Oldfield EH, *et al*. Penetration of recombinant interleukin-2 across the blood-cerebrospinal fluid barrier. J Neurosurg 1988; 69: 29 – 34.

12 Yu GD, Yin QZ, Hu YM, Yin ZW, Gu ZL, Qian ZN, *et al*. Effects of *Coriolus versicolor* polysaccharides peptides on electric activity of mediobasal hypothalamus and on immune function in rats. Acta Pharmacol Sin 1996; 17: 271 – 4.

白细胞介素-2 参与云芝糖肽引起的镇痛作用[1]

龚 珊, 张惠琴, 殷伟平, 印其章[2], 张 毅[3], 顾振纶[4], 钱忠明[5], 邓柏濂[5]

(苏州医学院神经生物学研究室, [3] 免疫研究室, [4] 药理学教研室, 苏州 215007, 中国; [5] 香港理工大学应用生物学及化学科技系, 九龙, 香港, 中国)

关键词 彩绒革盖菌; 多糖; 肽; 中部下丘脑; 白细胞介素-2; 镇痛

目的: 研究白细胞介素-2 (IL-2) 和下丘脑内侧基底部(MBH)在云芝糖肽(PSP)引起镇痛中的作用. **方法**: 采用脑室注射或腹腔注射的方法给 IL-2 抗血清, 采用电解法损毁 MBH. **结果**: 连续 6 日 ig PSP 1 $g \cdot kg^{-1} \cdot d^{-1}$ 能提高大鼠的电刺激鼠尾引起的嘶叫阈. PSP 引起的这种镇痛作用能被 icv IL-2 抗血清所翻转, 但 ip 没有作用. 电解损毁 MBH 后, 该镇痛作用消失. **结论**: PSP 能激活 IL-2, 后者进入脑内作用于 MBH 的 IL-2 受体而发挥镇痛作用.

BIBLID: ISSN 0253-9756 Acta Pharmacologica Sinica 中国药理学报 1998 Jan; 19 (1): 70 – 73

Effects of indometacin on joint damage in rat and rabbit

WANG Bin[1], YAO Yu-You, CHEN Min-Zhu

(*Institute of Clinical Pharmacology, Anhui Medical University, Hefei 230032, China*)

KEY WORDS indomethacin; adjuvant arthritis; interleukin-1; synovial membrane; proteoglycans; thymidine; lipopolysaccharides

AIM: To study the effects of indometacin (Ind) on joint damages. **METHODS**: The volume of noninjected hind paw and interleukin-1 (IL-1) production from peritoneal macrophages and articular synoviocytes induced by lipopolysaccharides were assayed in adjuvant arthritis (AA) rats. Measurements of synovial fibroblast proliferative response and proteoglycan synthesis of cartilage from rabbits were used. **RESULTS**: The secondary inflammatory reactions in AA rats on d 18, 21, and 24 were suppressed by ig Ind 2 $mg \cdot kg^{-1} \cdot d^{-1}$ for 9 d. Ind promoted IL-1 production from both macrophages and synoviocytes in AA rats. Ind 10 $\mu mol \cdot L^{-1}$ enhanced the proliferation of rabbit synovial fibroblasts and suppressed the proteoglycan synthesis of articular cartilage in response to IL-1 *in vitro*. **CONCLUSION**: Ind is unfavorable to the repair of joint destruction.

Rheumatoid arthritis (RA) is a chronic autoimmune disease characterized by joint swelling, synovial inflammation, and cartilage destruction[1,2]. Adjuvant arthritis (AA) in rat is an experimental immunopathy model that is thought to share many features of RA. Indometacin (Ind) is one of nonsteroidal anti-inflammatory agents that disrupts the biosynthesis of prostaglandins (PG) by inhibiting both cyclooxygenase-1 (COX-1) and COX-2, thus, may relieve the symptoms of arthritis and be accompanied by gastrointestional and renal toxicity[3,4]. But its effect on destructive process of the synovial joint is not fully understood. Here we observed the influence of Ind on joint damage in AA rats and on rabbit synovial fibroblast prolifertion and cartilage proteoglycan synthesis in response to human recombinant interleukin-1β (hrIL-1β) *in vitro*.

MATERIALS AND METHODS

Animals Wistar rats (2 – 3-month old, 161 ± *s* 17 g, clean grade, Certificate № 03) were provided by the Animal

[1] Phn 86-551-281-6602, ext 2055. Fax 86-551-281-3965.
Received 1996-12-25 Accepted 1997-07-09

Center of Anhui Institute of Medical Sciences. Inbred strain C57 BL/6 mice (6 – 8-wk old, 20.4 ± *s* 1.8 g) for IL-1 activity assay were purchased from Shanghai Experimental Animal Center, Chinese Academy of Sciences. New Zealand white rabbits (0.6 – 2.0 kg) were provided by the Animal Center of Anhui Medical University.

Drug and reagents Ind was purchased from Shanghai № 12 Pharmaceutical Factory. *Bacillus Calmette-Guérin* (BCG) was provided by National Institute for the Control of Pharmaceutical and Biological Products, Beijing. hrIL-1β was provided by Biotin Biomedicine Co, Beijing. Lipopolysaccharides (LPS) and collagenase (type Ⅱ) were purchased from Sigma Co. Sephadex G-25 medium was purchased from Pharmacia Co. RPMI-1640 medium and Dulbecco's modified Eagle's medium (DMEM) were purchased from Gibco Co. All RPMI-1640 and DMEM media were supplemented with HEPES buffer 10 mmol · L^{-1}, *L*-glutamine 2 mmol · L^{-1}, 2-mercaptoethanol 50 μmol · L^{-1}, benzylpenicillin sodium 100 kU·L^{-1}, streptomycin 100 mg · L^{-1}, and 10 % new-born bovine serum and were adjusted to pH 7.2. [^{3}H]Thymidine (TdR) and Na_2 $^{35}SO_4$ were obtained from Chinese Academy of Atomic Energy Science, Beijing.

AA induction Arthritis was induced as previously described[1]. Briefly, rats were immunized by intradermal injection into the left hind foot pad with heat-killed BCG 1 mg suspended in 0.1 mL paraffin oil. Right hind paw volume was determined with an MK-550 volume meter (Muromachi Kikai Co, Japan) on d 0, 15, 18, 21, and 24 before or after immunization. The paw volume difference between before and after immunization was expressed as the paw swelling (secondary inflammation).

Production of IL-1 by peritoneal macrophages Rat peritoneal lavage with cold Hanks' solution (calcium and magnesium free) was collected and resuspended in RPMI-1640 medium at a concentration of 2×10^9 cells · L^{-1}. The cell suspension (1 mL) was seeded on 24-well culture plate. After incubation at 37 ℃ in an atmosphere of 5 % CO_2 and 95 % air for 2 h, nonadherent cells were removed by gently washing twice with warm RPMI-1640 medium. The resultant cells were peritoneal macrophages. To monolayer cells, RPMI-1640 medium and LPS (5 mg · L^{-1}) were added to each well (final volume was 1 mL/well). Then the plate was incubated at 37 ℃ in a 5 % CO_2 atmosphere for 48 h. The supernatants were stored at – 20 ℃ until assay for IL-1 activity.

Synoviocyte culture Primary cultures of rat synoviocytes were established[11]. Synovium from rat knees was excised and dispersed with sequential incubations of collagenase and trypsin. Synoviocytes were resuspended in DMEM medium at a concentration of 1×10^9 cells · L^{-1}. The cell suspension (0.5 mL) and LPS (5 mg · L^{-1}) were added to 24-well culture plate (final volume was 1 mL/well). After incubation at 37 ℃ in a 5 % CO_2 atmosphere for 48 h, all the supernatants containing IL-1 were stored as above.

IL-1 assay IL-1 activity was measured by thymocyte proliferation assay[5]. IL-1 activity in supernatants was expressed as Bq of [^{3}H]TdR incorporated by 1×10^6 thymocytes at 1:80 dilution.

Synovial fibroblast proliferation assay Synovium from rabbit knees was excised. Fibroblast line was derived by explant culture of tissue fragment[11]. Cells were used at their 3rd passage. Fibroblasts were resuspended in RPMI-1640 medium at a concentration of 5×10^5 cells · L^{-1}. The cell suspension (0.1 mL) was added to 96-well microtiter plate. Following an overnight culture period to facilitate cell adherence, RPMI-1640, hrIL-1 or/and Ind were added to each well. The cultures were then incubated at 37 ℃ in a 5 % CO_2 atmosphere for 48 h. [^{3}H]TdR 18.5 kBq/well was added 6 h before the termination of incubation. The cells were then enzymatically detached (0.05 % trypsin – 0.02 % edetic acid, at 25 ℃ for 10 min) and harvested onto type-69 glass fiber filters. The radioactivity was determined by FJ-2107 liquid scintillation counter (Xi-an № 262 Factory, China). The results were expressed as means of Bq of triplicate wells.

Measurement of proteoglycan synthesis Articular cartilages of the knee and shoulder joints in rabbits were excised by sterile dissection. Pooled cartilage was cut into 1-mm^3 bits and 30 – 40 mg per well were placed in 24-well plate with 1 mL of DMEM medium. The cultures were then incubated in the presence or absence of hrIL-1β or/and Ind at 37 ℃ in a 5 % CO_2 incubator for 16 h. Proteoglycan synthesis was measured by the incorporation of Na_2 $^{35}SO_4$ in glycosaminoglycan chains[6,7]. The results were expressed as Bq per g cartilage.

Statistical analysis All values were expressed as $x \pm s$ and compared with *t* test.

RESULTS

Secondary inflammation and IL-1 production from peritoneal macrophages and articular synoviocytes induced by LPS in AA rats Rats were immunized on d 0 by intradermal injection of adjuvant to the left hind paw, which resulted in a gradual increase in volume of the right paw (secondary lesion). Arthritic animals on d 15 were divided randomly into AA and Ind groups. Ind 2 mg · kg^{-1} · d^{-1} was given intragastrically (ig) from d 15 to d 23. Ind suppressed the secondary inflammation in AA rats on d 18, 21, and 24. IL-1 activity was measured on d 24. Compared to normal control, IL-1 production from peritoneal macrophages and articular synoviocytes induced by LPS 5 mg · L^{-1} in AA rats was markedly enhanced. There was a positive correlation between IL-1 activity of macrophages and

paw volume difference ($\hat{Y} = 0.0027X - 0.7442$, $r = 0.953$, $P < 0.05$) or IL-1 activity of synoviocytes and paw volume difference ($\hat{Y} = 0.0015X - 0.3181$, $r = 0.977$, $P < 0.05$). Ind promoted IL-1 production from both macrophages and synoviocytes in AA rats (Tab 1).

Tab 1. Effects of Ind 2 mg · kg^{-1} · d^{-1} × 9 d on secondary inflammation and on interleukin-1 (IL-1) production from peritoneal macrophages and articular synoviocytes induced by LPS 5 mg · L^{-1} in adjuvant arthritis (AA) rats. $\bar{x} \pm s$. $^cP < 0.01$ *vs* **normal control;** $^eP < 0.05$, $^fP < 0.01$ *vs* **AA control.**

Parameter	Day	Normal	AA	Indomethacin
Paw swelling/mL ($n = 8$)				
	15	–	0.50 ± 0.15	0.50 ± 0.12
	18	–	0.73 ± 0.17	0.54 ± 0.14^e
	21	–	0.81 ± 0.22	0.49 ± 0.15^f
	24	–	0.84 ± 0.24	0.46 ± 0.16^f
IL-1 activity/Bq ($n = 4$)				
Macrophages	24	431 ± 58	604 ± 71^c	758 ± 88^{ce}
Synoviocytes	24	496 ± 84	794 ± 129^c	1 037 ± 145^{ce}

Proliferation of rabbit synovial fibroblasts and proteoglycan synthesis of cartilage in response to hrIL-1β Synovial fibroblasts were cultured with hrIL-1β 10 kU·L^{-1} or/and Ind 10 μmol·L^{-1} for 48 h. The proliferation of fibroblasts in response to hrIL-1β was much greater than that of control. When both hrIL-1β and Ind were added to the cultures, the proliferation was enhanced. Addition of hrIL-1β 30 kU·L^{-1} to cartilage cultures provoked a 68.1 % reduction of ^{35}S incorporation. Treatment of cartilage bits with hrIL-1β and Ind resulted in a more reduction in proteoglycan synthesis, which was 14.4 % of ^{35}S incorporation of control (Tab 2).

Tab 2. Effects of Ind 10 μmol·L^{-1} on proliferation of rabbit synovial fibroblasts induced by hrIL-1β (10 kU·L^{-1}) and on suppression of cartilage proteoglycan synthesis mediated by hrIL-1β (30 kU·L^{-1}) *in vitro*. $\bar{x} \pm s$. $^cP < 0.01$ *vs* **control.** $^eP < 0.05$ *vs* **hrIL-1β.**

Group	[^{3}H]TdR uptake ($n = 6$) (Bq/5 × 10^3 cells)	^{35}S uptake ($n = 4$) (kBq/g tissue)	(% of control)
Control	52 ± 8	276 ± 61	
hrIL-1β	93 ± 12^c	88 ± 28^c	31.9
hrIL-1β + Ind	112 ± 13^{ce}	40 ± 13^{ce}	14.4

DISCUSSION

Increasing evidence implicates IL-1 in the pathophysiology of RA[8,9]. Our present results demonstrated that IL-1 production from peritoneal macrophages and articular synoviocytes induced by LPS in AA rats was higher than that of normal control. Linear regression analysis showed the positive correlation between IL-1 productions from macrophages and synoviocytes and paw volume difference, suggesting that IL-1 may be involved in both inflammatory reaction and pathological lesion of joint. Treatment with Ind inhibited the inflammatory reactions, but further promoted IL-1 secretion by macrophages and synoviocytes in AA rats. The effects of Ind on the above may appear to conflict, because the celluar and molecular basis of the inflammatory process is complex and multifactorial. It is well accepted that PGE_2 could cause vasodilation, increase vascular permeability, and lead to eventual edema at inflammatory sites. IL-1 may promote several types of cells including macrophages, synoviocytes, and chondrocytes to secret PGE_2, which in turn, down-regulates IL-1 and other inflammatory mediator productions[9,10]. Because of inhibiting PGE_2 synthesis, Ind could not only suppress inflammatory response, but also abolish PGE_2 negtive feedback effect on IL-1, thus, result in large amounts of IL-1 release.

The hyperproliferation of synovial fibroblasts, which comprise the characteristic pannus of RA, is thought to be a key factor in the invasion of the joint spaces, resulting in destruction of cartilage and subchondral bone[11]. We observed the influence of Ind *in vitro* on the proliferation of rabbit synovial fibroblast and on the suppression of cartilage matrix synthesis mediated by IL-1. The results showed that Ind promoted the increase of fibroblast proliferation and suppression of matrix proteoglycan synthesis in response to IL-1. These *in vitro* studies further confirmed *in vivo* studies that Ind exacerbated destructive process of the synovial joint.

Though it is an effective anti-inflammatory agent, Ind is deleterious to gastrointestinal and renal functions as well as the repair of joint damage. These effects of

Ind limit its usefulness in RA and other inflammatory joint diseases.

REFERENCES

1 Wang B, Chen MZ, Xu SY.
Effect of total glucosides of paeony on synoviocyte function and splenocyte proliferation in adjuvant arthritis rats.
Chin J Pharmacol Toxicol 1994; 8: 128 – 32.

2 Kingsley G, Panayi G, Lanchbury J.
Immunotherapy of rheumatic diseases — practice and prospects.
Immunol Today 1991; 12: 177 – 9

3 Furst DE Comments on possible long-term consequences of non-steroidal anti-inflammatory use.
J Clin Pharmacol 1988; 28: 550 – 3.

4 Li JJ, Anderson GD, Burton EG, Cogburn JN, Collins JT, Garland DJ, *et al* 1, 2-Diarylcyclopentense as selective cyclooxygenase-2 inhibitors and orally active anti-inflammatory agents.
J Med Chem 1995; 38: 4570 – 8.

5 Shang HS, Deng YL, Lin ZB, Ding GF, Long ZZ. IL-1 bioassay and effect of *Lentinus edodes* (Berk.) sing polysaccharide in the release of IL-1 in the mouse. Chin J Immunol 1986; 2: 70 – 4.

6 Bates EJ, Kowanko IC, Ferrante A. Conditioned medium from stimulated mononuclear leucocytes potentiates the ability of human neutrophils to damage human articular cartilage.
Ann Rheum Dis 1988; 47: 1 – 9

7 Wang B, Yao YY, Li CY, Chen MZ.
Proteoglycan synthesis and degradation of cartilage from rabbit were measured by the incorporation of sodium [^{35}S]sulphate.
Chin Pharmacol Bull 1996; 12: 565 – 6

8 Chandrasekhar S, Harvey AK, Hrubey PS, Bendele AM.
Arthritis induced by interleukin-1 is dependent on the site and frequency of intraarticular injection.
Clin Immunol Immunopathol 1990; 55: 382 – 400.

9 Hulkower KI, Wertheimer SJ, Levin W, Colfey JW, Anderson CM, Chen T, *et al*. Interleukin-1β induces cytosolic phospholipase A_2 and prostaglandin H synthase in rheumatoid synovial fibroblasts: evidence for their roles in the production of prostaglandin E_2.
Arthritis Rheum 1994; 37: 653 – 61

10 Taskiran D, Stefanovic-Racic M, Georgescu H, Evans C. Nitric oxide mediates suppression of cartilage proteoglycan synthesis by interleukin-1. Biochem Biophys Res Commun 1994; 200: 142 – 8.

11 Gitter BD, Labus JM, Lees SL, Scheetz ME. Characteristics of human synovial fibroblast activation by IL-1β and TNFα.
Immunology 1989; 66: 196 – 200.

70-73

吲哚美辛对大鼠和兔关节损伤的影响

R971.1 R684.05

王 斌[1], 姚余有, 陈敏珠
(安徽医科大学临床药理研究所, 合肥 230032, 中国)

关键词 吲哚美辛; 佐剂性关节炎; 白细胞介素-1; 滑膜; 蛋白多糖; 胸苷; 脂多糖

目的: 研究吲哚美辛(Ind)对关节损伤的影响. **方法**: 检测佐剂性关节炎大鼠(AA)非致炎侧后足爪容积, LPS 诱导腹腔巨噬细胞和关节滑膜细胞产生白细胞介素-1 (IL-1), 以及兔滑膜成纤维细胞增殖反应和软骨蛋白多糖的合成. **结果**: Ind 2 $mg \cdot kg^{-1} \cdot d^{-1}$ ig 9 d, 可抑制 AA 大鼠 d 18, d 21, 和 d 24 继发炎症反应, 但促进巨噬细胞和滑膜细胞分泌 IL-1. Ind 10 $\mu mol \cdot L^{-1}$体外分别促进 IL-1 诱导兔滑膜成纤维细胞增殖反应以及抑制关节软骨蛋白多糖合成. **结论**: Ind 不利于关节损伤的修复.