# Effects of meloxicam on serum level of bone alkaline phosphatase,osteocalcin and bone density  in a sheep model

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## ABSTRACT

**Aim:** To examine the effect of meloxicam on serum bone alkaline phosphatase , osteocalcin  and bone mineral density in sheep model.

**Materials and Methods:** Ten apparently healthy mature male sheep aged 10-12 months and body weight of $25 \pm 2$kg from local market were included in the study. The animals were randomized into two groups of 5 animals: Group 1 not treated by any drug (control), Group 2 were  treated by intramuscular injection of meloxicam (Boehringer; Germany) in dose of 0.5 mg/kg/day for 90 days . Blood samples(10 ml) from each sheep in group 2 was drawn via jugular vein puncture before the drug administration and at the 10 ,20,30,40,50,60,70,80 days of treatment for analysis of BALP , OC by using ELISA kits from My Biosource company (USA) . All animals had been sacrificed after 90 days, a total of 20  tibial bone specimens (10 specimens/ group)  was  used to monitor  bone mineral density  by using Dual –energy x ray absorptiometry (DEXA)(STRATOS  dr, DMS, PSM / England)  with standard software (  Nero software version 5.5.10) .

 **Results:** Results of both ANOVA and Duncan˙s Multiple Range Tests showed that no significant differences in serum levels of bone alkaline phosphatase and osteocalcin between study times. Data analysis by using  Independent Samples Test showed that no significant differences between the mean level of DEXA scan readings between control and treatment groups($0.71 \pm 0.04$, $0.69 \pm 0.05$) respectively.

**Conclusion**: Treatment of sheep with meloxicam   for 90 days at a dose of 0.5 mg/kg/day caused  no significant changes  in serum level of bone alkaline phosphatase, osteocalcin and bone mineral density.

**Keywords:** Meloxicam , bone alkaline phosphatase, osteocalcin , DEXA scan.

## INTRODUCTION

 Several categories of prescription medications, when taken long-term, can  affect bone density and strength. Analgesic drugs are very commonly prescribed for long-term patients, such as those suffering from arthritis or chronic back pain and They could have such effect on bone[1]. Meloxicam is a very effective and safe NSAID with proven anti-inflammatory, anti-pyretic and anti-endotoxin activity. The pharmacokinetic and pharmacodynamic properties of the molecule create the advantage of single dose efficacy. Beside rather specific COX-2 inhibiting effects and the lack of a specific COX-1 inhibitory adverse side-effects, meloxicam is a potent inhibitor of oxyradical production at drug concentrations comparable with those encountered during therapy. [2] . In  humans and  many animal species, meloxicam has proven its curative and supportive, respiratory infections, diarrhea, mastitis, periparturient and during painful  surgical procedures.[3]

Meloxicam is  NSAID  acts by inhibiting prostaglandin synthesis. The molecule is highly plasma protein bound, when circulating in the body (95-99%). It has a long plasma half-life, enabling less frequent dosage schemes.. It is a derivative of oxicam, closely related to piroxicam, and falls in the enolic acid group of NSAIDs. Systemic meloxicam produces analgesia largely via peripheral mechanisms. The rapidity of its actions indicates a direct effect on sensitized  nociceptors,it starts to relieve  pain  30–60 minutes after administration.[4 - 6] .Meloxicam inhibits cyclooxygenase (COX2), the enzyme responsible for converting arachidonic acid into prostaglandin H2—the first step in the synthesis of prostaglandins, which are  mediators  of  inflammation.  Meloxicam  has  been  shown,  selectively  to  inhibit  COX-2  over  COX-1  [7] .After

intramuscular or subcutaneous administration, meloxicam is usually well absorbed. In general, the volume of distribution is low for most NSAID's in most species [8], this is probably caused by the extreme binding to plasma protein; meloxicam is no exception to this phenomenon. [9 – 10]. Meloxicam concentrations in synovial fluid range from 40% to 50% of those in plasma. The free fraction in synovial fluid is 2.5 times higher than in plasma, due to the lower albumin content in synovial fluid as compared to plasma which may account for the fact that it performs exceptionally well in the treatment of arthritis in animal models. [11-12]

The internal structure of bone is described in terms of quality or density which reflects a number of biomechanical properties, such as strength and modulus of elasticity. [13] Bone quality and quantity in the local environment can be evaluated by measuring biochemical bone markers like bone alkaline phosphatase (BALP) and osteocalcin (OC). Osteoblasts control mineralization and osteogenesis by regulating the passage of calcium and phosphate ions across their surface membranes. The latter contains alkaline phosphatase, which is used to generate phosphate ions from organic phosphates. So, BALP contributes to mineralization [14]. The level of BALP in serum indicates the metabolic status of osteoblasts. It is level in serum provides information useful in the evaluation and treatment of patients with bone diseases, also its level increase rapidly in response to anabolic therapy. The loss and deterioration of bone tissues is caused by a net imbalance in bone remodeling and this can be estimated by measuring bone mineral density, but this estimation is unable to provide direct information on the micro architectural deterioration of bone which is associated with deeper resorption sites .BALP can reflect this underlying remodeling process in a highly specific and sensitive way. Another bone formation marker is Osteocalcin, also known as bone gla protein (BGP), is a marker of late bone formation and appears during the mineralization phase. [15]

Animal model is necessary to evaluate new options of drug treatment modalities that could be related to bone. Sheep make excellent experimental animal for pharmacological researches . Their body weight and size approximates to that of a human, and they adapt rapidly and extremely well to a laboratory situation. Their size also enables sufficient blood to be withdrawn for chemical analysis.[16]. The serum values of bone turnover parameters in sheep could be of great value in research and could provide complementary non-invasive information on the bone density. [17]

The aim of this study was to examine the effect of meloxicam on serum BALP, OC and bone density in a sheep model .

## MATERIALS AND METHODS

This study was carried out in the department of Basic Science, College of Dentistry, University of Mosul .The study protocol was approved by the scientific committee /department of basic science /college, of Dentistry/University of Mosul. Ten apparently healthy mature male sheep of 10-12 months old and body weight of $25 \pm 2$kg purchased from local market were included in the study. During the entire period of the study, the animals were permanently housed indoors in animal house of College of Dentistry/University of Mosul .They were kept in group housing under photoperiod cycle of light: from 6:00 to 18:00 h and dark: from 18:00 to 6:00 at temperature $20 \pm 2$C° .This protocol was used in view of published reports of seasonal variation in bone remodeling and circadian rhythm variation of the biochemical markers of bone turnover. The animals were fed twice daily with standardized diet with tap water. Each sheep was subjected to a clinical examination, also they were examined daily by veterinarian until slaughtering .The animals were randomized into two groups of 5 animals: Group 1 not treated by any drug, Group 2 were treated by intramuscular injection of meloxicam (Boehringer;Germany) in adose of 0.5 mg/kg/day for 90 days .

**Blood sampling**: Animals that are treated by meloxicam (Group 2) were blood sampled before the drug administration and at the 10 ,20,30,40,50,60,70,80 days of treatment .The animals were prevented from feeding 12 hr before blood sampling ,then 5 ml of blood were drawn via jugular vein puncture between 10:00 - 11:00 am for the analysis of biochemical parameters , placed into plain tubes ,kept at room temperature for 30 minutes ,centrifuged for 15 minutes at 3000 rpm, then the separated serum was removed by micropipette, stored at -20°C till analysis. Bone alkaline phosphatase ELISA kit and Osteocalcin ELISA kit from My Biosource Company (USA) used for the quantitative determination of Sheep Bone alkaline phosphatase and Osteocalcin.

## ASSAY PROCEDURE

The desired numbers of coated wells in the holder have been secured then 100 μL of Standards or Samples were added to the appropriate well in the antibody pre-coated Microtiter Plate. 100 μL of Blank Solution (pH 7.0-7.2) was added in the blank control well, then 50 μL of Conjugate was added to each well (NOT blank control well), Mixed and covered then incubated for 1 hour at 37°C. After that the micro titer plate washed by using Automated Washing five times with diluted wash solution (400 μL/well/wash) using an auto washer and dried. A 50 μL Substrate A and 50 μL Substrate B were added

to each well including blank control well, subsequently, covered and incubated for 10-15 minutes at 20-25°C, followed by addition of 50μL of Stop Solution to each well including blank control well. Mixed well. Finally, the Optical Density (O.D.) at 450 nm was determined by using a microplate reader of ELISA device immediately.

### DEXA scan

All animals had been sacrificed after 90 days, a total of 20 tibial bone specimens (10 specimens/ group) were used to monitor bone mineral density by using Dual –energy x ray absorptiometry (DEXA)(STRATOS dr, DMS, PSM / England) with standard software ( Nero software version 5.5.10) .

Each bone sample was placed on a flat padded table and the arm of the instrument was passed over the whole length of the bone specimen .While the measurement was performed ,a beam of low- dose x- ray (< 0.025 μSV) from below the table passed through the area being measured .These x-rays were detected by the device in the instrument's arm .The machine converts the information received by the detector into an image of the bone and analyzed the quantity of bone .The results are usually reported as BMD expressed in $g/cm^2$ (the amount of bone per unit area)

### Statistical analysis

SPSS program version 19 was used to analyze the obtained data .Data expressed as a mean and standard deviation values . ANOVA test and Duncan's multiple rang test were used for the comparison between the study times . Independent Samples Test was used for comparison between 2 groups.

### Results

The results obtained in this study were represented as standard descriptive statistic of mean ± standard deviation (minimum-maximum) for the values of serum biochemical markers measured before the drug administration and at the 10 , 20 , 30 , 40 , 50 , 60 , 70 , 80 days of treatment.

### Bone alkaline phosphatase (BALP)

Standard descriptive statistic of BALP for each study time was shown in Table (1).Results of ANOVA test showed that no significant differences between study times as shown in Table (2) . Duncan's Multiple Range Test show no significant differences between all study time .Figure(1)

Table (1): Descriptive Statistics of Bone alkaline phosphatase for Meloxicam group during study times

| Study times | N | Mean ±SD pg/ml | Minimum | Maximum |
|---|---|---|---|---|
| Time 1 | 5 | 22.16 ±33.08 | 2.57 | 60.36 |
| Time 2 | 5 | 15.10 ±17.19 | 4.58 | 34.94 |
| Time 3 | 5 | 11.70 ±3.69 | 8.39 | 15.69 |
| Time 4 | 5 | 10.33 ±4.41 | 5.36 | 13.80 |
| Time 5 | 5 | 10.97 ±5.27 | 5.44 | 15.95 |
| Time 6 | 5 | 10.45 ±5.05 | 4.98 | 14.94 |
| Time 7 | 5 | 12.29 ±4.85 | 7.88 | 17.48 |
| Time 8 | 5 | 11.88 ±6.75 | 6.16 | 19.33 |
| Time 9 | 5 | 9.38 ±4.19 | 5.94 | 14.05 |

Table ( 2) :ANOVA Test comparison for Bone alkaline phosphatase within Meloxicam group

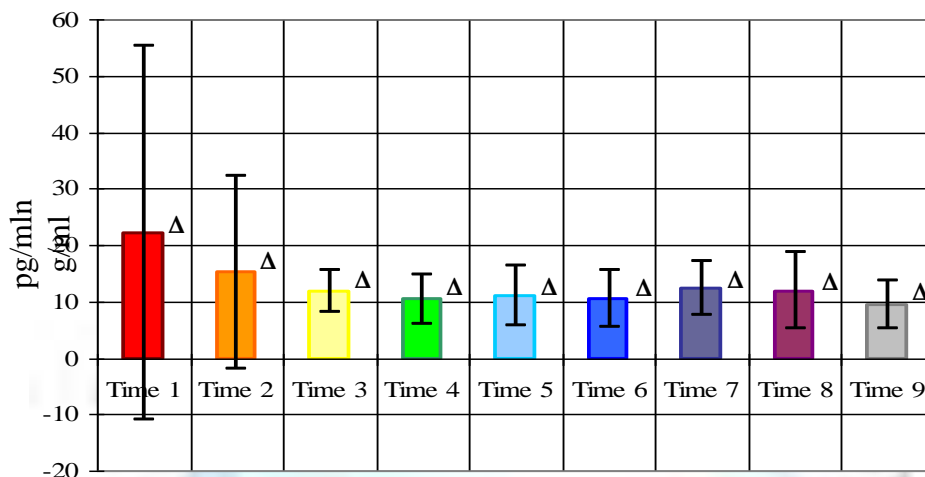| Meloxicam group | Sum of Square | df | Mean Square | F | p–value |
|---|---|---|---|---|---|
| Between Groups | 365.704 | 8 | 45.713 | 0.263 | 0.970 |
| Within Groups | 3126.627 | 18 | 173.701 | | |
| Total | 3492.331 | 26 | | | |



Figure (1): Duncan's Multiple Range Test for Bone alkaline phosphatse Measurments among Study times for Meloxicam group.Means with the same letters were statistically non significant (p>0.05).

**Osteocalcin (OC)**

Standard descriptive statistic of OC for each study time was shown in Table (3).Results of ANOVA test showed that no significant differences between study times as shown in Table (4) . Duncan's Multiple Range Test show no significant differences between all study time .Figure (2)

Table (3): Descriptive Statistics of Osteocalcin for Meloxicam group during study times

| Study times | N | Mean ±SD pg/ml | Minimum | Maximum |
|---|---|---|---|---|
| Time 1 | 5 | 4.81 ±1.11 | 3.74 | 5.96 |
| Time 2 | 5 | 5.07 ±0.72 | 4.58 | 5.89 |
| Time 3 | 5 | 4.60 ±1.41 | 3.45 | 6.18 |
| Time 4 | 5 | 5.05±1.97 | 3.03 | 6.97 |
| Time 5 | 5 | 4.37 ±1.36 | 2.86 | 5.48 |
| Time 6 | 5 | 5.11 ±1.68 | 4.12 | 7.05 |
| Time 7 | 5 | 4.51 ±0.91 | 3.81 | 5.54 |
| Time 8 | 5 | 4.73 ±0.97 | 3.82 | 5.76 |
| Time 9 | 5 | 4.24±0.72 | 3.67 | 5.05 |

Table (4 ):ANOVA Test comparison for Osteocalcin within Meloxicam group

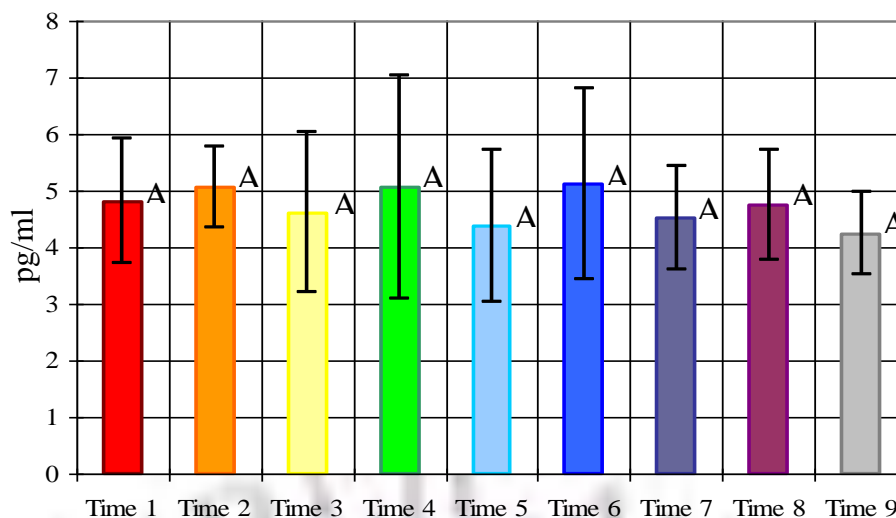| Meloxicam group | Sum of Square | df | Mean Square | F | p–value |
|---|---|---|---|---|---|
| Between Groups | 2.397 | 8 | 0.300 | 0.185 | 0.990 |
| Within Groups | 29.187 | 18 | 1.622 | | |
| Total | 31.584 | 26 | | | |

Figure (6): Duncan,s Multiple Range Test for Osteocalcin Measurments among Study times for Meloxicam group.Means with the same letters were statistically non significant (p>0.05)

### DEXA scan

Data analysis by using Independent Samples Test showed that no significant differences between the mean level of DEXA scan readings between control and treatment groups .Table (5)

Table (5): Independent Samples Test comparison between control and treatment groups for the mean level of DEXA scan values

| Study Groups | N | Mean±SD g/cm$^2$ | $p$–value |
|---|---|---|---|
| Control | 10 | 0.71 ±0.04 | 0.058 |
| Meloxicam | 10 | 0.69±0.05 | |

* Significant differences existed at 0.05 level.

### Discussion

In the present study  the changes in serum level of BALP and OC were not significant during study times in treatment group ,this was accepted by other studies   which is carried out to determine the ability of bone turnover markers to monitor the  bone mineral density and course of  callus consolidation during bone healing  ,based on the levels of those markers ,no effects were seen on bone formation [18-21]. This can be explained by the fact that these markers can effectively detect changes in bone formation after 3-6 months from the changes in bone mineral density [22]. For example ,the adaptation of BALP  to bone turnover could take more time ,because its concentration will depend on the bone remodeling process which is about three months in healthy individuals [23]. Delanaye P et al ;2013 found that significant change in serum  levels of BALP in patients with hemodialysis could only seen after 6 months . Large discrepancies were present between different bone markers and the strength of their association with bone mineral density .[24 -25]

In serum OC measurements ,it is necessary to take into account its short half life of approximately 20 min in the circulation ,as its rapidly eliminated by the kidneys .In addition the circadian rhythm of this molecule must taken into consideration and its high level of instability ,the intact molecule of OC represent one –third of the serum immunoreactivity of this protein that can be detected by ELISA test [26]

In the present study ,DEXA scan analysis showed no significant differences in bone mineral density  of tibial bones between two study groups .This was in line with other studies  showed that COX-2 selective inhibitors was associated with normal bone mineral density  at whole body  [27]. Bone mineral density  was normal in user of COX2 inhibitors  without

effect on serum levels of bone resorption markers . [28]   ,while other studies were   in disagreement with results of this study [29-34].

As bone mineral density of tibial bone could not determined using strandard human regions and method of examination , they were analysed  using two fixed sub regions (R1 and R2) along the tibial shaft [35]. The literature supports, as a satisfactory approach, DEXA analysis of excised bones, in vitro, for assessement of bone mineral density in longitudinal and end-point studies[36]respectively for animals the size of rats and larger . Jeffrey D. Kearbey (2009) evaluated the skeletal effects of propionamide in an osteopenic rat model by using regional bone mineral density analysis of excised bones  using DEXA scan[37] .For rodents bigger than mice, e.g. rats, hamsters and guinea-pigs which have a much higher bone mass ,DEXA analysis appears to provide use of an acceptable and reliable procedure which offers validated output data [38] . DEXA has been used with species of agricultural concern including sheep [39-43].In dogs , DEXA was used to quantify changes in bone mineral density at the site of fracture following ostectomies of various widths [44-45]. The results of these and other studies suggest that DEXA can be used to effectively monitor changes in bone density.

## Conclusion

Treatment of sheep with meloxicam  for 90 days at dose of 0.5 mg/kg/day caused  no significant changes  in serum level of bone alkaline phosphatase  ,osteocalcin and bone mineral density.

## Acknowledgements

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