LOCAL INHIBITION OF MATRIX-INDUCED BONE FORMATION BY CHEMOTHERAPEUTIC AGENTS AND REVERSAL BY RECOMBINANT BMP-7

INTRODUCTION
Demineralized bone matrix (DBM) is osteoinductive and may be useful in treating the skeletal defect resulting from a wide margin resection of malignant bone tumors. Some chemotherapeutic agents used in adjunctive chemotherapy for these tumors, however, might disrupt the osteoinductive potential of DBM. [1,2]. A system of matrix-induced enchondral bone formation has been well characterized in rats, and is useful for studying the various stages of bone formation [3]. Demineralized bone matrix, after subcutaneous implantation in the thoracic region, induces cartilage formation on day 7, bone formation on day 11, bone remodeling on day 14, and bone marrow formation on day 21. The purpose of this study is to investigate to what extent matrix-induced bone formation will be affected by the local application of anti-neoplastic agents in rats, and to examine the role of BMP-7 as an agent to overcome or compensate for any adverse changes in osteoinduction.

METHODS
Pellets were prepared using 30 mg of demineralized bone matrix, 300µg of rat-tail tendon collagen, and various concentrations of cisplatin (40 - 400 µg), methotrexate (100 - 400 µg), and/or BMP-7 (3 µg). These pellets were implanted subcutaneously on the thoracic region of male Long-Evans rats (100-150 g), age 23-25 days. Each rat received two implants: DBM with drug (cisplatin, methotrexate, and/or BMP-7) on the right side, and DBM with normal saline (control) on the left side of thoracic area. The day of implantation was designated day 0, and the matrix-induced plaques (implants) were removed on day 11. Part of each plaque was fixed in Bouin’s fluid for histological analysis and the remainder was analyzed biochemically by determining alkaline and acid phosphatase activity, and calcium content in acid-soluble extracts of the implant.

RESULTS
Alkaline and acid phosphatase activity from the implant declined in dose-dependent fashion when DBM pellets were prepared with increasing amounts of cisplatin (40 - 400 µg), and methotrexate (100 - 400 µg). Four-hundred µg of cisplatin per implant inhibited alkaline and acid phosphatase activity (83% and 32% respectively) in all stages of bone formation, as did 400 µg of methotrexate (78% and 45% respectively). The inhibition of alkaline and acid phosphatase activity by 400 µg of cisplatin or methotrexate was overcome by the addition of 3 µg of BMP-7 (Figure). Calcium content in matrix-induced plaques decreased when the implant was treated with cisplatin or methotrexate. This inhibitory effect induced by cisplatin and methotrexate was also overcome when BMP-7 was added to the implant. Histology of tissues from the implant correlated well with alkaline and acid phosphatase levels reflecting bone formation and bone remodeling respectively, and with calcium content representing de novo mineralization.

DISCUSSION
Local administration of chemotherapeutic agents after resection of malignant skeletal tumors to prevent local recurrence is a useful alternative to systemic administration because much smaller doses of drugs can be used and systemic complications are less frequent. An unfortunate side effect of these agents, however, is inhibition of osteoinduction within the healing defect site. This study suggests that although local administration of cisplatin and methotrexate inhibits bone formation and remodeling, recombinant BMP-7 can overcome this inhibitory effect. Further studies need to be conducted on the efficacy of local administration of anti-neoplastic agents.

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Figure: Alkaline phosphatase activity of DBM treated with 400 µg of chemotherapeutic agent, with or without 3 µg of recombinant BMP-7.

REFERENCES