Colchicine Inhibits Heterotopic Ossification: Experimental Study in Rabbits

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Abstract

Background: Heterotopic ossification is a common complication of hip surgery and musculoskeletal or brain trauma.

Objectives: To confirm by *in vivo* study that colchicine inhibits osteoblast cell proliferation with marked decrease in tissue mineralization.

Methods: Heterotopic ossification was induced in three groups of New Zealand white rabbits (females, 6 months old, weight 3–3.5 kg) by injecting 2 ml bone marrow drawn from the iliac crest into their right thigh muscle. To prevent heterotopic ossification, colchicine (0.25 mg/day) was administered orally for 4 weeks to two groups of adult rabbits: group A (preload group) – 1 week preceding bone marrow injection; group B – on day of injection; and group C – control group.

Results: After 4 weeks the rabbits were evaluated by radiographs and ultrasound for evidence of heterotopic ossification. At the end of the study histologic samples were taken from all the thighs. Imaging and histologic studies showed, with statistical significance, almost complete prevention of heterotopic ossification formation in group A (preload) and a marked decrease in group B, when compared with the controls where large new bone had formed at the injection site. These results indicated the inhibitory effects of colchicine on a bone-forming process in soft tissue such as heterotopic ossification.

Conclusions: The role of colchicine in preventing heterotopic ossification in other bone-forming conditions, such as hip arthroplasty or pelvic trauma, and after brain trauma, remains to be evaluated in a clinical setting.

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Heterotopic ossification is a common complication of injury to musculoskeletal tissues, such as direct trauma, burns, head trauma, pelvic fractures, and hip arthroplasties [1,2]. The exact mechanism responsible for each of these pathologic new bone formations is not clear [3,4].

Various medications, e.g., aspirin, indomethacin, diclofenac and/or radiation protocols with 500–1,000 rads of radiation, have been tried, especially after hip arthroplasty, to decrease the occurrence and implications of heterotopic ossification [5–7]. These treatment modalities have side effects and complications [5,8–10].

Currently, colchicine is used most frequently for the treatment of gout (Paget's disease) and familial Mediterranean fever. Previous clinical reports have shown that patients who received colchicine on a daily prophylactic basis against attacks of FMF did not develop heterotopic ossification after hip arthroplasty [11]. A recent publication reported that, *in vitro*, colchicine caused strong inhibition of osteoblast cell proliferation with marked decrease in tissue mineralization [12]. The present controlled animal study was conducted to confirm *in vivo* studies of these clinical and *in vitro* observations.

Materials and Methods

The study comprised three groups each of 12 female adult New Zealand white rabbits (6 months old, weight 3–3.5 kg). Authorization was obtained from the local Helsinki Committee for Animal Research.

Induction of heterotopic ossification in the animals was performed under general anesthesia and sterile conditions by intramuscular injection of 2 ml autologous bone marrow (aspirated from the iliac crest) into the middle of the lateral part of the right thigh muscle (the length of the thigh was measured by roller). This amount of bone marrow assures a high concentration of stem cells, which is a modification from other known models [4,6,13–16]. Group A (the preload group) started colchicine 1 week before heterotopic ossification induction, group B began colchicine on the day of heterotopic ossification induction, and group C (control group) received no colchicine after heterotopic ossification induction. Colchicine (0.25 mg/day) was administered orally via a feeding tube to ensure complete consumption.

Standard radiographs and ultrasound, using the Aloka SSD-500 apparatus (Japan) of 5–7.5 mHz, were performed after 4 weeks. A preliminary pilot study found this period to be the shortest time in which heterotopic ossification can be clearly diagnosed radiographically or sonographically.

Blood samples for $Ca+^{2}$, alkaline phosphatase, bone-forming factors, i.e., osteogenic growth peptide and basic fibroblast growth factor were obtained on days 0, 1, 3, 7, and 28 of the study [17]. Histologic specimens from the injected thigh were obtained on day 28.

FMF = familial Mediterranean fever

After 4 weeks the animals were sacrificed by i.m. injection of overdose of pentothal. The amount of heterotopic ossification was evaluated qualitatively by radiographs and quantitatively by ultrasound. The heterotopic ossification on the radiographs was interpreted independently and blindly by three radiologists, as a) no bone formation, if there was no radiographic evidence of heterotopic ossification whatsoever; b) mild, if there were just scant signs of heterotopic ossification; and c) marked heterotopic ossification in cases of clear radiographic evidence of new bone formation [Figures 1–3].

Ultrasound facilitated good qualitative measurements of the size (volume) of the heterotopic ossification, because the differences in the medium of cortical bone, muscle, and heterotopic ossification are easily distinguishable on sonography. Sonographic volumetric studies were performed by expert radiologists, with three measurements for each sample. Cross-sections and longitudinal cuts of the samples were stained with hematoxylin and eosin and with alizarin red.

Continuous variables are presented as mean $\pm$ standard deviation. Student's *t*-test was used for comparison between the groups. Fisher's exact test was used for comparison of the non-parametric variables.

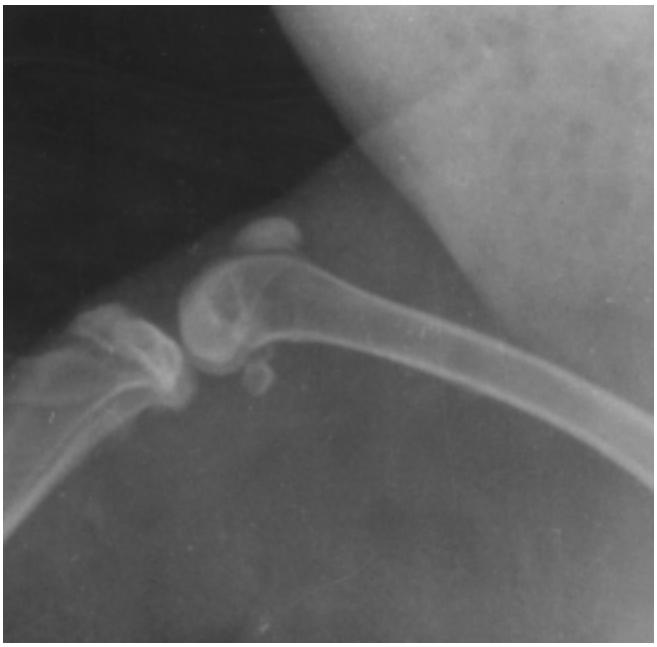

Figure 1. Radiograph of the femur of an animal from Group A (preload) shows no bone formation.

Results

All the animals survived the experimental period and were available for study. No adverse reactions to colchicine occurred in the rabbits.

Imaging studies in group A showed no bone formation in 11 of 12 rabbits [Figure 1] and only one had mild heterotopic ossification. In group B, mild bone formation was detected in 3 of 12 rabbits and no bone formation occurred in the remaining 9 [Figure 2]. In group C, marked bone formation was noted in 10 of 12 rabbits, and mild heterotopic ossification was discernible in the other 2 animals [Figure 3]. Ultrasonographic measurements of the volume of new bone formation revealed mean 8.3 $\pm$ 0.7 ml in group C (control, non-treated group) compared with mean 3.2 $\pm$ 0.31 ml in the only rabbit in group A, and mean 3.7 $\pm$ 0.23 ml in group B (only three rabbits).

The differences in the formation of heterotopic ossification in the three groups were statistically significant ($P < 0.05$). The interobserver variability was not significant. These findings were confirmed by histologic studies of tissue taken from the thighs of the affected limb on day 28. The histologic studies showed new bone formation in the muscles of the control group [Figure 4] with new bone marrow, compared with only a mild inflammatory reaction in group A.

The results of the biochemical assays were not consistent. A large variability within the groups reduced any statistical validation. This inconsistent pattern was seen throughout the study in the Ca^{+2} and alkaline phosphatase results, and in BFF assays.

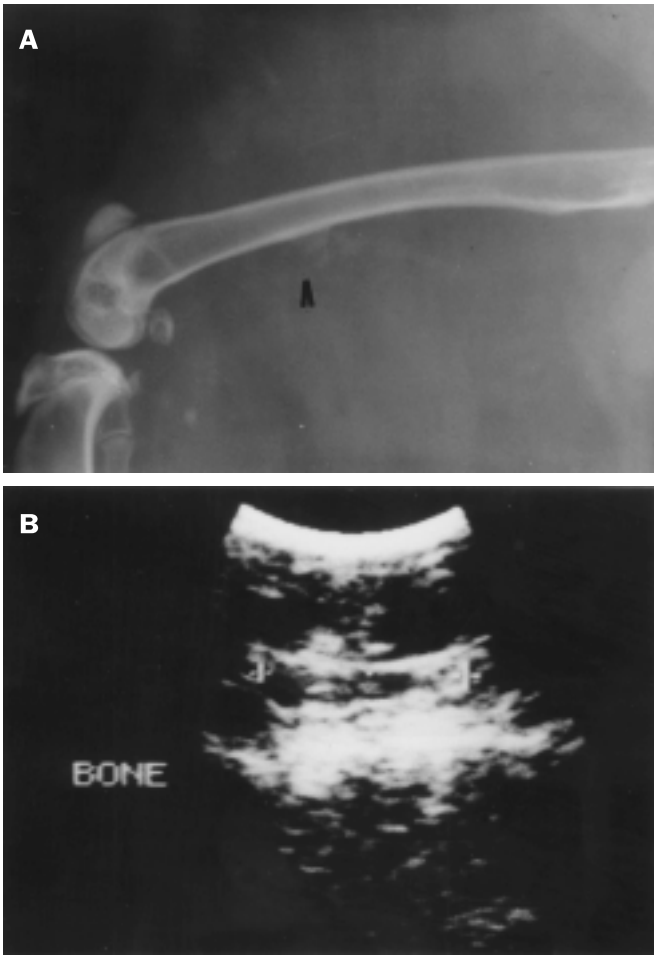

Figure 2. Mild bone formation is shown in an animal from group B in the **[A]** radiograph (arrow) and in the **[B]** ultrasonogram.

Discussion

The exact mechanism of heterotopic ossification formation is vague. While extensive clinical experience and data on the formation of heterotopic ossification were collected, the exact cellular processes involved remain undetermined [16,18].

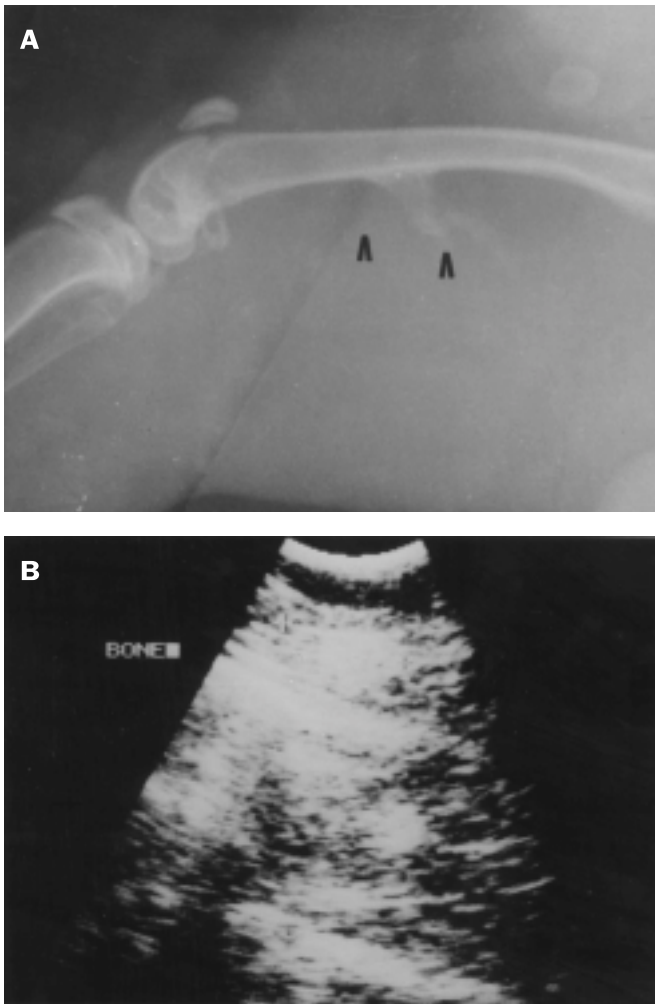

Figure 3. Marked bone formation can be seen in an animal from the control group in the **[A]** radiograph and in the **[B]** ultrasonogram.

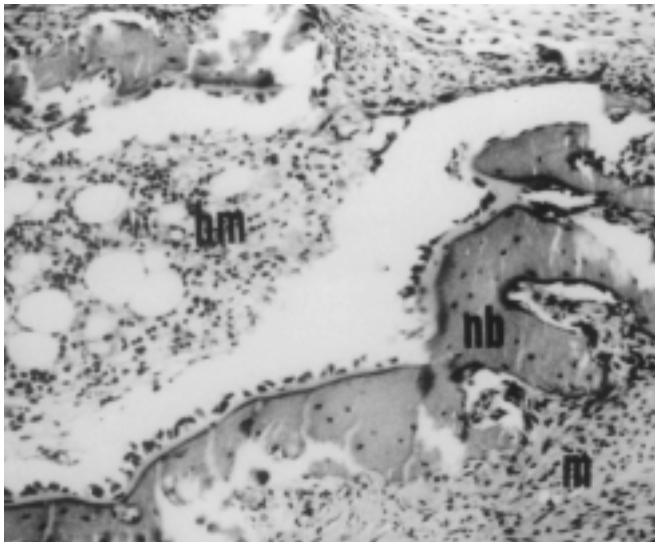

Figure 4. Bone formation in the muscle of an animal from the control group is shown, stained with hematoxylin and eosin, magnification x150. bm = bone marrow, nb = new bone, m = muscle.

In hip surgery (arthroplasty or pelvic fracture repair) it has been assumed that tissue osteoinductive factors, such as bone morphogenic proteins, activate the spilled bone marrow stem cells and are responsible for heterotopic ossification formation. The mechanism is even more obscure in cases of brain traumas, or trauma to soft tissue without fractures where no marrow spillage has occurred but heterotopic ossification nevertheless developed [15]. Accordingly, the treatment regimens currently in use are not specifically directed towards the process of heterotopic ossification formation. Radiation is a non-selective cell proliferation inhibitor, whereas the non-steroidal anti-inflammatory drugs inhibit the inflammatory process that precedes it, e.g., callus formation. Each of these treatment modalities has its inherent risks. First, the use of NSAIDs carries considerable risk of gastrointestinal irritation and bleeding [8,13,19,20]. Second, a single or fractionated dose of irradiation, as much as 1,000 rads, presumably prevents the pleuri-potential mesenchymal cells from proliferation into bone-forming cells; however, this method carries the potential risk of malignancy, i.e., post-irradiation sarcomas, and jeopardy of fracture unions. Third, diphosphonates were reported to prevent temporary hydroxyapatite crystal growth when administered for a long period; nonetheless, diphosophantes bear the risk for osteomalacia and gastrointestinal irritation [21].

Colchicine, used in the current study, presumably approached heterotopic ossification formation in soft tissue from other stages of its development, namely, selective interference with mineralization and non-selective prevention of cell division at the stage of metaphase, as also seen in an experimental *in vitro* study by Salai et al. [12]. Colchicine also exerts anti-inflammatory effects through interference with leukocyte mobilization during the inflammatory phase of heterotopic ossification [22].

As shown in the current study, prevention of heterotopic ossification formation was more pronounced when colchicine was administered prophylactically (group A, preload), as previously observed in patients with FMF [11].

Colchicine treatment is inexpensive, simple, and safe in terms of its potential side effects, mainly hemorrhagic gastroenteritis, leukopenia, aplastic anemia, myopathy, or peripheral neuropathy. The authors' clinical experience with colchicine treatment in patients with FMF demonstrated that these potential side effects are rare and easily controlled, usually by reducing the daily dosage of the drug. No such side effects were seen in the rabbits in this study. Blood samples, especially for osteogenic growth peptide and basic fibroblast growth factor, obtained during the study showed no correlation between biochemical changes and the clinical, radiographic and histologic changes among the three study groups. These findings may indicate that a different process involving various cells and tissue factors may be responsible for heterotopic ossification formation in soft tissue, compared with juxta-osseal bone formation or fracture healing, although the final outcome of bone formation is the same.

The potential clinical applications of our findings are: a) prophylactic treatment of patients with previous medical history

NSAIDs = non-steroidal anti-inflammatory drugs

of heterotopic ossification, after hip arthroplasty; b) immediate treatment of patients with pelvic fractures; c) for patients with extensive soft tissue injury to skeletal muscles prone to development of heterotopic ossification, thigh muscles or burns; and d) for head injury in patients in whom the mechanism of heterotopic ossification formation is even more obscure.

Controlled clinical research is necessary to confirm the results of the present study for the clinical treatment of patients with the potential for development of heterotopic ossification.

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Capsule

Epidural and cesarean – no thanks

In a meta-analysis of seven randomized controlled trials that compared epidural analgesia versus parenteral opioids, the odds of cesarean section were not different between the two groups. Risk for instrumental vaginal delivery (forceps, vacuum) was higher in the epidural group but without effect on neonatal outcome.

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E. Zimlichman

Capsule

Preventing *Helicobacter pylori* infection?

Helicobacter pylori infects about one in every two individuals and can cause gastric malignancy and stomach ulcers. However, given the large population that is infected, the small fraction that exhibit significant pathology suggests that mucosal defense in the stomach normally contains the pathogenic activity of *H. pylori*. Kawakubo et al. found a component of the gastric mucins, the mucin-type O-glycan, that occurs within deeper regions of the gastric mucosa and that can inhibit growth and motility of *H. pylori* in culture by interfering with cell-wall biosynthesis. Thus, cells of the gastric mucosa appear to protect themselves from *H. pylori* infection by secreting O-glycans that possess strong antibiotic activity.

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E. Israeli