Research Article

EFFECTS OF D-003 ON FORMALDEHYDE-INDUCED OSTEOARTHRITIS IN RATS

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Accepted on: 11-09-2011; **Finalized on:** 29-09-2012.

ABSTRACT

Osteoarthritis (OA) is a syndrome that affects hundreds of millions around the world. The treatment of OA is addressed to pain alleviation and functional status improvement. Non-steroidal anti-inflammatory drug are widely prescribed for OA, but produce gastrointestinal (non selective) and cardiovascular (ciclooxygenase–2 inhibitors) adverse effects. Then, the search for new substances is justified. D-003 is a mixture of higher aliphatic sugarcane wax acids with antiresorptive and antioxidants effects. The aim of this study was to investigate the effects of D-003 on formaldehyde-induced OA in rats. OA Animals were orally dosed with either D-003 (100 and 400 mg/kg) or naproxen (NAP) (3 mg/kg), as reference drug. Controls comprised negative and vehicle-treated rats. Treatments were administered for 10 days. The effects were assessed by measuring the changes on the diameters of rat ankle and paw. The formaldehyde injection significantly increased the diameters of rat paw and ankle as compared to the negative control group, changes that were significantly decreased by naproxen. D-003 significantly reduced these increases as compared to the positive control and baseline. The effects of the highest doses were lower than that of NAP in the paw. D-003 (100 and 400 mg/kg) significantly decreased the formaldehyde-induced increases of rat paw and ankle enlargement.

Keywords: Osteoarthritis, Sugarcane wax, D-003, NSAIDs, formaldehyde.

INTRODUCTION

Osteoarthritis refers to a syndrome of joint pain accompanied by functional limitation and reduced quality of life,[1] that affects hundreds of millions worldwide, mainly the elderly.[2] It is characterized by progressive cartilage loss, accompanied by secondary changes such as osteophyte formation and calcium deposition with inflammatory process associated, leading to stiffness and pain,[3] for these reasons patients require non-pharmacological[4-6] and/or pharmacological interventions.[7-10]

Non pharmacological interventions include life-style changes, such as weight reduction, specific exercises, patient education, physical and occupational therapy, joint unloading, among others.[2,4-6]

Pharmacological treatments of osteoarthritis (OA) are focused in alleviate the pain and improve the functional status of the subjects. Current guidelines recommend use analgesics or non-steroidal anti-inflammatory drugs (NSAIDs) to provide symptom relief despite they do not solve the underlying causal pathological process.[7-10]

Non selective non-steroidal anti-inflammatory drugs and ciclooxygenase-2 (COX-2) inhibitors, provide symptom relief in OA, but produce several adverse effects (AE), mainly gastrointestinal (non selective NSAIDs) and cardiovascular (COX-2 inhibitors).[11-14]

Other options of treatment are intra-joint corticosteroid injection and viscosupplementation that alleviate the pain and inflammation in affected area, but may not prevent the loss of the cartilage.[15]

Glucosamine and chondroitin are regularly used by patients with OA. Both agents are produced endogenously and are essential components of cartilage. Nevertheless, there not enough evidence that support the use of these agents either alone or in combination for pain relief and as disease-modifying agents in OA. In such regard, not all the results have been uniformly positive.[3,16,17]

New evidences point to the role of bone resorption in degenerative process, involving subchondral bone. Antiresorptive drugs, such as bisphosphonates, reduce the progression of the cartilage damage in experimental models of OA.[18] Risedronato treatment relief the pain and other symptoms in patients with OA of the knee, but this effect has not a radiological support.[19]

On the other hand, sinovial damage have been correlated with oxygen pressure fluctuations and increased free radicals production.[9] Reactive oxygen species (ROS) produced in the joint tissue have been associated with OA ethiology since the increase on lipid peroxidation and decrease on antioxidant defense in patients with OA.[20] In such regard, vitamin C, vitamin E and y β-carotenes intake reduce the progression of the disease in patients with knee OA[21] meanwhile deficiency of antioxidant substance on the diet have been associated with an increase on the incidence and progression of OA.[20]

Taking into account this background, the search for new substances for treatment of OA is justified. D-003 is a mixture of higher aliphatic acids (C24, C25, C26, C27, C29, C30, C31, C32, C33, C34, C35, and C36) purified from sugar cane wax.[22] D-003 inhibits mevalonate formation through regulation of HMG CoA reductase activity.[23] This is on line with its effects on bone, due to

the implication of mevalonate pathway on bone metabolism. In this sense, D-003 increases osteoclast apoptosis, thus prevents bone loss and bone resorption in rats with ovariectomy[24-26] and prednisolone-induced[27] osteoporosis. In addition, D-003 treatment (10 mg/day) for 6 months reduced urinary excretion of deoxypyridinoline/creatinine (DPD/Cr), a bone resorption marker, in postmenopausal women[28] and produced significant increases in bone mineral density (BMD) in the lumbar spine after 3 years on treatment, improving osteoporosis-related quality.[29]

Antioxidant effects of D-03 have been demonstrated in rats. D-003 (5-200 mg/kg) reduces lipid peroxidation induced by $FeCl_3$ and Cl_4C in active or inactive liver homogenates[30] and reduces production of carbonyl groups induced by 2'2'-azobis-2-amidinopropane (AAPH).[31] Clinical evidences of antioxidant effects of D-003 on healthy volunteers and elderly have shown that prevents plasmatic LDL oxidation and increases antioxidant total status.[32-34]

All these evidence support the study of D-003 in experimental models of OA. The aim of this study was to investigate the effects of D-003 on formaldehyde-induced OA in rats, a simple experimental model of OA.[35-40]

MATERIALS AND METHODS

Male Wistar rats (190- 230 g) from the National Centre for Laboratory Animal Production (CENPALAB) (Havana, Cuba) were adapted to laboratory conditions for seven days (20-25°C), humidity 60 ± 10 %, light/darkness cycles of 12 hours, with food and water provided *ad libitum*. Animal care and handling were in accordance with the Principles of Laboratory Animal Care and the Cuban Regulations for the use of laboratory animals. The study protocol was consistent with our approved Standard Operational Procedures.

The batch of D-003 used in the experiment was supplied by Plants of Natural Products (Havana, Cuba) after confirm its quality specifications.[23] For administration, D-003 was suspended in 2% Tween 20/H_2O vehicle. Naproxen (QUIMEFA, Havana, Cuba) was dissolved in sodium bicarbonate 5%.

Animals were distributed into 5 groups of 8 animals each: a negative vehicle control and five groups that received formaldehyde injection: one positive control, treated orally with the vehicle, two treated with D-003 (100 and 400 mg/kg), and other with naproxen (3 mg/kg) (NAP), the reference drug. All treatments were administered once daily by gastric gavage (5 mL/kg) for 10 days.

On day one, rats of all groups, except the negative control, were administered with the respective treatments. One hour later, arthritis was induced by injecting 0.1 ml of 2.0% formaldehyde solution into the right hind, and this procedure was repeated on day 3. The diameter of the paw and that of the right ankle were measured on day one, before the induction of the damage, and after 10 days on treatment. Measurements were done by determining the perimeter, using a tied thread around the surface and a graded rule. The anti-arthritic activity, assesses as the anti- inflammatory response, was calculated as follows: %=(1-T/C) x 100; where T and C are the mean of diameter increases of the treated and positive control groups, respectively.

Comparisons were done with the T test for independent samples. The level of statistical significance was chosen at α = 0.05. Data were processed with the Statistic software package for Windows (Release 6.1, StatSoft Inc, Tulsa, OK, USA).

RESULTS AND DISCUSSION

Formaldehyde injection induces a significantly increase in the diameters of rat paw and ankle as compared to the negative control group (Table 1). These changes were significantly decreased by naproxen, used as reference drug. These results, confirm the validity of the model in our experimental conditions, and are consistent with those reported by other authors.[38]

Table 1: Effects of D-003 on the rat paw and ankle diameters (X ±SD) (mm)

Treatments	Diameter increases	Inhibition (%)
Effects on the rat paw diameter		
Negative vehicle control	0^{+++}	-
Positive control (formaldehyde + vehicle)	2.1 ± 1.2	-
Formaldehyde +D-003 (100 mg/kg)	1.4 ± 0.8	33.3
Formaldehyde +D-003 (400 mg/kg)	1.1 ± 1.3	47.6
Formaldehyde +naproxen 3 mg/kg	$0.8 \pm 0.6^{+}$	61.9
Effects on the rat ankle diameter		
Negative vehicle control	0^{++}	-
Positive control (formaldehyde + vehicle)	1.7 ± 1.2	-
Formaldehyde +D-003 (100 mg/kg)	0.8 ± 1.1	52.9
Formaldehyde +D-003 (400 mg/kg)	$0.2 \pm 0.4^{+}$	88.2
Formaldehyde +naproxen 3 mg/kg	$0.4 \pm 0.5^{+}$	76.5

+ p<0.01; ++ p< 0.01; +++ p< 0.001, Comparisons with positive controls Test T for independent samples

Treatment with D-003 (100 and 400 mg/kg) reduced (33.3 and 47.6%, respectively) formaldehyde-induced increases of rat paw as compared to the positive control. The effect of the highest dose was lower than that of NAP, that significantly reduced the increase on such diameter (61.9 %).

D-003 (400 mg/kg) and NAP reduced (p<0.01) the increases of ankle diameter (88.2 and 76.5 %, respectively), as compare with positive control group. The effect of the highest dose of D003 was similar to NAP, meanwhile the effect of D-003 100 mg/kg moderately reduced the increases of ankle diameter but it did not reach statistical significance.

The formaldehyde-induced model of arthritis, useful to assess the potential anti-arthritic and anti-inflammatory effects of a substance, partially resembles the

characteristics of human arthritis. Formaldehyde injection causes a chronic inflammation of the rat foot, which involves the proliferation phase of inflammation elicited by COX mediators.[35-40]

Then, it is logical that naproxen, non selective NSAIDs that inhibits both COX-1 and COX-2 enzyme isoforms, effectively reduced the formaldehyde-induced increases in the size of the rat paw and ankle, particularly on this last one (61.9 and 76.5 %, respectively). Other NSAIDs had effects on this model. Thus aspirin and indometacin reduced the diameter of the paw after 10d on treatment (56.8 and 67.2 %, respectively).[38,40]

The effects of D-003 (100 and 400 mg/kg) on this model, are consistents with its antiresorptive effects. In such regard, D-003 prevents bone loss in ovariectomized and prednisolone treated rats, modifying resorption parameters through the inhibition of apoptosis of osteoclasts. Bone loss, affecting subchondral bone, have been associated with osteoarthritis progression,[18,19] them, a contribution of antiresorptive effects of D-003 on this model cannot be discarded.

In addition, antioxidant effects of D-003, at these doses, have been demonstrated in rats.[32,33] In this sense, D-003 inhibits lipid peroxidation of plasmatic proteins and production of malondialdehyde on rat livers and reduces production of carbonyl groups. Keeping in mind that an increase on lipid peroxidation and a reduction on antioxidant defenses, such as vitamin C and superoxide dismutase activity, have been established in osteoarthritis, it could possible that antioxidant effects of D-003 are contributing, at least in part, with these results.

CONCLUSION

Oral administration of D-003 (100 and 400 mg/kg) significantly decreased the formaldehyde-induced increases of rat paw and ankle enlargement. These results are consistent with previous reports of the anti-resorptive and anti-oxidant effects of D-003, and encourage investigating its effects on other experimental models of osteoarthritis.

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