SPATIAL AND TEMPORAL COLLAGEN GENE EXPRESSION IN OP-1-INDUCED LUMBAR INTERTRANSVERSE PROCESS FUSION IN RABBITS

+*Yamazaki, M; *Hisamitsu, J; *Suzuki, H; *Hashimoto, M; *Nakazawa, T; *Moriya, H.
+*Department of Orthopaedic Surgery, Chiba University Graduate School of Medicine, Chiba, Japan.
+81-43-226-2117, Fax: +81-43-226-2116, masashiy@ho.chiba-u.ac.jp

INTRODUCTION: Recombinant human osteogenic protein-1 (OP-1), also known as BMP-7, has been evaluated as a potential bone graft substitute. Previous experiments showed that OP-1 induced solid lumbar intertransverse process fusion in rabbits with high union rates (1, 2). These analyses were, however, undertaken mainly based on biomechanical testing, and little attention has been paid for the molecular mechanisms by which OP-1 induces posterolateral spine fusion (PLF). We have therefore analyzed the process of OP-1-induced PLF by means of in situ hybridization (ISH) in a rabbit model, and evaluated the spatial and temporal gene expression for type-specific collagen in each postoperative phase. The aim of this study was to clarify the details of molecular and cellular events in OP-1-induced PLF.

MATERIALS AND METHODS: Lumbar intertransverse process fusion: One-year-old male New Zealand white rabbits weighing 3.5-4.5 kg were used. The rabbit were divided into three groups: OP-1 group (n=18), carrier group (n=9) and autograft group (n=18). In OP-1 group, intertransverse process fusion at bilateral L5-L6 was performed as previously described (1, 2). The grafting material was 1 cc OP-1 putty (provided by Stryker Biotech) per side. The OP-1 putty included 1.2mg OP-1 with carrier (0.3g bovine collagen matrix and 77mg carboxymethylcellulose). In control group, only the carrier was placed. In autograft group, bone chips from iliac crest were grafted as previously described (3). The rabbits were euthanized at 2, 4, 6 and 8 weeks after surgery, and L5-L6 vertebrae were dissected. Sample preparation: After soft-radiographs were taken, the samples were fixed, decalcified and embedded in paraffin. Sagittal sections 6µm thick were stained with toluidine blue (pH 4.1), and used for ISH. ISH: DIG-labeled RNA probes (sense and antisense) for pro-α1(I) collagen (COL1A1), pro-α1(II) collagen (COL2A1), pro-α1(IX) collagen (COL10A1) were prepared, and ISH was carried out as previously described (4).

RESULTS: Radiological findings: In OP-1 group, fusion mass bridging over the L5 and L6 transverse process was observed at 6 weeks after surgery (Fig. 1). In autograft group, union of the grafted bone was found at 6 weeks. In control group, however, no bone formation was seen. Histological findings and ISH: In OP-1 group at 2 weeks, abundant cartilage tissues were multi-focally formed (Fig. 2). At this stage, a strong signal for COL2A1 was widely distributed not only near the transverse processes but also at the central portion of the intertransverse process area (Fig. 3). In contrast, only small cartilage tissues were present adjacent to the L5 and L6 transverse processes in autograft group at 2 weeks (Fig. 4), and a weak signal for COL2A1 was restricted near the transverse processes. After 4 weeks, however, endochondral ossification progressed from the L5 and L6 transverse processes toward the central portion in both OP-1 and autograft groups. At 6 weeks, cartilage was present like an island at the mid-portions of the intertransverse process area in both OP-1 and autograft groups (Fig. 2, 4). At this stage, COL2A1 signal was restricted at the front of endochondral ossification (Fig. 3). COL1A1 was diffusely expressed in osteoblastic cells, and COL10A1 was localized in hypertrophic chondrocytes at the endochondral ossification sites. Their expression patterns at 6 weeks were similar between OP-1 and autograft groups.

DISCUSSION: In the present study, gene expression pattern for collagen type II was markedly different between OP-1 and autograft groups at 2 weeks. This suggests that OP-1 vigorously stimulates cells and induces chondrogenesis in the early phase of PLF. In the late phase of OP-1-induced PLF, however, endochondral bone formation progressing toward the central portion became the major process. This process was almost similar to that occurred in autograft group. We suggest that, during the process of OP-1-induced PLF, osteogenic action of OP-1 is quite intensive in the early phase, but not in the late phase. In clinical application of OP-1, therefore, we should not overestimate its osteogenic potential. For accomplishing spine fusion, we should also pay attention for the local environment around the grafted OP-1 such as less invasive approach and adequate decortication of transverse process.


Fig. 1: Radiographs showing OP-1-induced PLF

Fig. 2: Toluidine blue stainings of PLF with OP-1 plus carrier and carrier alone (TP: transverse process)

Fig. 3: In situ hybridization for COL2A1 at the central portion of intertransverse process area of OP-1-induced PLF

Fig. 4: Toluidine blue stainings of PLF with autograft (TP: transverse process)