

## THE USE OF COLCHICINE IN ARTHROSCOPIC WASH-OUT FLUID IN EARLY DEGENERATIVE ARTHRITIS OF THE RABBIT KNEE JOINT

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*SUMMARY: The effects of arthroscopic wash-out method with colchicine was studied in early degenerative arthritis of rabbits knee joint induced by sulphurus. Histopathologic changes and PGE<sub>2</sub>, LTB<sub>4</sub> like activities of articular tissue were the main parameters of evaluation in this study. Elevation of the mediator levels and histologic changes induced by the injection of 2% solution of sulphurus were improved following arthroscopic wash-out with solutions containing colchicine and diclofenac sodium. Those findings are taken as an evidence for the therapeutical value of arthroscopic wash-out in the treatment of early degenerative arthritis. In conclusion, early knee joint degenerative arthritis induced by intraarticular injection of sulphurus, both PGE<sub>2</sub> and LTB<sub>4</sub> activities were increased and closely correlated to the histopathological findings. Intraarticular wash-out with colchicine and diclofenac sodium significantly inhibited the elevated activities of both mediators and improved the regeneration of altered tissue.*

*Key Words: Osteoarthritis of the knee, colchicine, arthroscopic wash-out.*

### INTRODUCTION

The early degenerative changes in cartilage and synovial tissues of knee joint are manifested by pain and limitation of motion. Degenerative changes are frequently seen in hyaline cartilage. Cell death ensues because of the calcification of cartilage. This degenerated cartilage has a very limited capacity of regeneration.

Arachidonic acid metabolites PGE<sub>2</sub> and LTB<sub>4</sub> plays important roles in inflammation. PGE<sub>2</sub> synthesis increases in inflamed synovial tissue and makes a tonic inhibition of T lymphocytes (11). It is also reported that the most potent chemotactic lipoxigenase product on polymorphonuclear leucocytes in the infected area is LTB<sub>4</sub> (7).

The aim of this study is to investigate a new treatment in the treatment of early degenerative arthritis, and the role of arachidonic acid metabolites in the pathogenesis of experimentally induced degenerative arthritis. The

changes in their activities by the use of diclofenac sodium and colchicine and the histopathological changes were also studied.

### MATERIALS AND METHODS

Thirty four knees of seventeen New Zealand white rabbits, 1.6 to 2.3 kg in weight and 10+/-5 months old were used for the experiments carried out of the Surgical Research Center of Hacettepe Medical School. Knee joints of six rabbits were injected 2% sulphurus suspension in carboxymethylcellulose in the dose of 100 mg/kg. Knee joints of the control group (6 rabbits) were injected carboxymethylcellulose in similar volumes. Three weeks later, specimens from cartilage and meniscal tissues were surgically removed under Sodium pentobarbital anesthesia (30 mg/kg IV) in order to determine histopathological changes and PGE<sub>2</sub>, LTB<sub>4</sub> like activities.

### Wash-out with colchicine

The joints were washed-out with colchicine (5 mg/kg in lactated Ringer's solution) one week after sulphurus injection. The wash-out of the knee joints were performed with two puncture

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Table 1: Wash-out with colchicine.

Rabbit (Number)	Knee (number)	Wash-out time	Investigated time
3	6	1st and 2nd week	3rd week
3	6	3rd and 4th week	5th week

needles inserted into the joint, one from medial the other from lateral. Wash-out fluid was given from one side and aspirated from the other. The wash-out was continued for one week and the rabbits were sacrificed in the 3rd week under anesthesia. Histopathologic examination of the joint cartilage and meniscal tissue were carried out and PGE<sub>2</sub> and LTB<sub>4</sub> like activities were measured. Six rabbit knee joints injected with colchicine 3 weeks after the injection and that was repeated at the end of the 4th week. These rabbits were sacrificed in the 5th week for investigation (Table 1).

#### Wash-out with diclofenac sodium

A similar experimental design was used for the wash-out with diclofenac sodium (3.75 mg/kg in lactated Ringer's solution).

#### PGE<sub>2</sub> and LTB<sub>4</sub> measuring

PGE<sub>2</sub> and LTB<sub>4</sub> like activities were determined by using the method of Vane (18) and Anderson *et al.* (1), Samhoun and Piper (1987), (1, 15) respectively. The tissues were divided into two parts, extraction was done by using the methods of Gilmore *et al.* (9) for PGE<sub>2</sub>, Folco and Sala (6) for LTB<sub>4</sub>. The results were statistically analyzed using Student's t-test.

#### Histopathological study

Bone tissues were stored in 10% formalin for 24 hours. They were held in 10% formic acid after softening and washed in tap water for 24 hours. Since were obtained from the thickest portion of the articular cartilage. Slices from particular soft tissues were

Table 2: PGE<sub>2</sub> and LTB<sub>4</sub> like activities of the knee joint tissues (mean±SEM of 6 experiments).

	PGE <sub>2</sub> like activity equivalent to PGE <sub>2</sub> (ng/g)	LTB <sub>4</sub> like activity equivalent to LTB <sub>4</sub> (ng/g)	Ratio PGE <sub>2</sub> /LTB <sub>4</sub>
Normal tissue	6.6±0.4	0.3±0.009	22
Carboxy-methylcellulose 20%	6.4±	0.4±	16
Sulphurus 100 mg/kg	23.2±	0.8±	29
Colchicine 5 mg/kg	1.8±0.2	0.4±0.07	4.5

Figure 1: The histopathological view of the carboxymethylcellulose injected knee joint shows the atrophy of the articular cartilage.

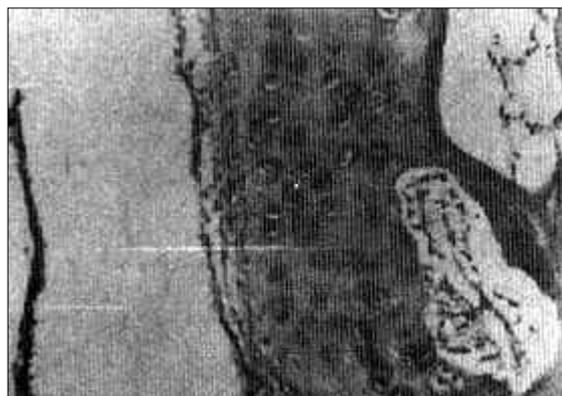
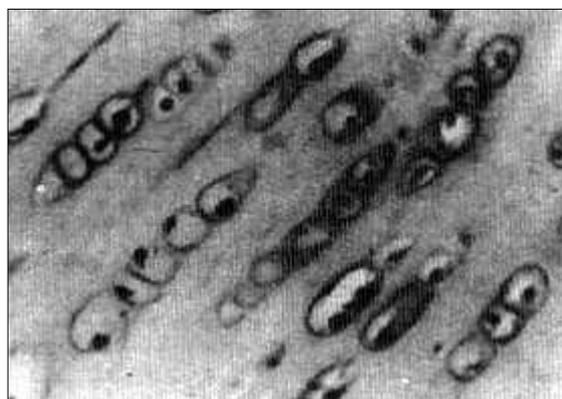


Figure 2: The healthy appearance of the chondrocytes.



also prepared. Those were dehydrated in alcohol, put in xylose and placed in paraffin. The 5 micron thick slices were stained with Hematoxylin-eosin for light microscopic analyze.

## RESULTS

PGE<sub>2</sub> and LTB<sub>4</sub> like activities of the tissues are summarized in Table 2.

#### Histopathological changes

Figures 1 and 2 represent the histological structure of joint from intact and carboxymethylcellulose injected knees. No changes were seen in the structure of the cartilage. Regional disappearance of cartilage and increase of cell count in the lacunae and columnar arrangement which means active degeneration of chondrocytes were seen in the sulphurus injected knee joints (Figure 3). The articular cartilage became thinner and the vertical splits were observed.

Figure 3: The histopathological view of 2% sulphurus injected rabbit knee joint shows gross articular disruption and the cartilage-subchondral bone interface.

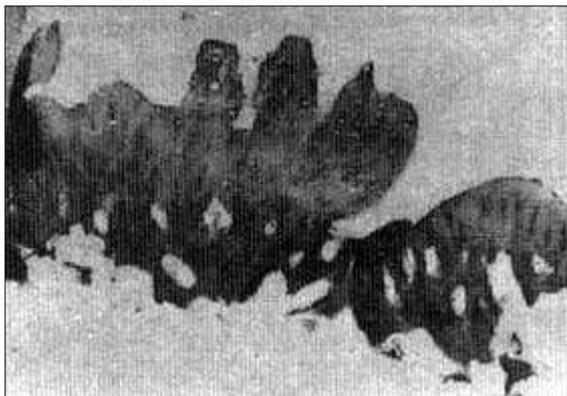


Figure 6: Typical columns and regeneration symptoms in the 3rd week in a joint treated with colchicine (5mg/kg intraarticularly).

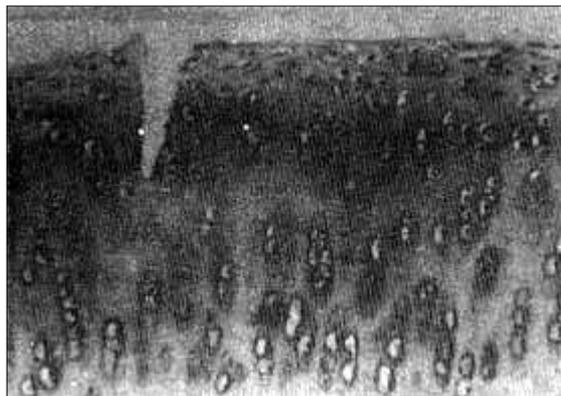


Figure 4: The pathologic changes in 2% sulphurus injected joint pretreated with diclofenac sodium (3.75 mg/kg intraarticularly). See the excessive regenerative activity as depicted by the appearance of double nuclear and column formation of the chondrocytes.

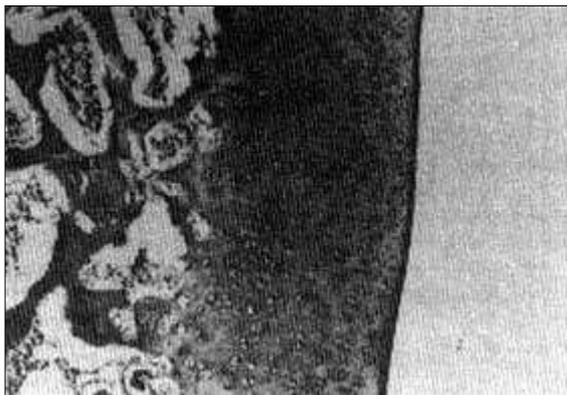
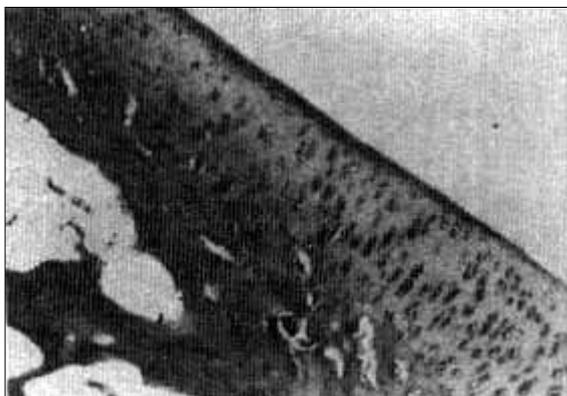


Figure 7: Attempts of regeneration of the chondrocytes are seen.



Figure 5: The thinning of the articular cartilage and the initial attempts of regeneration are seen.



Histological examinations of diclofenac sodium pretreated joints have shown double nuclei in cells, increase in cell count and recovery of the normal articular cartilage thickness (Figure 4).

Histological examination of colchicine pretreated joints in the week have shown the recovery of the columnar appearance and other regenerative signs (Figures 5-7).

DISCUSSION

Various methods of producing degenerative changes in joints have been described. Local trauma, infection, intraarticular injection of papain are well known methods (17). Our studies have confirmed that intraarticular injection of sulphurus is capable of producing degenerative changes. Similar histopathologic changes were observed in our study as well.

The results of the present study indicate that there is a close correlation between PGE<sub>2</sub> and LTB<sub>4</sub> like activities of tissues and evidence of degenerative changes.

Wash-out with colchicine and diclofenac sodium significantly inhibit the increased mediator activities. Both drugs also stimulate the recovery of the degenerative changes.

This effect of colchicine may be due to the inhibition of leukocyte migration, glycoprotein production and secretion in granulocytes (2, 3, 6, 8,12,14,16). It has been shown that parenteral injection of colchicine inhibits LTB<sub>4</sub> a real mediator of vertebral disc diseases (2).

According to histological examination, double nuclei in cells and cell columns were observed in colchicine and diclofenac sodium washed knees at the end of the second week. The count of double nuclei and columns exceeded those in unwashed knees. These results confirm our knowledge (20, 21).

In conclusion, early knee joint degenerative arthritis induced by intra articular injection of sulphurus, both PGE<sub>2</sub> and LTB<sub>4</sub> activities were increased and that closely correlated with the pathologic data. Intra articular wash-out with colchicine and diclofenac sodium significantly inhibited the elevated activities of both mediators and improved the regeneration of the tissues.

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