Percutaneous Application of Panax notoginseng Extract Improves the Strength of a Healing Ligament

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ABSTRACT

This study compared the effects of Panax notoginseng (PN) extract with a composite herbal application on the healing of medial collateral ligaments (MCL) in rats. Twenty-nine rats receiving surgical tensile rupture to their right MCLs were tested. Ten rats were treated with an alcohol pad application (Group 1), 9 were treated with a composite herbal application (Group 2) and 10 were treated with an alcohol extract of PN to their right knees (Group 3). The treatments were applied percutaneously over the medial side of their right knee with adhesive plaster stabilization. The plaster and medication in all groups was changed every other day throughout the study to maintain moisture of the medication. The MCLs were harvested and tested for the biomechanical properties at 2 weeks after injury. Results revealed that the normalized ultimate tensile strength and structural stiffness of Group 3 were higher than those of Groups 1 and 2 (p=0.029 and 0.062 respectively), whereas Groups 1 and 2 were not different from one another. We conclude that PN extract application improves the mechanical strength of repairing MCLs at 2 weeks after injury.

Keywords: healing, herbal application, injury, tissue biomechanics

INTRODUCTION

Soft tissue injuries such as ligament and tendon ruptures frequently occur in athletes during sports training or competitions. Complete rupture of ligaments and tendons requires extensive rehabilitation and the joints may not function at its optimal level for a long time. Furthermore, soft tissues usually heal by scar formation and the scar tissue may not attain normal mechanical properties even at a few years after injury (Frank et al. 1992; Ng et al. 1996; Reddy et al. 1999). An important aim of rehabilitation is to hasten the healing process and improve the strength of the tissues.

The use of herbal remedies in Chinese medicine has a history of more than three thousand years (Wong and Dahlen 1999). A number of herbal prescriptions have been reported to be effective in reducing swelling, pain and improving joint mobility (Lin 2001; Kao and Wang 2002). However, effects of herbal treatment on the actual tissue healing process have not been well investigated, thus the use of herbal medications is often dependent upon empirical experience.

Recent studies on the effects of a composite Chinese herbal formula on medial collateral ligament (MCL) healing in rats revealed that herbal treatment could improve the tissue strength and ultrastructural collagen morphology of the repairing MCL (Fung and Ng 2004, 2005). When comparing the herbal treatment to low level laser therapy, the two groups were comparable in most mechanical parameters, but the herbal treatment group was better in terms of structural stiffness (Fung et al. 2005).

However, with a composite herbal formula that contained different ingredients with some reportedly good for pain relief, some for inflammatory control, some for improving the penetrating effects of the herbs, it is not known which of the individual ingredients was primarily responsible for the repair process (Fung et al. 2005). According to the literature, an ingredient in the formula: Panax notoginseng (PN), has been reported to contain a molecule similar to the basic fibroblast growth factor (bFGF) in human (Takei et al. 1996), which could stimulate collagen production and facilitate healing (Fukui et al. 1998; Tang et al. 2001). This herb could be largely responsible for the repairing process which needs to be examined. Furthermore, in these previous studies (Fung and Ng 2004, 2005), a transection injury model on the MCL was used but that did not simulate the normal injury pattern of tensile failure of ligaments. Therefore, the aims of the present study were to test the effects of a PN extract in treating injured rat MCLs and to compare this herbal extract with the herbal formula that had been previously proved to be effective.

MATERIALS AND METHODS

Twenty-nine Sprague-Dawley female rats with mean weight of 257 g (range 239-308 g) aged 12 weeks at the time of surgery were studied. The Animal Subjects Ethics Review Committee of the administering institution approved this study. The animals were randomly allocated into three groups (Table 1).

Preparation of composite herbal application and PN extraction

In preparing the composite herbal plaster for the treatment of Group 2, we adopted a commercially available herbal remedy used in the studies by Fung and Ng (2004, 2005) (Chongqing Peidu Pharmaceutical Factory, Chongqing, China). The formula contained 12 herbal ingredients (Table 2).

In preparing the herbal extract for the treatment of Group 3, six hundred grams of high quality PN roots were used. The PN

Table 1 The animal grouping of the study.

<table>
<thead>
<tr>
<th>Group</th>
<th>Right MCL</th>
<th>Treatment</th>
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<tbody>
<tr>
<td>1 (n=10)</td>
<td>Ruptured</td>
<td>Alcohol</td>
</tr>
<tr>
<td>2 (n=9)</td>
<td>Ruptured</td>
<td>Composite herb</td>
</tr>
<tr>
<td>3 (n=10)</td>
<td>Ruptured</td>
<td>Panax notoginseng extract</td>
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roots were washed with 50% ethanol and subsequently shredded into small pieces of approximately 1 mm thick and soaked in 2.5 litres of 50% ethanol at 37°C for 24 hours. The solution was then kept at room temperature for two weeks. Debris of the mixture was removed by sedimentation using centrifugation at 6000 × g for 20 minutes. Supernatant of the mixture was collected and used in this study.

Surgical and treatment procedures

All surgical procedures were carried out under general anesthesia with intra-peritoneal injection of a mixture of 80 mg/kg ketamine (Alfasan International, Woerden, The Netherlands) and 8 mg/kg xylazine (Alfasan International, Woerden, The Netherlands). The MCL of the right knee was exposed, a fine nylon wire was threaded transversely underneath the ligament and the MCL was completely ruptured by a quick and forceful medial pull on the wire. The stumps of the ligament were realigned but not sutured with a size of 3.5 cm × 5 cm at the medial aspect of the injured knees. All the medications were secured with adhesive zinc oxide tape of the x-y plane was built for the lower cross-head of the MTS machine, so that the tibial clamp could be adjusted and the free ends of femur and tibia were potted in small m

Biomechanical testing procedures

The biomechanical testing procedures followed that of Fung et al. (2002) and Ng et al. (2004). In brief, at 6 hours before testing, the specimens were retrieved from the freezer and allowed to thaw at room temperature inside the plastic bag. Each specimen was carefully dissected, leaving only the femur-MCL-tibia complex intact. The length of the MCL was measured with a pair of vernier calipers. The room temperature was controlled at 25°C and the specimens were retrieved from the freezer and allowed to thaw at 5° of knee flexion. A sliding cylinder with a non-exothermic easy setting polymer. The cylinders were then mounted on two specially designed clamps, fixed on a material testing machine (MTS Synergy 200, MTS Systems Corporation, Minnesota, USA) at 5° of knee flexion. A sliding table of the x-y plane was built for the lower cross-head of the MTS machine, so that the tibial clamp could be adjusted and aligned with the femur. An extensometer (MTS 634.12F-24, MTS Systems Corporation, Minnesota, USA) was attached to the lower end of femur and upper end of tibia for measuring local displacement. After the specimen was mounted on the machine, it was pre-conditioned with 10 oscillation cycles of 2.5% strain at a rate of 10 mm per minute so as to minimize the effect of deep freezing (Woo et al. 1986). Immediately after pre-conditioning, the specimen was loaded at an elongation rate of 500 mm per minute until failure. The data of load and displacement were recorded at a sampling rate of 50 Hz. The maximum load recorded represented the UTS, and the gradient in the linear portion of the load-displacement curve represented the structural stiffness. The UTS and stiffness values of the right legs were normalized against the left leg of each animal before statistical analyses.

Statistical analyses

One-way analysis of variance (one-way ANOVA) was used to analyze the normalized UTS and stiffness data. Post-hoc linear contrast was conducted for the significant ANOVA results and α was set at 0.05 for all the tests.

RESULTS

The ANOVA results showed a significant difference among groups for UTS (p=0.029) (Fig. 1). Post-hoc linear contrasts revealed that Group 3 had higher UTS than Groups 1 and 2 (p<0.05) (Fig. 1). No difference was found between Group 1 and Group 2 in the outcome measures. This result implied that the MCLs treated with PN extract had attained higher structural strength than those treated with the composite herbal remedy. A similar pattern of finding was shown in the stiffness value but the statistical result was marginally insignificant (p=0.062) (Fig. 2).
CONCLUSION

We conclude that percutaneous application of panax notoginseng extract can improve the mechanical strength of repairing MCL after complete rupture in rats at 2 weeks post-injury.

ACKNOWLEDGEMENTS

We thank The Hong Kong Polytechnic University Faculty Area of Strategic Development Fund for financial support of this study. The technical supports of Dr Xia Guo and Mr Roy Lau of The Hong Kong Polytechnic University are deeply appreciated.

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