The Effects of Orally Administered Diacerein on Cartilage and Subchondral Bone in an Ovine Model of Osteoarthritis

SU-YANG HWA, DANIEL BURKHARDT, CHRISTOPHER LITTLE, and PETER GHOSH

ABSTRACT. ***Objective.*** An ovine model of osteoarthritis (OA) induced by bilateral lateral meniscectomy (BLM) was used to evaluate *in vivo* effects of the slow acting antiarthritic drug diacerein (DIA) on degenerative changes in cartilage and subchondral bone of the operated joints.

Methods. Twenty of 30 adult age matched Merino wethers were subjected to BLM in the knee joints and the remainder served as non-operated controls (NOC). Half of the BLM group (n = 10) were given DIA (25 mg/kg orally) daily for 3 mo, then 50 mg/kg daily for a further 6 mo. The remainder of the meniscectomized (MEN) group served as OA controls. Five DIA, 5 MEN, and 5 NOC animals were sacrificed at 3 mo and the remainder at 9 mo postsurgery. One knee joint of each animal was used for bone mineral density (BMD) studies. Osteochondral slabs from the lateral femoral condyle and lateral tibial plateau were cut from the contralateral joint and were processed for histological and histomorphometric examination to assess the cartilage and subchondral bone changes.

Results. No significant difference was observed in the modified Mankin scores for cartilage from the DIA and MEN groups at 3 or 9 mo. However, in animals treated with DIA, the thickness of cartilage ($p = 0.05$) and subchondral bone ($p = 0.05$) in the lesion (middle) zone of the lateral tibial plateau were decreased relative to the corresponding zone of the MEN group at 3 mo ($p = 0.05$). At 9 mo subchondral bone thickness in this zone remained the same as NOC but BMD, which included both subchondral and trabecular bone, was significantly increased relative to the NOC group ($p = 0.01$). In contrast, the subchondral bone thickness of the outer zone of lateral tibial plateau and lateral femoral condyle of both MEN and DIA groups increased after 9 mo, while BMD remained the same as in the NOC.

Conclusion. DIA treatment of meniscectomized animals mediated selective responses of cartilage and subchondral bone to the altered mechanical stresses induced across the joints by this procedure. While subchondral bone thickness in tibial lesion sites was reduced, cartilage and bone proliferation at the outer joint margins, a region where osteophyte formation occurred, were enhanced, suggesting that DIA supported the processes of repair and endochondral ossification.

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Key Indexing Terms:
OSTEOARTHRITIS MENISCECTOMY SUBCHONDRAL BONE
DIACEREIN UNCALCIFIED CARTILAGE PLATE BONE MINERAL DENSITY

Osteoarthritis (OA) is a multifactorial disorder in which pathological changes become established in articular cartilage, synovium, and subchondral bone[1-3], eventually resulting in impaired joint function and pain. For many years pharmacological intervention in OA was largely confined to analgesics, steroidal and nonsteroidal antiinflammatory drugs (NSAID). While these agents have provided an important means of controlling the inflammation and pain in OA, their application has been limited by their deleterious side effects on cartilage[4,5] and the gastrointestinal tract[6-8]. More recently, agents have been described that are reported to provide symptomatic relief by targeting the underlying pathology of OA, particularly in cartilage and subchondral bone, whose structural integrity is essential for the normal mechanical function of diarthrodial joints. Such agents have been classified as structural modifying osteoarthritis drugs (SMOAD)[9] and would be expected to retard, stabilize, or reverse the pathological changes that occur in OA joints, thereby limiting progression of the disease. Since structural joint changes in human OA generally take several decades to develop and methods to evaluate

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such changes are still the subject of investigation, animal models of OA have been employed to study potential SMOAD and identify their mechanisms of action[10-12].

In humans, meniscectomy is a common surgical procedure that is often accompanied by cartilage degeneration and the onset of OA because of the high focal stresses imposed on articular cartilage and subchondral bone due to excision of the meniscus[13-15]. Our previous studies had shown that the similar sequelae occur in dogs[16,17] and sheep when they were subjected to total medial[18-23] or lateral meniscectomy[24-26]. Moreover, the biochemical and histological changes that occur in cartilage and subchondral bone of bilaterally meniscectomized sheep joints were shown to exhibit many similarities to those described for human OA[24-26].

Diacerein (4,5-diacetoxy-9, 10-anthraquinone-2-carboxylic acid) is an anthraquinone derivative that is reported to produce symptomatic relief when administered orally to patients with OA[27]. It is also known to inhibit the production of interleukin 1 (IL-1) and reduce cartilage breakdown in a murine granuloma model[28], and slow the progression of cartilage lesions in the canine cruciate deficiency model of OA[29] as well as in a rabbit joint contusion model[30]. *In vitro* diacerein inhibits collagenase production by lapine chondrocytes[31,32], modulates IL-1 inhibition of proteoglycan (PG) synthesis[31,32], and enhances transforming growth factor (TGF)-ß1 and ß2 expression in articular chondrocytes[33]. Collectively, these reports suggest that diacerein could be classified as a SMOAD with respect to its effects on cartilage. However, its ability to influence remodelling of the subchondral bone in OA joints had not been previously investigated.

We investigated whether diacerein possessed such activity using an ovine model of OA induced by bilateral lateral meniscectomy (BLM).

MATERIALS AND METHODS

Animals and surgical protocol. Thirty adult (2–3-yr-old) purebred Merino wethers obtained from the University of Sydney farm at Camden, NSW, were used for this study. The animals were divided into 6 age and weight matched groups. Ten animals served as non-operated normal controls (NOC group), while the remaining 20 animals underwent BLM of the knee joints as described[25]. However, to minimize discomfort and facilitate rehabilitation after the first operation the contralateral knee joint was meniscectomized 2 weeks after the first. All animals were allowed 2 weeks recovery in the animal house, then maintained in an open grassed paddock (100 × 22 m) with supplementary hay feeding and water *ad libitum* for the duration of the study. The surgical procedures and the program of postoperative care were approved by the Animal Care and Ethics Committee of the University of Sydney.

Ten of 20 BLM sheep were used as OA controls without diacerein treatment (MEN group). The remaining 10 BLM animals were treated with diacerein (DIA group).

Drug administration. A weighed amount of pharmaceutical grade diacerein powder (Negma Laboratories, Paris, France) was stirred with 2.5% methyl cellulose (BDH Chemicals, Australia Pty. Ltd) in water in a ceramic mortar until the suspension became homogenous. A predetermined volume of this methyl cellulose suspension of the drug corresponding to the required dose was administered to the DIA group commencing 2 weeks after the last operation. The MEN group received an equivalent volume of aqueous methyl cellulose via the same route. Both prospective 3 month and 9 month diacerein treated groups received 25 mg/kg of the drug daily for the first 3 months, thereafter the 9 month experimental group received 50 mg/kg DIA daily for the remainder of the 6 month period.

Clinical observations. To assess possible complications of BLM and to evaluate any side effects of the diacerein treatment, such as on wound healing, gait, daily activities and feeding, stool consistency, and body weight, animals were monitored weekly over the period of the study.

Processing of specimens. At the end of each 3 month or 9 month experimental period, animals were euthanized by an intravenous overdose of sodium pentobarbitone (Letharb®, Virbac, Sydney, Australia). The stifle joints were removed intact and immediately transferred to the laboratory on ice. After removing the periarticular soft tissues, the joints were opened and photographed. Midcoronal bone slabs (3–4 mm thick) were cut through the central weight bearing region of the tibial plateaus and the femoral condyles with a band saw. The full width of medial and lateral osteochondral sections of 1.0 cm depth were obtained. The slabs were defined as regions of the lateral femoral condyle (LFC) and lateral tibial plateau (LTP). Each of these regions was further subdivided into 3 zones corresponding to the inner joint margin (I), the middle area (M), and the outer (O) zone of articular cartilage (Figure 1). Anatomically, the location of the O zone of the tibial plateau was normally fully covered (or protected) by the meniscus, the M zone was partially covered, and the I zone was totally exposed.

For the subchondral bone studies, the osteochondral section of each region was trimmed again to obtain a 4 mm thick and 5–8 mm deep slab that included articular cartilage, the underlying bone trabeculae, and marrow spaces. These specimens were fixed, decalcified, dehydrated, and embedded in paraffin as described[25]. Care was taken to ensure that the specimens were properly oriented in the mould to allow cutting of full thickness sections, extending from the cartilage surface to the underlying subchondral bone and marrow spaces.

Assessment of gross morphological changes of articular cartilage. The gross morphology of articular cartilage of all freshly opened femorotibial joints was assessed using the scoring system described by Ghosh, *et al*[21].

Histological/histochemical and histomorphometric studies. Two 7 µm thick sections were cut from each paraffin block for staining with toluidine blue and Masson trichrome using standard procedures. However, for the toluidine blue/fast green counterstaining procedure, the method of Getzy, *et al*[34] was employed. The advantage of using the toluidine blue/fast green counterstain was that subtle differentiation of the difference in metachromasia associated with the variation of PG contents from the surface to the deep layer of articular cartilage was more evident. The Masson trichrome stain was used to distinguish the amount and distribution of collagen in calcified cartilage and subchondral bone from uncalcified cartilage. A modification[25] of the original Mankin scoring system[35] was used to assess the histological/histochemical grading of sections. This included scoring for cartilage structure (scores 0–10), cellularity and viability (scores 0–4), cell cloning (scores 0–4), territorial toluidine blue staining (scores 0–4), and interterritorial toluidine blue staining (scores 0–4). In addition for each section, the tide mark/calcified cartilage/subchondral bone changes were also scored (scores 0–3). For each section the most severely affected region (the lesion area) only was used for scoring. A maximum possible score for each section was 29.

Cartilage thickness. The uncalcified cartilage thickness of sections was determined using a 5× calibrated eyepiece graticule with 25 µm graduations (Graticules Ltd., Kent, England) and the 4× objective lens of a light microscope. The uncalcified cartilage was determined as the distance (µm) from the cartilage surface perpendicularly to the level of the most advanced tide mark. Ten measurements were recorded at regular intervals in each zone of the different regions (Figure 2).

Subchondral bone plate (SCP) thickness. The SCP thickness included the

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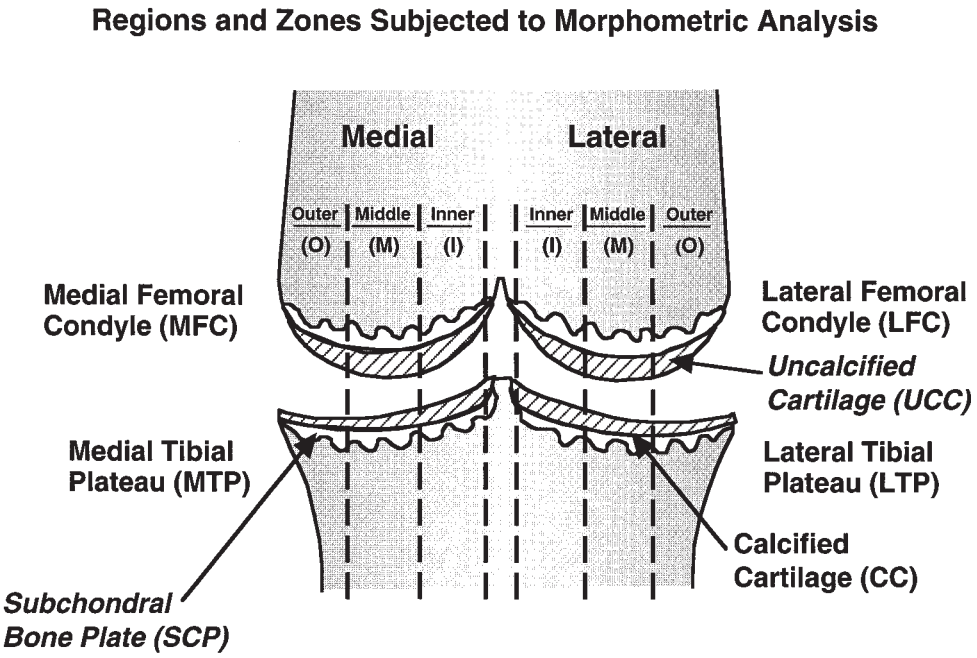

Figure 1. Coronal section through an ovine knee joint showing zonal regions of the joint compartments: inner (I), middle (M). and outer (O). Morphometric measurements of the uncalcified cartilage and subchondral bone plate, which included calcified cartilage, were made at 10 equally spaced points across each zone (see Figure 2).

whole layer of the calcified cartilage and subchondral cortical bone; it was measured as the distance below the level of the most advanced tide mark to the marrow space where the SCP ended and the trabecular bone network began (Figure 2). For differentiation of the vascular tunnels in the SCP from the marrow spaces, the horizontal width of the vascular tunnel that was greater than 500 µm in diameter was subjectively defined as the marrow space (Figure 2). When the trabeculae of the SCP extended farther down into the marrow space, the point of intersection of the tangents drawn to adjacent marrow spaces was considered to be the SCP lower boundary (Figure 2). This method was modified from that originally described by Armstrong[36]. The SCP thickness (µm) consisted therefore of the mean value of 10 readings in each zone (I, M, O) determined from the level of the most upper tide mark in the calcified cartilage to the upper boundary of the marrow spaces in the subchondral bone (Figure 2).

Bone mineral density (BMD) assessment. The BMD of a defined area in the tibial plateau and femoral condyle was measured and analyzed by a Hologic model QDR 4500/W X-ray bone densitometer (Hologic Inc., Waltham, MA, USA). Before measurement, a human lumbar spine phantom was used as a standard model to ensure that the correlation variance and confidence interval for the instrument were within specification. Because the sizes of the ovine tibial plateaus and femoral condyles were comparable to those of a human lumbar vertebral segment, the existing Hologic clinical software could be used to analyze BMD of the ovine specimens. To simulate the soft tissue background contribution of the human spine, rice granules were used both to position the specimen and to serve as the substitute analog of the periarticular soft tissues. Each ovine knee joint specimen was scanned from the top of the articular surface to 2.5 cm below the subchondral bone, tangentially in a supero-inferior direction. From the resulting image, the area of interest for BMD was selected as a rectangular area of 5 × 3 mm positioned in the center of each of the I, M, and O zones of the LTP and 3 × 3 mm in the corresponding zones of the LFC. The areas selected did not include osteophytes.

Statistical analysis. All histological scores and other measured variables were expressed as mean ± standard error of the mean (SEM) for each experimental group. For detecting the difference between data from the OA controls and diacerein treated OA animals, a nonparametric Mann-Whitney U test was used. The level of difference between groups was considered to be significant when $p \leq 0.05$.

RESULTS

Clinical assessment. After meniscectomy, wound healing was progressive and predictable except that some wounds initially developed subcutaneous seroma, which subsided within one week. Lameness or minor joint effusion was observed in some operated sheep; however, surgically induced wounds showed complete healing. Gait and feeding patterns returned to normal levels within 2 weeks of surgery. During the course of diacerein administration in the first 3 months, no gastrointestinal disturbances were observed. However, 2 sheep of the 9 month diacerein group given a daily dose of 50 mg/kg developed frequent diarrhea and poor feeding during the last month before sacrifice. For these animals diacerein treatment was terminated. One animal recovered from diarrhea, but the body weight had decreased from 45.5 to 40.0 kg over the 1 month prior to sacrifice. The other animal was euthanized due to intractably severe diarrhea accompanied by significant body weight loss (from 53.0 to 45.5 kg). This animal was excluded from the study. Since all the remaining animals in the 9 month diacerein treated group also showed a decrease in body weight (mean 3.7 ± 1.2 kg) in the final month before sacrifice, the 8 month drug treated animal was included in the study. The decision to include this animal was also influ-

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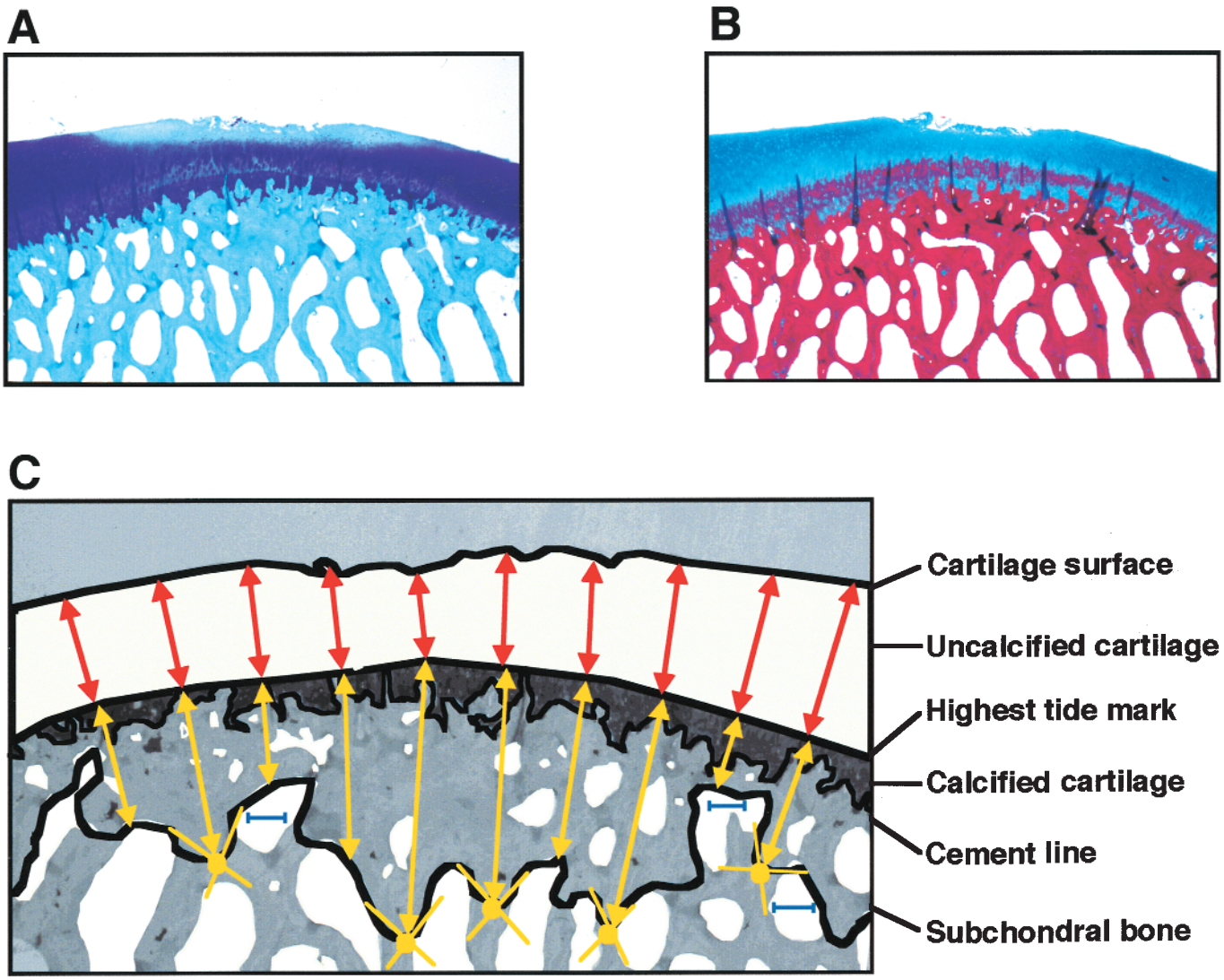

Figure 2. (A) Toluidine blue stained histological section of the middle (M) (lesion) zone of a lateral femoral condyle from a stifle joint of a 9 month diacerein treated animal showing surface fibrillation and loss of staining for proteoglycans and thickened subchondral bone plate (magnification ×16). (B) Equivalent Masson trichrome stained section of (A). (C) Diagram of the osteochondral section shown in (B), illustrating the method used to determine uncalcified cartilage, calcified cartilage, and SCP thickness. Red arrows perpendicular to the highest tide mark indicate where measurements of uncalcified cartilage thickness were determined. Yellow arrows perpendicular to the lower margin of the highest tide mark identify where the thickness of the SCP was assessed. The blue bars represent the arbitrary width used to distinguish a vessel space (≤ 500 µm) from a marrow space (≥ 500 µm), allowing charting of the lower edge of the SCP. The intersect points (yellow dots) of each pair of yellow lines drawn tangentially from the neighboring edges of the marrow spaces closest to the trabeculae of the SCP were taken as the lowest boundary of the trabeculae in the SCP.

enced by the need to preserve the statistical power of analysis. The non-diacerein treated meniscectomized and NOC control groups maintained their body weights over the 9 months of the study.

Gross morphology of articular cartilage. The cartilage surface of the knee joints from the meniscectomized groups (MEN and DIA) at 3 month and 9 month followup was typical of early OA changes and consistent with previous reports[21,25]. These changes included cartilage fibrillation, fissures, erosion, hypertrophy, and marginal osteophyte formation in the LTP and LFC. Topographically, the cartilage surface of the middle (M) zone, which was partially protected by the meniscus before surgery, appeared more severely fibrillated and locally eroded than other zones (Figure 2). The outer (O) zone, normally totally protected by the lateral meniscus, appeared more irregular and thickened. Osteophyte/chondrophyte formation was consistently noted around the inner and particularly the outer periarticular regions of the tibial plateaus of the meniscectomized joints. Using the gross morphological scoring system[21], no significant difference between the cartilage of the joints of diacerein and non-diacerein OA animals could be observed (data not shown). Apart from the scars arising from surgery the appearance of the synovium from the meniscectomized

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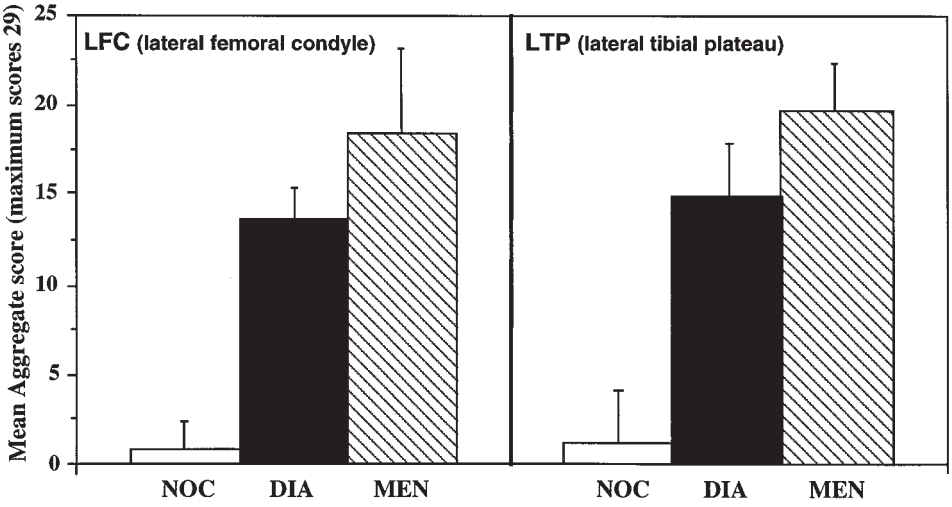

Figure 3. Mean (± SEM) aggregate modified Mankin scores[25] (maximum possible scores 29) for the middle (lesion) region of lateral femoral condyles and lateral tibial plateaus from non-operated controls (NOC) (white bars), OA animals treated with diacerein (DIA) (black bars), and non-drug-treated OA animals (MEN) (shaded bars) at 9 months.

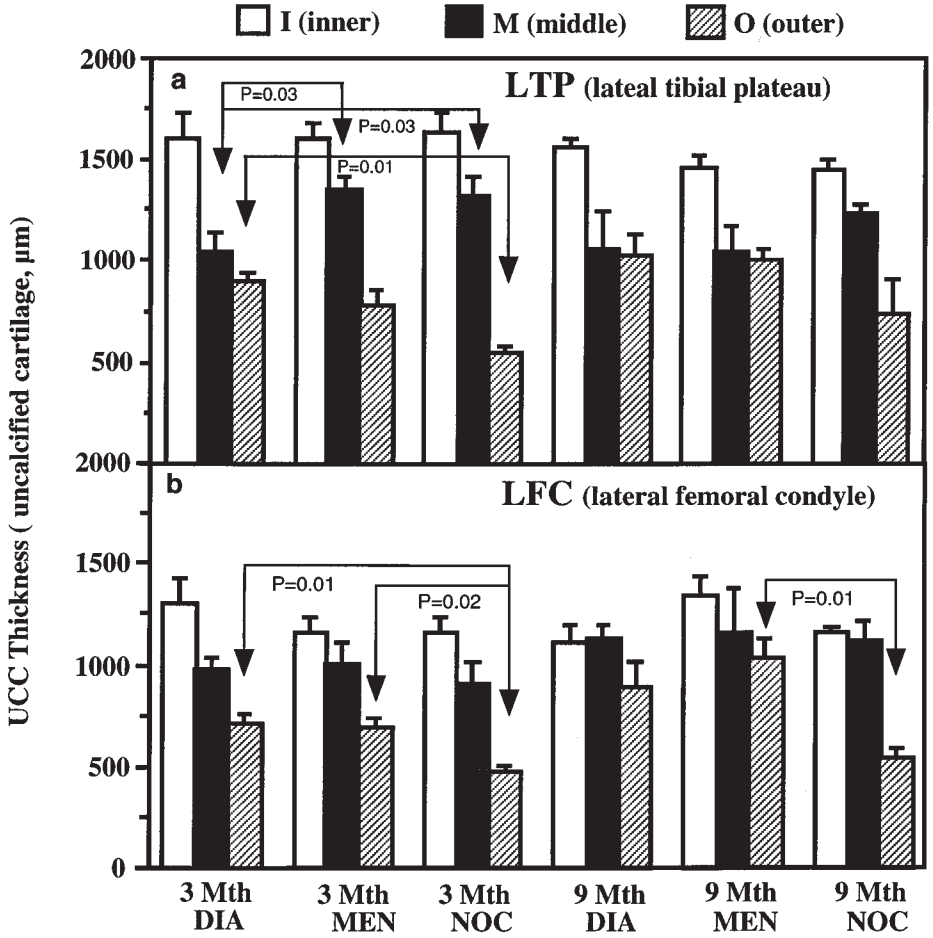

Figure 4. Histogram showing zonal changes in uncalcified cartilage thickness (as mean µm ± SEM) in the LTP (a) and LFC (b) of sheep joints from non-operated controls (NOC), OA controls (MEN), and diacerein treated OA group (DIA) post-meniscectomy. At 3 months, uncalcified cartilage thickness measurement of the O zone in the OA joints (LTP and LFC) of the DIA and MEN animals was significantly higher than the NOC, and uncalcified cartilage thickness of the M zone in LFC of the DIA treated animals decreased significantly more than the MEN group, but was not different from the NOC group. At 9 months, only the uncalcified cartilage thickness of the O zone in LFC of the MEN group was still significantly greater than the NOC group. P values indicate significant difference of the mean uncalcified cartilage thickness between groups for the same zones.

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joints showed no abnormalities or gross inflammatory changes (data not shown).

Histological grading scores of cartilage (Figure 3). Using the modified Mankin histological grading system[25], there was no statistical difference between the scores observed for the MEN and DIA groups, but the mean values for both groups were higher than the NOC means (data not shown). At 9 months, the mean aggregate histological scores of the LTP in the DIA were decreased relative to the MEN group, but again no statistical difference between groups could be determined.

Uncalcified cartilage (UCC) thickness (Figure 4). (A) In the lateral tibial plateau (LTP) at 3 months post-meniscectomy, the UCC thickness of the O zone in both the DIA and MEN groups increased significantly compared to the NOC group ($p = 0.01$; $p = 0.05$). However, the UCC thickness of the M zone in the DIA group was decreased compared to the MEN and NOC groups ($p = 0.03$; $p = 0.03$, respectively). At 9 months, there was no difference in the UCC thickness between the experimental groups. (B) In the lateral femoral condyle (LFC) at 3 and 9 months post-meniscectomy, the UCC thickness of the O zone in both the DIA ($p = 0.01$) and MEN ($p = 0.02$) groups was significantly increased relative to the NOC group, but there was no difference between the DIA and MEN groups.

Subchondral plate (SCP) thickness (Figure 5). (A) In the

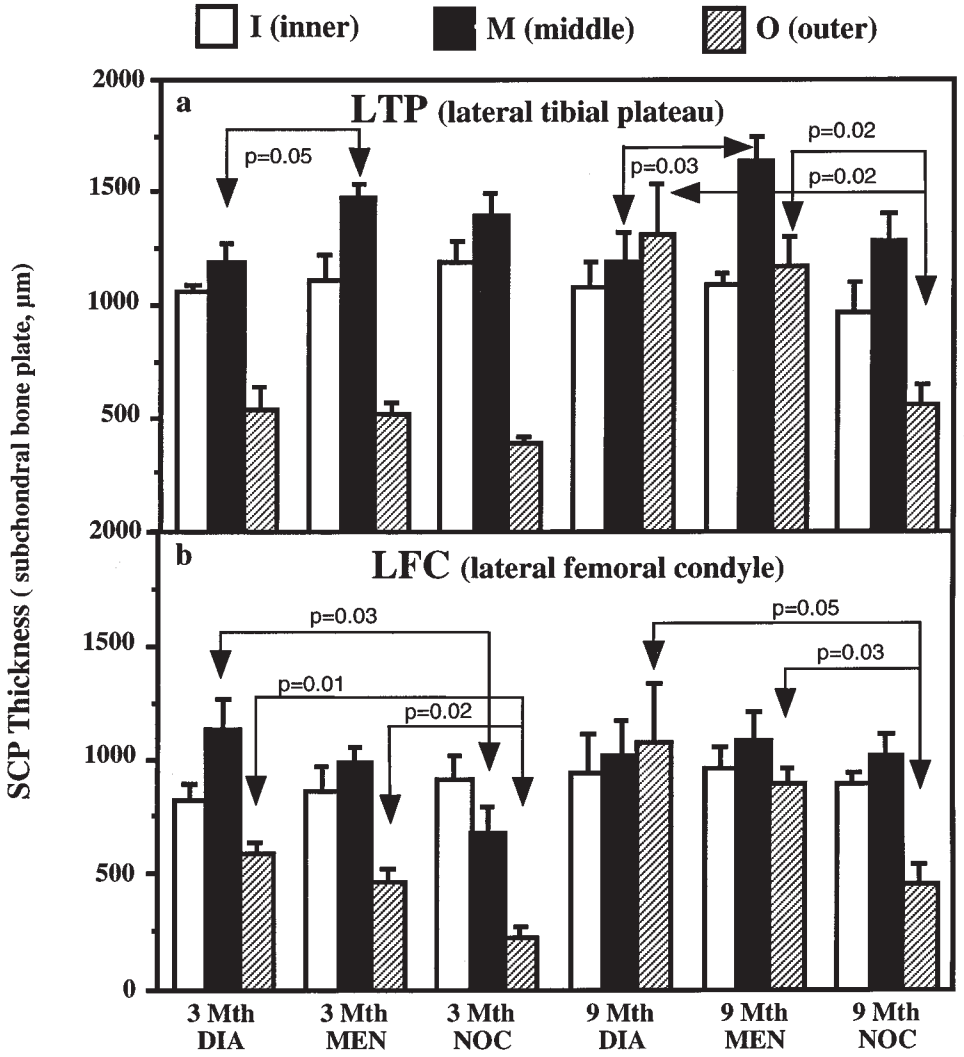

Figure 5. Histogram showing zonal changes of SCP thickness (as mean µm ± SEM) in the LTP (a) and LFC (b) of sheep joints from the non-operated controls (NOC), OA controls (MEN), and diacerein treated OA groups (DIA) post-meniscectomy. At 3 and 9 months, the SCP thickness of the M zone in the LTP of the DIA group decreased significantly more than in the MEN group, but was not different from the NOC group values. However, at 9 months, as for the LFC at 3 months, the SCP thickness of the O zone in the operated joints (LTP and LFC) of the DIA and MEN increased significantly more than the NOC group, but there was no difference between the DIA and MEN groups. P values indicate significant difference of the mean SCP thickness between the groups for the same zones.

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lateral tibial plateau (LTP) at 3 and 9 months post-meniscectomy, the SCP thickness of the M zone in the DIA group was significantly less (p = 0.05) than the same zone of the MEN group, but was not different from the NOC. At 9 months, the SCP thickness of the O zone in both the DIA (p = 0.03) and MEN (p = 0.02) groups was significantly greater than the NOC, but no difference between the DIA and MEN groups was observed. (B) In the lateral femoral condyle (LFC) at 3 months, the SCP thickness of the M zone in the DIA was significantly higher than for NOC (p = 0.03), but was not different from the MEN group. At 3 months post-meniscectomy, the SCP thickness of the O zone in both the DIA and MEN was significantly higher than the NOC group (p = 0.01; p = 0.02) as well as at 9 months (p = 0.05; p = 0.03). There was no statistical difference between the NOC, DIA, or MEN O zones in the LTP or LFC at either time period.

Bone mineral density (Figure 6). (A) In both the lateral tibial plateaus and lateral femoral condyles there was no significant zonal difference in BMD for the experimental groups after 3 months (data not shown). At 9 months, the BMD of the outer (p = 0.01) and middle (p = 0.05) zones in the MEN group was elevated relative to BMD of the same zones of the NOC group. However, the BMD of the O zone of LTP of the DIA group was not significantly different from that in the NOC and MEN groups. (B) In the LFC, at 9 months, the BMD of the O zone in the MEN group increased relative to the DIA (p = 0.05) and the NOC (p = 0.01) groups, but there was no difference between the DIA and NOC group BMD in this zone. BMD in the middle zone of the LFC was less than in the same zone for LTP of the meniscectomized groups (p = 0.05).

DISCUSSION

These studies show that bilateral lateral meniscectomy induced profound morphological and histological changes in cartilage and subchondral bone of ovine joints that were consistent with the early pathology of human OA[1-3]. Using a modification[25] of the histological grading system originally described by Mankin, *et al*[35], aggregate scores for the lesion areas (M zone) of the operated groups were significantly higher than the NOC, confirming loss of PG, cartilage fibrillation, cell cloning, and cell death. However, no statistical difference between the DIA and MEN at 3 or 9 months post-meniscectomy could be observed. In contrast, using a more comprehensive methodology in which cartilage and subchondral bone thickness (UCC and SCP) and BMD were determined zonally across the entire joint surface, significant effects of the drug could be observed. These observed changes in both UCC and SCP reflected the coordinated response of these tissues to the altered mechanical stresses introduced across the joint by excision of the lateral meniscus. This methodological approach also highlighted the advantages of simultaneously examining structural changes in joint cartilage and subchondral bone during the initiation and progression of OA, rather than studying cartilage alone.

As expected from the increased mechanical loading introduced by lateral meniscectomy, both cartilage and the SCP thickness in the outer region of the lateral compartment, which is normally covered by the meniscus, showed increases corresponding to a hypertrophic response of the resident cells to overloading. However, in these adult male animals, the cartilage reaction to the increased mechanical stresses appeared to precede the response that occurred in

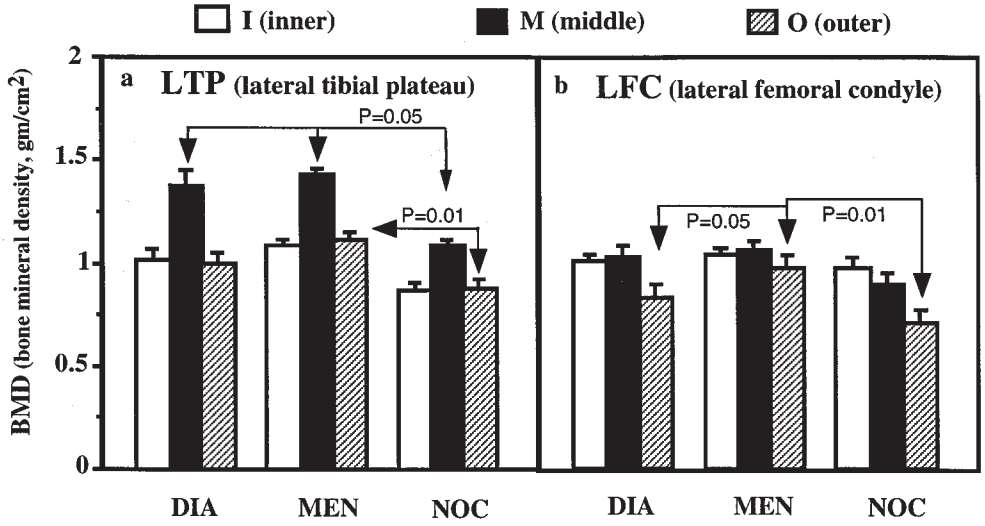

Figure 6. Histogram showing zonal changes of BMD (g/cm^2 ± SEM) in the LTP (a) and LFC (b) of sheep joints from the non-operated controls (NOC), OA controls (MEN), and diacerein treated OA groups (DIA) post-meniscectomy. There was no zonal difference of BMD at 3 months (data not shown). At 9 months, BMD of the middle zone of the LTP in the MEN and DIA groups increased significantly more than the NOC group, while BMD of the outer zone of the MEN group was also elevated. In the LFC, BMD of the O zone in the DIA and MEN groups was greater than the NOC group. P values indicate the significant difference of mean BMD values between the groups for the same zones.

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the SCP during the first 3 months post-meniscectomy. This was particularly evident for the LTP. By 9 months, proliferation of the SCP was well advanced in the outer zone and middle regions, both of which are normally protected by the overlying lateral meniscus. The observed subchondral bone changes were supported by an increase in BMD in the outer and middle zones of the LTP at 9 months post-meniscectomy. This finding was consistent with the more rapid progression of OA on the tibial plateau than on femoral condyles as reported by others[37].

The SCP thickness in the middle zone or the LTP of animals treated with diacerein did not exhibit the increased thickness observed for the corresponding region of non-drug-treated OA animals. These differences were not observed for the LFC, again illustrating the variable tissue response across the joint[37]. The mechanism(s) responsible for this effect of DIA on subchondral plate reaction to supra-physiological mechanical stresses is unclear. It is possible that the diacerein had a direct effect on bone cell metabolism since it is reported to reduce interleukin 1 (IL-1) levels[38,39] while stimulating prostaglandin activity[40,41]. The IL-1 family of cytokines are potent stimulators of osteoclastic bone resorption[42] and any reduction in this activity by diacerein could be beneficial to bone formation. Prostaglandins of the E series are also known to both promote bone resorption and attenuate osteocyte activity, but the relative effects are concentration and species dependent[42]. Moreover, the BMD studies confirmed that generalized bone resorption was not associated with use of the drug. The reported ability of diacerein to increase TGF-ß production by chondrocytes[33] could also contribute to the maintenance of bone metabolism if the drug produced a similar effect in bone cells. Additional studies will be necessary to resolve these speculative alternatives.

However, it should also be noted that osteophytes also arise as a physiological response by joint tissues to abnormal mechanical stresses[1-3]. Their formation following meniscectomy must be seen as an adaptive reaction that would serve to stabilize the joint in the absence of the meniscus. Diacerein did not appear to prevent osteophyte/chondrophyte formation at the margins (outer region O) of the joint, and BMD values in these regions were also marginally higher than in the NOC controls, suggesting that the drug did not inhibit the metabolic process involved in endochondral ossification in contrast to subchondral plate remodelling.

Other studies with diacerein in animal models of OA have shown a variable response to the drug. Carney,[43] reported that the diacerein preserved levels of collagen in femoral joint cartilage of a beagle OA model, but did not significantly affect the synthesis of this protein or glycosaminoglycans. Moore, *et al*[28] reported that diacerein had a chondroprotective effect in a murine model of granuloma induced cartilage breakdown and concluded that this effect was mediated by a reduction in proinflammatory cytokines. Brandt, *et al*[44] failed to observe any significant effect of diacerein in cartilage matrix metalloproteinase (MMP) activity in a rapidly progressive canine model of OA induced by transection of the anterior cruciate ligament (ACL). However, in some diacerein treated animals fibrillation and erosion of cartilage and levels of collagenase in extracts were lower than in non-drug-treated OA controls[44]. In a later study, Smith, *et al*[29] reported that diacerein retarded the progression of the cartilage lesions in the canine ACL transection model.

In vitro studies have shown that diacerein could potentially provide some protection to articular cartilage by decreasing the production of collagenolytic proteinase induced by IL-1[31-33]. This anti-IL-1 activity was more recently confirmed and extended by Martel-Pelletier and coworkers using synovial cells and chondrocytes from human OA joints[38]. This group showed that diacerein and its metabolite, rhein, reduced the synthesis of IL-1 but not tumor necrosis factor-α (TNF-α) in synovial cells and attenuated the binding of IL-1 to OA chondrocytes. In a more recent study[45] it was shown that diacerein reduced the levels of IL-1 and IL-18 in human OA cartilage by decreasing the availability of the IL-1 converting enzyme, as shown by immunolocalization of the protein and ELISA. Yaron, *et al*[39] examined human synovial and cartilage explants from OA joints and confirmed that diacerein and rhein not only diminished IL-1 synthesis but also lowered nitric oxide free radical production when used at concentrations of 10^{-6} M in their culture system.

Interestingly, diacerein and its metabolite rhein, in contrast to NSAID, do not suppress prostaglandin E_2 (PGE_2) production by chondrocytes and other cells when used *in vitro* at therapeutic equivalent concentrations. Indeed, in rat and guinea pig tissues PGE_2 synthesis is stimulated[40,41]. Since PGE_2 is an important regulator of cell activity, including the downregulation of IL-1ß, nitric oxide synthase, and MMP[46,47] and increased production of collagen by chondrocytes[48], it is possible that diacerein exerts its pharmacological effects on cartilage and bone by increasing PGE_2 production while decreasing IL-1ß levels. However, the ability of diacerein and related anthrones to stimulate prostaglandin production in mucosal tissues may also account for the diarrhea and weight loss observed for some of the 9 month treated animals[49].

The recommended human oral dose of diacerein for symptomatic relief in OA is 100 mg per day. While this dose is reported to be statistically superior to placebo in longterm double blind trials[27,50], 8–50% of patients receiving this dose experienced diarrhea, loose stools, and sometimes abdominal cramps[50]. It is not unexpected therefore that with the very high dose of diacerein used in our study (roughly 25 times the human dose over 6 months) chronic diarrhea was a common side effect. Notwithstanding this problem, diac-

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erein was shown in 4/5 of the animals who completed the study to exhibit some selective effects on cartilage and subchondral bone that were suggestive of disease modification. While the drug failed to diminish the hypertrophic response of cartilage or prevented subchondral bone proliferation at the outer joint margin, in the middle zone of the tibial plateau, a region of high focal stress following meniscectomy, both cartilage and subchondral plate thickness were lower than in the non-diacerein-treated OA group.

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