Effect of Tobacco on Knee Joint Recovery of College Students After Sports

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Objectives: By analyzing the protective effect and mechanism of tobacco on knee joint cartilage in rats, this paper studies the effect of tobacco on knee joint recovery of college students after sports. Methods: Firstly, the main subunits of nAChRs were systematically studied by using the rat knee arthritis model α 7 and α 4 and β To clarify the correlation between nAChRs and the occurrence and development of OA. Then, the OA rat model prepared by iodoacetic acid was used as the experimental object to observe the protective effect of nicotine on knee osteoarthritis cartilage in rats. Results: The histological changes of rats in MIA group were obvious after operation. The results of light microscope score and Mankin's score at 15 and 30 days were significantly higher than those in con group. Of right knee cartilage in rats in MIA group α 7、 α 4 and β The expression of 2 did not change significantly on the 15th day, but increased significantly on the 30th day compared with the blank control group. Conclusion: Nicotine has a protective effect on knee bone and joint cartilage and promotes the accelerated recovery of knee bone and joint after exercise..

Key words: nicotine, knee joint, cartilage, recovery after exercise.

Tob Regul Sci.™ 2021;7(4):400-407
DOI: doi.org/10.18001/TRS.7.4.17

Nicotine, commonly known as nicotine, belongs to the alkaloid family of Solanaceae and is the main component of tobacco. In recent years, nicotine is mainly used to treat smoking addiction[1]. OA is a common joint degenerative disease. There are many predisposing and risk factors, such as heredity, obesity and women[2]. At the same time, some living environment and behavior habits are also closely related to this disease[3]. Early studies have noted the correlation between smoking and OA. In the first NHANES I, when studying the association between OA and obesity and occupation, the incidence rate of OA in smokers was lower than that in non-smokers[4-6]. Many studies showed that the incidence rate of OA in smokers was lower than that in non-smokers, suggesting that nicotine might have some protective effects on OA.

Clinical symptoms and signs of OA, including joint swelling and joint effusion, are all related to inflammatory reaction[7-9]. Histologically, OA inflammation is characterized by synovial hyperplasia, accompanied by increased lining cells and infiltration of mixed inflammatory cells, mainly macrophages[10]. With the development of molecular biology, scholars at home and abroad have made in-depth research on the effect of inflammatory cytokines on OA cartilage. Cholinergic anti-inflammatory pathway, as an important neurophysiological mechanism of regulating immune system, is closely related to pathological processes such as sepsis, rheumatoid arthritis, Crohn's

disease and autoimmune diseases[11-13]. In recent years, it has been found that cholinergic anti-inflammatory pathway can antagonize some inflammatory cytokines, including TNF-α, IL-1β and HMGB1, which is helpful to regulate the occurrence and development of inflammatory reaction[14-15].

METHODS

Experimental Instruments And Equipment

All molecular biology experiments, including RNA extraction, reverse transcription reaction, Real-Time PCR and PCR reaction system preparation, are operated in biosafety cabinet (ME04073, The Baker Company). The frozen high-speed centrifuge (Centrifuge 5417R, Eppendorf), Real-Time PCR instrument (StepOnePlus, ABI), PCR instrument (GeneAmp PCR System 9600, ABI), electrophoresis instrument (Power PAC 300, BIO-RAD), gel electrophoresis imaging system (Tanon 3500). Dissecting microscope (ZOOM645, SingSun) was used for gross observation of SD knee joint specimens.

Nicotine Intervention In Oa Animal Model

Forty SD rats were randomly grabbed and divided into two groups: blank group (Con group), 10 rats; 10 rats in model group (MIA group). Low dose intervention group (MIA+Nic 0.25mg·kg^{-1}, N1 group) and high dose intervention group (MIA+Nic 0.5mg·kg^{-1}, N2 group).

SD rats in N1 and N2 groups were pretreated with Nic for one week before operation, and the pretreatment method was that every SD rat was injected with Nic once a day (0.5 mg kg^{-1}).

After pretreatment, rats in each group were anesthetized by intraperitoneal injection of 2.5% pentobarbital sodium (35 mg kg^{-1}-40 mg kg^{-1}), and then were put in supine position, skin was prepared routinely, and the operation area was disinfected with iodophor. The right knee incision was made in rats, and the skin was cut to expose the joint capsule and patellar ligament. Under direct vision, a 50ul microinjector was used to penetrate the knee joint cavity through the patellar ligament, and 50 μl MIA (1 mg) was injected. The operation should be gentle to avoid damage to articular cartilage. After rinsing with normal saline, suture the skin layer by layer with 3-0 mousse thread, and scrub the wound with iodophor cotton ball. Con group entered the joint cavity in the same way, injected with 50μl of normal saline, and sutured after irrigation (refer to the first part for surgical pictures). Three days before operation, 200,000 penicillin units were injected into gluteus maximus muscle of each SD rat every day to prevent infection. After operation, they were put in cages and allowed to move freely, paying close attention to wound infection and other complications.

SD rats in each group were operated once a week for 4 weeks. In this period, NIC (0.25 mg kg^{-1}) was injected intraperitoneally to each SD rat in N1 group and NIC (0.5 mg kg^{-1}) was injected intraperitoneally to each SD rat in N2 group.

SD rats in each group were killed on the 15th and 30th days after operation (cervical dislocation after anesthesia), and 5 rats in each group were killed for gross observation, histological and pathological sections and molecular biology experiments (see Figure 1).

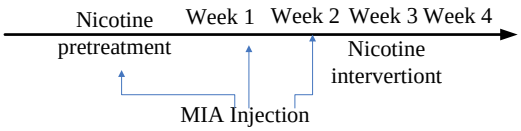


Figure 1 Flow chart of experimental design

Gross Light Microscopic Observation Of Knee Joint In Sd Rats

SD rats in each group were killed by cervical dislocation after anesthesia. They were disconnected from the proximal end of the right femur of SD rats and kept

away from the right knee as far as possible to avoid mechanical injury. Remove the surface skin, muscles and other surrounding tissues. Carefully cut the suprapatellar capsule, separate the patella from both sides of the patella, and expose the knee joint cavity. Disconnect the internal and external collateral ligament of the right knee joint, flex the knee joint at the maximum angle, expose and carefully disconnect the cruciate ligament, and separate the internal and external femoral condyle and tibial plateau. Carefully remove the internal and external meniscus and fully expose the articular surface of tibial plateau and femoral internal and external condyle. The articular cartilage of the right knee was observed under an anatomical microscope. The whole process time was controlled within 5 minutes to avoid degeneration and necrosis of cartilage after too long in vitro. Operate carefully and gently to avoid mechanical damage to articular cartilage. The subjects were patellar articular surface, femoral medial and lateral condyles, femoral intercondylar sulcus and tibial plateau. Refer to table 1 below for the scoring standard of OA under general light microscope.

Table 1

Oa Scoring Standard Of Sd Rats Observed By Gross Light Microscope

Fraction	Project
0 points	The joint surface is smooth and the color is as usual
1 point	The joint surface is rough, with small cracks and gray color
2 points	The cartilage defect reached the middle layer of cartilage
3 points	The articular surface ulcer was formed, and the cartilage defect reached the deep layer of cartilage
4 points	Cartilage exfoliation, subchondral bone exposure

Histological And Pathological Observation Of Knee Joint In SD Rats

Excess femur and tibia were removed from the above-mentioned severed knee joint, and the joint samples were quickly put into 4% paraformaldehyde for fixation for 24 hours. Then put the fixed specimen into the prepared EDTA decalcification solution for decalcification for 3 weeks, and change the decalcification solution once every 3 days. After decalcification, the samples were taken with surgical blade, cut perpendicular to articular cartilage surface, cut into 1.0 cm×0.5 cm×0.2 cm, dewatered by automatic dehydrator, embedded in paraffin, and sectioned by routine pathology and Sum continuously. After conventional dewaxing and dehydration, hematoxylin-eosin (HE) staining and rapid toluidine blue staining were performed. The scoring standard refers to Mankin's score.

Table 2

The Mankin's Score Was Observed By Histological And Pathological Sections

Observation object	Describe	Score
Cartilage structure	Finishing as usual	0 points
	Surface irregularity	1 point
	Irregular surface and dark blood vessels	2 points
	The fracture depth reaches the transitional layer	3 points
	The crack depth reaches the radial layer	4 points
	The crack depth reaches the calcified layer	5 points
	Cartilage layer abscission	6 points
Cartilage cells	Quantity as usual	0 points
	Diffuse increase in number	1 point
	A large number of clustered cell clusters appear	2 points
	The number decreased significantly (evaluated by toluidine blue staining)	3 points
Proteoglycan content	Normal staining	0 points
	Staining decreased slightly	1 point
	Moderate decrease in staining	2 points
	Severe decrease of staining	3 points
	The staining disappeared completely	4 points
Tidal line integrity	Complete	0 points
	Vascular crossing	1 point

Data Statistics And Analysis

Gross OA score and Mankin's score data of histological and pathological sections were expressed by means of X±SEM, and analyzed by SPSS 13.0 statistical software. Kruskal-Wallis rank sum test was used for comparison between groups. $P<0.05$.

RESULTS

Oa Model Established By Injecting Mia Into Knee Joint Cavity

No wound infection, delayed healing and animal death occurred in SD rats in each group. SD rats in each group could resume normal knee movement 2-5 days after operation. The activity gait of SD rats in con group did not change significantly, and the activity was as usual. The right knee joint of SD rats in 1MIA group, N1 group and N2 group was gradually swollen, the gait of the right lower limb was slow, and the activity was slightly limited.

Gross Light Microscopic Observation Of Right Knee Joint In Sd Rats

SD rats in each group were killed and sampled on schedule. The articular surface of right knee skeleton, femoral interosseous sulcus, internal and external skeleton and cartilage of each articular surface of tibial plateau were observed under anatomical microscope.

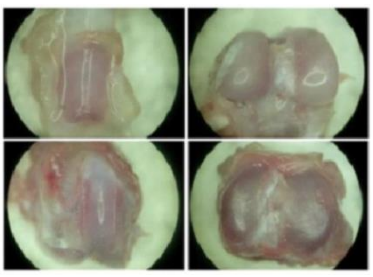
No obvious abnormality was found in the right knee specimens of SD rats in Con

group on the 15th and 30th day. The synovium has no obvious hyperemia and thickening, the articular surface is clear, flat, smooth and shiny, the articular cartilage is transparent and clear without damage, and there is no osteophyte at the edge of the joint. Gross OA score: the scores of Con group on day 15 and day 30 were 0.2 ± 0.447.

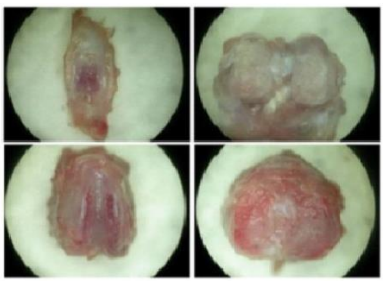
On the 15th day, SD rats in MIA group saw synovial hyperplasia of right knee joint specimen, slightly uneven joint surface of skeleton and cartilage defect. The internal and external femoral bones, interskeletal sulcus and tibial plateau cartilage permeability decreased, and cartilage defects appeared in some areas. On the 30th day, the synovium of the right knee joint specimen was congested and proliferated, the joint surface of the skeleton was uneven and tarnished, and the cartilage defect was serious. Obvious wear and tear also occurred in the inner and outer bones and interosseous sulcus of the femur, the articular surface lost luster, the cartilage defect was serious, and more osteophytes were formed. The articular surface of the tibial plateau is blurred and tarnished, the cartilage defect is serious, the subchondral bone is exposed, and the formation of annular osteophytes can be seen on the inner and outer platforms. Gross OA score: the score of MIA group on the 15th day was 12.2 ± 0.447, and that of 1mia group on the 15th day was 15.8 ± 0.447. Compared with con at the same time point, the score of MIA group was significantly higher ($P < 0.01$).

Compared with MIA group, the symptoms of cartilage destruction in N1 group were improved on the 15th and 30th day. It can be seen that there are still obvious cartilage defects on the articular surface of bones, internal and external bones of femur, interskeletal sulcus and tibial plateau, but the peripheral synovial inflammation was reduced, and the articular cartilage was proliferated and repaired to a certain extent. Gross OA score: the score of N1 group on the 15th day was 10.2 ± 0.837, and the score of N1 group on the 30th day was 12.4 ± 0.548. At the same time point, the score was higher than that of MIA group ($P < 0.05$).

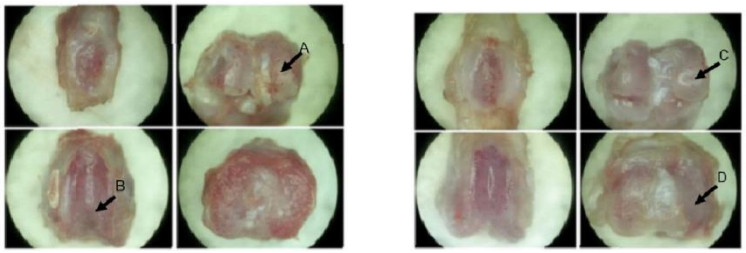
N2 group was significantly better than MIA group on day 15 (see Figure 2) and day 30 (see Figure 3). Obvious cartilage hyperplasia and repair can be seen on the articular surface of the skeleton, the inner and outer bones of the femur, the interskeletal sulcus and the articular surface of the tibial plateau, and the articular surface tends to be flat and smooth. Synovitis was significantly reduced and the structure of articular cavity was clear. The severity was significantly less than that in MIA group, and obvious repair marks were visible. General OA score results: the score of N2 group was 6.6 ± 1.14 on the 15th day and 5.4 ± 1.67 on the 30th day. At the same time point, the score was significantly lower than that of MIA group ($P < 0.05$).



Con group



MIA group



N1 group N2 group

Figure 2 Gross light microscopic observation on day 15

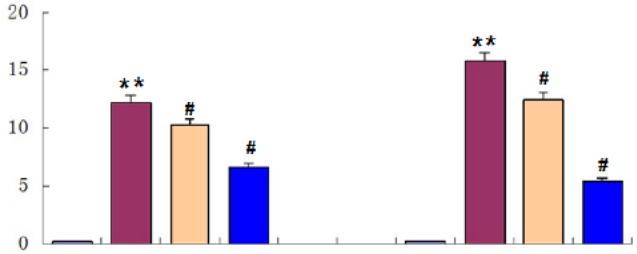


Figure 3 The right knee joint samples of SD rats were generally scored

Expression Changes Of Type II Collagen And Aggrecan In Knee Joint Of Sd Rats

After the rats in each group were killed on schedule, the RNA of cartilage tissue was extracted, and the expression changes of type II collagen and aggrecan after nicotine intervention were detected by RT-PCR.

RT-PCR showed that after the OA model was established by intra-articular injection of MIA into the right knee of SD rats, the expression of type collagen and aggrecan decreased significantly, and the trend of this decrease became more and more obvious with the extension of time. After nicotine intervention, the low-dose (0.25mg) nicotine intervention group has shown a certain protective effect, and the expression of type collagen and aggrecan has increased. The protective effect of high-dose (0.5mg) nicotine intervention group is more obvious, and the expression of type collagen and aggrecan has increased significantly.

On the 15th day, the expression of type II collagen and aggrecan in knee cartilage of SD rats in MIA group was significantly lower than that in con group ($P < 0.05$), the expression of type II collagen and aggrecan in N1 group was higher than that in MIA group ($P < 0.05$), and the expression of type II collagen and aggrecan in N2 group was significantly higher than that in MIA group ($P < 0.05$).

On the 30th day, the expression of type II collagen and aggrecan in MIA group was significantly lower than that in con group ($P < 0.05$), the expression of type II collagen and aggrecan in N1 group was increased ($P < 0.05$), and the expression of type II collagen and aggrecan in N2 group was significantly increased ($P < 0.05$).

DISCUSSION

NAChRs are a large family with pattern recognition receptor function. They can affect inflammatory response in neuroendocrine and immune regulation through cholinergic anti-inflammatory pathway. They are an important anti-inflammatory pathway. At the sub physiological level, the activation of toxin like receptor can slightly inhibit the activity of macrophages, while nicotine is more effective than ACh in inhibiting the

production of macrophage inflammatory cytokines. Therefore, the anti-inflammatory effect of ACh on macrophages seems to be mediated by nicotine receptors, and nicotine seems to be a high affinity agonist regulating the production of pro-inflammatory cytokines.

Nicotine, commonly known as nicotine, is an alkaloid of Solanaceae family and the main component of tobacco. In recent years, nicotine is mainly used to treat smoking addiction. The characteristics of neuronal nicotinic receptors promote the application of nicotine in different neurological diseases (such as depression, autosomal dominant frontal lobe epilepsy, attention deficit hyperactivity disorder, Parkinson's disease and Alzheimer's disease). Similarly, the functional characteristics of nicotine receptors in immune cells provide a new research idea for the clinical study of infection and inflammatory diseases. The finding of different nAChRs subtypes in these immune cells suggests that different nicotinic receptors have different effects on inflammatory cells due to different affinity of receptors. Interestingly, many experiments have proved that the cholinergic regulation of macrophage activity is related to α7 nicotine receptor, and the anti-inflammatory effect of nicotine in macrophages can be blocked by selective α7-nAChRs antagonists. Selective α7-nAChRs agonists can significantly reduce the production of cytokines in macrophages and inhibit inflammation in animal models of pancreatitis, colitis and intestinal obstruction induced by DSS.

One of the most important clinical evidences for nicotine treatment of infections and inflammatory diseases is the epidemiological study of the incidence rate of ulcerative colitis in smokers. Ulcerative colitis is a typical inflammatory bowel disease, which is prone to cause colon cancer. This continuous spontaneous inflammation of colonic or rectal mucosa is usually related to the reduction of normal intestinal flora, abnormal humoral and cell-mediated intestinal immunity and / or widely enhanced response to intestinal bacterial antigens. 90% of ulcerative colitis deaths are non-smokers. Among the non smoked Mormons, ulcerative colitis incidence rate was 5 times higher than that of the normal people. Smokers who had history of smoking were sick after they stopped smoking. Patients who smoke intermittently often feel that their colitis symptoms are improved when they smoke. Among those who quit smoking, the incidence of the disease increased significantly after stopping smoking. Smoking seems to block the occurrence and development of this disease. The use of specific α7 nicotinic agonists has a good effect on the maintenance and treatment of active ulcerative colitis. It is worth noting that the same mechanism can be used for different joint diseases. Smoking seems to have a protective effect on osteoarthritis, but it increases the risk factors of rheumatoid arthritis.

In this subject, we used the method of intra-articular injection of MIA to establish the animal OA model. It was found that the establishment of this animal model would lead to limited knee movement and limb swelling, and it was found that the knee cartilage on the operation side was significantly damaged under the observation of gross light microscope and pathological section. The application of nicotine intervention can effectively alleviate this destructive effect. It is worth noting that the degree of cartilage damage and the expression of nAChRs subunits in rats are interrelated. With the increase of the degree of cartilage damage, the expression of nAChRs also increases, and this trend becomes more and more obvious over time. After the application of nicotine intervention, the expression of type collagen and aggrecan increased to a certain extent, and the trend of increased

expression became more and more obvious with the extension of nicotine intervention time, suggesting that nAChRs can intervene in OA inflammation and play a certain role in controlling inflammation. Further studies are needed to identify the receptor subtypes in the anti-inflammatory mechanism of nicotine receptor in OA model.

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