COMPARISON OF THE EFFECTIVENESS OF HYALURONIC ACID, PREDNISOLONE AND DOXYCYCLINE ON THE PROGRESSION OF THE POND-NUKI MODEL OF OSTEOARTHRITIS IN DOGS

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Köpeklerde Pond-Nuki Tipi Osteoarthritislerin Gelişimi Üzerine Hyaluronik asit, Prednisolon ve Doxycyclinin Etkisinin Karşılaştırılması

SUMMARY

The purpose of this study was to evaluate the benefit of Hyaluronic Acid (HA), prednisolone and doxycycline on OA which was induced experimentally by ACLT bilaterally in 3 and unilaterally in 9 crossbred dogs. While the left stifle joints of 3 cases with bilateral ACLT were used as control (group 1) their right joints were given intraarticularly 0.7 ml HA (group 2) with one week intervals. The remaining 9 dogs were equally allocated to 3 groups termed groups 3, 4 and 5. Group 3 cases received intraarticularly 5 mg prednisolone per week and groups 4 and 5 cases orally 0.25 mg/kg/day prednisolone and 50 mg b.i.d doxycycline, respectively. The treatment started 5 weeks after surgery and continued for 7 weeks. During intaarticular examination of the euthanized cases, the synovial thickness appeared to be greater in group 2 than group 1 cases whose synovial thickness was than other three group cases. Although statistically non-significant ($P > 0.05$), the degree of the synovitis increased successively as groups 3, 5, 4, 2 and 1. The joint surfaces of group 1 cases contained ulcerations bigger that those of experimental groups. In this respect, there was no difference between experimental groups. According to the histologic data, the degree of the cartilage degradation was found to increase sequentially as groups 2, 5, 3, 4, and 1. It was determined from both morphologic and quantitative results that OA tended to progress more slowly in groups 2 than group 5 which was successively than groups 3, 4 and 1. Doxycycline like HA and prednisolone appears to have an anti-arthritic feature as compared to control. In this respect, it was concluded to be used as an alternative to other two drugs.

Key Words: Dog, Osteoarthritis, Prednisolon, Hyaluronic Acid, Doxcycline.

ÖZET

Bu çalışmada OA'in sağaltımı üzerine HA, prednisolon ve doxycyclinin etkilerinin araştırılması amaçlandı. OA deneysel olarak 3 yerli köpekte bilateral ve 9'unda ise uniateral (sağ) anterior cruciate ligament transeksiyonuyla (ACLT) oluşturuldu. Bilateral ACLT gerçekleştirilen 3 olgunun sol dizleri kontrol (grup 1) olarak kullanılırken sağ dizlerine intraartilülar olarak birer hafta arayla 0.7 ml hyaluronic asit uygulandı (grup 2). Diğer 9 olgu eşit olarak grup 3, 4 ve 5 olarak adlandırılan üç gruba ayrıldı. Grup 3'teki olgular intraartikülar olarak hafta da bir kez 5 mg prednizolon, grup 4 ve 5'tekiler ise oral olarak sırasıyla günde 0.25 mg/kg prednizolon ve günde iki kez 50 mg doxycyclin aldılar. Tedaviye operasyondan 5 hafta sonra başlandı ve 7 hafta süreyle devam edildi. Ötenazi edilen olgularda yapılan intraartiküler incelemede, sinovyadaki kalınlığın birinci gruptaki olguların ikinci gruptakilere, bu grubunda diğer gruplardaki olgulara göre daha kalın olduğu görüldü. İstatistiksel olarak önemli olmamasına karşın sinovitisin derecesinin sırasıyla grup 3, 5, 4, 2 ve 1 şeklinde artığı gözlendi. Birinci grubun eklem yüzlerinde deney gruplarına göre daha büyük ülserasyonların şekillendiği ve bu açıdan deney grubu olgular arasında önemli bir farkın olmadığı saptandı. Histolojik verilere göre eklem kıkırdağında oluşan degradasyonun derecesinin sırasıyla grup 2, 5, 3, 4 ve 1 şeklinde artığı görüldü. Gerek

morfolojik ve gerekse de kalitatif bulgulardan grup 2'deki OA'in gelişim hızının grup 5'den, bununda sırasıyla grup 3, 4 ve 1'de daha yavaş olduğu saptandı. Kontrol grubuyla karşılaştırıldığında HA ve prednisolon gibi doxycyclinin de anti-artritik özelliğe sahip olduğu ve bu açıdan diğer iki ilaca alternatif olarak kullanılabileceği kanısına varıldı.

Anahtar Kelimeler: Köpek, Osteoarthritis, Prednisolon, Hyaluronic Asit, Doxcycline.

INTRODUCTION

Osteoarthritis (OA) is a slowly progressing degenerative disease of the articular cartilage in human and animals (6, 17). Although all pathologic mechanisms involving in OA are not fully understood, matrix metalloproteinases (MMPs), i.e collagenase (MMP-1), gelatinase (MMP-2) and stromelysin (MMP-3) are believed to possess an important role on its development (6, 12, 31). These cartilage destructive enzymes are laid down as proenzymes largely by chondrocytes and converted into active form by proinflammatory cytokines such as interleukin-1 and tumor necrosis factors (5, 12, 19, 31).

In OA, the viscosity of the synovial fluid decreases, which may initiate or propagate a chain of events leading to or exacerbating degenerative changes. Therefore, reestablishing normal synovial viscosity is a mandatory (2, 18). Hyaluronic acid (HA), also known as sodium hyaluronate, is a linear polydisaccharide (1) and forms the major component of the viscoelastic properties of the synovial fluid (14). It has been used in clinical (20) and experimental cases of OA (7, 25, 26) with different degrees of successes. Prednisolone has long been used for OA treatment, yet its true mechanism of action is still unclear. Some authors (17) suggested that prednisolone may target the chondrocyte activity possibility either through their receptors or intracellular activities. Another possibility is that it may inhibit synthesis and/ or release of chondrocyte activity factors present in the inflamed synovium. Doxycycline (Doxy), a tetracycline group antibiotic (27, 30), is believed to have other functions beyond its antibiotic action (9, 14, 22). It may prevent cartilage degradation possible via chelation of zinc and calcium (4, 14, 22, 31) which maintain normal structural conformation of MMPs (31). Doxy is reported to prevent proinflammatory cytokines to convert into active form (10). Additionally, it may decrease cartilage degradation through inhibiting phospholipase, scavenging oxygen free radicals and altering immune response (10, 22).

For OA treatment purposes, many drugs with various characteristics have been advocated (e.g., 10, 14, 17, 20). While seeking experimentally the benefit of a drug on OA it should be carried out under a condition as near as to clinical reality (6). Pond-Nuki model of OA based on the experimental transaction of the anterior cruciate ligament (ACL) is suggested to represent this situation better than other techniques proposed for this particular purpose (17, 21, 31). Because, morphologic and biochemical alterations occurring in the joint cartilage with this model were found to be similar to those in naturally occurring OA (6). The purpose of the current study was to evaluate the benefits of prednisolone, HA and doxcycline on the treatment of Pond-Nuki model OA by evaluating clinical findings associated with gross, radiographic and histomorphometric examinations of the knee synovium and cartilage in a controlled trial.

MATERIALS AND METHODS

In this study, 7 male and 5 female crossbred dogs weighing between 15-39 kg (median 22 kg) and aging between 1-9 years (median 3.6 years) were used (Table 1). Anesthesia was induced with tiopenthal sodium (Pental sodyum, İ.E. Ulugay) of i.v. dose of 20 mg/kg and maintained with 1.8 % isoflurane (Forane, Abbott). The operation sites were prepared for aseptic surgery and the Pond-Nuki model of the anterior cruciate ligament transaction (ACLT) was performed bilaterally on the stifle (knee) joints of 3 dogs and unilaterally on the right stifle joints of 9 dogs through a stab wound (Fig 1). The left stifle joints of the latter 9 cases also underwent a sham operation leaving their ACLs intact. As a result, ACLT was carried out on a total of 15 joints from 12 dogs. The presence and degree of the joint instability were tested postoperatively by radiographic findings and, the cranial drawer and tibial compression tests. All animals were fed ad libitum and housed in 1.5 m^2 cages. However, they were allowed to walk freely approximately 4 hours a

day in a large shelter to induce further the joint lesions. All cases received i.m. 10 mg/kg/day Alfasilin (Abfar) for 7 days. Prior to the treatment, the operated knees of these dogs were divided into five groups. While the left stifle joints of 3 dogs with bilateral ACLTs were left as control (group 1), their right counterpart joints were given intraarticularly 0.7 ml (8.2 mg) HA (Healon, Pharmacia & Upjohn) (group 2). The right knees of the remaining 9 dogs were allocated equally to three groups called groups 3, 4 and 5. Group 3 received intraarticularly 5 mg prednisolone (Prednisolon., Fako) per week, groups 4 obtained orally 0.25 mg/kg/day prednisolone (Neocorten, Şanlı) and group 5 orally 50 mg b.i.d. doxycycline (Monodoxs, Deva). The treatment was initiated 5 weeks after the ACLT and continued for 7 weeks with a total of 12 weeks of follow-up period. All clinic findings were noted during the follow-up period. Lateromedial and caudocranial radiographs (120 FFD and 75 kV) were taken from the operated knees of each cases immediately after operation and one mount intervals until the end of the study. Finally, all dogs were killed with an overdose of tiopenthal sodium and their stifle joints were exposed for examining the synovial fluid, appearance and thickness of the synovium (synovial membrane) and presence of cartilage lesions and osteophyte formation. After macroscopical evaluation, full-thickness articular cartilages including underlying bones from the medial femoral condylus and the synovium specimens from the parapatellar pouch were obtained for histopathologic examination (Fig 2).

These tissue samples were fixed in 10 % formalin solution, embedded in paraffin, cut perpendicular to the articular and synovial surfaces circa 6 µm thick and stained with Alican blue and hematoxylin-eosin (H-E). The severity of OA and synovitis was graded according to criteria of Table 2 which was modified from the scales of Goldenberg and Cohen (8) and Mankin et al (11). Statistical comparison was made with ANOVA if the data were parametric and with Kruskal-Wallis test if they were non-parametric. The results were considered as significant at $P < 0.05$.

RESULTS

Clinical, radiographic and intraarticular findings:

Figure 1. Operation sites and the area where the stab wound was created, are observed. The subcutanous tissues are being sutured.

All 12 dogs recovered smoothly from anesthesia and survived until the end of the study. A visible swelling coupled with a mild local temperature was determined in the right operated knee of case 1. It resolved in two weeks without medial intervention. One case (no: 4) had removed its sutures.

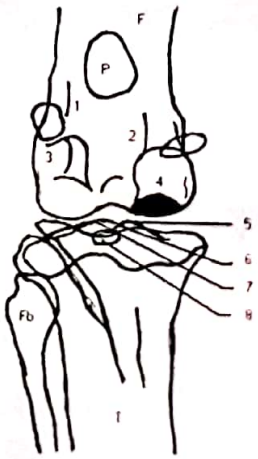

Figure 2. Diagramatic representation of the stifle joint. The shadow area shows the area where the joint cartilage samples were taken for histhophatologic examination. Fm: Femur, t: Tibia, f: Fibula, Lsb: Lateral sesamoid bone, Msb: Medial sesamoid bone, 1. Lateral crista, 2. Medial crista, 3. Condylus Lateralis, 4. Condylus Medialis, 5. Tuberculum intercondylare mediale, 6. Area Intercondylaris, 7. Tuberculum intercondylare laterale, 8. Os. Sesamoideum.

The region was cleaned daily with betadine until the healing was completed. A patellar luxation was detected in case 2, which was replaced on notice and no relapse occurred afterward. Dogs introduced

oral doxycline and prednisolone did not have any alimentary discomfort during the treatment. Postoperatively, all dogs showed various degrees of lameness whose degree, duration and distribution in different cases and groups have been noted in Table 1 where the results of postoperative stifle joint instability have been recorded. The displaced joints were readily differentiated radiographically (Fig 3).

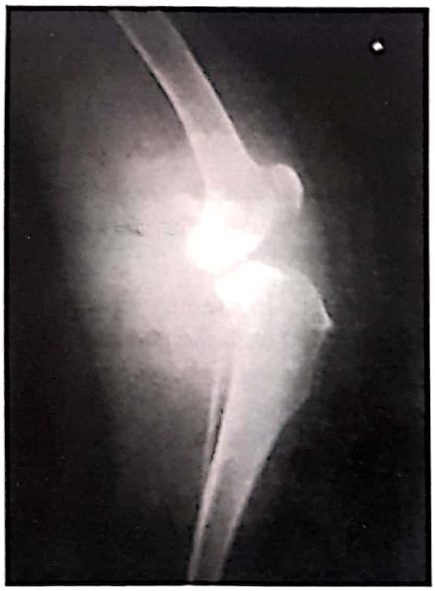

Figure 3. Case 4, group 5. This radiograph taken 3 months after surgery shows cranially dislocated the tibial head and caudally displaced femur head. A marked ostephyte on the tibial platue is seen.

Figure 4. Case 2, group 3. The transected surface of the ACL has increased markedly in thickness and its central core appears hyperemic. The ligament has been surrounded by a thick synovial sheath (arrow).

The cross sections of the ACL increased in sizes (Figs 4) .

The sectioned ACLs obtained reddish and edematous appearance as compared to the white purple appearance of the normal ligaments (Figs 5). The articular surfaces of the stifle joints in control group consisted of ulcerations (Fig 6) bigger that those of the experimental groups (Fig 5).

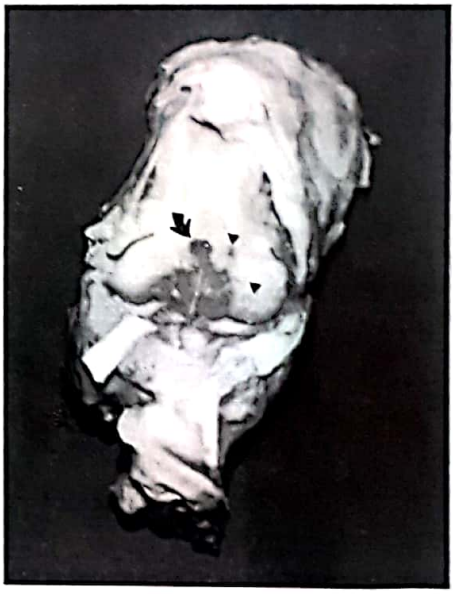

Figure 5. Case 9, group 4. Showing a large and deep (arrow) and some minor and superficial (arrow heads) abrasions on the femoral condylus. The cut end of ACL appears swollen and hyperemic.

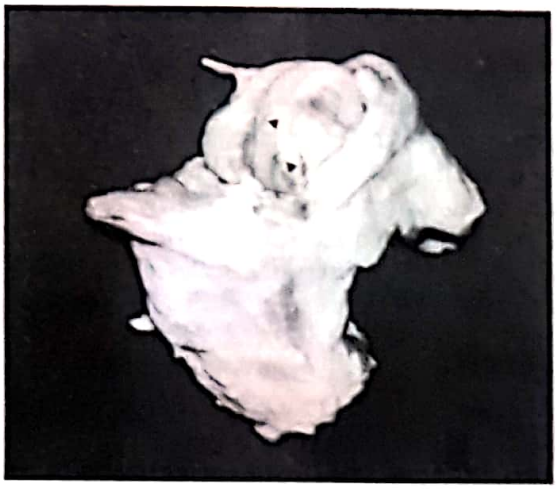

Figure 6. Case 1, group 1. Note the presence of one large and one small cartilage abrasion (arrow heads) on the central weight-bearing region of the medial femural condylus

The synovium of group 1 (control) appeared to be thicker than group 2 whose synovial thickness was higher than groups 3, 4 and 5, respectively. HA

treated joint capsules (group 2) were far thicker than their counter-lateral normal joints (Fig 7). In this group, amount of synovial fluid increased and obtained a yellowish color. However, the joint surfaces did not have major damages (Fig 7).

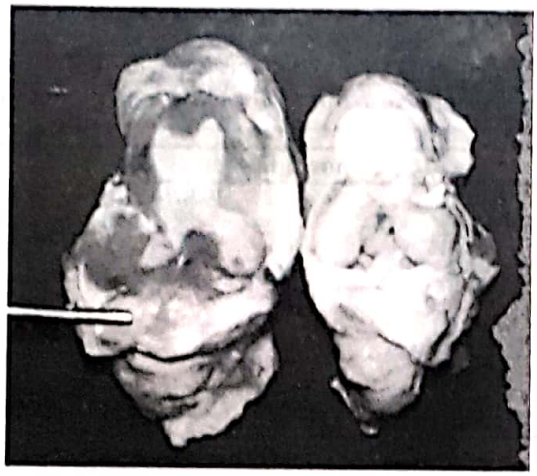

Figure 7. Case 1, group 2. Notice marked difference between the thickness of the synovial membranes of right and left stifle joints of case 8 in group 2. Inflamed synovium has yellowish red color. A pin point erosion (arrow head) on the medial condylus is observed

Regarding to gross intraarticular findings, no difference was determined between oral (group 3) and intra-articular (group 4) cortisone applications. Amount and color of synovial fluid and appearance and thickness of the synovial membrane were indistinguishable from those of the control. The articular surface of the joints of these two groups included superficial and focal erosions. Intrarticular osteophytic developments was found on case in group 5 (Tablo 1), which was also determined radiographically (Fig 3). Distribution of OA lesions observed during intraarticular examination is summarized (Table 1).

Table 1. Details about cases (C), statues of joint instability, durations of lamness and marked intraarticular lesions are shown in 5 groups (Gs). G1: Weight was loaded on the operated leg with a great care, G2: Weight was loaded on the operated leg through finger points, G3: Operated leg was hanged up, R. Right, L: Left, t: Total, MFC: Medial femural condylus.

C No	Signalmants			Gs	Leg	Joint instability	Duration of lameness (Days)				Marked IA lesions
	Age (Y)	Weight (kg)	S				G 1	G 1	G2	t	
1	1	22	F	2	R	Clinically detectable	69	21	-	90	Minor irregularities articular surface
				1	L	Clinically detectable	83	7	-	90	Large ulcer in MFC
2	1,5	21	M	3	R	Undetectable	90			90	Swollen ACL
3	1	-	M	4	R	Detectable by test (T)	90	-	-	90	Small abrasions
4	5	20	M	5	R	Clinically detectable	82	8	-	90	Pin-point abresion, ostheopytic develop.
5	1,5	15.5	M	3	R	Detectable by T	72	16	2	90	Small abrasions
6	4	25	F	4	R	Detectable by T	75	10	5	90	Small abrasions
7	5	20	F	5		Detectable by T	90	-	-	90	Synovitis, thick synovium
8	3	27	M	2	R	Detectable by T	86	4	-	90	Focal cartilage loss in MFC, thick synovium
9	9	22	F	4	R	Detectable by T	76	14	-	90	Deep focal ulcus, swollen ACL
				1	L	Detectable by T	76	14	-	90	Increased thickness in synovium, focal ulcus
10	6	39	M	5	R	Clinically detectable	55	25	10	90	Swollen ACL
11	4	30	M	2	R	Detectable by T	90	-	-	90	Thick synovium, Small abrasions
12	2	24	F	3	R	Detectable by T	90	-	-	90	Small abrasions
				1	L	Detectable by T	85	5	-	90	Focal abrasions

Histopathologic findings:

One case in group 3 (no 2) had totally intact synovium. In other cases, the synovial lining epithelium underwent the different degrees of increase in thickness. Villus hyerplasia was determined in 6 cases (Fig 8). The synovium showed major variations regarding to WBC infiltration rates.

Table 2. Criteria for evaluating the severity of the OA and synovitis: ABSL (Alcian blue stain loss), CD (cartilage degradation), SLCH (Synonial lining cell hyperplasia), VH (Villus hyperplasia), WBCI (White blood cell infiltration), HPF: High power field.

Score	Articular cartilage		Synovium		
	ABSL (%)	CD	SLCH	VH	WBCI/HPF
0	0	No	1-2 cell thick	Not present	No
1	25%	Superficial	3-5 cell thick	Few, scattered and short	1-5
2	50%	Profound including the underlying bone	5<cell thick	Marked and finger-like	5-10
3	75%	-	-	Marked and diffuse	10<
4	100%	-	-	-	-

The infiltration cites consisted of mononuclear leukocyte and plasma cells (Fig 8).

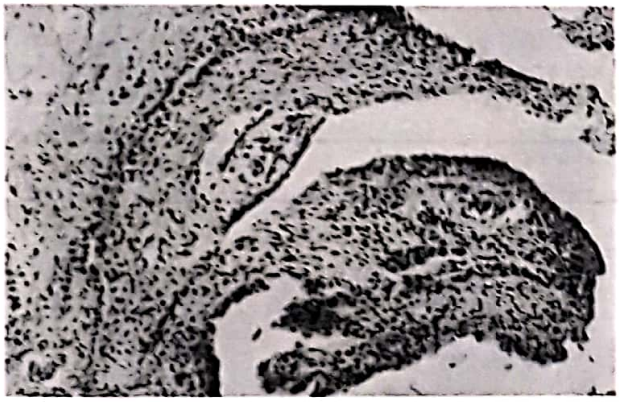

Figure 8. Case 8, group 2. Finger like villus hyperplasia associated with mononuclear leukocyte infiltration in the synovium. H-E, X200

When the quantitative data of these parameters were evaluated, a non-significant difference between groups was found. However, when mean values of these data were put in order, the degree of synovitis increased as groups 3, 5, 4, 2 and 1 (Table 3). The surfaces of the intact cartilages were smooth and contained peripherally a thin amorphous layer determined in three out of 15 operated joints. OA cartilages contained different degrees of undulations from minor depressions to deep and large defects (Figs 9, 10 and 11).

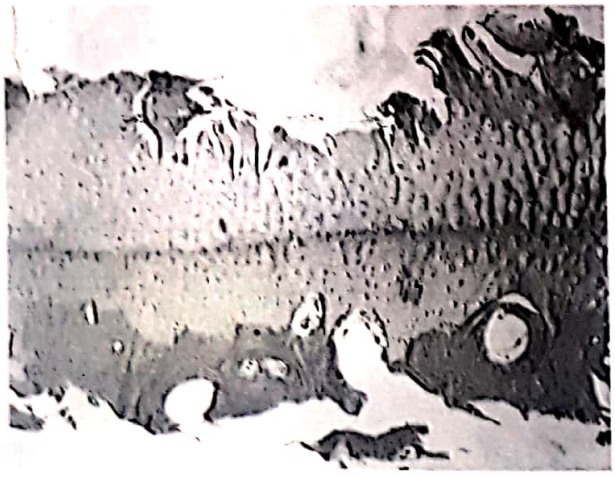

Figure 9. Case 1, group 1. Cartilage destruction extended almost half way down its whole thickness. This site contains marked irregularities. H-E, X80

There were great variations between the different levels of OA cartilage of an individual owing to the ration of Alcian blue staining. When these variables were evaluated statistically, the difference between groups was non-significant ($P > 0.05$). However, when mean values of these variables were put in order, groups 2 and 5 had lowest degree of the lesions followed successively by groups 3, 4, and 1 (Table 3).

Table 3. Mean (M) and standard error of mean (SE) obtained from the histopathologic scoring of synovial and cartilage lesions. Groups have been put in order from the smallest to the highest value.

Groups	Synovium		Cartilage	
	M ± SE	Order	M ± SE	Order
1	5.00± 2.14	3	3.00± 1.0	2
2	5.00± 1.0	5	2.00± 1.0	5
3	1.67± 1.2	4	3.67± 1.3	3
4	2.94± 2.52	2	1.67± 1.5	4
5	3.67± 3.22	1	4.00± 1.0	1
P values	0.167		0.580	

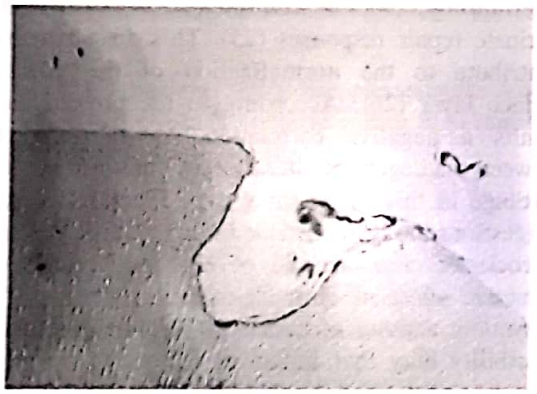

Figure 10. Case 6, group 4. A large ulcer in the joint cartilage is observed. H-E, X80

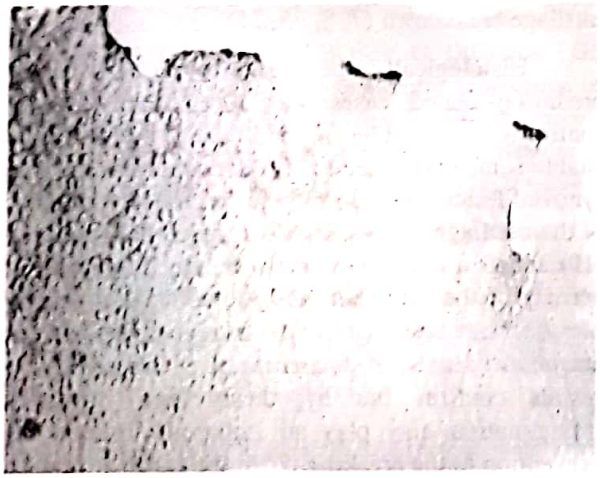

Figure 11. Case 5, group 3. In this micrograph, cartilage damage is widespread, forming marked irregularities on the joint surface. This tissue is weakly stained with Alcian blue. X80

DISCUSSION

The stifle is a complex hinge joint with a two functionally distinct articulation. This joint is stabilized by a combined function of cruciate and collateral ligaments, meniscus and joint capsule (29).

In clinical cases, ACL rupture is usually associated with partial or total disintegration of the joint stabilizing members depending on the extent of the external force. In cases with experimentally induced OA, although ACLTs are performed the other joint stabilizing members remained intact. These intact structures may initially compensate for the function of cruciate ligaments to some extent and prevent an abnormal joint mobilization to occur. The inflicted animals appeared to contribute to this compensatory function by carefully using their operated limbs. However, with the reduction of the pain resulting from operation in due course they may start to full bear their weights on the stifle joint. This action associated with the joint instability due to ACLT may force the joint capsule to wear and tear (19) with a consequence of gradually increasing laxity in the joint capsule. The enhanced laxity may increase abnormal joint motion and its related complications including cartilage erosions, osteophytes and synovitis. The reason for delaying medical treatment in this study was expected to enhance the degree of joint instability and thus to produce larger damages on the joint surfaces so that the distinction between groups should be apparent. Some authors (15, 31) have accelerated the occurrence of the lesion by performing the dorsal root ganglionectomy coupled with ACLT. Other (6, 17) had induced OA lesion in cases with ACLT by allowing totally free walking environment, which let the cases walk more with a results of large lesions. For this purpose, Yu et al (31) and the present authors kept the cases in an indoor pen with a condition of exercise in certain times of the day.

The dosage of doxycline used in this study was the same as that used by previous studies (30, 31). As was noted by them and supported by the results of the present study, this drug with this dose is well tolerated by dogs. Because, during the treatment period, no dog presented any alimentary problems like diarrhea and vomiting. Oral administration of

this amount of doxycline was found to reach a satisfactory level in articular fluid to accomplish its function (31). A study (31) on time and response relationship of doxy on OA demonstrated that its prophylactic application reduced markedly cartilage degradation. It has been claimed that anti-destructive effect of the doxycycline on the knee cartilage was determined to be regional specific, being particularly effective on the central weight bearing region of the femoral cartilage. The cause of this variation is unclear (31). The cartilage lesions of this group cases were focal and superficial, which were encountered to scatter randomly over the joint surface rather than located in a specific site noted by the previous authors.

In this study, ACLT was performed on 15 joints but osteophitic formation was observed in just one case treated with doxycycline. As other joints treated with doxycycline were free from such a lesion, it is hard to associate this development to the negative consequence of the treatment. According to other studies (9, 26) this formation is not due to the limitation of this drug. It was attributed to the creation of excessive forces on mechanically instable joint during walking. Therefore, if one expect to have a satisfactory outcome with this treatment they should combined it with the mechanical stability of the joint (16). Works on gait analysis showed no significant difference between the vertical forces of limbs treated and untreated with doxy, indicating that this drug has no effect on gait function and joint motility. These findings imply further that ostheophitic development is independent from the enzymatic activity of the OA joint (31). In this part of this study, the enzymatic activity of the OA joint was not evaluated, but the present study examined quantitative degree of inflammatory changes in synovium and cartilage using the histologic parameters. These results showed that the inhibitory effect of doxycycline on the inflammatory process of synovium was more marked than that of other drugs used in this study.

The operated knees of the dogs showed increased synovial fluid, thick synovial membrane and focal cartilage lesions in group 2. The results of synovial scoring considering histomorphometrik analysis were higher in HA treated cases than control and other groups in this study. Additionally, macroscopic alterations in synovium with HA treated knees were more apparent than other 4 groups. These findings indicate that HA may have no or limited antiinflammatory effect on the synovial tissues. Therefore, the results of this study may not totally be compatible with suggestion that HA mediates anti-inflammatory properties (23) and has direct chemotactic inhibition on lymphocytes, macrophages and polymorphnuclear cells and their enzymes (3, 24, 28). On the other hand, macroscopic results of the articular cartilage in this group were fully correlated with their histomorphometric analyses. Efficacy of intraarticular HA treatment was found to be evident in this established OA. HA may have an inhibitory effect on the cartilage structure breakdown possible determining favorable conditions for extrinsic and intrinsic repair responses (25). This drug may also contribute to the normalization of the articular surface layer (26). According to the present study results a negative correlation was also present between the degree of alteration in the synovium and cartilage in this treatment group. There are several suggestions likely to be made in this regard. First, the viscoelastic characteristic of HA may reduce the inductive effect of synovial origin products on the enzymatic activity of cartilage origin. The second possibility may that inductive factors of synovium may be inactivated by HA itself or by increased density of the synovium during HA application. In this respect, the last possibility may that there may be no relation between synovial inflammation and cartilage breakdown (7, 8, 25, 26).

Histologically, the degree of the synovitis in prednison-treated cases was lower than that of control cases (Table 3), indicating that steroids enable somehow to reduce the synovial lesions. The synovial lesions were seen to be correlated with those of the cartilage lesions. In this respect, Pelletier et al (19) found a correlation between the inflammatory activity of the synovium and the collagenic activity of the cartilage, but no relationship between enzymatic levels of the respective tissues. These results confirm the hypothesis that synovial inflammation may play an important role in the prevention of the breaking down the cartilage matrix. Prednisolone can effectively decrease the level of cartilage collagenolytic activity. Assuming that chondrocytes are responsible for an increased synthesis of cartilage collogenolytic enzymes, there are two possible explanations for the action of the prednisolone. The first is that it directly inhibits chondrocyte enzyme synthesis by either acting on the level of cellular receptors, or doing so intracellularly. The second possible explanation is that prednisolone may inhibit synthesis and/ or release of chondrocyte activating factors from the inflamed synovium (13).

The results of the present study indicate that HA, prednisolone and doxycycline may have different degrees of ant-arthritic effect as compared to control. This effect appears to be greater in HA than doxycycline which is higher than prednisolone. Therefore, it was concludeded that doxycycline can be used for the treatment of OA as an alternative to other drugs.

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