Microcrack density and length in the proximal and distal metaphyses of the humerus and radius in dogs

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Objective—To compare microcrack density and length in the proximal and distal metaphyses of the humerus and radius in dogs.

Sample Population—Left humerus and radius from each of 10 dogs of medium to large size.

Procedure—Metaphyseal specimens were bulk stained in 1% basic fuchsin in graded alcohols and embedded in methylmethacrylate. For quantification of fatigue-induced microscopic damage, transverse sections were prepared from proximal and distal metaphyseal regions, and length and density of microcracks were determined, using light microscopy.

Results—Bone region, age, and body weight were not significantly associated with microcrack density or length.

Conclusions and Clinical Relevance—The hypothesis that fatigue-induced injury (increased microcrack density and length) caused by cyclic loading associated with daily activity is greater in bone regions prone to development of osteosarcoma was not supported by data from this study. (*Am J Vet Res* 2000;61:6–8)

Fatigue-related microdamage in cortical bone has been revealed by detection of microcracks with light microscopy and is caused in vivo by repetitive physiologic loading.[1,2] Microcracks in cortical bone may develop first in regions of high local strain[3]; accumulation and coalescence of microcracks will eventually result in fatigue or stress fracture of bone.[4] Haversian bone is considered to be a fiber-reinforced composite material in which osteonal cement lines inhibit crack growth and contribute to fatigue resistance.[5] Although induction of microcracks provides a stimulus for cellular differentiation and microcrack repair via formation of secondary osteons,[1,6] fatigue-induced microcracks accumulate with increasing age in human bone,[7] because remodeling of fatigue-induced microdamage is incomplete.[8]

Osteosarcoma in dogs shares many features with osteosarcoma in humans, including male sex predilection and large patient size; in 75% of cases, appendicular sites are affected. However, osteosarcoma is more common in dogs and typically affects middle-aged dogs, in contrast to adolescent humans.[9] Large patient size is considered an important biological feature in dogs; the pattern of primary bone neoplasia in toy-breed dogs is quite different.[10] Although many risk factors for osteosarcoma in dogs and humans have been studied, including familial incidence, preexisting bone defects, multiple cartilaginous exostoses, and fracture-induced trauma, the cause of osteosarcoma in dogs is unknown.[11]

Osteosarcoma develops most commonly in long-bone metaphyses with late-closing physes in limbs that receive the greatest weight-bearing stresses during daily activity; these are the pelvic limb in humans and the thoracic limb in dogs.[11] The distal portion of the femur and the proximal portion of the tibia are the 2 most common sites in humans, and the proximal portion of the humerus and the distal portion of the radius are the 2 most common sites in dogs.[11,12] It has been hypothesized that cyclic loading of bone associated with daily activity may be an important risk factor for induction of osteosarcoma in dogs.[12] The objective of the study reported here was to test this hypothesis by comparing microcrack density and microcrack length in the proximal and distal metaphyses of the humerus and radius in dogs.

Materials and Methods

Specimens—The left humerus and radius were collected from 10 adult dogs of medium to large size and of various breeds; dogs were euthanatized for reasons unrelated to the study. Body weight and age were recorded.

Histomorphometry—Proximal and distal metaphyseal segments were excised from each bone, defatted in chloroform for 2 days, and fixed in 70% ethanol. Specimens were then bulk-stained in 1% basic fuchsin[13,14] by sequential staining in 80, 90, and 100% ethanol for 18 days under a vacuum of 20 mm Hg. Bone segments were rinsed in 100% ethanol, infiltrated with methylmethacrylate monomer for 4 hours under a vacuum of 20 mm Hg, and embedded in methyl methacrylate.

Transverse sections were cut perpendicular to the long axis of the shaft of the bone from each metaphysis with a diamond saw[a] and reduced to a thickness of 150 µm by use of a grinder.[b] Transverse sections were cut at 15 and 85% of bone length. Cortical areas were measured by computerized image analysis[c] after images of entire transverse sections were captured, using a color videocamera. The entire surface of single transverse sections was examined for microcracks by a single observer (PM). Microcrack length was measured by use of an eye-piece grid. Stained microcracks (**Fig 1**) were identified by detection of sharp borders, observance of depth of field, and permeation of the stain into the bone matrix, forming the walls of the crack to create a halo of basic fuchsin staining[2,13,15] and categorized into 4 locations[7] (within interstitial or lamellar bone, extending from interstitial or lamellar bone into or across cement lines, extending along cement lines, within osteons). Microcrack number and microcrack length (µm)

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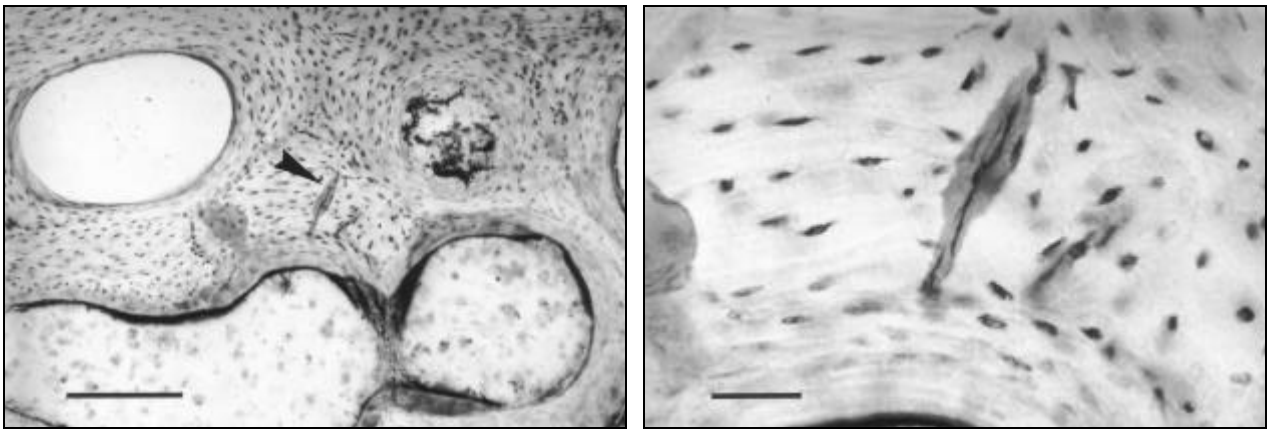

Figure 1—(A) Photomicrograph of a 150-μm-thick transverse cross-section of the proximal humeral metaphysis of a dog. Notice stained microcrack in interstitial bone (arrowhead). Basic fuchsin stain. Bar = 250 μm. (B) High-magnification photomicrograph of microcrack in A. Notice stained microcrack that consists of an array of smaller microcracks that extend through osteocyte lacunae. Basic fuchsin stain. Bar = 50 μm.

were determined. Because the bone specimens were bulk-stained before preparation of transverse sections, microcracks that were induced in vivo were stained, whereas microcracks associated with tissue processing were unstained.[13,14]

Statistical analyses—Microcrack density (microcracks/mm^2), microcrack length, and prevalence of microcrack type were determined for each bone region (proximal humeral metaphysis, distal humeral metaphysis, proximal radial metaphysis, and distal radial metaphysis). Data were expressed as mean ± SD. Repeated-measures ANCOVA was used to evaluate association of bone region with microcrack density and length. Dog age and weight were analyzed as covariates. Repeated-measures ANOVA also was used to evaluate association of cortical location with microcrack length. The Friedman test was used to compare prevalence of crack location between and within bone regions. Differences were considered significant at $P < 0.05$. When certain differences were not significant, the **percentage difference between populations (D)** that would yield a power of 0.8 was calculated.[16]

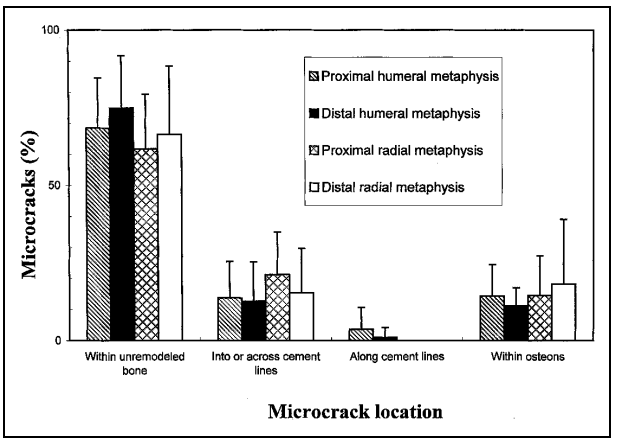

Figure 2—Percentage distribution (mean ± SD) of location of microcracks in the proximal and distal metaphyses of the radius and humerus in dogs.

Results

All dogs were skeletally mature. Age (99 ± 34 months) ranged from 46 to 144 months, and body weight (22.5 ± 11.2 kg) ranged from 11.5 to 44.0 kg. None of the dogs had undertaken regular athletic training or racing activity.

Cortical bone observed in metaphyseal sections was lamellar bone with secondary osteonal remodeling. Microcrack densities (microcracks/mm^2) were determined: proximal humeral metaphysis, 0.20 ± 0.09; distal humeral metaphysis, 0.16 ± 0.08; proximal radial metaphysis, 0.17 ± 0.08; distal radial metaphysis, 0.21 ± 0.13. Microcrack lengths (μm) were determined: proximal humeral metaphysis, 74 ± 16; distal humeral metaphysis, 82 ± 10; proximal radial metaphysis, 80 ± 13; distal radial metaphysis, 87 ± 22. Bone region did not have a significant association with microcrack density (power = 0.8 at Δ = 45%) or microcrack length (power = 0.8 at Δ = 16%). Furthermore, age and body weight did not have significant association with microcrack density or microcrack length. As evaluated by high magnification of stained histologic sections, microscopic bone damage often appeared as an array of small microcracks (Fig 1). Location of microcracks with reference to osteons was not significantly different between bone regions (**Fig 2**). Cortical location had a significant effect on number of microcracks; numbers of microcracks located within interstitial or lamellar bone were increased, compared with other locations. Numbers of microcracks extending along cement lines were decreased, compared with other locations. Intraosteonal microcracks were significantly shorter than microcracks located within interstitial or lamellar bone.

Discussion

In vivo microscopic cracks in compact bone stained by basic fuchsin were first described in 1960 by Frost,[2] who suggested that these microcracks represented failure of the bone matrix because of fatigue. Other investigators have subsequently detected in vivo microscopic damage caused by physiologic loading in human bone.[7] Microscopic damage caused by experimental cyclic loading has been detected in canine bone,[1,6] and we have identified microdamage in canine bone resulting from physiologic cyclic loading. In vitro loading of compact bone causing strain similar to physiologic values causes development of microcracks similar to those seen in human bone.[17]

Our results indicated that, in dogs, fatigue microcrack density in bone was not significantly higher in metaphyseal regions that are considered to have predilection for development of osteosarcoma than other regions. Therefore, although fatigue injury to compact bone as a result of cyclic loading has been suggested as a possible mechanism for induction for canine osteosarcoma,[12] other risk factors, such as familial incidence, association with late-closing physes, and bone trauma may be more important and require further study.[11,12]

Many authors have suggested that bone remodeling is a repair process intended to remove microcracks in bones that develop in vivo because of cyclic loading[8]; this process has been termed bone maintenance.[18] Mean microcrack lengths of 58 μm for canine bone, 88 μm for human bone, and 80 μm for bovine bone[6,13,17] have been measured in transverse bone sections; values reported in our study were similar to those reported for human and bovine bone and higher than published canine values.[6] The direction of microcracks may influence their 3-dimensional shape and their length as measured by use of standard light microscopy techniques, but recently, 3-dimensional imaging of microcracks using confocal microscopy has been described.[19] Theoretically, the lengths of microcracks in bone will be constant for given stress conditions.[18] Mean length of microcracks in the bones of the study reported here were within the expected range that would result from physiologic stresses that stimulate adaptive remodeling for bone maintenance.[18]

Microcrack densities of human rib bones (0.11 to 0.14/mm^2),[13,20] human femoral diaphysis (6/mm^2),[7] and canine radial diaphysis (0.06/mm^2) after experimental bone loading[1,6] have also been measured in transverse bone sections. Microcrack densities of the long bone metaphyses of dogs reported here were similar to densities reported for human bone, but they were higher than densities reported for canine bone, possibly because of differences between metaphyseal and diaphyseal bone or differences in histomorphometric assessment techniques.

There is evidence that microcracks accumulate with increasing age in human bone[2,7] because of incomplete remodeling of fatigue-induced microscopic damage; however, results of our study did not indicate accumulation of microcracking with increasing age. Although cortical bone from elderly dogs has many histologic features similar to those of bone from elderly humans,[21] elderly dogs, unlike humans, are not commonly affected with fractures that result from minimal trauma; species differences in completeness of microcrack remodeling in osteonal bone have not been investigated. Higher activation frequency of osteonal remodeling in canine bone could explain our failure to detect significant differences in microcrack densities between metaphyseal regions.

Distribution of microcracks within cortical bone was similar to that observed in other studies[7]; most microcracks were located in interstitial or lamellar bone or extended into cement lines. Microcracks appear to develop preferentially in areas of old bone tissue that remain from previous modeling and remodeling cycles.[7] Cement lines are a weak interface within compact bone and may serve as sites of crack initiation as well as crack arrest.[3,5,17]

[a]Isomet 2000, Buehler, Lake Bluff, Ill.
[b]Motopol 2000, Buehler, Lake Bluff, Ill.
[c]Optilab Image Analysis, Graftec, Mendon-La-Foret, France.

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