of the canonical Wnt signaling pathway and it is secreted primarily by osteocytes [7].

Certolizumab-pegol (CMZ) is a biologic TNF inhibitor (TNFi) that has been shown to be effective in RA, especially in combination with methotrexate (MTX) [8]. According to the current recommendations, treatment with TNFi in patients affected by RA should be considered after failure with MTX monotherapy in the presence of poor prognostic risk factors, such as: moderate to high disease activity scores, presence of rheumatoid factor (RF) and/or anticitrullinated protein antibody (ACPA), presence of early erosions [9].

Given the interesting link between TNF-dependent inflammation, Wnt dysregulation and bone metabolism impairment in RA, we wanted to investigate the acute influence of TNFi treatment on Wnt modulators (Dkk-1 and sclerostin) and bone turnover markers (BTM), including intact N-terminal propeptide of collagen type I (PINP) and C-terminal telopeptide of type I collagen (CTX-I) in patients affected by RA.

2. Materials and methods

2.1. Study population and design

This was a 2-months longitudinal, uncontrolled study. Inclusion criteria were: a diagnosis of RA according to the 2010 ACR/EULAR criteria, anti-citrullinated protein antibodies (ACPA) positivity, and

* Corresponding author.
E-mail address: Angelo.fassio@yahoo.it (A. Fassio).

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non-responsiveness to a 3-month treatment course with at least 15 mg weekly MTX. All patients were started on CMZ with the following schedule: 200 mg weekly subcutaneous administration for the first 4 weeks, followed by 200 mg every 4 weeks. CMZ was co-administered through a weekly subcutaneous MTX injection (dose range: 10 to 15 mg). Exclusion criteria were: a previous treatment with bisphosphonates or denosumab within the last 2 years, or with other drugs known to affect bone metabolism or fracture risk; onset of menopause within 2 years or onset of the climacteric period during the study; and the presence of renal, liver, endocrine, heart, and metabolic bone diseases. Every patient received also 800 UI vitamin D and 1 g calcium per day; the dose could not be changed.

Standard radiographs of the hands and feet obtained at baseline were assessed using the Simple Erosion Narrowing Score (SENS) method [10], and the total and erosion scores were calculated.

Disease activity was assessed at baseline, week 4, and week 8 by means of the 28-joint Disease Activity Score (DAS28) with erythrocyte sedimentation rate (ESR).

This study was approved by the institutional and/or national research committee and was conducted in accordance with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Written informed consent was obtained from all individual participants included in the study.

2.2. Biochemical assessment

Blood samples were collected at each time point. They were stored upon collection at −50 °C and were assayed for PTH, 25OH-vitamin D, PINP, CTX-I, sclerostin, and Dkk-1. All samples were processed in the laboratory of the Rheumatology Unit at the University of Verona. PINP and CTX-I levels were measured using the IDS-iSYS Multi-Discipline Automated Analyzer (Immunodiagnostic System, Boldon, UK) based on chemiluminescence technology. Serum Dkk-1 and sclerostin levels were measured by ELISA (Biomedica Medizinprodukte, Vienna, Austria). 25OH-vitamin D and PTH levels were measured by ELISA (IDS Ltd. Boldon, UK). ESR was determined by the standard technique and it was measured at each time point.

2.3. Statistical analysis

All statistical analyses were performed using SPSS software, Version 22 (SPSS, Inc., Chicago, IL, USA). A paired sample *t*-test was used to compare the values at baseline *versus* the various time points. Spearman's rho test was used to assess the strength of the association between the total and erosion score (SENS) and the biochemical parameters. Two-sided p values of 0.05 or lower were considered significant. Data are presented as mean ± SD.

3. Results

Seventeen patients who met the selection criteria were enrolled. The mean (± SD) age was 65 (± 10) years and the mean disease duration was 1.7 (± 0.4) years.

The mean total SENS score was 15.29 (± 10.4) and the mean SENS score for erosions was 5 (± 5.3). A strong positive correlation was found between the erosion score and baseline values of PTH ($r = 0.544$, $p = 0.024$). No other correlations were found between the radiographic scoring and other serum biomarkers at baseline.

Baseline levels and at each observation point of ESR, DAS28, PTH, 25OH-vitamin D, CTX-I, PINP, Dkk-1, and sclerostin are reported in Table 1.

CTX-I decreased significantly at week 1 (−25.0 ± 20.6%, $p < 0.01$) and remained stable thereafter. PINP increased at week 1 (+43.2 ± 61.5%, $p < 0.01$) and remained stable thereafter. We observed a transient increase of PTH after 4 weeks (+20.1 ± 32.3%, $p < 0.05$), but it then decreased to baseline values at week 8.

Serum Dkk-1 levels almost halved after one week of CMZ treatment (−49.1 ± 17.1%, $p < 0.01$), and then remained stable. Finally, there was a decrease in sclerostin levels, which reached significance at weeks 1 and 8 (−28.4 ± 29.3% and − 25.4 ± 34.7%, respectively; $p < 0.01$), but not at week 4 (−23.5 ± 43.4%, $p = 0.05$).

4. Discussion

In this study, we demonstrated that treatment with TNFi in RA patients is associated with a rapid decline in Dkk-1 and sclerostin serum levels, along with an acute increase in PINP (a marker of bone formation) and a decrease in CTX-I (a marker of bone resorption) serum levels. These data support the relevance of the dysregulation of the Wnt pathway in RA and suggest its link with TNF-dependent inflammation *in vivo*.

One hypothesis for the development of bone erosions in RA is that, through TNF-dependent Dkk-1 increase [1], there is either an increase in bone resorption and an inhibition of bone formation and repair, both locally (erosions) and systemically (RA-associated osteoporosis) [1,6,7]. Indeed, there is now consistent evidence from animal studies showing that Dkk-1 is able to down-regulate osteoprotegerin and to up-regulate the expression of receptor activator of nuclear factor-kB ligand (RANKL) [11].

One striking finding from our study is the rapidity and the extent of the reduction in serum Dkk-1 levels, that occurred soon after one week from the first administration of CMZ. Considering the consistent evidence available showing increased levels of Dkk-1 in patients with RA [2,12] and the demonstration *in vitro* of TNF-induced Dkk-1 expression [6], the close proximity of the changes we observed in serum Dkk-1 to the initiation of TNFi treatment may support the hypothesis of the strict dependence of Dkk-1 production on TNF also *in vivo* in patients with RA.

In addition, the observed decrease of a marker of bone resorption (CTX-I) and the increase in a marker of bone formation (PINP), may indeed contribute to further support this hypothesis.

The correlation between the baseline serum PTH levels and the erosion scores on X-rays is a point of interest; it represents a confirmation of previous findings [3] and an additional clue of the possible role of PTH in certain rheumatic diseases [13].

Our study has several limitations. First, there was no control group. This limitation was mainly due to ethical reasons; all patients were non-responsive to MTX, with moderate to high DAS28 values, all had positive RF and/or ACPA, and some of them presented early erosions. Therefore, they were all eligible to receive treatment with TNFi, as per current clinical practice [9]. Second, the sample size was small, and the follow-up period limited. Third, the serum levels of BTM and Wnt inhibitors are surrogate markers of a local process, and their value in assessing the risk for radiographic progression has yet to be demonstrated through long-term studies that include imaging data.

In conclusion, this is the first study, to our knowledge, that has analyzed the acute changes in Wnt inhibitors and BTMs after the initiation of treatment with TNFi in patients with RA. Our findings reveal that effective TNFi treatment may induce a very rapid decline in serum Dkk-1 and sclerostin that are accompanied by dramatic changes in bone turnover. Further studies should aim to investigate if the extent of Dkk-1 changes induced by TNFi treatment can be predictive of better outcomes in terms of radiographic progression.

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Table 1
Serum levels of biomarkers at baseline and at each time point. Data reported as mean ± SD. The reference range (expressed as mean ± SD) was calculated in a healthy age and sex-matched control group.

	Baseline	Week 1	Week 4	Week 8
ESR (mm/h)	26 ± 16	17 ± 13*	16 ± 12*	16 ± 12*
DAS28	4.1 ± 0.9	NA	2.8 ± 1.2*	2.6 ± 1.4*
PTH (ng/mL) [29.2 ± 11.6]	31.8 ± 11.0	35.9 ± 16.7	40.0 ± 20.3#	33.0 ± 16.7
25OH vitamin D (ng/mL) [27.6 ± 8.6]	33.7 ± 16.7	32.5 ± 15.3	32.0 ± 12.7	34.4 ± 13.8
CTX-I (ng/mL) [0.367 ± 0.136]	0.317 ± 0.2	0.223 ± 0.158*	0.220 ± 0.148*	0.243 ± 0.162#
PINP (ng/mL) [47.6 ± 12.5]	36.9 ± 17.8	52.9 ± 29.3*	54.0 ± 29.4*	48.2 ± 20.3*
Dkk-1 (pmol/L) [25.7 ± 9.8]	40.7 ± 16.2	18.7 ± 4.7*	18.8 ± 5.4*	17.7 ± 6.0*
Sclerostin (pmol/L) [34.2 ± 17.9]	31.1 ± 10.3	23.3 ± 12.4*	25.0 ± 17.2	24.0 ± 14.0#

# $p < 0.05$.
* $p < 0.01$.

Authors' roles

Study design and execution: AF, GA, DG. Data collection: EV, CB, IL, GO, GA, AG.

Data analysis: AF, DG. Data interpretation: AF, OV, DG, MR. Drafting manuscript: AF. Revising manuscript content: All. Approving final version of manuscript: All. AF and DG take responsibility for the integrity of the data analysis.

Disclosure statement

Maurizio Rossini reports personal fees from AbbVie, Abiogen, Amgen, Biogen, Celgene, Eli-Lilly, Janssen, Merck Sharp & Dohme, Mundipharma, Novartis, Pfizer, Sanofi, Sandoz, UCB, BMS, outside the submitted work.

Angelo Fassio, Giovanni Adami, Luca Idolazzi, Ombretta Viapiana, Davide Gatti, Camilla Benini, Elisabetta Vantaggiato, Giovanni Orsolini and Alessandro Giollo have no conflict of interest to declare.

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