

EFFECTS OF HYPERBARIC OXYGEN AND PLATELET GROWTH FACTOR-BB ON LIGAMENT HEALING

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Introduction

Hyperbaric oxygen (HBO) therapy has been shown to enhance bone, muscle, skin, and wound healing, particularly in conditions of ischemia and low oxygen tension (1, 2). Platelet growth factor-bb (PDGF-bb) increased medial collateral ligament (MCL) healing (3). The synergy effects of HBO and platelet growth factor-bb (PDGF-bb) on rabbit ear ischemic ulcers have been reported (1) but there has been no study on the combination effects of HBO and PDGF-bb on ligament fibroblast. We investigated the effects of HBO, PDGF-BB, and their combination effect on the fibroblast from MCL. Cell growth and collagen alteration have been tested.

Materials and Methods

MCL fibroblast isolated from New Zealand white rabbits. Control cells were maintained in 5% CO₂ / 95% air through the experimental protocol. All hyperoxic cells were exposed to 100% O₂ at 2.5 ATA (atmospheres absolute) in a hyperbaric chamber with a total treatment of 120 minutes per 48 hours. At intervals of 48 hours, 96 hours, and 144 hours, cell growth was measured by increase in cell number after hyperbaric or normobaric treatments. The PDGF-bb treatment cells were exposed to medium with various concentrations of PDGF-bb (0.1 ng/ml, 1 ng/ml, 10 ng/ml, and 25 ng/ml). The combined treatment cells were exposed to 1 ng/ml of PDGF-bb plus HBO treatment. After 48 hours, cell growth was measured by increase in cell number. Type I collagen or Type III collagen were separated by 10% SDS-PAGE. The density of the band was quantified with use of a computerized image-analysis system. Differences between two groups were compared by two-tailed Student's t test. Values were expressed as the mean ± SD; p < 0.05 was considered statistical significantly.

Results

Dose Dependent Effect of HBO on MCL cell growth. HBO treatment dose dependently increased MCL cell number as compared with the control cells cultured under the same medium (Fig. 1, *p<0.05)

Dose Dependent Effect of PDGF-bb on MCL cell growth. PDGF-bb treatment dose dependently increased MCL cell number as compared with the control cells (Fig. 2, C:control, *p<0.05).

Additive Effect of PDGF-bb plus HBO treatment on MCL cell growth. HBO treatment and 1 ng/ml of PDGF-bb treatment were with significant effect on MCL cell growth. Combined treatment with HBO plus 1 ng/ml of PDGF-bb was with additive effect on MCL cell growth as compared with the HBO treatment and 1 ng/ml of PDGF-bb treatment cells, respectively (Fig.3, C:control, H:HBO, P:PDGF-bb, HP:HBO+PDGF-bb, *p<0.05).

Effects of HBO or PDGF-bb on Type I collagen and Type III collagen synthesis. After quantified by immuno blotting and imagine analyzer, Type I collagen containing was without significant changes after HBO treatment or PDGF-bb treatment or HBO combined with PDGF-bb treatment (Fig. 4, H/C ratio:101.6% ± 4.1%, P/C ratio:99.7% ± 4.7%, HP/C ratio:103.8% ± 3.2%). Type III collagen containing was significantly decreased after HBO treatment or HBO combined with PDGF-bb treatment but without significant changes after PDGF-bb treatment alone (Fig.4, H/C ratio:90.6% ± 2.1%, P/C ratio:100.7% ± 5.7%, HP/C ratio:92.3%±2.2%). HBO treatment decreased the Type III collagen/Type I collagen containing ratio.

Discussion

Previous studies on MCL healing have shown that Type III collagen levels (4) and Type V collagen levels (5) increased during ligament healing in relation to Type I collagen. A high concentration of Type III collagen relative to Type I collagen is suspected to result in smaller collagen fibrils which are mechanically weaker than those with the normal collagen fibrils. In our previous study showed that HBO treatment increased the mechanical strength of MCL (unpublished data). In this study we suggested the additive effect of HBO and PDGF-bb treatment on MCL cell proliferation. Furthermore, HBO treatment decreased the Type III collagen/Type I collagen containing ratio which may result in mechanically stronger collagen fibrils. HBO therapy can be suggested as a potential mode of treatment for MCL healing, although further studies are required.

Fig. 1

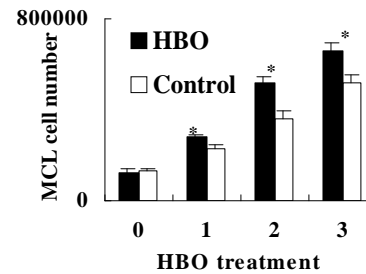


Fig. 2

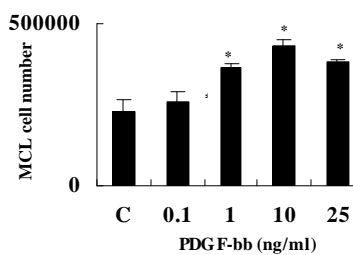


Fig. 3

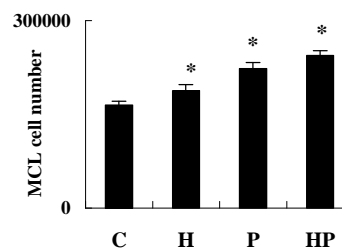
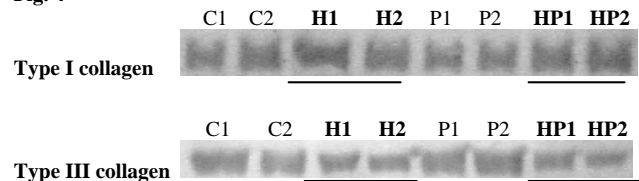


Fig. 4



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