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ORIGINAL PAPER

Yang Tian Fu · Wang Guang Lin · Zhao BaoCheng
Gong Quan

The effect of T-2 toxin on IL-1β and IL-6 secretion in human fetal chondrocytes

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Abstract The effects of T-2 toxin on IL-1β and IL-6 secretion in human fetal chondrocytes in vitro were investigated. The evaluation is realised on primary monolayer culture of human fetal epiphyseal chondrocytes with or without PMA stimulation. The levels of supernatant IL-1β and IL-6 were analyzed by ELISA. As compared with their respective controls, we observed a significant increase of IL-Iβ and IL-6 in supernatants of chondrocytes cultivated for 24 h with T-2 at 8 ng/ml after PMA stimulation; in the absence of PMA, IL-Iβ was increased alone after 48 h. The results demonstrated that T-2 toxin could superinduce IL-1β and IL-6 secretion in chondrocytes. All these data suggested that *superinduction* of cytokines might be one of the key mechanisms of chondrocyte injuries by T-2 toxin.

Résumé Le but de cette étude est d'étudier les effets de la toxine T-2 sur la sécrétion de l'IL-Iβ et IL-6 par les chondrocytes foetaux humains in vitro. Les effets de la Toxine T-2 sur la sécrétion de l'IL-Iβ et IL-6 sont évalués sur une culture monocellulaire primaire de chondrocytes épiphysaires de foetus humain avec et sans stimulation par du PMA. Les concentrations d'IL-Iβ et IL-6 dans le surnageant sont dosés par ELISA. Comparé aux contrôles respectifs, nous avons observé une augmentation significative de l'IL-Iβ et de l'IL-6 dans le surnageant des chondrocytes cultivés pendant 24 h en présence d'une concentration de 8 ng/ml de T-2 après stimulation par le PMA. En l'absence de PMA, IL-Iβ est seul augmenté après 48 h. Les résultats démontrent que la toxine T-2 peut activer la sécrétion d'IL-Iβ et IL-6 par les chondrocytes. Ces observations suggèrent que l'augmentation de la production des cytokines peut être l'un des mécanismes d'action de la Toxine T-2 sur les chondrocytes.

Y.T. Fu (✉) · W.G. Lin · Z. BaoCheng · G. Quan
Department of Orthopaedics Surgery of First Hospital
of West China, University of Medical Science,
Chengdu, SiChuan, PR China, 610041
e-mail: tfyang@yeah.net

Introduction

Kashin-Beck's disease (KBD) is an endemic disease. The main pathological features are degeneration and necrosis of articular and epiphyseal cartilage and T-2 toxin is one of the suspected elements causing KBD [10]. Interleukin-1 (IL-1β) and Interleukin-6 (IL-6) are involved in the degeneration of the cartilagenous matrix but the effects of T-2 toxin on secretion of IL-1β and IL-6 in human fetal chondrocytes are unknown. Primary chondrocyte culture and ELISA methods were used in this study to investigate the effects of T-2 toxin on the secretion of IL-1β and IL-6 in human fetal chondrocytes.

Materials and methods

Dulbecco's Modified Eagle Medium (DMEM; Gibco), type II collagenase (Sigma), T-2 toxin (Chinese Military Medical Research Centre) and phorbol 12-myristate 13-acetate (PMA; Biochemtech) were used.

Isolation and culture of chondrocytes was performed after collection of articular and epiphyseal cartilage from the proximal epiphysis of humeri, the proximal and distal epiphysis of femurs and from the proximal epiphysis of tibiae of ten 4- to 6-month-old aborted fetuses. Tendons, ligaments, fat and other connective tissues were carefully removed from the cartilage under sterile conditions. The fibroblasts were removed from the cartilagenous surface by trypsin. The cartilage was digested by type II collagenase at 37°C for 6 h, and the chondroblasts were then washed and counted.

The effects of T-2 toxin on the proliferation of chondrocytes were analysed as follows. Chondrocytes were plated at 2×10^4 cells/cm^2 in 24-well cell culture plates. After 24 h T-2 toxin was added to the culture medium at 0, 1, 2, 4 and 8 ng/ml. Wells without T-2 toxin were used as the control group. Chondrocytes were counted in three wells at random for each group each day.

The effects of T-2 toxin on IL/1β and IL-6 secretion were assessed by the following techniques. Chondrocytes were plated at 1×10^5/cm^2 in 24-well cell culture plates, PMA was added to one group at a concentration of 50 ng/ml, and this group was marked as PMA+. The other group without PMA was marked as PMS÷. T2 toxin was added at concentrations of 0, 1, 2, 4 and 8 ng/ml to each of the two other groups. The upper-layer culture media were collected after 2 days, centrifuged for 10 min at 1500 rpm, and stored at –70°C.

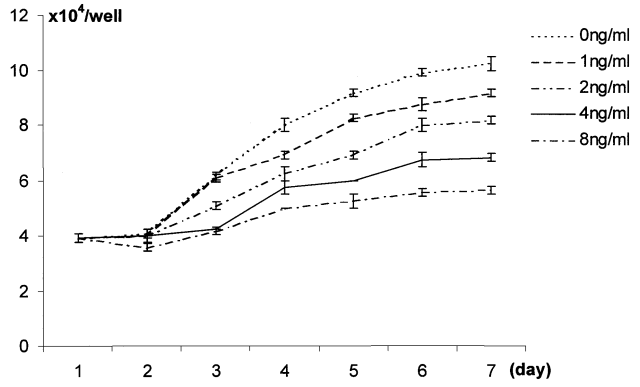

Fig. 1 The number of chondrocytes per well in relation to time for different concentrations of T2-toxins

ELISA methods (Genzyme) were used to measure the concentrations of IL-1β and IL-6. All data were analysed by the ANOVA and Newman-Keuls tests using the PeMS 2.0 software.

Results

The number of chondrocytes in culture increased with time in each group. When T-2 toxin was added to the culture medium, the proliferation of the chondrocytes decreased in the presence of T-2 toxin, indicating that T-2 toxin could inhibit the proliferation and that this effect was related to the concentration of T-2 toxin (Fig. 1).

With or without PMA stimulation and at a concentration of T-2 toxin of 8 ng/ml, the secretion of IL-1β by chondrocytes was significantly increased with regard to the control groups (Table 1). The secretion of IL-6 by chondrocytes was increased only with PMA stimulation, even at a concentration of T-2 toxin of 8 ng/ml. The effect of T-2 on the secretion of IL-1β and IL-6 was apparently biphasic. When the concentration was lower than 4 mg/ml, T-2 toxin inhibited secretion of IL-1β and IL-6, and when the concentration was higher than 4 ng/ml, T-2 toxin could increase the synthesis and secretion of IL-1β and IL-6 (Table 1).

Discussion

Since 1967, chondrocytes, isolated from rigid cartilage matrix using trypsin and collagenase have been cultured in vitro in order to study the pathology of articular cartilage diseases. In recent years studies have found that T-2 toxin can cause articular damage through protein and DNA synthesis, metabolic alteration, cell membrane injury, glycoprotein and collagen synthesis [1, 3]. In this study where chondrocytes were isolated and cultured from articular and epiphyseal cartilage, T-2 toxin was found to influence their proliferation. T-2 toxin can inhibit DNA synthesis during the early growth of chondrocytes [2].

Our experiments show that the effect of T-2 on the secretion of IL-1β and IL-6 was apparently biphasic. When the concentration was lower than 4 ng/ml, T-2 toxin inhibited secretion of IL-1β and IL-6, but when the concentration was higher than 4 ng/ml the secretion of IL-1β and IL-6 was increased. The reason could be that T-2 toxin might have two effects on protein synthesis: inhibition of the synthesis of DNA, or superinduction [5, 7].

Superinduction, that is to say inhibition of protein synthesis, can increase either transient gene expression or the protein synthesis induced by stimulating mitogen. Vomitoxin and other toxins can superinduce IL-1β, IL-2, IL-4, IL-5, IL-6 and TNFα mRNA gene expression and protein synthesis [8, 9]. Superinduction is caused by transcription and post-transcription mechanisms, increasing the bio-ability of NF-KB, promoting cytokine gene transcription, enhancing stability of cytokine mRNA, and finally by increasing the secretion of cytokines [4, 6]. When the concentration was lower than 4 ng/ml, T-2 toxin could not superinduce cytokine gene expression or protein synthesis and only had an inhibiting function, so the secretion of IL-1β and IL-6 was decreased. When the concentration was 8 ng/ml, T-2 toxin had two roles: the function of superinduction was stronger than inhibition, and the secretion of IL-1β and IL-6 was then increased.

IL-1β and IL-6 can promote catabolism of cartilage matrix as well as inhibiting synthetic metabolism of chondrocytes. IL-1β can inhibit type II and IX collagen, lycoprotein synthesis and secretion of TIPM-1 and 2, but may promote type I and III collagen synthesis and secretion of MMPs. It may also enhance Pas and PGE_2 synthesis, which will take part in the inflammatory response of joints, regulate immune responses and stimulate both resorption of bone and osteoblast proliferation.

IL-1β and IL-6 can promote synovioblasts to secrete MMPs, PGE_2 and induce an inflammatory reaction of the synovium, which in turn causes a deterioration of chondrocyte survival. IL-6 can also inhibit the chondro-

Table 1 The effect of T-2 toxin on secretion of IL-1β and IL-6. The concentration of PMA was 50 ng/ml (*PMA*÷ without PMA, *PMA*+ with PMA)

T-2 toxin (ng/ml)	IL-1β (pg/ml)		IL-6 (pg/ml)	
	PMA÷	PMA+	PMA÷	PMA+
0	17.37±0.98	17.51±0.46	2.53±0.11	9.29±0.21
1	16.67±0.14	14.01±0.30	2.35±0.11	8.26±0.32
2	15.34±0.65	12.91±0.07	1.79±0.11	7.26±0.18
4	18.31±0.60	17.13±0.29	2.11±0.11	6.17±0.10
8	19.56±0.65	19.03±0.34	2.47±0.21	18.83±0.63
P	<0.05	<0.01	<0.05	<0.01

cyte synthesis of glycoprotein and in cooperation with 9IL-6R increase osteoclast proliferation and ICAM-1 expression. IL-6 and 9IL-6R may enhance damage to the cartilage by increasing the sensitivity of chondrocytes to the catabolic effect of cytokines. Much attention has been paid to cell necrosis in KBD whereas the change of gene expression has been ignored. Our study showed that T-2 toxin could superinduce chondrocytes to secrete IL-1β and IL-6, and that these cytokines could induce cartilage degeneration and necrosis. So, T-2 toxin can cause cartilage damage directly as well as superinduction of IL-1β and IL-6 secretion in chondrocytes. These two effects may play an important role in the generation and development of Kashin-Beck's disease.

T-2 toxin is an important factor in the aetiology of KBD. In our study we found that T-2 toxin could both inhibit the growth of cultured chondrocytes as well as increase secretion of IL-1β and IL-6, two important cytokines involved in degeneration and necrosis of cartilage. So, the T-2 role in KBD might result from promoting the synthesis and secretion of IL-1β and IL-6 by the chondrocytes. An ability to block the synthesis and secretion of IL-1β and IL-6 might be helpful in the treatment of Kashin-Beck's disease.

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