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Original Article

Influence of coblation nucleoplasty on IL-1β expression and phospholipase A2 activity in a degenerative goat model

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Abstract: The aim of this study was to explore the effect of coblation nucleoplasty (CN) on interleukin 1 beta (IL-1β) expression and phospholipase A2 (PLA2) activity, as well as the mechanisms whereby CN aids the treatment of discogenic low back pain. Twenty-one 6-month-old goats were randomly assigned to a control group (CG) and a study group (SG). An artificial degenerative model was established using an osteotome to penetrate the posterior paraspinal approach and injure the L4/5 disc. The success of this procedure was confirmed by X-ray and magnetic resonance imaging, as well as hematoxylin-eosin staining. Four goats in the SG were selected randomly for CN treatment and classified as the therapy group (TG). The remaining 13 SG goats were designated as the non-therapy group (NTG). Two goats each from the CG, TG, and NTG were sacrificed on the second and fourth weeks after CN treatment. The L4/5 intervertebral discs were investigated for IL-1β expression and PLA2 activity. PLA2 activity and IL-1β expression in the SG were significantly higher than those in the CG and significantly lower in the TG than in the NTG. PLA2 activity and IL-1β expression during the fourth week of CN treatment were significantly lower than those during the second week. CN treatment inhibited PLA2 activity and IL-1β expression in the goat lumbar intervertebral disc degenerative model. These data may reflect 2 possible mechanisms whereby CN treatment alleviates discogenic low back pain.

Keywords: Intervertebral disc, coblationnucleoplasty, animal model, IL-1β, PLA2

Introduction

Coblation nucleoplasty (CN) uses low-temperature plasma layers generated by an ion vaporization rod to treat lumbar intervertebral disc degeneration by breaking molecular bonds within tissues. The method is used to vaporize and ablate tissues within 1 mm around the plasma layer at low temperatures. Afterwards, part of the nucleus in the intervertebral discs is removed and reconstructed. Heat coagulation generated by vaporization constricts and solidifies the collagen in the nucleus, decreases disc pressure, retracts the bulging annulus fibrosus, relieves pressure on nerve roots, and moderates symptoms immediately [1]. Recent data have shown that inflammatory reactions induced by chemical mediators are closely correlated with low back pain caused by disc degeneration [2, 3]. The opinion that pain is caused by chemical mediators released from damaged tissues is generally accepted.

In the current investigation, we established a goat lumbar intervertebral disc degeneration model for CN treatment. We aimed to determine the molecular-level mechanisms of CN in the treatment of disc-derived low back pain by comparing the differences in interleukin 1 beta (IL-1β) expression and phospholipase A2 (PLA2) activity between a control group (CG), a non-therapy group (NTG), and a therapy group (TG).

Methods

Animals and grouping

This study was conducted in accordance with the declaration of Helsinki. This study was conducted with approval from the Ethics Committee of Tianjin Union Medical Center. Twenty-one healthy goats were provided by the Tianjin Aoyi Experimental Animal Rearing Company (Shanghai, China). The goats were 6 months old, randomly distributed in gender,

and 25-35 kg in weight. Four goats were randomly assigned to the CG, whereas the remaining 17 goats were assigned to the study group (SG).

Establishment of the L4/5 disc degenerative model

Goats were deprived of food and water at approximately 6 p.m. on the day before the operation. Weighing of animals and anesthetic dosage calculations were also performed at this time. The approach was constructed in the superficial vein inside the forelimb. Goats were administered cefradine (0.5 g, *iv. gtt*.). Before the operation, an intramuscular injection of 2% midazolam solution and sumianxin was given, and the extent of anesthesia was determined 2 min later. A pathway through the right side of the posterior paraspinal approach was chosen for the operation. The skin, approximately 12 × 15 cm^2 in size at the right side of the lumbar spine, was sheared and prepared before the operation.

All animals were kept in a left-lateral position when constructing the posterior paraspinal approaches. A 1-cm straight longitudinal incision was made along the right side of the L4-L7 processus spinalis. Ten-centimeter-long incisions were made until the discs were exposed. Disc segments were then verified. An osteotome was used with the right posterolateral disc to make a 5-mm deep incision into the L4/5 intervertebral space (the average depth of the annulus fibrosus is approximately 7.5 mm) [4]. The depth was controlled by the limited-depth chamber in the osteotome, and a portion of the annulus fibrosus tissue was cut.

A 0.5% chlortetracycline hydrochloride eye ointment was applied to the incision after the operation and for the next 3 days. The model animals were placed into rearing cages and allowed to roam free. Animals were subjected to absolute diet and water deprivation during the day of operation. Normal diet and water allocation were resumed on the day following the operation.

At 2, 3, and 4 weeks after modeling, 9 animals from the SG were sacrificed to observe whether successful models had been established. After successful model establishment was confirmed, the 8 remaining animals in the SG were divided randomly into the TG and the NTG. The L4/5 discs of the animals in the TG were treated with CN, whereas those in the NTG were not given any treatment.

CN procedure

Animals in the TG were intramuscularly anesthetized with 2% midazolam solution (Jiangsu Nhwa Pharmaceutical Co., Ltd., Xuzhou, China) and sumianxin (Jilin Huamu Animal Health Products Co., Ltd., Changchun, China). The animals were sheared to expose the skin at the left-lateral position. The L4/5 disc interspace were located and marked under C-arm X-ray scanning. A lumbar puncture needle (Arthrocare Company, USA) was used, with the needle tip located at the 2/3 intervertebral disk interspace (lateral position perspective), not passing the midline (orthophoria perspective). After puncturing, the needle was removed, and then the plasma knife was inserted for CN. The coblation variable was adjusted to a grade of 1. The head point of plasma knife entered the inner layer of the fiber ring, with the end point entering the inner layer of the inner ring on the opposite side. The “coblation” key was pressed, and coblation was performed for 3-5 s. The bubble spilled out from the puncture orifice. Then the “coagulation” key was pressed, and the knife head was withdrawn slowly. According to the 6, 8, 10, 12, 2 and 4-point marks at the puncture orifice, this process was repeated for 6 times. Thus, multi-point coblation was performed. The treatment path was controlled within a distance of 5 mm. During the operation, cefradine (0.5 g, Sigma-Aldrich Corp., MO, USA) was dripped in the forelimb superficial medial vein. After the operation, the incision site was coated with 0.5% chlortetracycline hydrochloride eye ointment (Guangzhou Baiyun Mountain Pharmaceutical Co, Ltd., Guangzhou, China) for 3 days. The animals were housed in cages, with freedom of movement. On the day of operation, the animals were subjected to fasting and water deprivation. Subsequently, they were allowed access to water and food *ad libitum*. The animals’ overall conditions, nerve functions, and puncture healing were observed after the operation.

Specimen preparation

Two animals in the CG, TG, and NTG were sacrificed during the second and fourth week after

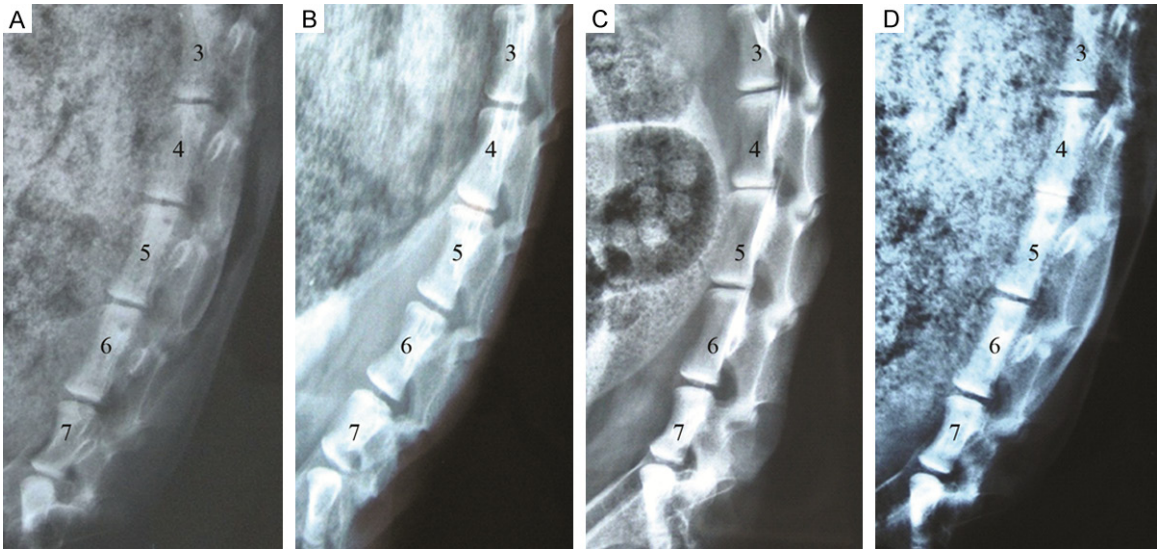

Figure 1. A. Lateral position imaging of preoperative goal lumbar vertebrae discs. B. Lateral position imaging of discs 2 after operation. C. Lateral position imaging of discs 3 weeks after operation. D. Lateral position imaging of discs 4 weeks after operation.

the operation. Under aseptic conditions, a complete L4/5 disc was sampled and preserved at -80°C in a profound hypothermia refrigerator.

Determination of IL-1β expression

Frozen L4/5 disc tissues were warmed to room temperature and cut into 5-mm sections with a microtome. After natural, open-air drying, routine immunohistochemical procedures were performed. Other procedures such as application, reaction, and coloration were conducted using commercial kits, according to the manufacturers' recommended protocol. The samples were preserved with neutral resins. The positive cells in each section were counted using a light microscope.

Determination of PLA2 activity

Disc tissues were weighed, cut into 1 mm^3 cubes, and homogenized in a glass motor-operated homogenizer. PLA2 was added to the resulting homogenate at a concentration of 4 mL/g. Supernatant fluid was drawn after centrifugation for 10 min at 4°C at a rotation speed of 400 rpm. A 0.4-mL PLA2 dilution was added to the solution and centrifuged for 20 min at a rotation speed of 8000 rpm. Supernatant fluids were drawn after complete cell disruption and stored at 4°C. PLA2 activity was calculated by trace acid titration.

Statistical analysis

Two personnel from the Department of Pathology examined all immunohistochemical-staining sections in a blinded manner, counted the number of positive cells in the visual fields, and expressed the data as the mean ± SD. Results were processed using SPSS 13.0. Homogeneity tests were applied for variance. A group t test was used for the simultaneous analysis of data from different groups, whereas a paired t-test was used to analyze data from similar groups obtained at different times. $P < 0.05$ was considered as a statistically significant, whereas $P > 0.05$ was considered as not statistically significant.

Results

Radiographic changes in animal models

On the second, third, and fourth weeks post-operation, progressive stenosis and cartilage end-plate calcification were observed in the L4/5 intervertebral spaces of the model animals (**Figure 1**). However, changes in the physiological arcuation of the lumbar vertebrae, narrowing of the intervertebral space, formation of bony outgrowth, and calcification of the cartilage end plate were not observed in the L5/6 and L6/7 intervertebral spaces at any time point (**Figure 1**).

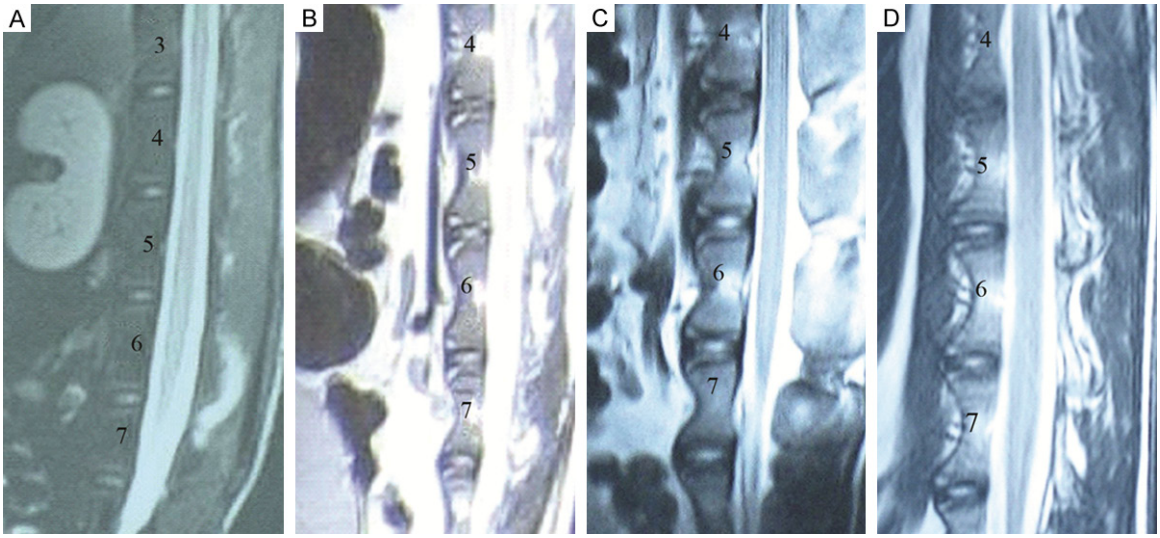

Figure 2. A. MRI of preoperative goal lumbar vertebrae discs. B. MRI of discs 2 weeks after operation. C. MRI of discs 3 weeks after operation. D. MRI of discs 4 weeks after operation.

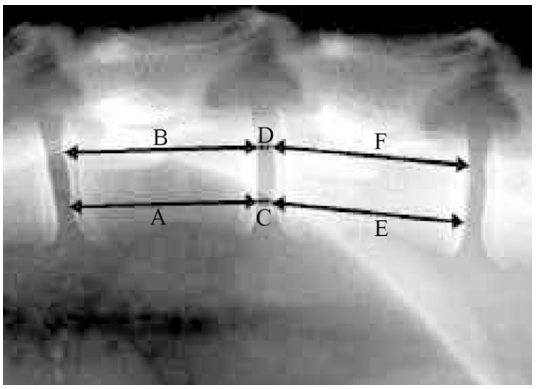

Figure 3. Detection of DHI: A. The anterior height of the epistasy vertebral body. B. The posterior height of the epistasy vertebral body. C. The anterior disc height (height of 1/4 of the anterior disc). D. The posterior disc height (height of 1/4 of the posterior disc). E. The anterior height of the lower vertebral body. F. The posterior height of the upper vertebral body, the data were accurate to 0.1 mm. DHI = 100 × 2 × (C + D)/(A + B + E + F).

Preoperative magnetic resonance imaging (MRI) determinations showed that no animals had axial skeleton congenital deformities or disc degeneration. T2 weighted images (T2WIs) showed bright, uniform nucleus signals in the sagittal view and distinct demarcations between the nucleus and annulus fibrosus in the horizontal position (**Figure 2**). T2WIs from MRI determinations on the second, third, and fourth weeks showed a gradual decrease in the areas of the nucleus in the L4/5 disc, with a gradual increase occurring in the area of the annulus fibrosus. T2WI signals from the MRI determinations of the inner nucleus pulposus decreased and darkened progressively. However, no such changes were observed in the L5/6 and L6/7 intervertebral spaces (**Figure 2**).

Variations in disc height index and T2WI signals

Statistical analysis of the disc height index (**Figure 3**) and T2WI signals of the modeled vertebral segments was conducted at different periods. The results indicated that the data for L4/5 on the second and third weeks showed no significant differences compared with the preoperative data ($P > 0.05$), but varied significantly compared with the data from the fourth week ($P < 0.05$). No statistical differences were found between the L5/6 and L6/7 data either at the preoperative or postoperative stage ($P > 0.05$).

Pathological changes in the intervertebral disc

Three modeled animals were sacrificed after imaging examinations during the second, third, and fourth weeks post-operation by lethal injections with phenobarbital sodium. Sampled L4/5, L5/6, and L6/7 disc tissues were cut into 5-mm thick sections by using a freezing microtome after 72 h of profound hypothermia cryofixation in liquid nitrogen. Histological morphologies of the patches were naturally open

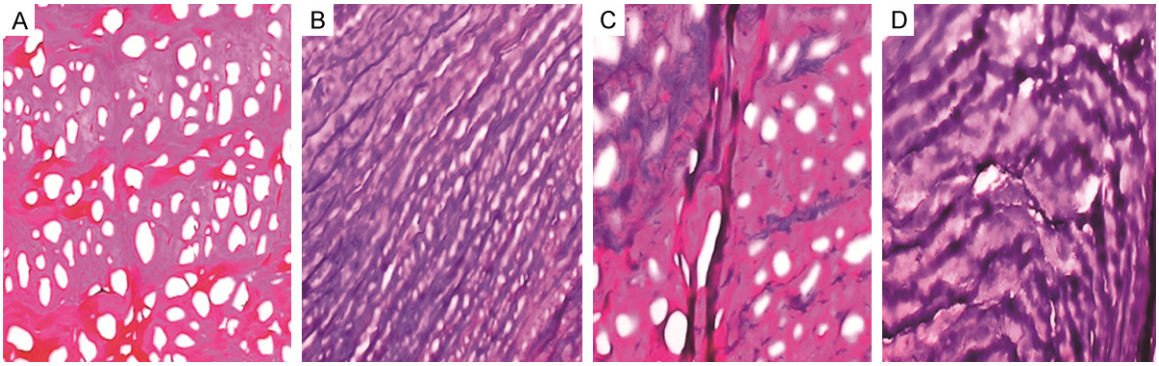

Figure 4. A. The nucleus tissue of L6/7 at 4 weeks after operation (HE 10 × 20). B. The annulus fibrosus of L6/7 at 4 weeks after operation (HE 10 × 20). C. The nucleus tissue of L4/5 at 4 weeks after operation (HE 10 × 20); D. The annulus fibrosus of L4/5 at 4 weeks after operation (HE 10 × 20).

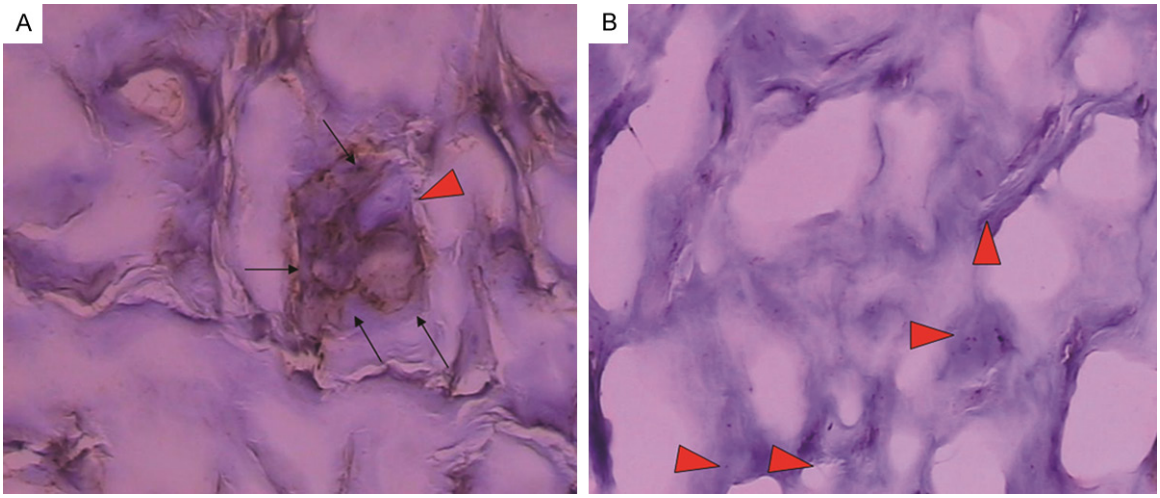

Figure 5. Positive cells by staining were signaled with arrows (10 × 40), cytoplasm was turn to brown by staining, indicated the expression of IL-1β; Negative cells were signaled with triangles, cytoplasm was turn to blue by staining not brown. (A) Immunohistochemisty section from therapy group 4 weeks after operation, (B) immunohistochemisty section from control group 4 weeks after operation.

air-dried, stained with HE, and observed under a light microscope. During the second, third, and fourth weeks post-operation, both the L5/6 and L6/7 discs presented characteristics similar to those of normal discs. Such characteristics included uniformly aligned annulus fibrosus and nucleus pulposus tissues. The annulus fibrosus tissues consisted of tiny inner and massive outer layers composed of fibrochondrocytes and fibroblasts. No demarcations were observed between the inner layer and the nucleus pulposus, which were tightly constructed. The nucleus pulposus was located in the middle rear of the disc with a large degree of extracellular matrix and chondrocyte-like nucleus pulposus cells (**Figure 4**). Fibroplasia was observed at the incision point of the L4/5 disc by light microscopy with HE staining. The annulus fibrosus did not show any signs of healing, but local swelling was observed in some samples. With time, similar histological characteristics such as human intervertebral disk degeneration gradually appeared. The number of notochord cells and chondrocytes in the nucleus pulposus decreased progressively and was replaced gradually by indiscriminate and hypocellular fibrous-like tissues. The reticular formation of collagen fiber in the nucleus pulposus was constructed indiscriminately. Some samples exhibited extensive vacuole-like degeneration, a folded annulus fibrosus, and damaged structures. The delimitation between

Table 1. The expression of IL-1β in each groups at both time ($\bar{x}$±s %)

Group	Time (after operation)	
	2nd week	4th week
Control	1.327±0.241	1.268±0.339
Non-therapy	60.476±7.828①	83.934±9.452①
Therapy	32.583±5.078①,②	15.625±4.705①,②,③

①Compared with control group ($P < 0.05$); ②Compared with Non-therapy group ($P < 0.05$); ③Compared with the same group at posterior 2 weeks ($P < 0.05$).

Table 2. The activity of PLA2 in each groups at both time ($\bar{x}$±s)

Group	Time (after operation)	
	2nd week	4th week
Control	19.390±2.895	20.060±1.933
Non-therapy	175.476±14.387①	193.579±18.091①
Therapy	142.637±11.145①,②	124.053±12.259①,②,③

①Compared with gontrol group ($P < 0.05$); ②Compared with non-therapy group ($P < 0.05$); ③Compared with the same group at posterior 2 weeks ($P < 0.05$).

annulus fibrosus and nucleus pulposus tissues was not observed. Fibroblast multiplication along the edge of the incision was observed in the outer layer of the annulus fibrosus. The fibroblasts proliferated and formed fibrocartilage tissues (**Figure 4**).

The decreased HDIs observed in X-ray images and the low intensity of T2WIs were initial, frequent signs of disc degeneration [5-7]. These findings indicated that all models were successfully established.

Variations in IL-1β expression in different groups

IL-1β expression in the SG increased significantly at all time points compared with that in the CG ($P < 0.05$), as signified by brown cytoplasmic staining that was typical of positive cells (**Figure 5**). IL-1β expression levels in the TG decreased significantly at all time points compared with those in the NTG ($P < 0.05$). IL-1β expression in the TG decreased significantly on the fourth week compared with that on the second week ($P < 0.05$; **Table 1**).

Variations in PLA2 activity in different groups

PLA2 activity in the SG increased significantly at all time points compared with that in the CG ($P < 0.05$), whereas it decreased in the TG decreased significantly at all time points compared with that in the NTG ($P < 0.05$). PLA2 activity in the TG on the fourth week decreased significantly compared with that on the second week ($P < 0.05$; **Table 2**).

Discussion

Low back pain is a common health problem in all countries around the world. Epidemiological investigations indicate that ~75% to 85% of people experience back pains at some point in their lives. Meanwhile, 18% of people suffer from low back pain at any given time [8]. The intervertebral disc critically affects the occurrence of low back pain [9-12]. The mechanisms of disc degeneration-induced low back pain are postulated in 3 theories: the theory of mechanical compression, the theory of chemical neuritis, and the theory of apoptosis. In recent years, the opinion that pain is induced by chemical mediators released by injured tissue has become generally accepted [13]. Some researchers do not consider mechanical factors to be the fundamental reasons underlying spinal nerve root pain. Rather, some researchers suggest that the painful sensation at the nerve might become much more sensitive to mechanical factors when nerve fibers are demyelinated following local inflammatory stimulation and immunity reaction [14].

Numerous mediators of inflammation are involved in disc herniation. Among these mediators, IL-1β and PLA2 have been frequently investigated for their intensive pain-causing effects in radiculitis. The IL-1 signaling pathway includes IL-1α, IL-1β, and IL-1 receptor antagonists. The acceptors and biological effects of IL-1α and IL-1β are nearly the same. IL-1β serves an important function in blood circulation. The prostaglandin E2 content in the disc increases in the presence of IL-1 [15], which is a significant transmitter that increases nerve ending sensitivity by activating certain metabolic processes, decreasing the threshold of sensation, and inducing tissue hyperalgesia. IL-1 also increases and extends the effects of pain factors induced by histamine, serotonin, and bradykinin to nerve endings. IL-1 activates PLA2 [16] and increases pain sensitivity through positive feedback mechanisms [17].

PLA2 is a fat-splitting enzyme first implicated in disc herniation by Saal in 1990 [18]. During stimulation, factor effects, or cell damage, cellular PLA2 is activated and specifically combines with the acyl group at the 2-position of glycophospholipids in the phospholipid membrane, thereby releasing arachidonic acid, which further degrades into proinflammatory cytokines such as PGs, TX, LT, and PAF. These activity factors induce the generation of more inflammatory factors, eventually inducing an inflammation cascade. PLA2 is recognized as a rate-limiting enzyme in chain reactions [18]. Therefore, PLA2 is not only a significant mediator in inflammation and is associated with factors that cause pain, but is also recognized as a special signaling molecule promoting inflammation in local tissues. Changes in the concentration and activity of PLA2 are critical factors in intervertebral discs. PLA2 is greatly correlated with degenerated discs or intervertebral disc herniations, nerve root-derived symptoms, peripheral nerve damage, and disc-derived low back pain. PLA2 is the first link in a series of pathological changes induced by disc lesions [19].

In this study, differences between IL-1β and PLA2 in discs of normal and degenerative model tissues were significant. IL-1β expression and PLA2 activity were significantly higher in degenerative models compared with those in normal tissues. Thus, IL-1β expression and PLA2 activity are closely related to disc degeneration or disc herniation.

CN is a newly developed micro-operation method that generates a curative effect by vaporizing small quantities of nucleus tissues with radio frequency energy, forming porous channels inside the nucleus, thereby decreasing disc pressure. The mechanism of CN principles may be as follows: the low-temperature plasma layer is formed surrounding ion vaporization rod, which can break molecular bonds in tissues, with subsequent tissue ablation and removal of part of the nucleus pulposus. The nucleus pulposus in the intervertebral disc is remodeled. The hot-coagulation formed during vaporization leads to nucleus pulposus collagen contraction and solidification. Thus, the intradiscal pressure is rapidly reduced, and the bulging fiber loop is retracted. The nerve root compression is removed, and the symptoms are rapidly relieved.

Azzazi et al. demonstrated that the curative effects of CN are satisfactory as a disc-derived low back pain treatment [1]. However, during clinical treatment with CN, the imaging data (CT and MRI) from patients suffering from low back pain showed no apparent nerve root compressions. T2WI showed high local signals in the posterior border of the annulus fibrosus, indicating the occurrence of tearing. The pain experienced by patients were moderated or relieved by CN treatment, which could not be explained by the theory of decompression. We presumed the existence of other possible mechanisms in the CN treatment of disc-derived low back pain.

In the present study, animal intervertebral disk-degenerative models were treated with CN, after which IL-1β expression and PLA2 activity were determined during the second and fourth weeks post-operation in the TG. IL-1β expression and PLA2 activity decreased more significantly in the TG than in the NTG. Thus, CN decreases IL-1β expression and PLA2 activity and generates clinical effects. IL-1β expression and PLA2 activity in the TG decreased more significantly on the fourth week than on the second week, indicating that CN can inhibit the cascade effects generated by inflammation.

The results of this study demonstrate that IL-1β expression and PLA2 activity can be decreased by CN. The reduction of these 2 factors may be potential mechanisms whereby CN treatment alleviates discogenic low back pain.

Disclosure of conflict of interest

None.

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