## EXPERIMENTAL STUDY

## Effects of hyperbaric oxygen on gene expressions of procollagen, matrix metalloproteinase and tissue inhibitor of metalloproteinase in injured medial collateral ligament and anterior cruciate ligament

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Abstract Animal experiments were performed to investigate whether and how the administration of hyperbaric oxygen (HBO) affects gene expressions of procollagens, matrix metalloproteinases (MMPs) and tissue inhibitors of metalloproteinases (TIMPs) in injured medial collateral ligament (MCL) and anterior cruciate ligament (ACL). In 64 Sprague-Dawley rats, the MCL of the left knee was lacerated at the midsubstance, and the ACL of the left knee was lacerated adjacent to the tibial insertion in another 64 rats. Of these, 32 rats with lacerated MCL and 32 rats with lacerated ACL were housed in individual cages at normal atmospheric pressure (Groups MC and AC, respectively), while the remaining 64 rats were exposed to 100% oxygen at 2.5 atmospheres absolute for 2 h for 5 days a week (Groups MH and AH, respectively). Rats were sacrificed at 3, 7, 14 and 28 days postoperatively. After macroscopic examination, bilateral MCLs were harvested from Groups MC and MH, and bilateral ACLs from Groups AC and AH. Total RNA

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H. Ohtake Department of Physiology, Dokkyo University School of Medicine, Tochigi, Japan was extracted from each specimen and gene expressions of type I and type III procollagens, MMP-2, -9 and -3, and TIMP-1 and -2 were estimated using semiquantitative reverse transcription-polymerase chain reaction (RT-PCR). Macroscopically, lacerated MCL healed by scar tissue formation, the amount of which appeared to be greater in Group MH than in Group MC. In contrast, no lacerated ACLs united, and little, if any, differences were apparent in macroscopic findings between Groups AH and AC. Gene expression of type I procollagen was significantly greater in Group MH than in Group MC at 7 days postoperatively and was also significantly greater in Group AH than in Group AC at 28 days (P < 0.05). No significant differences in type III procollagen gene expression were noted between Groups MH and MC or between Groups AH and AC. In addition, no significant differences in gene expressions of MMPs were seen in either ligament, except that gene expression of MMP-13 was significantly lower at 7 days in Group MH than in Group MC (P < 0.05). Gene expressions of TIMPs did not differ significantly between Groups MH and MC in each time interval, whereas gene expressions of TIMPs were significantly greater in Group AH than in Group AC at 7, 14 and 28 days for TIMP-1 and at 3, 7 and 14 days for TIMP-2 (P < 0.05). RT-PCR results suggested that HBO enhances structural protein synthesis and inhibits degradative processes by enhancing TIMP activities in the lacerated ACL. However, none of the lacerated ACLs united macroscopically despite administration of HBO, indicating that the effect of HBO is insufficient for healing of the injured ACL. If HBO therapy is used as an adjunctive therapy after primary repair of the injured ACL, the success rate of surgery seems likely to be increased.