Clinical changes in sodium monoiodoacetate–induced stifle osteoarthritis model in dogs

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Abstract

In six dogs, experimental model of osteoarthritis (OA) was reproduced by intraarticular injection of sodium monoiodoacetate (MIA) in left stifle joints. Contralateral joints served as control. The clinical status and some goniometric parameters were monitored before MIA introduction and at post injection days 30, 60 and 105. The results showed convincingly that the used experimental chemical OA model reproduced successfully the disease in canine stifle joints. The studied clinical indices correlated with the severity of disease.
Key words: dogs, stifle joint, sodium monoiodoacetate, osteoarthritis

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Introduction

Osteoarthritis (OA) is a degenerative articular cartilage disease encountered in all mammals, birds and men (Lipowitz, 1993). Ehrlich (2003) and Haima (2005) describe it as a "wear-and-tear" process, while Bennett (1990) defines OA as the final stage of a prolonged accumulation of biochemical disorders, hence, as adaptation of the joint to abnormal stress. Gardner (1994); Grainger & Cicuttini (2004) support the hypothesis for the multifactorial etiology of OA resulting in cartilage loss, bone remodeling, pain, effusion and inactivity.

The incidence of degenerative joint conditions in dogs is about 78%, with increasing share of primary OA with age (May, 1994). According to Anderson (1994) and Vasseur (1993), in small animals, the stifle joint is the most commonly affected (more than 20% of all OA cases), a substantial part of alterations being bilateral (Tirgari & Vaughan, 1975).

The experimental reproduction of OA is of both scientific and applied importance. Sometimes, this is the only means to confirm a working hypothesis. Orthopaedics uses frequently *in vivo* experimental animal models (Bendele, 2001; Murray, 2002). The reproduction of all disorders and symptoms of OA is however challenging, so a model possessing the most important signs of natural disease and that could be reproduced, is good enough for the purpose of research (Brandt, 2002; Carlson, 2005). The interest towards reproduction of OA of the knee is due to its anatomical features and the increasing prevalence of natural degenerative events (Bendele, 2001; Carlson, 2005). The numerous existing experimental models of OA are classified by Witter (1999) and Schaller *et al.* (2005).

The metabolic inhibitor sodium monoiodoacetate **(**MIA) destructs the joint cartilage by blocking glyceraldehyde-3-phosphate dehydrogenase in chondrocytes and inhibition of glycolysis. The rapid depletion of ATP results in cellular death. The number of cells is progressively reduced, the synthesis of proteoglycans for the articular matrix is stopped (Kalbhen, 1987; Van

der Kraan *et al.* 1989). MIA-induced degenerative processes, typical for natural OA, appear within 6 to 8 weeks (Kalbhen & Blum, 1977; Janusz *et al.* 2001; Guzman *et al.* 2003). The time of occurrence of changes and the used MIA doses are very different. Fourteen weeks after two intraarticular injections of 0.6 mg MIA in the stifle joints of hens, Kalbhen & Jansen (1990) detected radiological and gross anatomical changes. Gencosmanoglu *et al.* (2001) have achieved a chondrotoxic effect in rats after 8 weekly injections of 1 mg MIA. Permanent histological injuries were reported to occur after 2 to 8 weeks of application of 1 to 4 mg MIA in rats; they were progressive and similar to OA lesions in humans (Saied *et al.* 1997; Guinamp *et al.* 1997; Bove *et al.* 2003; Guzman *et al.* 2003). The rat OA model (2 mg MIA) was found useful for monitoring of chronic OA pain over 10 weeks (Combe *et al.* 2004). In mice, Boileau *et al.* (2004) have used a single dose of 0.1 mg MIA. The histological changes were detected 7-14 days later.

In rabbits, the described events occurred until the 12th week (Regling *et al.* 1989), with correlative changes in alkaline phosphatase and lactate dehydrogenase activities (Horn *et al.* 1988). Gustafson *et al.* (1992) have evaluated the changes in equine carpal joints with regard to the applied MIA dose as mild (0.09 mg/kg), moderate (0.12 mg/kg), and severe (0.16 mg/kg) after a 12-week period. Moreover, it turned out that high MIA doses (60-100 mg/ml in 2 ml) were able to provoke a chemical arthrodesis of equine tarsal joint after 13-51 months (Penraat *et al.* 2000; Bohanon, 1995). Therefore, the MIA dose and the number of intraarticular applications were essential for the reproduction of the generative process.

Stobie *et al.* (1994) were the only researchers having applied twice MIA in dogs at doses of 0.375 mg/kg and 0.500 mg/kg MIA at a 2-week interval, but the result was an insignificant lameness without permanently affected locomotion and biochemical profile until the 12th post injection week. The authors did not support their findings with histological evidence, but nevertheless they suggested that most probably, higher doses and/or multiple injections would result in more consistent OA changes in this animal species.

The clinical evaluation of lameness, pain, joint effusion and stifle joint motility is subjective (Budsberg & Thomas, 2006). Therefore, several authors (Cross *et al.*, 1997; Grisneaux *et al.*, 1999; Budsberg *et al.*, 1999) introduced scoring systems for cumulative assessment of these parameters. The range of motion is an objective although non-specific parameter, while goniometry is a non-invasive method for quantitative estimation of the range of motion by measurement of specific angles formed by bones (Lipowitz, 1993; Jaegger *et al.*, 2002). They could be also used to assess the efficacy of therapy (Crook *et al.*, 2007). These measurements are routinely performed in men, but literature data about dogs are very limited (Millis & Lavine, 1997; Jaegger *et al.* (2002).

The purpose of the present study was to attempt to reproduce experimentally stifle osteoarthritis in dogs by means of intraarticular administration of sodium monoiodoacetate and to evaluate the success or failure of the model by means of 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. The experiment was approved by the Committee on Animal Experimentation at the Trakia University, Stara Zagora, Bulgaria and was performed in strict compliance with animal welfare regulations (Directive 86/609/EEC).

Reproduction of osteoarthritis: In the left stifle joint of each dog, ten intraarticular injections of sodium monoiodoacetate (MIA) (MERCK # S05800 228) were performed once weekly at doses of 0.12, 0.14, 0.16, 0.26, 0.36, 0.96, 1.28, 3.00, 5.00 and 10.00 mg/kg in 1 ml 0.9% saline solution. The right joint served as control. The clinical parameters were monitored on days 0, 30, 60 and 105.

Clinical evaluation and goniometric analysis: Three clinical scoring systems – according to Grisneaux *et al.* (1999), Cross *et al.* (1997) and Budsberg *et al.* (1999), originally used for drug therapy efficacy evaluation, were parallelly used for gait, behaviour and pain analysis (Tables 1, 2, 3).

Table-1. Criteria for pain and behaviour evaluation of dogs with osteoarthritis (Grisneaux *et al*., 1999).

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 (Cross *et al*., 1997)

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 response	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

Table-3. Scoring system for evaluation of hindlimb use in dogs with OA (Budsberg *et al*., 1999).

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 hindlimb; ** replacement of the contralateral limb in < 10 s *** replacement of the contralateral limb in < 5 s

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 (Robins, 1990; 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 90^{o} flexion, ROM was measured between the longitudinal axes of the femur through trochanter major and the tibia through maleolus lateralis. 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., 1994-1995). 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 61 points in the beginning of the experiment to 14 ± 2 (day 30) and 12 ± 2 (day 60) (Table 4). The

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

Parameter		Days after the first MIA injection			
		0	30	60	105
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

Table-5. Correlation coefficients between clinical and goniometric parameters in dogs with experimental monoiodoacetate model of osteoarthritis

	GSc	CSc	BSc	TCir	SCir	ROM
GSc	-------	r = 0.88 p<0.001	r = 0.83 p<0.001	r = –0.27	r = –0.61 p<0.001	r = 0.30 p<0.001
Csc	r = 0.88 p<0.001	-------	r = 0.92 p<0.001	r = –0.37 p<0.001	r = –0.63 p<0.001	r = 0.25
Bsc	r = 0.83 p<0.001	r = 0.92 ***	-------	r = –0.32 p<0.01	r = –0.61 p<0.001	r = 0.33 p<0.001
Tcir	r = –0.27	r = –0.37 ***	r = –0.32 **	-------	r = 0.36 p<0.001	r = 0.46 p<0.001
Scir	r = –0.61 p<0.001	r = –0.63 ***	r = –0.61 ***	r = 0.36 p<0.001	-------	r = –0.33 p<0.001
ROM	r = 0.30 p<0.01	r = 0.26	r = 0.33 ***	r = 0.46 p<0.001	r = –0.33 p<0.001	-------

GSc – clinical score according to Grisneaux *et al*. (1999); CSc - clinical score according to Cross *et al*. (1997); BSc - clinical score according to Budsberg *et al*. (1999); TCir – thigh circumference; SCir – stifle joint circumference; ROM – range of motion.

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 105) (p<0.01 vs baseline). Average Budsberg score before the first MIA 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). A strong positive correlation was observed between scores obtained by the three systems (Table 5).

Goniometric analysis provided evidence for thigh muscles atrophy of the left hindlimb (Table 4) as thigh circumference decreased significantly from 32±1 cm in the beginning to 23±1 cm by the 105th day (p<0.01). The values between left and right limb were also statistically significantly different (p<0.05 by days 30 and 60 and p<0.01 by day 105). 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 105, respectively (p<0.01). ROM of the left joint exhibited a negative correlation with clinical scores: r=–0.61, p<0.001 with Grisneaux's and Budsberg's scores; r=–0.63, p<0.001 with Cross' score. The same relationships were observed for thigh and joint circumferences (Table 5).

Discussion

The obtained results showed that OA in dogs could be successfully reproduced by intraarticular injection of the glycolysis inhibitor sodium monoiodoacetate in higher doses and multiple applications unlike Stobie *et al.* (1994), which did not manage to induce OA with 0.5 mg/kg and 0.375 mg/kg MIA in this animal species. According to Beyreuther *et al.* (2007), a single low MIA dose results only in transient synovitis, resolving by the 14th day. Our experiments evidenced that higher and repeated doses (5-10 mg/kg) succeeded to reproduced all symptoms of osteoarthritis: acute inflammation at onset, progressive degeneration, transition to chronic atrophic phase, similarly to conclusions of Guinamp *et al.* (1997), that only a sufficient amount of MIA resulted in rapid decline in the locomotor function of rat stifles and that low doses provoked a transient effect. Bove *et al.* (2003) demonstrated a relationship between the

locomotor impairment in rats, MIA concentrations and the cumulative effect occurring with time.

Animal behaviour trials showed that joint pain and allodynamic were commonly observed after experimentally induced OA and therefore, they were recommended for OA pain assessment (Bove, 2006). They are even more appropriate for chemical OA model, as the one used in the present study, because the joint instability obtained after the mechanical models could be ruled out as a cause for occurring kinematic changes.

Since the structural integrity of cartilage relies on the normal functioning of chondrocytes, intra-articular injection with MIA produces cartilage degeneration and subchondral bone disorders corresponding to histopathology of OA. As this degenerative model progresses, the subchondral bone becomes exposed joint impairment and mechanical hypersensitivity associated with pain are generated (Harvey and 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, 2003).

In order to minimize the subjectivity of clinical examination, we have used three pain and gait scoring systems in dogs (Grisneaux *et al.* 1999; Cross *et al.*, 1997, Budsberg *et al.*, 1999) including numerous different parameters. The observed strong positive correlation between individual scores confirmed the opinion of Gardner (1994), that clinical symptoms in the course of OA development were consistent.

The used goniometric analysis provided an objective measure of the presence of early joint effusion and progressing inactivity and muscle atrophy of treated limb, combined with limited joint motility, specific for this chemical model as early as the first month.

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.

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Conflict of interest

Authors declare that they have no conflict of interest.

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