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Reduction in the structural changes of experimental osteoarthritis by a nitric oxide inhibitor

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Summary

Objective: To evaluate the in-vivo therapeutic efficacy of *N*-iminoethyl-L-Lysine (L-NIL), a selective inhibitor of inducible nitric oxide synthase (iNOS) in a dose response study, on the progression of lesions in the experimental osteoarthritic (OA) dog model.

Design: The sectioning of the anterior cruciate ligament of the right stifle joint of mongrel dogs was done by a stab wound. Dogs were separated into experimental groups: Group 1 received no treatment, Groups 2, 3, and 4 received oral L-NIL (0.3, 1 or 10 mg/kg/day, respectively) starting immediately after surgery. The OA dogs were killed at 12 weeks after surgery.

Results: Macroscopically, L-NIL decreased the size of the cartilage lesions on condyles and plateaus. The histologic severity of the cartilage lesions was decreased in the L-NIL-treated dogs. This effect was more pronounced at the highest dosage tested (3 and 10 mg/kg/day).

Conclusions: This study confirms the effectiveness of L-NIL, a selective inhibitor of iNOS, in attenuating the progression of experimental OA. It also clearly shows that the effect is dose-dependent.

Key words: Osteoarthritis, iNOS, L-NIL, Structural changes.

Introduction

Osteoarthritic (OA) lesions are believed to result from an imbalance in the anabolic and catabolic processes that occur during the development of the disease. A decreased synthesis of aggrecan, possibly induced by an excess production of nitric oxide (NO), has been associated with the catabolic events of OA.[1] The nitric oxide synthase (NOS) is responsible for the production of NO. The inducible form of NOS (iNOS), which can be upregulated by cytokines, is the main enzyme involved in the excess production of NO in arthritic disorders.[1,2] Moreover, NO can reduce proteoglycan synthesis and enhance metalloprotease (MMP) activity, which are likely factors contributing to cartilage damage in OA.[3,4] A high level of nitrite/nitrate has been found in the synovial fluid and serum of patients,[5] and an elevated level of iNOS synthesis/expression in OA cartilage.[6]

There has been little investigation into the potential of the NOS inhibitor on the progression of OA. We recently conducted a study examining the in-vivo effect of L-N^6-iminoethyl-L-lysine (L-NIL), a potent and selective inhibitor of iNOS, on the progression of experimental OA using the anterior cruciate ligament dog model.[7] This one-dose study demonstrated that a selective inhibition of iNOS could reduce the progression of early lesions in an experimental OA dog model under prophylactic conditions. It was therefore concluded that the suppression of NO production and the formation of peroxinitrite, a toxic, highly reactive oxidant, may help in the preservation of cartilage.

The aim of this study was to examine, by means of a dose-range study, the in-vivo effect of the potent NO synthase inhibitor, L-N^6-iminoethyl-L-lysine (L-NIL), on the progression of lesions in an experimental dog model of OA under prophylactic conditions.

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Materials and methods

EXPERIMENTAL GROUPS

Adult crossbred dogs (2–3 years old), weighing approximately 25 kg each, were used in this study. Surgical sectioning of the ACL of the right knee was performed on 12 dogs, as previously described.[8,9] Following surgery, the dogs were kept in animal-care facilities for 1 week, then sent to a housing farm, where they were free to exercise in a large pen.

A total of four groups of dogs (N=3 per group) were included in the study. Group I was operated on and received no treatment (OA group). Groups II, III, and IV were operated on and received L-NIL orally (0.3 mg/kg/day, 1 mg/kg/day, and 10 mg/kg/day, respectively) (Monsanto/Searle, St Louis, MO), beginning at the time of anterior cruciate ligament (ACL) transection of the right knee. The four groups of operated animals were killed 12 weeks after surgery.

Table I
Cartilage macroscopic lesions

Group*	No. of animals	Femoral Condyles		Tibial Plateaus	
		Size (mm^2)	Grade (0–4 scale)	Size (mm^2)	Size (mm^2)
OA (12 week)	3	17.7±7.0	1.8±0.4	30.7±5.2	2.2±0.4
L-NIL (0.3 mg/kg/day)	3	19.0±8.3	2.0±0.4	39.8±5.8	1.8±0.3
L-NIL (1 mg/kg/day)	3	14.2±8.8	1.3±0.6	32.7±7.5	2.0±0.4
L-NIL (10 mg/kg/day)	3	14.3±6.9	1.5±0.6	11.5±5.5	1.0±0.2

*Animals were killed and tissue examined at 12 weeks. L-NIL was given orally for 12 weeks beginning immediately after surgery.

MACROSCOPIC GRADING OF LESIONS

Immediately after killing, the right knees of the dogs were dissected. Each knee was examined for gross morphologic changes, as previously described.[10] Examinations were performed by two independent, blinded observers (JF, DJ). The cartilage changes on the medial and lateral femoral condyles and tibial plateaus were graded separately under a dissecting microscope (Stereozoom: Bausch & Lomb, Rochester, NY). The depth of erosion was graded on a scale of 0–4.

HISTOLOGIC GRADING

Histologic evaluation was performed on sagittal sections of cartilage from the lesioned areas of each femoral condyle and tibial plateau as described.[8–10] Specimens were dissected, fixed in 10% buffered formalin, and embedded in paraffin for histologic evaluation. Serial sections (5 μ) were stained with Safranin *O*. The severity of the OA lesions was graded on a scale of 0–14, by two independent observers (JF, DJ), using the histologic/histochemical scale of Mankin *et al.*[11]

Results

EXPERIMENTAL ANIMALS

All dogs in each experimental group completed the study. No sign of drug toxicity was noted in the group of dogs treated with L-NIL. The level of daily activity was similar in all dogs from the three experimental groups, and there was no change in the body weight of the dogs during the study period.

MACROSCOPIC FINDINGS (TABLE I)

There was a trend in dogs treated with L-NIL for the lesions on femoral condyles, and even more for tibial plateaus, to be less severe in size and grade in all groups but the 0.3 mg/kg/day one, as compared to untreated OA group.

MICROSCOPIC FINDINGS

Both for femoral condyles and tibial plateaus there was a trend for the histologic lesions to be less severe in the 1 mg/kg/day and 10 mg/kg/day treated dogs (4.6±0.8 and 4.1±0.7) and (4.8±0.7 and 3.4±0.7), as compared to the untreated OA group (5.0±0.6 and 5.4±0.8). These differences did not reach statistical significance.

Table II
Histologic-histochemical grading of cartilage lesions

Group*	No. of animals	Femoral Condyles	Tibial Plateaus
OA (12 week)	3	5.0±0.7	5.6±1.4
L-NIL (0.3 mg/kg/day)	3	6.8±1.0	5.5±1.4
L-NIL (1 mg/kg/day)	3	4.2±0.9	4.0±0.6
L-NIL (10 mg/kg/day)	3	4.7±0.9	3.3±0.7

*Animals were killed and tissue examined at 12 weeks. L-NIL was given orally for 12 weeks beginning immediately after surgery. Statistical analysis done by Mann-Whitney U-test; *P*-values compared to 12 week OA group.

Discussion

The results from this preliminary study demonstrate that, in the experimental dog model of OA under prophylactic conditions, treatment with L-NIL can, to a certain extent, reduce the structural changes of OA, an effect that is dose-related, with the best results achieved at the higher dosages tested. Treatment with L-NIL reduced the progression of macroscopic cartilage lesions, both on femoral condyles and on tibial plateaus. Again, the best results were obtained at the higher dosages tested (1 and 10 mg/kg/day). These differences were also observed in the histologic study which revealed less severe lesions on the tibial plateaus of dogs treated with the highest dosage of L-NIL. It should be noted, however, that the differences between the control and treatment groups did not reach statistical significance because of the small number of animals in each group. More precise information should be obtained with the completion of the definite study.

This study confirms our previous study on the chondroprotective effect of L-NIL, and clearly shows that this effect is dose-dependent. The exact mechanism by which L-NIL exerts this effect remains to be determined, but, as previously demonstrated, may be related to the reduced synthesis of IL-1β and metalloproteases.

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