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Clinical Changes Evaluations in Amphotericin B Induced Osteoarthritis in Canine Models

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Abstract: The experimental model of osteoarthritis (OA) was replicated in Six dogs induced osteoarthritis by intra-articular injection with amphotericin B (AB) in the left stifle joints. Right joints served as control. Medical status and certain goniometric parameters were tracked prior to the initiation of AB and on the 30th, 60th and 90th days after injection. The findings showed compellingly that the OA model used by the experimental chemical effectively replicated the disease in the stifle joints in dogs and showed a progressive disease progression, just as it does in the reported cases of arthritis that naturally affects dogs depending on the time of inflammation. Medical indications studied were associated with the seriousness of the disease.

Keywords: Dogs, Stifle joint, Amphotericin B, Osteoarthritis, Canine Models.

تقييم التغيرات السريرية في التهاب المفاصل العظمي الناجم عن الأمفوتيريسين ب في نماذج الكلاب

المستخلص: تم تكرار النموذج التجريبي لالتهاب المفاصل العظمي **(**OA**)** في ستة كلاب من التهاب المفاصل العظمي الناجم عن الحقن داخل المفصل مع الأمفوتريسين B (AB) في مفصل الركبة اليسرى. كانت المفاصل اليمنى بمثابة السيطرة. تم تتبع الحالة الطبية وبعض المعلمات الهندسية قبل بدء AB وفي الأيام الثلاثين والستين والتسعين بعد الحقن. أظهرت النتائج بشكل مقنع أن نموذج الزراعة العضوية الذي استخدمته المادة الكيميائية التجريبية كرر المرض بشكل فعال في المفاصل الخانقة لدى الكلاب وأظهر تطورًا تدريجيًا للمرض، تمامًا كما هو الحال في حالات التهاب المفاصل المبلغ عنها والتي تؤثر بشكل طبيعي على الكلاب اعتمادًا على وقت الالتهاب. وارتبطت المؤشرات الطبية المدروسة بخطورة المرض.

الكلمات المفتاحية: الكلاب، مفصل الركبة، الأمفوتيريسين ب، هشاشة العظام، نماذج الكلاب. .

INTRODUCTION

Osteoarthritis (OA) is a serious and chronic disease of movable joints in domestic dogs. (Johnson et al., 2016; Kraus et al., 2015) The prevalence of canine OA in the adult dog population is around 20 % to 30 %. (Muir et al., 2004). Osteoarthritis is characterized by various changes in the metabolism of joint tissue, degradation of cartilage, modified bone remodeling, formation of osteophytes, inflammation of the joint and loss of normal joint function. (Clements et al., 2006; Kraus et al., 2015).

The clinical signs of naturally occurring canine OA include, for example, reduced pain-free range of motion in affected synovial joints, reduced muscle flexibility, modified limb weight-

bearing during standing or moving, reduced performance level in daily living activities such as running, walking, rising, climbing, and gradual changes in the behavior of dogs e.g. various social contexts (Hyytiäinen, 2015; Wiseman et al., 2001).

However, pain and disability do not always correlate with structural joint changes detected by radiography, i.e. in joint space narrowing, osteophyte formation, bone sclerosis and bone cysts, pathological bone contour alterations and joint malalignment (Gordon et al., 2003; Kraus et al., 2015; Lascelles et al., 2012).

Several assessment steps should be introduced to cover the full range of pain sensitivity and the health status of osteoarthritic dogs. Quantitative sensory assessment methods have been used to examine neuronal changes in dogs with OA-related pain. (Knazovicky et al., 2017; Williams et al., 2014).

Assessment methods based on the structure and function of the body, e.g. joint range of motion, should preferably be used in combination with valid activity and performance measures, e.g. functional test batteries and quality of life associated with health (Stokes, 2010).

Pain is the most common sensory and emotional uncomfortable experience in dogs, and the inability of dogs to communicate their experience in words makes it difficult to use self-reporting methods to specifically measure pain (Mathews et al., 2014).

The choice of the measurement method needs to be focused on a specifically defined variable for clinical practice and study. That is, we have to know what to measure first. In addition, how the variable is evaluated is referred to an evaluation process. Psychometric examination includes determining the characteristics of the assessment, i.e. the validity, reliability and responsiveness of an evaluation system (Mokkink et al., 2010).

Amphotericin B intraarticular injection has an immediate toxic effect on chondrocytes, led to the development of osteoarthritis in the joint anesthetic (Fahmy et al., 1994). Symptoms of lysosomal disruption and release of inflammatory mediators resulting in joint inflammation, capsulitis and synovitis (Fahmy et al., 1994). Amphotericin has several advantages in that it generates moderate intensity of lameness, is transient in nature with limited articular changes, avoids significant systemic side effects and causes arthritis (Marttinen et al., 2006).

The aim of this study was to attempt to reproduce experimentally stifle osteoarthritis in dogs intra articular. with administration of amphotericin B to determine the model's performance or failure through gait and pain analyses.

MATERIALS AND METHODS

Experimental animals:

Six clinically healthy mongrel dogs from both sexes were used (body weight 15±2 kg). They were housed indoor in individual boxes and had free access to drinking water and dry canine food for maintenance.

Experimental induction of arthritis:

Amphotericin B preparation:

Amphotericin B (Photericin B, CIPLA LTD., Verna industrial estate; India) was prepared under strict aseptic preparation with a concentration of 10 mg / ml by adding directly into the lyophilized

cake a 5 ml sterile distilled water using a 20-gage sterile needle with a syringe. The vial was well and immediately shaken until there was clear colloidal solution.

The clinical evaluation and goniometric analysis:

Three clinical scoring systems, according to Budsberg et al. (1999); Cross et al. (1997); Grisneaux. Emmanuelle et al. (1999), Used typically for drugs Effectiveness assessment of therapy was used in particular, For study of gait, behavior and pain (Tables 1, 2, 3).

The thigh circumference (TC), the stifle joint circumference (SC) and the range of motion of the stifle joint (ROM) between full flexion and extension were measured in all dogs (Ashraf. abdel-hamed. Hegazi & Khaled. M. A. Hussin, 2008; Millis & Levine, 1997) with goniometer and a band. The animals were in lateral recumbency, with the studied limb on the top.

The thigh circumference was determined in the middle of the thigh, the stifle joint circumference in 90o flexion, ROM was measured between the longitudinal axes of the femur through trochanter major and the tibia through maleolus lateralis.

Table: (1). Criteria for pain and behaviour evaluation of dogs with osteoarthritis.

Parameter	Score	Clinical sign
Behaviour	1	Apathetic or indifferent
	2	Friendly
	3	Nervous, submissive behaviour
	4	Very nervous, tries to move away
	5	Aggressive
Compliance with restraint	0	No objection
	1	Recognizes manipulations, no complaint
	2	Objects but does not try to bite
	3	Tries to bites and struggles
Heart rate	0	0 to 10% greater than normal
	1	11 to 30% greater than normal
	2	31 to 50% greater than normal
	3	>50% greater than normal
Respiratory rate	0	Normal
	1	Mild abdominal assistance
	2	Marked abdominal assistance
Vocalization	0	No crying
	1	Crying but responds to calm voice
	2	Crying but does not respond to calm voice
Agitation	0	Asleep or calm
	1	Mild agitation
	2	Moderate agitation
	3	Severe agitation
Response to manipulation	0	No response
	1	Minimal response
	2	Turns head toward site, slight vocalization
	3	Turns head to bite, howls

Table: (2). Kinetic gait analysis system for clinical evaluation of lameness, pain and joint effusion of knee osteoarthritis in dogs.

Parameter	Score	Clinical sign
Standing lameness	1	Normal weight-bearing
	2	Partial weight-bearing
	3	Intermittent toe touching
	4	No weight-bearing
Trotting lameness	1	Normal weight-bearing
	2	Marked lameness with partial weight-bearing
	3	Marked lameness with intermittent toe touching
	4	No weight-bearing
Pain re-sponse	1	Absence of pain and response
	2	Slight pain, allowing manipulations of the limb within the normal range of motility, manifested by turning the head and pulling the limb away
	3	Moderate pain, not allowing manipulations of the limb within the normal range of motility, manifested as described for score 2
	4	Significant pain, not allowing manipulations of the limb Joint
effusion	1	Normal – palpatory compression upon the patellar ligament
	2	Weak – slight increase, the patellar ligament is palpated
	3	Moderate – marked increase, slightly perceptible ligament
	4	Significant – the patellar ligament is not palpated

(Cross et al., 1997).

Table: (3). Scoring system for evaluation of hindlimb use in dogs with OA.

Parameter	**Score**	**Clinical sign**
Lameness	1	Stands and walks normally
	2	Stands normally, slightly lame at walk
	3	Stands normally, severely lame at walk
	4	Abnormal stance, slightly lame at walk
	5	Abnormal stance, severely lame at walk
Weightbearing	1	Normal at both rest and walk
	2	Normal at rest, favours affected limb at walk
	3	Partial at both rest and walk
	4	Partial at rest, no weightbearing at walk
	5	No weightbearing at rest and walk
Response to contralateral limb lift*	1	Accepts displaced weight
	2	Mild resistance to displaced weight
	3	Moderate resistance to displaced weight**
	4	Strong resistance to displaced weight***
	5	Refusal to lift the contralateral limb
Response to affected limb extension	1	No response
	2	Mild response (turning head toward the affected limb)
	3	Moderate response (withdrawal of affected limb)
	4	Severe response (vocalization, aggression)
	5	Disallows manipulation or palpation of affected limb

* response of the affected hind limb; ** replacement of the contralateral limb in < 10 s *** replacement of the contralateral limb in < 5.
(Budsberg et al., 1999).

All results were compared to the contralateral joint.

Statistical analysis:

The results were statistically processed by the non-parametric Friedman and Mann-Whitney tests using statistical software (Statmost for Windows, Datamost Corp). Differences were accepted as statistically significant at $p<0.05$. Relationships between parameters were estimated by the Pearson correlation analysis test.

RESULTS

The average Grisneaux's score increased statistically significantly at $p<0.01$ from 6±1 points in the beginning of the experiment to 14±2 (day 30) and 12±2 (day 60) (Table 4).

The respective score according to Cross et al. yielded 4±0 points (day 0), 13±1 (day 30), 12±1 (day 60) and 9±1 (day 90) ($p<0.01$ vs baseline). Average Budsberg score before the first AB administration was 4±0 with considerable increase by day 30 to 17±1, followed by reduction to 13±1 and 11±1 by days 60 and 10±5, respectively ($p<0.01$ vs day 0).

Goniometric analysis provided evidence for thigh muscles atrophy of the left hind limb (Table 4) as thigh circumference decreased significantly from 32±1 cm in the beginning to 23±1 cm by the 90th day ($p<0.01$). The values between left and right limb was also statistically significantly different ($p<0.05$ by days 30 and 60 and $p<0.01$ by day 90). The stifle joint circumference did not show significant differences with time. The range of motion (ROM) of left joints decreased

considerably from 115±2 in the beginning to 95±7, 91±2, and 83+4 by days 30, 60 and 90, respectively ($p<0.01$). ROM of the left joint exhibited a negative correlation with clinical.

Table: (4). Clinical scores and goniometric parameters in dogs with experimental (AB) model of stifle joint osteoarthritis (mean + SEM; n=6).

Parameter		Days after first AB injection			
		0	**30**	**60**	**90**
Grisneaux' score		6±1	14±2**	12±2**	11 ± 3
Cross' score		4±0	13±1**	12±1**	9±1**
Budsberg' score		4±0	17±1**	13±1**	11 ±1**
Thigh circumference, (cm)	left	32±1	27±1*	27±1**	23±1**
	right	32±1	32±1#	30±1#	30±1##
Stifle joint circumference, (cm)	left	25±1	27±1	26±1	25±1
	right	25±1	23±1	23±1	23±1
Range of motion, (o)	left	115±2	95±7**	91±2**	83±4**
	right	115±2	111±5	113±3##	110 ±4##

*p<0.05;**p<0.01 vs baseline (day 0); #p<0.05; ##p<0.01 between left (OA) and right (control) joints.

DISCUSSION

This is one of the first studies proving the degree and duration of canine amphotericin B-induced synovitis arthritis. The results indicated that OA in dogs could be successfully by injecting higher doses of AB into the joints and using duplicated applications. In this study, Amphotericin-B was administered to induce arthritis. In (Kotschwar et al., 2009). used an amphotericin B-induced transient arthritic model to evaluate the effects of The amphotericin B-induced lameness paradigm has also been shown to produce a transitory synovitis arthritis that was expected and mild in degree.

They were significantly reduced in Kotschwar et al. (2009) study than it was in the equine literature, with equine studies showing moderate to severe lameness (grade 3-4/5) lasting 3 to 2 weeks (Marttinen et al., 2006) Other research has found a reduction in the severity and duration of lame-

ness in cattle. compering with canine and equine model, indicated a little dose of AB was enough to cause a rapid decrease in rat stifle locomotor activity, and that low dosages only had a temporary impact. may be due to several reasons, including that only one injection of amphotericin B was administered as opposed to several injections in the majority of the equine and canine studies When Amphotericin B was injected in the distal interphalangeal joint rather of one of the carpal or tarsal joints, the severity and duration of lameness may be changed. Furthermore, cattle may transmit a significant amount of weight from one claw to the other, reducing pain-related lameness in the afflicted claw (Kotschwar et al., 2009).

Similar to Bove et al. (2003) findings' larger and repeated dosages successfully replicated all signs of osteoarthritis: acute inflammation at beginning, increasing degeneration, and transition to chronic atrophic phase. They're significantly ideal for chemical OA models, including the one used in this study, because the joint instability identified after the mechanical models could be ruled out as a source of biomechanical abnormalities.

Since chondrocytes are required for cartilage structural stability, intra-articular injection of AB causes cartilage degradation and subchondral bone formation. The goniometric study that was utilized provided, this chemical model as Amphotracin B-induced osteoarthritis has an objective measure of the appearance of early joint effusion and progressing inactivity and muscle atrophy of the treated limb, combined with limited joint motility. abnormalities that match the pathology of OA The subchondral bone becomes exposed as the degenerative model proceeds, resulting in joint dysfunction and mechanical hypersensitivity associated with pain (Harvey & Dickenson, 2009).

The pain-related behaviour in this model is thought to be characterised by an early acute inflammatory phase resulting from a fluid expansion of the synovial membrane followed by a persistent phase where the inflammation is largely resolved and is not thought to contribute to the pain pathogenesis (Bove et al., 2003). We utilized three pain and gait rating methods in dogs to reduce the subjectivity of clinical assessment (Grisneaux et al. 1999; Cross et al., 1997, Budsberg et al., 1999).

Although these methods for measuring pain have been presented, they are very useful in determining the intensity and duration of pain in addition to Various parameters are included. Gardner (1994) observed that clinical symptoms in the course of OA development were regular, which was verified by the high positive correlation between individual scores. As early with the first month, the utilized goniometric analysis provided an objective assessment of the appearance of early joint effusion, progressive inactivity and muscle atrophy of the treated limb, associated with restricted joint mobility, particular for this chemical model.

CONCLUSION

Clinical evaluations, goniometric data, and the correlation between them indicated that the utilized experimental model of OA causing metabolic problems in articular cartilage had a duration course comparable to that of naturally occurring disease in dogs. Amphotericin B model was successful in producing acute synovitis during the first period of study and joint pain in addition to degenerative joint disease. This model could be useful to those studying the pathophysiology of joint disease and may be an ideal method to test the efficacy of new drugs intended for the treatment of joint disease. AB model need more investigation and studying of the

appropriate AB dose and the number of injections. The used experimental model of OA provoking metabolic disorders in articular cartilage exhibited a time course similar to that of naturally occurring disease in dogs, confirmed by clinical scores, goniometric data and the correlation between them effective method to study the disease process in dogs during the early stages of the disease.

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