Tian Fu Yang · Guanglin Wang · Wenseng Tong
Quan Gong · Yongqing Cheng

Measurement of the bioactivity of interleukin and tumour necrosis factor in synovial fluid of Kashin-Beck disease

Accepted: 16 January 2001 / Published online: 6 April 2001
© Springer-Verlag 2001

Abstract We studied the bioactivity of interleukin-1 (IL-1) and tumour necrosis factor (TNF) in the synovial fluid of 12 patients with Kashin-Beck disease (KBD), 8 patients with osteoarthritis (OA) and 8 normal controls. C57 female mouse T cell proliferation method was used to test the bioactivity of IL-1, and lethality method with L929 cells to test the bioactivity of TNF. The bioactivities of IL-1 and TNF in the synovial fluid from patients with KBD were higher than those in the synovial fluid of osteoarthritis and normal controls, indicating the participation of IL-1 and TNF in the pathogenesis of KBD.

Résumé Nous avons étudié la bioactivité de l'interleukin-1 (IL-1) et du facteur de nécrose tumorale (TNF) dans le liquide synovial de 12 patients atteints de la maladie de Kashin-Beck (KBD), 8 patients d'arthrose et 8 sujets témoins. La bioactivité de IL-1 est évaluée par la méthode de prolifération des cellules T de souris femelles C57 et le TNF par la mortalité des cellules L929. L'activité d'IL-1 et de TNF dans le liquide synovial des patients atteints de KBD est plus élevée que pour l'arthrose et les sujets normaux, révélant la participation d'IL-1 et de TNF dans la pathogénèse de la maladie de Kashin-Beck.

Introduction

Kashin-Beck disease (KBD) is an endemic disease in Tibet of unknown aetiology and pathogenesis [15]. Interleukin (IL-1), tumour necrosis factor (TNF) and other cytokines can interfere with normal metabolism and collagen synthesis in the chondrocytes of articular cartilage. They can also promote chondrocyte synthesis and the secretion of metalloproteinase in order to enhance cartilaginous degeneration. IL-1 and TNF can also stimulate chondrocytes to produce PGE₂, inducing inflammation and a local immuno-

reaction. There are no reports on IL-1 and TNF concentrations in the synovial fluid of KBD-affected subjects. In this study, the bioactivity of IL-1 and TNF was tested in order to evaluate their participation in the pathogenesis of KBD.

Materials and methods

The first group contained 12 KBD patients (8 men and 4 women) whose mean age was 49 years (range: 35–72 years). The diagnosis was established according to the “Diagnosis Standard of KBD” used in China [2]. All 12 patients were in the second degree. No treatment or drug was given before surgery and synovial fluid was collected during an arthrotomy. The second group consisted of eight patients suffering from osteoarthritis whose mean age was 62 years (range: 51–78 years). Synovial fluid was collected during total knee arthroplasty (three cases) and arthroscopy (five cases). The third group (eight subjects) served as controls, and their mean age was 38 years (range: 24–47 years). They had no history of bone and joint disease and their synovial fluid was collected just after death.

Measurement of the bioactivity of IL-1 was done by the C57 female mouse T cells proliferation method. Measurement of the bioactivity of TNF was done using L929 cells and the lethality method. The biuret solution terminal end method was used to measure the amount of total protein.

All the samples were marked at random and tests were performed using a double-blind procedure.

Results

The total amount of proteins in the synovial fluid of KBD-affected patients and those with OA was 3.38 ± 0.08 g/ml and 3.4 ± 0.12 g/ml, respectively. The difference is not statistically significant (Table 1).

The bioactivity of IL-1 in KBD-affected patients ($n=12$), in OA patients ($n=8$) and in the control group ($n=8$) showed a significant difference ($P<0.05$), indicating that the bioactivity of IL-1 in the synovial fluid of KBD-affected patients was higher than in OA patients and the control group. IL-1 in the synovial fluid of OA patients was also higher than in the control group (Table 2).

The bioactivity of TNF in KBD-affected patients ($n=12$), OA patients ($n=8$) and in the control group ($n=8$) showed a significant difference ($P<0.05$), indicating that

T.F. Yang (✉) · G. Wang · W. Tong · Q. Gong · Y. Cheng
Department of Orthopaedic Surgery of the First Hospital of the
West China University of Medical Science, Chengdu, 610041,
SiChuan, PR China
e-mail: tfyang@yeah.net

Table 1 Amount of total protein in synovial fluid

Group	Cases	Total protein (g/ml)	<i>P</i>
1 KBD	12	3.38±0.08	NS
2 OA	8	3.14±0.12	

Table 2 The bioactivity of IL-1 in synovial fluid

Group	Cases	IL-1 (ng/ml)	Range (min/max)	<i>P</i>
1 KBD	12	0.425±0.109	0.25–0.62	(1–3) 0.01
2 OA	8	0.279±0.032	0.23–0.33	(1–2) 0.05
3 Control	8	0.244±0.044	0.19–0.29	(2–3) 0.05

Table 3 The bioactivity of TNF in synovial fluid

Group	Cases	TNF (IU/ml)	Range	<i>P</i>
1 KBD	12	31.25±4.92	25–43	(1–3) 0.01
2 OA	8	25.25±3.01	21–30	(1–2) 0.05
3 Control	8	21.62±3.11	18–26	(2–3) 0.05

the bioactivity of TNF in the synovial fluid of KBD-affected patients was higher than in OA patients and in the control group. TNF in the synovial fluid of OA patients was also higher than in the control group (Table 3).

Discussion

In 1983 Wood et al. [14] reported the presence of IL-1, TNF and other cytokines in the synovial fluid of patients with rheumatoid arthritis or osteoarthritis, and animal experiments confirmed that IL-1 can induce joint disease. Intra-articular injection of IL-1 concentrated in the middle and upper cell layers of the cartilaginous matrix in rheumatoid and osteoarthritis [6, 9] and it interferes with normal cartilage metabolism [11, 13]. TNF and IL-1 are two different cytokines with nearly the same biological action. They also have a synergistic effect.

The aetiology of KBD is unclear, and recent studies have shown that T-2 toxin association with selenium deficiency may be responsible for this disease. When given to rats a low selenium nutrient mixed with T-2 toxin produces cartilage metabolic alteration, a decrease in the synthesis of collagen and proteoglycan and induced degeneration and necrosis of cartilage similar to that seen in KBD [8].

The serum of a KBD-affected patient may inhibit ³⁵S-labelled proteoglycan absorption. T-2 toxin can promote IL-1 and IL-6 synthesis and in this study we found an increased bioactivity of TNF and IL-1 in the synovial fluid of KBD-affected patients (TNF: 31.25 IU/ml; IL-1: 0.425 ng/ml), and this was significantly higher than in the control group. These results show that the TNF and IL-1 are involved in the pathogenesis of KBD.

Caron et al. found that injection of interleukin-1 receptor antagonist (IL-1Ra) into the joints of dogs can protect cartilage and inhibit quantitatively the action of collagenase [1, 5].

As the degeneration and necrosis of cartilage seems to involve IL-1 in KBE, it seems likely that IL-1Ra may protect cartilage. Gene therapy to increase IL-1Ra in synovial fluid may therefore be useful in the management of KBD [12]. Two methods of gene therapy have been used in experimental tests. One is a direct method using the injection of IL-1Ra cDNA into knee joints employing a combination of adenovirus, IL-1Ra cDNA trans-infection in synovial macrophages and fibroblasts. The production of IL-1Ra persists for 4 weeks.

The other method is indirect, using IL-1Ra cDNA trans-infection through a retrotranscriptive virus. This is used to infect synovioblasts which are then transplanted into the joint cavity. The effect lasts for 5 weeks. The inflammation induced by IL-1 can be reduced [3, 4, 7, 10]

References

1. Caron JP, Fernandes JC, Martel-Pelletier J, et al (1996) Chondroprotective effect of intra-articular injections of interleukin-1 receptor antagonist in experimental osteoarthritis. Suppression of collagenase-1 expression. *Arthritis Rheum* 39(9):1535–1544
2. Diagnostic Criteria of Kashin-Beck Disease (1994) *Chin J Dom Dis* 13(5):309–317
3. Evans CH, Robbins PD, Ghivizzani SC, et al (1996) Clinical trial to assess the safety, feasibility and efficacy of transferring a potentially anti-arthritis cytokine gene to human joints with rheumatoid arthritis. *Hum Gene Ther* 7:1262–1280
4. Firestein GS, Boyle DL, Yu C, et al (1994) Synovial interleukin-1 receptor antagonist and interleukin-1 balance in rheumatoid arthritis. *Arthritis Rheum* 37:644–651
5. Guo Chuanyou, Chao Junling, Zhang Shiyuan (1995) Serum of young patient of KBD interference of anabolism of proteoglycans of hemostasis cultured ox cartilage. *Chin J Dom Dis* 16(3):143–144
6. Martel-Pelletier J, Cloutier JM, Pelletier JP, et al (1990) Cytokines, interleukin-1 and the tumour necrosis factor in human osteoarthritic tissues. *Trans Orthop Res Soc* 15:111–118
7. Otani K, Nita IM, Macaulay M, et al (1996) Suppression of antigen-induced arthritis in rabbits by in vivo gene therapy. *J Immunol* 156:3558–3562
8. Pang Wei, Wang Zhilun, Li Jinqiao, et al (1997) Low selenium and T-2 toxin interfere with metabolism of rat cartilage. *Chin J Dom Dis* 12(6):341–343
9. Pelletier JP, Fauer MP, Dibattista JA, et al (1993) Coordinate synthesis of stromelysin, interleukin-1 and oncogene proteins in experimental osteoarthritis. An immunohistochemical study. *Am J Pathol* 142:95–105
10. Pelletier JP, Caron JP, Evans C, et al (1997) In vivo suppression of early experimental osteoarthritis by interleukin-1 receptor antagonist using gene therapy. *Arthritis Rheum* 40:1012–1019
11. Ratcliffe A, Billingham MEJ, Saed-Nejad F, et al (1992) Increased release of matrix components from articular cartilage in experimental canine osteoarthritis. *J Orthop Res* 10:350–358
12. Schalwijk J, Joosten LAB, Van Den Berg WD, et al (1989) Insulin-like growth factor stimulation of chondrocyte proteoglycan synthesis by human synovial fluid. *Arthritis Rheum* 32(1):66–72
13. Venn G, Lauder RM, Billingham MEJ, et al (1990) Experimental canine osteoarthritic cartilage responds to rh-IL-1β and rh-TNF-α similarly to control cartilage. *Orthop Trans* 14:424
14. Wood DD, Ihrie EJ, Dinarello CA, Cohen PL (1983) Isolation of an interleukin-1-like factor from human joint effusions. *Arthritis Rheum* 26:975–983
15. Wu Jiaiping, Qiou Fazhua (1992) Surgery of Hong Jiashi, 5th edn. People's Health Pub Beijing:2392