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Effect of Intraarticular Ketamine Administration on the Histopathological Changes of Knee Joint in the Rat Models

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Abstract

Objective- Postoperative pain management in patients that undergone arthroscopy is one of the most important procedures for their rehabilitation. Analgesic injection intra-articularly can facilitate pain control in such patients. The aim of this study was to evaluate the effects of ketamine on articular cartilage in rat model.
Design- Experimental study.
Animals- Twenty Wistar Rats
Procedures- Rats were included in this study and were randomly divided into 4 groups. Ketamine was injected at doses of 0.1 (Group 1), 0.25 (Group 2), 1.0 (Group 3) and 2.5 (Group 4) mg/ml to their right knee intra-articularly. Isotonic saline (NaCl) was injected into their left knee as the control group. A surface scoring system applied to evaluate the superficial zone of the joint histopathologically. Additionally, morphometric values were considered for the evaluation of the cartilage cellular changes.
Results- Results showed that in articular cartilage, there was a surface intact till discontinuity in most cases, when compared groups 1 to 4 with the control group. In addition, in the group 4, one of the cases showed a focal fissure on the articular cartilage surface. Morphometric results showed that in all groups injected with ketamine, measured parameters was variable than to the control group. But there was no significant difference between all experimental groups compared with the control group ($P>0.05$).
Conclusion and Clinical Relevance- Although in this study a teeny change was observed in one of the cases, but it seems that ketamine can be applied as an anesthetic representative with very few complications.
Key Words- Rat, Ketamine, Histopathology, Knee joint, Scoring.

Introduction

The goal of postoperative pain management in the arthroscopy surgery is to relieve pain while keeping side effects to a minimum. A comprehensive approach to pain control should include preoperative, intraoperative, and postoperative efforts. Almost all the arthroscopic knee surgeries are done on an outpatient basis in which improves patients' functional outcome. Usually patients, who undergo knee surgery often, use nonsteroidal anti-inflammatory (NSAID) drugs for successful pain management.[1-5]

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Ketamine is a noncompetitive NMDA receptor (NMDAR) antagonist. More specifically, ketamine binds to the allosteric site of the NMDA receptor, effectively inhibiting its channel [6-9] and gently allays stimulated pain by blocking postoperative hyperalgesia.[10, 11] As, Menigaux et al. (2000) showed that injection of the intraoperative small-dose ketamine decrease postoperative morphine requirements and improve movement 24 h after arthroscopic anterior ligament repair.[12] The administration of analgesic drugs may block sensitization of the central nervous system before trauma of tissue.[13] The risk of the opioid drugs dependence and their side effects on the respiratory and cardiovascular systems would prevent their general use.[14] Therefore, the importance of the treatment management for postoperative procedures has been described for this purpose.[15]
Although some drugs i.e. morphine and or tramadol are often chosen, with or without local anaesthetics, for intra-articular administration;[16-19] but also ketamine

could be used for decreasing pain intra-articularly. Therefore, the goal of the current study was to evaluate the effect of the ketamine intra-articularly on the characteristics of the tissue structure at the articular cartilage level of knee in rat.

Materials and Methods

Animals and surgical procedures

Twenty healthy and adult male Wistar rats (*Ratus norvegicous*) of 280±10 g body weight were used in this study. Involving animals maintained in a room with controlled temperature (22±1°C) and cycle of light-dark. Animals were maintained on the normal pellets and water *ad libitum*. The experimental protocol was approved by the Animal Care and Experiment Committee of the Shahrekord University.
Animals were randomly divided into four groups (each n=5). The skin of injection site was clipped and scrubbed with 10% povidon iodine and 70% alcohol for aseptic preparation. The articular cavity was recognized by extension and flexion movements of femur and tibia bones and then the synovial fluid aspirated. The right knee joints of rats were considered for the intra-articular injection of the ketamine. Value 1 ml of different concentrations of ketamine, 0.1 (group 1), 0.25 (group 2), 1 (group 3) and 2.5 mg/ml (group 4) was injected by means of sterile syringes into the articular space of the knee. Their left knee (the control group) was injected with an equivalent isotonic normal saline (NaCl) amount.

Histological assessment

Just after injection, animals were immediately placed in separate cages to obtain normal conditions and postoperative evaluation. One week after the injection, all animals were killed with minimal pain by Ether overdose prior to sampling. The knee joints were detached, labeled and get ready for histological examinations. The joints were fixed in 10% buffered formalin for 1 wk at laboratory temperature and then decalcified in solution including of 300 ml absolute alcohol, 670 ml distilled water, 30 ml 70% nitric acid and 50 g chloral hydrate. Tissue samples were dehydrated in decrease degrees of ethanol, cleared and embedded in paraffin. Sections of 5 µm were stained with haematoxylin and eosin (H&E). Four morphometric parameters were considered to forecast tissue changes in articular cartilage included the cartilage thickness, the cells number (the average number of cells per field was calculated, ×400 magnification), the cell diameter and the cell number in graticule grade. To calculate the cells number in graticule grade, a test grade was laid on articular cartilage and the number of intersections hit with cells was counted.
Superficial zone of the articular cartilage was histopathologically examined based on a scoring system to define surface changes.[20] This scoring was based on surface intact, surface discontinuity and vertical fissures for each group.

Statistical analysis

All statistical analyses were carried out with SPSS software (Version 11.5, Statistical Analysis System) with data presentation as means ±SD, LSD test, *P* value was <0.05.

Results

There was no intra-operative and post-operative death drug injection. Macroscopical results obtained from the present study showed that there was no hematoma into the knee joints. Histological scoring results of knee joint are shown in detailed in Tables 1 and 2. Figure 1 shows histological property of the articular cartilage in experimental rats.
The scoring system results showed that in rats injected with saline, there were a normal architecture in matrix and cells. In groups 1 to 3, twelve specimens showed surface intact in articular cartilage and only three specimen showed surface discontinuity (discontinuity at superficial zone). In group 4, three specimens showed surface intact, one specimen showed discontinuity in surface and one of the cases had focal fissure in the articular cartilage.

Table 1- Histopathological scoring system

Histopathological indices	Grade
Surface intact, cartilage morphology intact	0
Surface discontinuity	1
Focal fissures (cleft)	2

Table 2- Surface intact, surface discontinuity and vertical fissures scores for each group

Treatment group	Doses	scoring		
		0	**1**	**2**
ketamine	**G1** (0.1 mg/ml)	4	1	-
	G2 (0.25 mg/ml)	4	1	-
	G3 (1 mg/ml)	4	1	-
	G4 (2.5 mg/ml)	3	1	1
Saline	control	5	-	-

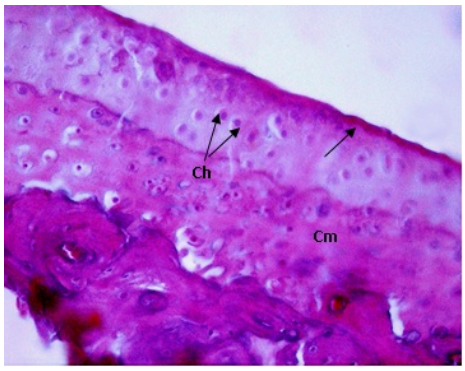

Figure 1- Light photomicrograph of the articular cartilage in control rats with scoring 0; Note surface intact (arrow), regularity of the chondrocyte (Ch) layers was observed into cartilaginous matrix (Cm); (H&E, ×200).

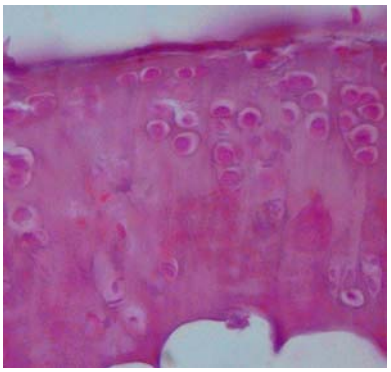

Figure 1- Light photomicrograph of the articular cartilage in experimental rats with scoring 1; Note discontinuity in surface (arrow), irregularity of the chondrocytes (Ch) was observed into cartilaginous matrix (Cm); (H&E, ×400).

Morphometric parameters for each group were shown in detailed in Table 3. It should be noted that there was no significant difference in the thickness of the articular cartilage in all the groups, however a typical increase had occurred in all experimental groups in comparison with the control group (P>0.05).

The value of the chondrocytes diameter were increased to 1.31±0.14 in group 4 as compared with the control group (1.13±0.47), nevertheless there were not significant difference between all the experimental groups than to each other and to the control group (P>0.05).

The statistical survey showed that the cell number per microscopic field was increased slightly in group 1, as it reached from 64.1±6.1 in the control group to 66.5±6.7 in group 1. An ascending trend in the cell number per microscopic field occurred in groups 2, 3 and 4 than to the control group, as reached to 70.76±7.9, 78.8±6.2 and 79.2±6.6, respectively. However, there was no significant difference between experimental groups than to each other and to the control group (P>0.05).

In experimental groups, a upward trend were observed in cells number in graticule grade, as this parameter from the lowest value 8.36±4.24 in group 1 increase to 9.31±4.01 in group 4. However, there was not significant difference between treatment groups than to each other and the control group (P>0.05).

Table 3- The mean measured parameters of articular cartilage in different concentrations of ketamine

Parameter	Group				
	1 (0.1 mg/ml)	**2 (0.25 mg/ml)**	**3 (1 mg/ml)**	**4 (2.5 mg/ml)**	**Control**
The thickness of articular cartilage (µm)	13.17±1.2	13.28±0.9	13.24±1.33	13.63±1.27	13.05±1.08
The diameter of chondrocytes (µm)	1.18±0.12	1.28±0.14	1.22±0.14	1.31±0.14	1.13±0.47
Cell number per field	66.5±6.7	70.76±7.9	78.8±6.2	78.2±6.6	64.1±6.1
Cell number per graticule	8.36±4.24	8.53±2.62	8.84±3.6	9.31±4.01	8.8±3.42

The values are means ± SD

Discussion

Special considerations were given to microstructure of the articular cartilage in knee joint of rats with regard to effect of ketamine different concentrations on the knee joint structure, in which important amount of information was acquired for experimental groups. A great reason for curiosity of current study in these levels was that findings represented alteration values of tissue characteristics in encounter with intra-articular injection of drug. Each drug has effects of the histopathology when administer locally; hence, drugs that inject intra-articularly affect articular cartilage and its synovium.[21, 22] Our findings revealed that ketamine injected intra-articularly in the different dosages in level of the articular cartilage causes very low histo-structural alterations. Although in scoring system, there was a focal fissure in one of the cases at superficial zone, we did not find dramatic differences between rats injected with saline and ketamine. Also, there were no significant differences between the all experimental groups in terms of thickness of the articular cartilage, chondrocytes diameter, cell number per field and cell number in each graticule. According to our results, ketamine can be used as an anesthetic agent after arthroscopy without much complication.

The knee joint is considered as a clinical model which effect of analgesic drugs with different concentrations on it are studied and these effects evaluate the structural changes of articular cartilage.[23, 24, 2] Gupta et al. (1999) demonstrated that in articular capsule, tendons, ligaments, synovium and periosteum there is a combination from terminals of the sensory nerve.[25] By using handlings of the surgical instruments and or tensional statuses in knee joint occurs a mechanical stimulation that cause neural response. Some of the neural bundles no respond to stimuli in usual situations but alterations such as inflammation of due to injury excites these nerves.[26] Therefore great number of fibers are non-responsive under typical situations but react after inflammation, however, it seen that potential for acute damage or swelling to stimulate nerves such that they respond even when the origincal ? stimuli is take off.[26] In another study that was close to our findings, Dogans et al. (2004) showed that after intra-articular injection of bupivacaine and neostigmine in the rabbit knee joints, there were not histopatological changes in joints in 24 h, 48 h and 10 days after injection.[27] Anderson et al. (2010) revealed that there was a causal link in the development of glenohumeral chondrolysis in relation with the intra-articular injection of bupivacaine and epinephrine.[28] A severe postoperative knee chondrolysis was observed in injection of the high or low-flow-volume intra-articular bupivacaine.[29]

In present study we showed that except for one exception, in the cell number per field in dose of 2.5 mg/ml, there were no significant differences when ketamine (experimental group) compared with saline (control) in all parameters of interest. David et al. (1992) reported effect NSAIDs in vitro cell culture studies. They obtained different effects of NSAIDs on cartilage metabolism and the synthesis of proteoglycans. However, they reported differences in cellular excretory functions and cellular metabolic activity between systemic use and intra-articular use.[21] While currently ketamine and or memantine (NMDA) are used for prophylactic analgesia in control of the arthritic pain in human, Zhang and colleagues showed effect of intra-articular pretreatment these drugs. Their findings demonstrated that intra-articular application of NMDA receptor antagonists inhibit formed arthritis associated with pain in knee joints.[30] In a pioneering work in postoperative pain for patients that is underwent meniscectomy, Ayoğlu et al. (2010) studied effects of intra-articular tramadol, ropivacaine, and a combination of these two agents with ketamine in eighty patients. They showed that the combination of the tramadol-ketamine was found to be the most effective in alleviating pain.[31] In another study, Sarıcaoğlu et al. (2008) studied histopathological effects of intra-articular lornoxicam on rat cartilage and demonstrated there was no significant difference between experimental group in the inflammation changes with the control group.[32] In this study we found that histological effects of ketamine on joint cartilage are admissible. Doses were used of ketamine in our study does not seem to be inconvenient.

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چکیده

ارزیابی اثر تجویز داخل مفصلی کتامین بر تغییرات هیستوپاتولوژیک مفصل زانو در مدل حیوانی رت

رحمت اله فتاحیان دهکردی*[۱]، امین بیغم صادق[۲] و علیرضا محمدزاده[۳]

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هدف- مدیریت درد پس از عمل در بیمارانی که متحمل آرتروسکوپی می شوند، یکی از مهمترین اقدامات برای توانبخشی این بیماران است. تزریق بی‌حسی به صورت داخل مفصلی می‌تواند کنترل درد را در چنین بیمارانی تهسیل نماید. هدف از این مطالعه ارزیابی اثرات کتامین بر روی غضروف مفصلی در مدل موش بود.

طرح- مطالعه تجربی

حیوانات- بیست سر موش نژاد ویستار

روش‌کار- موش‌های که در این مطالعه در نظر گرفته شدند به طور تصادفی به ۴ گروه تقسیم شدند. کتامین در دوزهای ۰/۱ (گروه ۱)، ۰/۲۵ (گروه ۲)، ۱/۰ (گروه ۳) و ۲/۵ (گروه ۴) میلی‌گرم/میلی‌لیتر به شکل داخل مفصلی تزریق شد. محلول سالین نرمال (کلرید سدیم) به داخل زانوی چپ آنها به صورت گروه کنترل، تزریق شد. یک سیستم امتیازبندی سطحی برای ارزیابی ناحیه سطحی مفصل به صورت هیستوپاتولوژیکی به کار رفت. به‌علاوه، مقادیر مورفومتریک برای ارزیابی تغییرات سلولی غضروف در نظر گرفته شد.

نتایج- نتایج نشان داد که وقتی غضروف مفصلی گروه‌های ۱ تا ۴ با گروه کنترل مقایسه شد، یک سطح سالم تا ناپیوسته در اغلب موش‌ها مشاهده گردید. علاوه بر آن، در گروه ۴، یکی از نمونه‌ها شیاری موضعی بر سطح غضروف مفصلی از خود نشان داد. نتایج موفومتریک نشان داد که در تمام گروه‌های تزریق شده با کتامین، پارامترهای اندازه‌گیری شده نسبت به گروه کنترل متغییر بودند؛ اما اختلاف معناداری بین تمام گروه‌های آزمایش در مقایسه با گروه کنترل مشاهده نگردید ($P>0/05$).

نتیجه‌گیری و کاربرد بالینی- اگرچه در مطالعه حاضر تغییر ناچیزی در یکی از نمونه ها مشاهده گردید، اما به نظر می‌رسد که کتامین می‌تواند به شکل معرف بی‌حسی با عوارض بسیار کم مورد استفاده قرار گیرد.

کلمات کلیدی- موش، کتامین، هیستوپاتولوژی، مفصل زانو، امتیازبندی.