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Original

Changing of Bone Resorption Marker by Treatment with Infliximab for Rheumatoid Arthritis

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In order to investigate which clinical factors are associated with bone improvement by treatment with infliximab in rheumatoid arthritis (RA), twelve cases using concise laboratory data were analyzed in terms of urinary NTx one year before and one year after treatment with infliximab. Urinary NTx changed from 41.55 ± 13.5 (20.9-62.8) (nM BCE/mM Cr) to 40.77 ± 16.34 (15-75.7) (nM BCE/mM Cr) and there was no significant difference between before and after treatment with infliximab (p=0.814). There was a significant correlation between improvement of urinary NTX and steroid or RAPA one year after treatment with infliximab (p=0.038 respectively). At less than 80 times RAPA, urinary NTx significantly decreased from 40.0 ± 15.0 (nM BCE/mM Cr) to 29.98 ± 8.65 (nM BCE/mM Cr) (p=0.043). Therefore, low RAPA and low dose of steroid was associated with improvement of urinary NTx after using infliximab. X-ray examination revealed that bone atrophy improved in 8 cases out of 12 (67%) and erosion improved in 1 case out of 12 (8.3%). MRI of the hand was assessed and synovium proliferation decreased in 1 case. Histological findings of the subchondral bone at the time of total elbow replacement during treatment with infliximab revealed newly formed fibrous woven bone, including osteoid material which filled the space of trabecular bone tissue. Therefore, the rheumatoid factor (RF) may be one of the points which indicates bone healing after using infliximab for RA.

Key words: infliximab, rheumatoid arthritis (RA), NTx, bone metabolism, tumor necrosis factor-alpha (TNF-α)

Introduction

Biological reagents, including anti-TNF-α therapy, are effective for RA not only to control inflammation but also to inhibit bone destruction. There are several reports about changes in bone metabolism after using infliximab[1)2)]. However, there have been no descriptions about which factors are correlated with bone healing clinically, even when X-ray or MRI examinations were improved[3)]. Some cases of RA show X-ray improvement of bone atrophy or bone erosion in early RA and improvement of bone resorption by markers such as the N-telopeptides of type I collagen (NTx) or bone alkaline phosphatase (BAP)[2)].

We treated 245 RA patients with infliximab from August, 2003 until January, 2008. In the clinical findings, we discovered that in peripheral bone such as in the hand and foot, bone erosion or joint space narrowing tend to improve compared with large joints such as knee or hip joints. In joint destruction we found that bone erosion or joint space narrowing had improved only by X-ray examination. In bone atrophy it is important to diagnose early RA before checking for bone erosion. It is reported that there is bone erosion and joint space narrowing in the hand and foot[3)4)].

Today several anti-TNF-α therapies are used, such as etanercept (a fusion protein consisting of the extra cellular ligand-binding domain of the 75kD receptor for tumor necrosis factor-alpha and the constant portion of human IgG1)[4)], adalimumab (a fully human monoclonal tumor necrosis factor-alpha

Table 1 Background of patients for analysis of bone metabolism

Patients No.	gender	年齢	尿中 NTx (pre)	尿中 NTx (post)	CRP (pre)	罹患期間 (months)	MTX (mg)	PSL (mg)	RAPA
1	F	69	44.1	75.7	3.62	384	6	10	640
2	F	50	50.1	58.8	1.3	216	6	10	320
3	F	57	26.8	41.8	2.94	228	8	5	640
4	F	45	41.2	45.2	2.12	84	8	5	160
5	F	51	36.6	33.9	3.97	18	4	5	40
6	F	74	37.9	36.4	1.88	70	4	0	40
7	F	47	41.8	34.2	5.67	20	4	0	40
8	F	48	62.8	30.4	2.54	360	6	5	40
9	F	27	30.4	36.6	0.18	144	6	5	320
10	F	66	39	25.3	5.44	144	4	0	160
11	F	68	67	56	5.82	108	4	0	320
12	M	54	20.9	15	1.46	36	6	5	80

antibody)[5], tocilizumab (anti-interleukin 6 receptor)[6], rituximab (anti-CD20)[7], and abatacept (cytotoxic T-lymphocyte antigen 4 immunoglobulin)[8] for the treatment of RA. However, the relationship between biologics and bone metabolism is not known. The primary aim of our study was assess the effect of bone metabolism after using inflixisimab for RA, and analyze the association with clinical factors and bone metabolism before treatment with infliximab. This is the first report to investigate the clinical factors associated with bone healing by the treatment of RA with infliximab.

Materials and Methods

We analyzed twelve cases who were treated with infliximab and underwent blood checks, urine examinations, and X-ray examinations routinely over one year. The patients who were treated with infliximab had a mean age of 54.7 (27-74 years old) with an average disease duration period of 151 (18-384) months. Four mg/week of MTX was used for 5 cases, 6 mg/week for 5 cases and 8 mg/week for 2 cases. The patients used an average of 4.2 (0-10) mg/day of prednisolone. Mean CRP before infliximab treatment was 3.08 (0.18-5.82) mg/dl. According to Steinbrocker's criteria[9], there were 5 stage II cases. All patients who took part in this study were diagnosed according to the classification criteria of the American College of Rheumatology (ACR)[10]. None of the patients had a history of hormone (estrogen) replacement therapy or had used any other bone-sparing material, including bis-phosphonate or calcium supplements. Infliximab was administered by intravenous infusion at a dose of 3 mg/kg at the outset, then at 2 and 6 weeks, and then every 8 weeks. Urinary NTx was taken to measure just before treatment of infliximab and one year after treatment of infliximab. Among the patients, urinary NTx was 41.55 ± 13.5 nM bore collagen equivalent/mM Cr (nM BCE/mM Cr) in the infliximab group and 73.4 ± 8 nM BCE/mM Cr in the control group (Table 1). One year after using infliximab with MTX, we measured urinary NTx, and then analyzed the relationship between clinical factors, including age, disease duration, pre NTx, prednisolone (PSL), rheumatoid factor using the rheumatoid arthritis particle-agglutination (RAPA), CRP and urinary NTx one year after treatment with infliximab.

We analyzed what factors correlated with NTx improvement after using infliximab. Histological examination of subchondral bone was performed by hematoxylin and eosin (H&E), alizarin-red, and safranin-o stains in patient number 6 at the time of total elbow replacement during treatment with infliximab by informed consent.

Statistical analysis

We used the Wilcoxon test to compare urinary NTx before and after treatment with infliximab and the chi-square test for categorical variables to analyze the correlation between NTx improvement (−, +) and age (<65 years, ≧65 years), disease duration (<5 years, ≧5 years), PSL (−, +) and RAPA

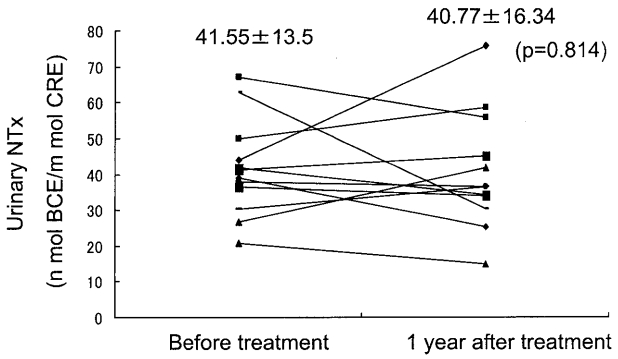

Fig. 1 Comparison of urinary NTx between one year before and one year after treatment with infliximab for RA
There was no significant difference between before and after treatment with infliximab (p = 0.814).

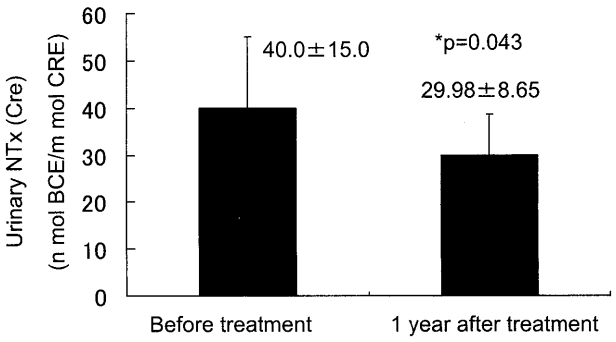

Fig. 2 Comparison of urinary NTx between one year before and one year after treatment with infliximab for RA in cases at less than 80 times RAPA
RAPA of less than 80 times, urinary NTx significantly decreased after one year (p = 0.043).

Table 2 Association between factors and NTx improvement after treatment of infliximab

Factors	p value	OR	95% CI
Age (> 65 years)	0.408	0.467	0.066-3.282
Disease duration	0.091	1.75	0.921-3.324
PSL	0.038 *	2.667	1.09-6.524
RAPA	0.038 *	2.667	1.09-6.524

PSL: prednisolone, RAPA: rheumatoid arthritis particle-agglutination, OR: Odds ratio, CI: credible interval, p values < 0.05 were considered to be significant.

(<80, ≧80) detecting Odds ratio and 95% Credible Interval (CI) by using SPSS version 15.0 software (Japan). P values <0.05 were considered to be significant.

Results

Urinary NTx changed from 41.55 ± 13.5 (20.9-62.8) (nM BCE/mM Cr) to 40.77 ± 16.34 (15-75.7) (nM BCE/mM Cr) . There was no significant difference between before and after treatment with infliximab (p=0.814, Fig. 1). After chi-square test for categorical variables, the p value, (odds ratio; 95% CI) of age was 0.408 (0.467; 0.066-3.282), disease duration was 0.091 (1.75; 0.921-3.324), PSL was 0.038 (2.667; 1.09-6.524), and RAPA was 0.038 (2.667; 1.09-6.524) (Table 2). There was a significant correlation between improvement of urinary NTX and steroid or RAPA one year after treatment with infliximab (p=0.038 respectively). Therefore, low RAPA and low dose of steroid was associated with improvement of urinary NTx after using infliximab. At RAPA of less than 80 times, urinary NTx significantly decreased from 40.0 ± 15.0 (nM BCE/mM Cr) to 29.98 ± 8.65 (nM BCE/mM Cr) (p=0.043, Fig. 2). X-ray examination revealed that bone atrophy improved in 8 cases out of 12 (67%) and erosion improved in 1 case out of 12 (8.3%). The histological findings in patient number 6 revealed that fibrous tissue contained fibroblasts among the osteoid as newly formed woven bone tissue (Fig. 3A, B). Calcium deposition was detected in osteoid by alizarin-red stain in bone marrow (Fig. 3C). Cartilage matrix (red) and osteoid (blue) were recognized in the newly formed fibrous tissue by safranin-o stain (Fig. 3D). Therefore osteocartilage formation in histology was formed in the RA patient using infliximab.

Discussion

Bone healing of joint destruction by X-ray observation occurred after undergoing biological therapy, infliximab, for the treatment of RA[11]. However, the kind of clinical factor related to bone improvement has not been confirmed yet. In this study, we detected that RAPA was associated with improvement of urinary NTx compared with other clinical factors such as age, disease duration, steroids and CRP.

The RF used in clinical practice in an IgM antibody directed against IgG. It has traditionally been detected by agglutination in sheep red blood cells (RF cross-reacts with IgG from other species or latex particles attached to human IgG[12]). The biological function of RF is still unknown. It is present

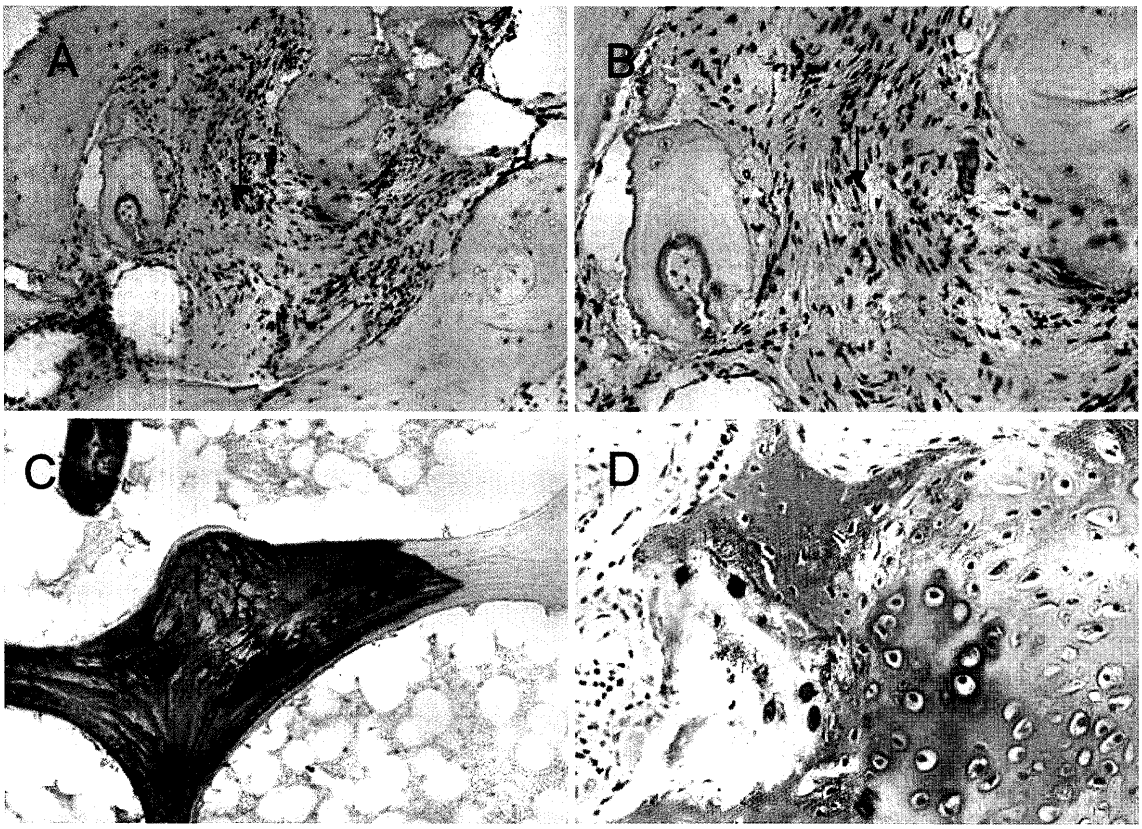

Fig. 3 Histological findings of subchondral bone after using infliximab
A: H&E, ×100, B: H&E, ×200, C: alizarin-red, ×100, D: safranin-o, ×200.
The histological findings in patient number 6 revealed that fibrous tissue contained fibroblasts among the osteoid as newly formed woven bone tissue (A, B, arrow). Calcium deposit was detected in red colour (C) in bone marrow and the osteocartilage formation were found in the newly formed fibrous tissue of subchondral bone (D).

most often in RA, the elderly, a number of rheumatic diseases other than RA, and neoplastic and infectious diseases. The sensitivity and specificity of RF were reported to be 80% and 95%, respectively[13]. Infliximab decreases serum RAPA, probably implying that it may be related to TNF-α cascades to produce RF. An association between rheumatoid factor (RF) titers and clinical disease activity has been reported, and because RF titers decrease with successful treatment, particularly MTX or parenteral gold, this suggests an indirect link with disease activity[14].

We experienced patients who had continuous morning stiffness with negative CRP and MMP-3 but a high level of RAPA. This means that disease activity is correlated with RA clinically. And also it has recently been reported that RF, but not anti-cyclic citrullinated peptide (CCP) antibodies, is modulated by infliximab treatment in rheumatoid arthritis[15]. Anti-CCP antibodies have been used recently to diagnose early RA but their range is limited and the threshold is too low to monitor the disease activity during infliximab treatment. We detected significant correlation of steroid dosage with urinary NTx one year after treatment with infliximab. Therefore, steroids may be one of factors in inhibiting bone remodeling, at least when using infliximab. Furthermore, age was not related to bone remodeling with an improvement of NTx. This means that age-dependent osteoporosis is not related to bone healing by infliximab. To achieve bone remodeling, it may be important to increase the metabolism of osteoclasts and osteoblasts for the formation of new bone marrow tissue. We recently reported that infliximab displayed histological evidence to improve bone marrow by the thickness of

the interstitial septum (TIS) compared with MTX alone[16]. In X-ray examination, we detected improvement in the bone atrophy of the hand against the background of this histological evidence.

In this study, we showed that subchondral bone remodeling can be seen without infliximab but the amount of new bone formation was much greater in RA treated with infliximab than without infliximab. It has been reported that bone metabolism markers, such as NTx and BAP, improved after 6 months of treatment with infliximab[2]. NTx is considered to be a more sensitive marker than BAP or deoxypyridinoline, which are bone formation markers; however, its mechanism is still unknown. In our data, NTx was improved in low-RAPA and low steroid cases after infliximab treatment. Therefore, low-RAPA cases and low steroid usage are susceptible to the effect of infliximab, especially for bone remodeling. The long-term results of bone remodeling by infliximab in terms of bone metabolism remain to be investigated.

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関節リウマチに対するインフリキシマブ治療による骨吸収マーカーの変化

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近年，関節リウマチの治療に対して骨破壊抑制および改善効果のある生物学的製剤の使用により治療が行われている．しかし，すべての症例で骨破壊改善が認められるわけではなく，その詳細については現在不明である．当科でインフリキシマブにより治療し1年以上経過して骨代謝マーカーのデータが詳細にとれた12例について尿中NTxの改善を解析したので報告する．当科でインフリキシマブを使用した12例（男1例，女11例），平均年齢54.7（27～74）歳，平均罹患期間151ヵ月，平均MTX 5.5 mg/week，平均Steroid 4.2 mg/day，平均BMI 31.4であった．投与前と投与後1年の尿中NTxクレアチニン換算値をWilcoxonの符号付き順位検定により比較し，投与後尿中NTxクレアチニン換算値の変化と年齢，罹患期間，steroid投与，リウマチ因子につきカイ二乗分析を行った．インフリキシマブ投与前の尿中NTxクレアチニン換算値は平均41.55±13.5から投与後平均40.77±16.34 n mol BCE/m mol CREであり有意差は認めなかった（p=0.814）．投与後尿中NTxクレアチニン換算値の改善はリウマチ因子とステロイド投与に有意に関連した（p=0.038）．すなわちリウマチ因子が低い，あるいはステロイド投与なしの症例では尿中NTxクレアチニン換算値は有意に低下を認めた．従って，リウマチ因子の低い，あるいはステロイド非投与の関節リウマチにおけるインフリキシマブ投与による治療は骨破壊改善を有意に導く可能性があると考えられた．