ANGIOGENIC EFFECTS OF TRANSCUTANEOUS TOPICAL INJECTION OF OXYGEN INTO THE HEALING LIGAMENT IN RATS

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Introduction
In our previous study, using systemic hyperbaric oxygen we have shown that oxygen supply in the healing ligament is closely related to increases in collagen synthesis (1,2). However, this system needs a large chamber and the technique cannot be applied in most hospitals. Therefore, simple methods of enhancing oxygen supply to the target organ are recommended. In this study, we have examined the method of transcutaneous oxygen injection into the site of ligament injury and evaluated its effect on angiogenesis during the healing process.

Materials and Methods
Approval for animal work was obtained from the Ethical Committee of the University of Tsukuba. Seventeen male Sprague-Dawley rats aged 7 weeks (220-250 g) were used in this study. Animals were anesthetized with pentobarbital intraperitoneally (60 mg/kg) and the oxygen monitor (Licox CMP, Germany) was connected to the subcutaneous tissue over the patellar ligament of the right knee. Partial oxygen pressure (pO2) was monitored before and after injection of (i) 0.5ml hyaluronan and (ii) 0.5ml hyaluronan mixed with haemoglobin and oxygen.

Following anesthesia a longitudinal incision was made over the lateral aspect of the right knee. The patellar ligament was exposed and a laceration (2 mm) was made with a razor-thin knife, transversely in the middle of the patellar ligament, leaving fibers on both sides of the laceration intact. The skin was then sutured with 4-0 nylon. After recovering from anesthesia, the animals were returned to their cages. The rats were divided into the following groups (n=5 per group). Group A: Control group, Group B: injection of 0.5ml hyaluronan to the wound transcutaneously, Group C: injection of 0.5ml hyaluronan mixed with haemoglobin and oxygen to the wound transcutaneously. In each of the two injection groups, application was once a day for 3 days (total 3 times).

Repair sites of the three groups were assessed 7 days post-surgery by gross appearance, histological studies (HE, Masson Trichrome) and the expression of VEGF mRNA by RT-PCR. Histologically the number and mean area of blood capillaries was evaluated using NIH image. The mean value of VEGF mRNA concentration in control animals was given as a relative unit value of 1 and results are calculated from a comparison with the mean value of the control group. Statistical analysis of the data was performed using ANOVA.

Results
pO2 was elevated to 334.6mHg (baseline of subcutaneous tissue 40-50mmHg) immediately after injection of 0.5ml hyaluronan mixed with haemoglobin and oxygen and recovered to baseline after 8min (Fig.1). However, the pO2 was 100mmHg following injection of 0.5ml hyaluronan.

From the gross appearance and histology, the gap portion of the patellar ligaments began to be filled with collagen fibers and fibroblasts in all three groups. There were no significant differences between the three groups in the number of blood capillaries (Table 1). However, the mean area was significantly higher in Group C than the other two groups (P<0.05). The relative levels of VEGF mRNA expression are shown in Fig 2. Levels in the injected groups were lower than the control group and the difference was significant between Group A and Group C (P<0.05).

Discussion
Following ligament rupture, the wound becomes hypoxic due to the disruption of circulation and edema. Collagen synthesis and fibroblast proliferation cannot proceed without adequate oxygen supply. Therefore, angiogenesis and oxygen supply are important for wound repair. Our results show that oxygen injection into the wound leads to down-regulation of VEGF mRNA expression but is effective in promoting angiogenesis histologically. Our results suggest that topical oxygen injection has the potential to accelerate wound healing and possibly benefit tissue engineering. Further studies will be needed to elucidate the underlying mechanisms.

References

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