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Original article

Hyaluronic acid induces the release of growth factors from platelet-rich plasma

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Abstract

Background/Objective: Platelet-rich plasma (PRP) and hyaluronic acid (HA) injection are both therapeutic options for osteoarthritis and chronic tendinopathy. Although several comparative studies on the two have been published, the effects of mixing PRP and HA are not fully understood. The purpose of this study is to investigate the influence of HA on platelets in PRP by measuring releasing growth factors.
Methods: PRP was produced from nine healthy adult volunteers (mean age, 32.8 ± 2.9 years; range, 29–37) with a commercial separation system. HA of weight-average molecular weight of 50–120 kDa was used. PRP group (PRP 1 mL + phosphate buffered saline 0.2 mL) and PRP + HA group (PRP 1 mL + HA 0.2 mL) were incubated at 37°C for 2 hours. The amounts of transforming growth factor β1 (TGF-β1) and platelet-derived growth factor (PDGF-AA) released from the PRP and PRP + HA samples were measured on Day 0, Day 3, and Day 5. In addition, the same growth factors on Day 5 were measured for PRP + high HA group (PRP 1 mL + HA 0.6 mL) with five donors. After collecting all of the samples on Day 5, the remaining gels were observed with Giemsa stain. Statistical analyses were performed using paired *t* tests to compare the PRP and HA groups at each time point, and a one-way analysis of variance (one-way ANOVA) with Tukey *post hoc* tests was used to compare the PRP, PRP + HA, and PRP + high HA groups.
Results: The TGF-β1 concentrations in the PRP and PRP + HA were 24.3 ± 7.2 μg/mL and 22.4 ± 1.8 μg/mL ($p = 0.689$) on Day 0, 17.2 ± 13.9 μg/mL and 25.4 ± 7.1 μg/mL ($p = 0.331$) on Day 3, and 12.7 ± 10.5 μg/mL and 33.7 ± 8.3 μg/mL ($p = 0.034$) on Day 5. The TGF-β1 concentrations on Day 5 were 24.1 ± 5.2 μg/mL (PRP group), 28.3 ± 2.4 μg/mL (PRP + HA), and 31.9 ± 4.8 μg/mL (PRP + high HA; one-way ANOVA: $p = 0.003$; *post hoc* PRP vs. PRP + HA: $p = 0.016$). The PDGF-AA concentrations in the PRP and PRP + HA groups were 2.30 ± 1.21 μg/mL and 2.32 ± 0.79 μg/mL ($p = 0.931$) on Day 0, 2.03 ± 0.53 μg/mL and 2.13 ± 0.73 μg/mL ($p = 0.500$) on Day 3, and 1.51 ± 0.40 μg/mL and 2.00 ± 0.52 μg/mL ($p = 0.003$) on Day 5. The PDGF-AA concentrations were 1.48 ± 0.46 μg/mL (PRP group), 1.94 ± 0.57 μg/mL (PRP + HA), and 2.69 ± 0.70 μg/mL (PRP + high HA; one-way ANOVA: $p = 0.0002$; PRP vs. PRP + high HA: $p = 0.002$; PRP + HA vs. PRP + high HA: $p = 0.011$) on Day 5. The PRP showed larger coagulated masses than the PRP + HA. The high concentration HA group had the smallest coagulated mass of all of the group.
Conclusion: The levels of growth factors released by PRP on Day 5 were increased by the addition of HA. A mixture of PRP and HA may be a more effective therapy than PRP or HA alone for osteoarthritis and tendinopathy.

Keywords: growth factor; hyaluronic acid; platelet-derived growth factor; platelet-rich plasma; transforming growth factor-β1

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Introduction

The use of platelet-rich plasma (PRP) to treat musculoskeletal soft tissue injuries,[1] bone grafts,[2] osteoarthritis (OA),[3,4] and even skin ulcers[5] is increasing. Although the long-term effects of PRP remain controversial, the high concentration of autologous growth factors in PRP is expected to reduce the time needed for healing based on the accumulated basic and clinical research. Therefore, assessment of the levels of growth factors released from PRP is important.

Hyaluronic acid (HA) is widely used to treat OA of the knee.[6] The beneficial effects of HA are attributed to its function as a viscosupplement and its anti-inflammatory activity. HA injection is also used to treat tendon and ligament injuries and after surgery.[7,8]

Several reports that compare the clinical outcomes achieved with HA and PRP for OA have been published.[3,4] However, the clinical results of simultaneous HA and PRP injections have not yet been reported.

Recently, Chen et al[9] published an *in vitro* study of the synergistic anabolic actions of HA and PRP on cartilage regeneration in OA. In that report, the combination of HA and PRP reduced the levels of proinflammatory cytokines and increased articular chondrocyte proliferation and chondrogenic differentiation. The authors concluded that the observed synergistic effects were the result of different molecular mechanisms: the HA-dependent Erk1/2 pathway and the PRP-dependent Smad2/3 pathway. However, the direct influence of HA on the platelets in PRP was not discussed. In the present study, we tested the hypothesis that the addition of HA increases the levels of growth factors released by PRP.

Materials and methods

The protocol for this study was approved by the Ethics Committee of Hirosaki University Graduate School of Medicine, Aomori, Japan.

Preparation of PRP

Nine healthy adult volunteers (2 women and 7 men) with an average age of 32.8 ± 2.9 years (range, 29−37 years) were included in this study. Only one patient was taking medication of any kind, and that person was taking purgative medicine. No impairment of liver or kidney functions was detected in the patient blood samples.

Forty-five mL of peripheral blood for PRP preparation and an additional 1 mL of blood for the whole blood cell count were collected from the median cubital veins of each donor using a 21-gauge needle. No anticoagulant or activation materials, such as calcium chloride, were used. The PRP was produced using a commercial PRP separation system (Arthrex ACP; Arthrex, Naples, FL, USA) using a double syringe system according to the manufacturer's instructions. From each donor, 10−12 mL of PRP was prepared. Blood counts for the PRP preparations were measured using 1 mL of PRP.

PRP culture and harvest of released growth factors

ARTZ-Dispo HA (Seikagaku, Tokyo, Japan) with a weight-average molecular weight of 50−120 kDa was used as the HA. Three replicate wells of 1 mL of PRP and 0.2 mL of phosphate buffered saline (PBS; PRP group), three replicate wells of 1 mL of PRP and 0.2 mL of HA (PRP + HA group), and one well of 1 mL of PRP and 0.6 mL of HA (PRP + high HA group) were incubated on noncoated six-well dishes (Nunc, Shanghai, China) in a cell culture incubator at 37°C with 5% of CO_2 immediately after PRP preparation. After 2 hours of incubation (defined as Day 0), all the specimens had formed gels. At that time, 8.8 mL of PBS was added to one well from the PRP and PRP + HA groups to a 10-fold dilution, and all the liquid was collected 1 hour later. Any remaining platelets were removed with gentle centrifugation for 15 minutes at 200*g* and then another centrifugation for 15 minutes at 10,000*g*. The samples were immediately frozen with liquid nitrogen and stored at −80°C until the growth factors were assessed. In the same way, samples from the PRP and PRP + HA groups were obtained on Day 3 and Day 5 after PRP preparation.

For five of the donor PRPs ($n = 5$ donors), the PRP + high HA group samples obtained on Day 5 were diluted with 8.4 mL of PBS because of the higher dose of 0.6 mL of HA. In addition, to confirm that the growth factors were continuously released from the PRP, 0.2 mL of PBS for the PRP group and 0.2 mL of HA for the HA group was added to the remaining gels ($n = 5$ per group) after sample collection on Day 0 and Day 3. The released growth factors were collected on Day 3 (Days 0−3) and Day 5 (Days 3−5) in a similar way as was done for the PRP and PRP + HA groups.

Gross appearance on Day 5

After collecting all of the samples, the remaining gels were fixed with absolute methanol for 5 minutes and Giemsa stained for 5 minutes. Microscopic images (Olympus IMT-2-21 RFM; Olympus Corp., Tokyo, Japan) were taken using a digital camera (Canon DS 126181; Canon Inc., Tokyo, Japan).

Haematology

The platelet, white blood cell, neutrophil, lymphatic cell, and red blood cell counts in the peripheral blood and PRP were determined using an automated cell count analyser (Sysmex XE-5000; Sysmex Corp., Kobe, Japan).

Transforming growth factor-β1 and platelet-derived growth factor-AA levels

After thawing the stored samples, quantitative determinations of the transforming growth factor-β1 (TGF-β1) and platelet-derived growth factor-AA (PDGF-AA) levels were performed using a commercially available enzyme-linked immunosorbent assay kit (R&D Systems, Minneapolis, MN, USA) according to the manufacturer's instructions.