Title

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possibility that rhBMP-2 and BMP-2 encoding gene can be anabolic agents for regenerating matrix of the intervertebral disc.
Disclosures: BMP-2: Investigational.
Conflict of interest: Hwan-Mo Lee, grant research support, Brain Korea 21 Project (government).

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2:20

The osteogenic activity of 14 types of bone morphogenic proteins: implications in bone regeneration and spine fusion

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Purpose of study: Although several bone morphogenic proteins (BMPs) have been shown to enhance bone healing and spinal fusion, the optimal BMPs or combination of BMPs to promote fusion are unknown. This study was designed to comprehensively elucidate the distinct and potentially synergistic osteogenic activity of 14 types of BMPs at various stages of osteogenesis.
Methods used: Recombinant adenoviruses expressing 14 human BMPs (ie, BMP-2 to BMP-15) were constructed and used to infect both pluripotent mesenchymal progenitor C3H10T1/2 cells and committed osteoblastic C2C12 cells. The osteogenic activity was determined by measuring the induction of alkaline phosphatase, osteocalcin and matrix mineralization on BMP stimulation.
Summary of findings: BMP-2, -4, -6, -7 and -9 significantly induced alkaline phosphatase activity in C2C12 osteoblastic cells. BMP-5, -8, -10, -11, -12 and -13 exerted weak induction of alkaline phosphatase. However, only BMP-2, -6 and -9 significantly induced alkaline phosphatase activity in C3H10T1/2 pluripotent cells, although weak induction by BMP4 and 7 was detected. Histochemical staining assays demonstrated that BMP-6 and -9 induced the greatest increase in alkaline phosphatase staining, whereas BMP-2 and -4 (and BMP-7 to lesser extent) induced a modest increase in alkaline phosphatase activity in C2C12 cells. BMP-2, -6 and -9 significantly induced osteocalcin expression (ie, a late marker of osteogenesis) in C3H10T1/2 cells, and BMP-4 and -7 slightly increased osteocalcin expression. In C2C12 cells osteocalcin expression was strongly induced by BMP-2, -4, -6, -7 and -9, whereas BMP-5, -10 and -14 slightly induced expression. Mineralized nodules were readily detected in the C3H10T1/2 cells infected with BMP-2, -6 and -9 vectors (and, to lesser extent, with BMP-4 -7 and -10). Finally, we observed strong synergistic effects among the BMPs that had an ability to activate the osteogenic markers. Interestingly, a strong synergistic effect on osteogenesis was detected in cells stimulated by BMP-5 plus -10, BMP-5 plus -12, BMP-5 plus -13, BMP-7 plus -10 or BMP-7 plus -13.
Relationship between findings and existing knowledge: We have conducted a comprehensive analysis of the osteogenic activity of 14 types of BMPs in osteoblastic progenitor cells and demonstrated that BMP-2, -6 and -9 are the most potent osteogenic factors, whereas BMP-4 and -7 exhibit reasonably strong osteoinductive activity. Further, certain combinations of BMPs exert a strong synergy on osteogenesis.
Overall significance of findings: These findings have important implications for the development of effective new formulas for bone healing and spine fusion.
Disclosures: No disclosures.
Conflict of interest: Tong-Chuan He, grant research support.

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2:26

Nicotine inhibits bone morphogenic protein–2–induced proteoglycan and collagen 2 synthesis of disc nucleus cells

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Purpose of study: We test the hypothesis that nicotine can reverse bone morphogenic protein (BMP)-2 induced upregulation of proteoglycan synthesis and collagen 2 expression but not collagen 1.
Methods used: Cells were isolated from nucleus pulposus of rat lumbar discs and were grown in monolayer culture. The cells were cultured in DMEM/F12 with 1% FBS and treated without added BMP-2 or nicotine (no treatment), 100 ng/ml of BMP-2 (BMP-2 only) or with 100 ng/ml of BMP-2 and increasing doses of nicotine (1, 10, 100 mcg/ml). The culture media was collected between day 4 and 7 after BMP-2/nicotine treatment and sulfated-glycosaminoglycan (s-GAG) content in the media was quantified using the DMMB to estimate proteoglycan production. The results were normalized by cell number at day 7. On day 7, mRNA was extracted, and reverse transcriptase polymerase chain reaction (RT-PCR) and real-time PCR were used to assess collagen 1 and 2 mRNA expression.
Summary of findings: By day 7, s-GAG production in the BMP-2–only group was increased 2.5-fold as compared with no treatment group. The s-GAG production monotonically decreased with increased concentration of nicotine down to 46% of BMP-2–only group (Fig. 1). No change in collagen 1 expression with the addition of BMP-2 or nicotine was noted. However, significant increase in collagen 2 mRNA in the BMP-2–only group compared with no treatment group was noted. Collagen 2 mRNA downregulation was noted with nicotine concentrations of 100 mcg/ml with RT-PCR. In order to better quantitate changes in collagen 2 mRNA abundance, real-time PCR was used. This showed collagen 2 mRNA that decreased to 69% and 27% of BMP-2–only group with the addition of nicotine at 10 and 100 mcg/ml, respectively. The effect of nicotine on sGAG production and the relative abundance of mRNA as percentage of BMP-2 only is noted in Fig. 1.

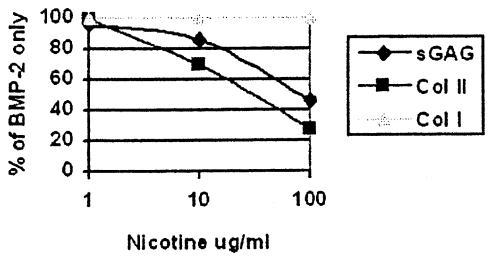

Fig. 1. Effect of nicotine on sGAG, collagen 1, and collagen 2.

Relationship between findings and existing knowledge: BMP-2 and its receptor are normally found in the intervertebral disc, and BMP-2 has recently been shown to enhance the chondrocytic phenotype of disc cells (increase proteoglycan and collagen 2 synthesis). Epidemiologic human studies and in vivo rat experiments indicate that smoking may lead to disc degeneration. We show here that nicotine, an important component of cigarette smoke, can inhibit proteoglycan and collagen 2 synthesis induced by BMP-2. Of note, collagen 1 is not regulated by BMP-2 or nicotine at the tested concentrations, indicating a certain specificity in nicotine activity.
Overall significance of findings: This study establishes the negative influence of nicotine on intervertebral disc cell response to BMP-2 at a cellular and molecular level.
Disclosures: No disclosures.
Conflict of interest: No conflicts.

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Effect of nutrient concentration and osteogenic protein–1 on the metabolism of intervertebral disc: in vitro organ culture study

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