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Research Paper

Detection of sonic hedgehog in patients undergoing orthognathic surgery

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ABSTRACT

Purpose: Sonic Hedgehog (SHH) is a regulatory protein involved in bone fracture healing. Orthognathic surgery involves surgical osteotomy of the mandible or maxilla to restore the proper anatomic and functional position in patients with dentofacial deformity. The purpose of this study was to analyze SHH local blood serum concentrations after osteotomy to gain further understanding of the molecular regulation of the initial stage of osteotomy healing.

Methods: Serum samples (local drainage and peripheral venous) of 34 patients (24 females and 10 males, mean age was 23.4 (16–42) years) who underwent orthognathic surgery were isolated from patients at different time points during the perioperative period. The levels of SHH, soluble receptor activator of nuclear factor-κB ligand (sRANKL) and osteoprotegerin (OPG) were measured using ELISA.

Results: SHH was detected in the local drainage immediately after osteotomy (309.5 ± 58.2 pg/ml), and decreased for 2 days after the operation (197.5 ± 43.6 pg/ml). The sRANKL local serum concentrations were at the maximum level immediately after the operation (141.4 ± 22.6 pg/ml) and decreased for 2 days (110.1 ± 23.4 pg/ml). On the other hand, the OPG concentration in the local serum was at a minimum after osteotomy (59.4 ± 4.6 pg/ml) and reached its maximum (181.5 ± 17.8 pg/ml, $P < 0.01$) at 2 days after osteotomy. SHH and OPG local serum levels on day 2 were associated with the amount of bleeding during the operation. The local drainage serum level of SHH of maxillary/mandibular osteotomy had a tendency to be higher than that of mandible-only osteotomy at 2 days after operation.

Conclusions: Elevated levels of SHH in local serum after osteotomy, especially during the initial stage of healing, indicates its importance in osteotomy healing.

1. Introduction

Mandibular osteotomy healing is a unique process that leads to bone regeneration, and several cytokines and growth factors are known to be involved in the critical initial stage of fracture healing. However, local and systemic concentrations of certain factors during the initial stage of mandible osteotomy are not well understood.

Fracture healing involves a delicate balance of bone formation and resorption [1]. Sonic Hedgehog (SHH) is a regulatory protein involved as a morphogen of embryonic development [2], fracture healing [3] and socket healing after tooth extraction [4]. SHH indirectly induces osteoclast formation by upregulating parathyroid hormone-related peptide (PTHrP) [5] and receptor activator for nuclear factor-κB ligand (RANKL) in osteoblasts and bone stromal cells [6]. RANKL stimulates differentiation and maturation of osteoclasts, leading to bone resorption. Osteoprotegerin (OPG), a decoy receptor of RANKL, is made by osteoblasts and blocks osteoclast formation and bone resorption. Soluble RANKL (sRANKL) and OPG in plasma and drainage fluid is involved in the fracture-healing process after surgical treatment of proximal humeral fracture [7]. However, it is not yet established whether the local serum concentration of SHH is increased after mandibular osteotomy.

In this study, we evaluated the local levels of SHH expression after mandible osteotomy along with RANK and OPG expression for better understanding of the role of SHH in the process of mandible osteotomy healing.

2. Materials and methods

2.1. Patients and specimens

Between 2012 and 2013, 34 patients (24 females and 10 males, mean age 23.4 (16–42) years) were admitted to our institution for

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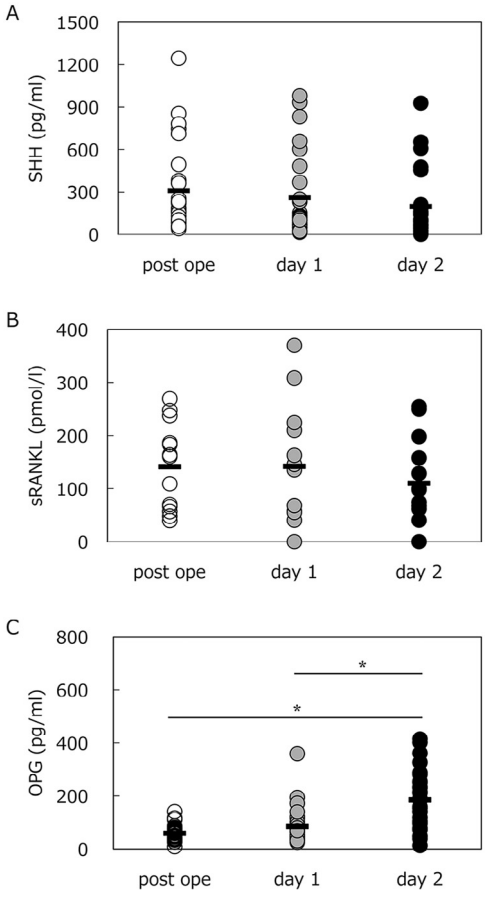

Fig. 1. Detection of SHH, sRANKL and OPG local drainage serum concentrations in mandible osteotomy. SHH (A) sRANKL (B) and OPG (C) measurements in local drainage serum for each considered time point. Values shown are means and S.E.s. Statistically significant differences (*P < 0.01) between the indicated groups are marked by asterisks.

orthognathic surgery (22 maxillary/mandibular, 12 mandible-only). Human serum from local drainage from the mandible osteotomy and peripheral venous blood was isolated from patients at different time points during the perioperative period (local drainage: day 0, 1, 2 and peripheral venous: day –1, 1, 3, 7). The protocols were reviewed and approved by The Human Research Ethics Committee of Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences (1327).

2.2. Serum measurements

Serum samples were stored after collection at –80 °C until analysis. SHH concentrations in the serum were determined using a sonic hedgehog N terminus (Human Shh-N) enzyme-linked immunosorbent assay (ELISA) kit from RayBiotech (Norcross, GA, USA). Total sRANKL (free and bound sRANKL) concentrations in the serum were determined using a sandwich enzyme-linked immunosorbent assay (ELISA) kit from BioVendor (Brno, Czech Republic). OPG concentrations in the serum were determined using an enzyme-linked immunosorbent assay (ELISA) kit from RayBiotech (Norcross, GA, USA). All measurements were performed in duplicate.

2.3. Statistical analysis

Data were analyzed using Tukey's test or Spearman's rank–order correlation coefficient. Results were expressed as means ± S.E.s P values < 0.01 were considered to indicate statistical significance.

3. Results

3.1. SHH, RANKL and OPG serum concentrations in patients with mandible osteotomy

The SHH local serum concentration was 309.5 ± 58.2 pg/ml immediately after osteotomy, and decreased time independently after osteotomy (Fig. 1A, day 1, 258.8 ± 55.9 pg/ml; day 2, 197.5 ± 43.6 pg/ml). sRANKL local serum concentrations remained unchanged during the considered time points (Fig. 1B, immediately, after osteotomy, 141.4 ± 22.6 pg/ml; day 1, 142.0 ± 30.8 pg/ml; day 2, 110.1 ± 23.4 pg/ml). On the contrary, OPG local serum levels significantly increased time-dependently after osteotomy (Fig. 1C, day 1, 85.7 ± 10.5 pg/ml, P < 0.01; day 2, 185.1 ± 17.8 pg/ml, P < 0.01).

3.2. Relationships of SHH, sRANKL and OPG local serum concentrations with the amount of bleeding during surgery

SHH local serum concentrations of patients with osteotomy were significantly associated with the amount of bleeding during the surgery on day 2 (Fig. 2A, r = 0.4415). At these time points, sRANKL local serum concentrations were not correlated with the amount of the bleeding (Fig. 2B). OPG local serum concentrations of patients were weakly related to the amount of bleeding on day 2, with statistical significance (Fig. 2C, r = 0.3062).

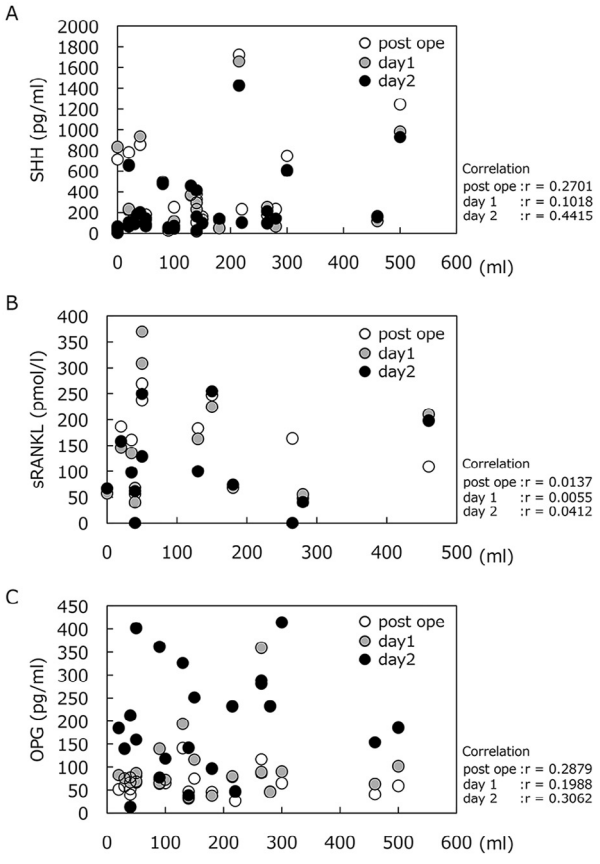

Fig. 2. Relationship between SHH, sRANKL and OPG local drainage serum concentrations and amount of bleeding during the surgery. The amount of bleeding and the concentrations of SHH (A), sRANKL (B), and OPG (C) in local drainage serum for each considered time point. Spearman's rank–order correlation was used to identify the strength of the relationship between the amount of bleeding and the factor measurements.