Effect of *Cynodon dactylon* and Tenoxicam on the Lysosomal Enzyme Activities in the Cartilage Tissue of Osteoarthritic Guinea Pigs

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Summary To estimate the chondroprotective effect of *Cynodon dactylon*, an indigenous drug, we measured the activities of lysosomal enzymes, β-glucuronidase, N-acetyl-β-D-glucosaminidase, and cathepsin D in cartilage tissues of guinea pigs with experimentally induced osteoarthritis (OA). Induction of OA was achieved by intra-articular injections of papain into the knee joints of guinea pigs. OA-induced animals were treated with either *Cynodon dactylon*, an indigenous drug, or tenoxicam, a known chondroprotective allopathy drug. The result showed that the activities of the lysosomal enzymes were increased in the cartilage tissue of OA-induced animals and that these values were reversed to normal by both of the interventional agents. The reason for this normalcy may be attributed to the anti-inflammatory effect of the drugs, probably due to stabilization of the lysosomal membrane.

Key Words: osteoarthritis, lysosomal enzymes, *Cynodon dactylon*, tenoxicam, cartilage

Several hypotheses have been advanced to explain the pathogenesis of inflammation and joint damage in osteoarthritis (OA). OA is a degenerative joint disease characterized by progressive cartilage degradation, subchondral bone thickening, and formation of subchondral bone cysts [1]. OA is the most common form of
arthritis. OA is no longer regarded as an ageing and cartilage degeneration process; rather it is now considered to be an active process that may be more reparative than destructive in nature.

Articular cartilage is a highly metabolically active tissue with a constant slow turnover of its macromolecules. Articular cartilage has two main functions: absorbing stress by deforming under mechanical load and providing a smooth load-bearing surface to permit low-friction movement of the joint. The functional properties of cartilage result from its unique structure of chondrocytes embedded in a matrix of collagen and proteoglycan. The presence of collagen renders stability to the cartilage [2].

Lysosomes play an important role in the destruction of connective tissue by discharging proteolytic enzymes. The lysosomal proteolytic enzymes play a prominent role in the degradation of connective tissue proteins, particularly by degrading the native collagen fibers into smaller fragments [3, 4].

In the present study, in guinea pigs used as the experimental animals, lysosomal enzymes such as, β-glucuronidase, N-acetyl-β-D-glucosaminidase (NAG), and cathepsin D were estimated in the cartilage tissue. Induction of OA was achieved by intra-articular (IA) injections of papain into the right knee joints of guinea pigs, and saline was injected into the left knees as a control to nullify the effect of the injection itself when given intra-articularly, papain, a proteolytic enzyme, has been shown to produce a reproducible model of OA that simulates the disease in human beings [5-7]. OA-induced animals were treated with an indigenous drug, namely, Cynodon dactylon, popularly known as Bermuda grass in English, or by a known chondroprotective allopathy drug, tenoxicam. The changes in the levels of lysosomal enzymes were studied following treatment of the OA-induced animals with these interventional agents.

MATERIALS AND METHODS

Adult male guinea pigs were used for the study. All animals were of the same age group and weighed 400-450 g. The animals were purchased from King Institute of Preventive Medicine, Guindy, Chennai, India, and were acclimatized to our animal house condition for one week. They were fed Gold Mohur commercial feed marketed by the Hindustan Lever, Bombay, and water was given ad libitum.

Experimental procedure. Animals were divided into six groups with six animals in each group.

Group I (n = 6): The first group served as the normal control.

Group II (n = 6): The second group was treated to induce OA, which induction was achieved by IA injections of papain (0.5 ml) of 0.1% in one knee and saline (0.5 ml) on the other knee as a control for two consecutive days. According to histopathological studies, induction of OA was achieved on the 15th day, but the animals were observed under the same condition for another 21 days and were sacrificed on the following day (i.e., on the 37th day).
Group III (n = 6): Interventional group I with *Cynodon dactylon*: The animals in this group were treated with the same dosage of papain as in the second group; and after the 15th day, oral administration of 0.5 ml of *Cynodon dactylon* extract (62.5 mg/kg body weight) was given once daily for 21 days. The animals were then sacrificed on the next day (i.e., on the 37th day).

Group IV (n = 6): Interventional group II with tenoxicam: The animals in this group were treated the same as those in group III except that tenoxicam (0.5 mg/kg body weight) was administered instead of *Cynodon dactylon* extract.

Group V (n = 6): These were another set of control animals which were administered the same dosage of *Cynodon dactylon* only as given in the group III animals for 21 days, but the animals were observed under the normal condition for another 15 days without administering the drug and were sacrificed on the next day (i.e., on the 37th day). The purpose of this group was to study whether the drug alone has any adverse effect when it was administered to the control animals.

Group VI (n = 6): These animals were the control animals and were administered the same dosage of tenoxicam only as given in the group IV for 21 days, but the animals were observed under the normal condition for another 15 days without administering the drug and were sacrificed on the next day (i.e., the 37th day). The purpose of this group was to study whether the drug alone has any adverse effect when it was administered to the control animals.

According to the histopathological report, the experimental animals were sacrificed on the corresponding periods, and the estimation of lysosomal enzymes was carried out spectrophotometrically. β-Glucuronidase was estimated by the method of Stahl and Fishman [8], NAG, by the method of Moore and Mooris [9]; and cathepsin D, by the method of Sapolsky *et al.* [10].

Statistical analysis was done by ANOVA, and the F ratios were computed. Multiple comparisons to detect significant differences between various groups were performed by means of Tukey's test. A value of \( p < 0.05 \) was considered statistically significant. Student's \( t \) test was done, and the \( p \) value was arrived at, to assess the statistical significance of the changes in lysosomal enzymes observed between the left (saline) and right (papain) knees of the same animal.

RESULTS

The values of β-glucuronidase, NAG, and cathepsin D in cartilage tissues of all the groups were tabulated. The lysosomal enzyme values were found to be increased (\( p < 0.001 \)) in the group II (Control + Papain) animals when compared with those of the group I (Control) animals. Comparisons were made between and within the groups by ANOVA and multiple Tukey's test. Decreases in the lysosomal enzymes were observed in group III (Control + Papain + *Cynodon dactylon*) and group IV (Control + Papain + Tenoxicam) when compared with the levels for group II animals. However, no changes were observed when group V (Control + *Cynodon dactylon*) and group VI (Control + Tenoxicam) animals were
compared with group I animals (Table 1).

Significant changes were observed between the saline-treated (left) and the papain-treated (right) cartilage of group II animals, and no significant changes were observed between the knees of group III and group IV animals when the data were analyzed by Student’s t test (Table 1).

**DISCUSSION**

Lysosomes are a distinct group of cell organelles characterized by their content of a variety of acid hydrolases that are active at low pH [11]. The lysosomal proteinases form an intracellular pathway for the breakdown of proteins. Among the lysosomal glycohydrolases, β-glucuronidase, and NAG are involved in the degradation of glycoproteins and GAG (glycosaminoglycans) by cleaving the β linkage of glucuronic acid [8] and N-acetylglucosamine residues, respectively [12]. Cathepsin D is the major lysosomal endopeptidase that plays an important role in physiological and pathological breakdown of intracellular and extracellular connective tissue proteins [13]. In 1971 Dingle [14], using an immunoinhibition assay, concluded that cathepsin D is the principal and probably exclusive agent of cartilage matrix degradation in organ culture. An increase in the extracellular acid hydrolases has been well established in a number of pathological
conditions involving inflammatory processes [15–17] and damage to the connective tissue matrix. The release of lysosomal hydrolases during cell injury or excessive phagocytosis may play a role in damaging the articular cartilage [18].

In the present study, a significant increase in the lysosomal enzymes was observed in the cartilage tissues of OA-induced group II guinea pigs compared with the activities of the control group. Comparisons were possible between left and right knees for the groups II, III, and IV, in which the left knees were given saline and the right knees were administered papain to rule out the effect of injection. This comparison was not applicable for groups I, V, and VI, as they did not receive any injections.

In India, many plant and herbo-mineral preparations are used for the treatment of different diseases [19, 20], and Cynodon dactylon also finds wide application in traditional medicine [21]. Tenoxicam is a thienothiazine derivative of the oxicam class of non-steroidal anti-inflammatory drugs, and is structurally related to piroxicam. In the present study, tenoxicam was chosen as a drug of choice because of its lower gastrotoxic potential than some other non-steroidal anti-inflammatory drugs [22].

The activity of the lysosomal enzymes were increased in the cartilage tissues of OA-induced animals. The increased activities of lysosomal glycohydrolases and cathepsin D may indicate the increased synthesis of the enzyme and instability of the lysosomal membrane during OA. This finding is supported by that of Van Caneghem [23]. The lysosomal values were found to be reversed to normal by the interventional agents. The reason for this normalcy may be attributed to stabilization of the lysosomal membrane by these interventional agents.

REFERENCES


