INTRODUCTION:
The prevention and management of heterotopic ossification (HO) is of significant clinical relevance. Current treatment methods include radiation and/or pharmacological therapy with cyclooxygenase (COX) inhibitors. Current NSAIDs inhibit both COX-1 and COX-2 enzymes, which can lead to serious side effects on the gastrointestinal (GI) tract, such as GI bleeding. Recently, the drug celecoxib has gained popularity due to its specificity toward the COX-2 enzyme. Because of its efficacy in reducing prostaglandin formation and the associated inflammatory response, it is expected that such a drug would be just as effective in reducing HO with fewer side effects than the current NSAID drugs. In fact, we have previously shown that COX-2 inhibitors do inhibit HO in a rat model. Currently, we are comparing both COX-2 and COX-1 specific inhibitors in their effects on PGE2 synthesis and HO formation in a single-injury rat model. Our objectives were the following: (1) To quantify the amount of HO using a microCT analysis procedure; (2) To quantify the amount of prostaglandin formation at different time periods following surgery in order to show that prostaglandin formation becomes elevated before HO occurs; (3) To compare the effects of COX-1, COX-2, and nonspecific COX inhibitors on PGE2 formation and ectopic ossification in the rat model. The overall goal was to (a) characterize the temporal changes in prostaglandin synthesis that precede ectopic bone formation in a new HO model; and (b) determine whether or not HO formed in this model could be reduced by the prophylactic administration of COX 1 and COX 2 inhibitors in order to define the roles of prostaglandin synthesis, COX 1 and COX 2 in the HO process.

METHODS:
The animal model that we used was an IUCAC approved Lewis rat model, in which rats were subjected to soft tissue injury in the area of the quadriceps to induce HO. Both the right and left lower limbs of rats underwent a surgical procedure in which the ventral side of the femur was scraped to injure the periosteum. Kocher clamps were then placed across the vastus intermedius for ten minutes in order to produce ischemic injury to the muscle. Homologous bone marrow from syngeneic donor rats was subsequently placed on the anterior surface of the femur to provide stem cells for osteogenesis leading to HO. Following surgery, separate groups of rats were administered two different dosages (high or low) ad libidum of either the nonspecific COX inhibitor ibuprofen (10 or 25 mg/kg), the COX 2 inhibitor celecoxib (1 or 10 mg/kg), or the COX-1 inhibitor SC560 (0.1 or 1 mg/kg) for (a) 28 days and rats followed by sacrifice at 42 days post surgery for the bilateral analysis of HO or, in a separate study group of animals, for (b) 1-21 days post surgery and rats followed by sacrifice at 1, 7, 14, or 21 days post surgery and prostaglandin levels in the quadriceps in both limbs were assayed. On the date of sacrifice, the quadriceps of each rat was homogenized, lipids were extracted, and quantification of prostaglandin using an ELISA methodology was performed. Femurs were taken from the sacrificed rats and examined by microCT scan for evidence of HO. Imaging involved cross sectional views of the femur from the proximal to distal ends. Grading was based on software volumetric analysis of the cross-sectional images using a metatmorph program.

RESULTS:
The data indicates that most PGE2 synthesis occurs within 7 days post injury. Negative control values show that negative control (no surgery) levels of PGE2 are approximately 500ng/g quadriceps muscle. During the first day after surgery, levels increased to 40 times control levels. Within the first week, PGE2 synthesis increased to a level of 24,000pg/g, almost 50 times the control level. At 2 weeks after surgery, PGE2 levels start to decrease and return to baseline. Data indicates that even at a low dosage, the COX-2 inhibitor reduced PGE2 to 25 % of control HO levels during the first day alone. At the lower dosage, the COX-1 inhibitor, SC560, was not as effective an inhibitor of prostaglandin synthesis as celecoxib in this model after one of treatment, although it did significantly reduce PGE2 levels at the high dosage. At the 7 day time point, all three drugs reduced PGE2 levels to roughly 25 % or less of control HO levels. It was also found that HO formation was reduced by 30 – 35 % following prophylactic treatment with either a COX 2 or a nonselective COX inhibitor. The failure of the COX 1 inhibitor to reduce HO levels at the low dosage is consistent with its lower potency compared to celecoxib as an inhibitor of prostaglandin synthesis.

DISCUSSION:
Our findings suggest that prophylactic treatment for HO is best given within 1-7 days after surgery. Micro CT scans show that HO occurs after injury to soft tissue, which is preceded by elevated prostaglandin levels. Recent data on COX-2 inhibitors demonstrate that they inhibit/delay bone healing. It is known that nonspecific NSAIDs (such as indomethacin) are effective in the prevention of severe forms of HO. A selective COX-2 inhibitor should thus be effective as an inhibitor of heterotopic bone development, with minor or no effects on the GI tract. The fact that COX-2 inhibition, following treatment with celecoxib or ibuprofen, is associated with reduced PGE2 synthesis and HO formation identifies a role for COX 2 in the pathophysiological mechanism of HO. These preliminary findings also suggest that COX-2 inhibitors are more effective than COX 1 inhibitors in reducing PGE2 synthesis and HO formation in a rat model.

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