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

Gabriel Ng[1*] • Dicky Fung[1] • Francis Kwok[2] • Mason Leung[1]

[1] Department of Rehabilitation Sciences, The Hong Kong Polytechnic University, Hung Hom, Hong Kong, China
[2] Formerly: Lee Wai Lee Hong Kong Institute of Vocational Education, Hong Kong, China

Corresponding author: * Gabriel.Ng@inet.polyu.edu.hk

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 responseble for the repair process (Fung and Ng 2004, 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 Pharmacentical 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.

Group	Right MCL	Treatment
1 (n=10)	Ruptured	Alcohol
2 (n=9)	Ruptured	Composite herb
3 (n=10)	Ruptured	*Panax notoginseng* extract

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Short Communication

Table 2 The ingredients of the herbal remedy plaster.

Herb ingredient	Plant part	% by dry weight
Panax notoginseng	Root	8
Dryobalanops aromatica	Resin	8
Eugenia caryphyllata	Flower bud	4
Cinnamomum cassia	Bark	6
Mentha haplocalyx	Leaf	13
Capsicum frutescens	Fruit	8
Ilex pubescens	Root	13
Sparganium stoloniferum	Rhizome	8
Aconitum kusnezoffli	Rhizome	8
Zanthoxylum nitidum	Root	8
Curcuma phaeocaulis	Rhizome	8
Aquilaria sinensis	Resin	8

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 and the skin wound was closed.

After injury, Group 1 was given an alcohol pad without other medication on the knee to act as control, Group 2 was treated with a composite herbal application (**Table 2**), and Group 3 was treated with a PN extract pad. All the pads were applied percutaneously 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 bandaging plaster and changed on alternate days to prevent drying. The animals were kept in cages with unlimited activities with water and food given *ad libitum*. They were sacrificed at 2 weeks post-injury with overdose of anesthetics. Both lower limbs were harvested by dis-articulating at the hip joint, sealed in a plastic bag and then stored in a freezer at -80°C for biomechanical testing later.

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 specimen was kept moist with normal saline throughout the tests.

The free ends of femur and tibia were potted in small metal cylinders with a non-exothermic easy setting polymer. The cylinders were then mounted on two specially designed clamps, fixed on a material testing machine (MTS Synergie 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 preconditioned 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**).

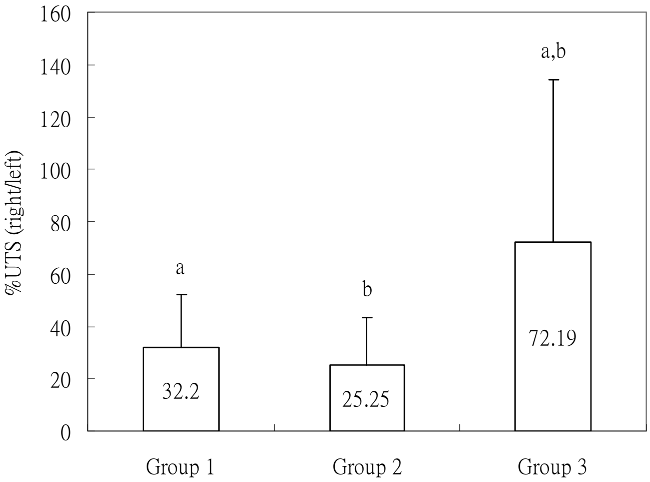

Fig. 1 Results of the normalized ultimate tensile strength (UTS) (p=0.029). The superscripts [a,b] indicate Group 3 has significantly higher tensile strength than both groups 1 and 2 respectively (p<0.05).

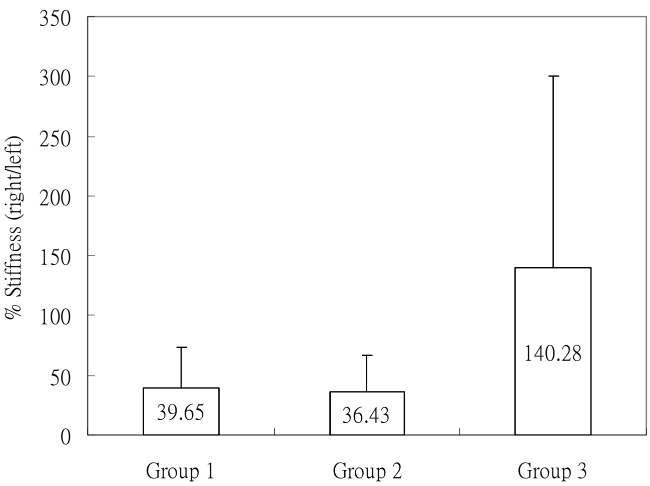

Fig. 2 Results of the normalized structural stiffness (p=0.062).

DISCUSSION

The present study has shown that PN extract can promote recovery of injured MCLs as early as 2 weeks after injury. The finding that the PN treated MCLs have significantly higher structural strength than those treated with the composite herbal remedy and the alcohol treated control is in line with a previous report on the therapeutic potential of PN in soft tissue healing (Li and Chu 1999).

For the composite herbal remedy of Group 2, the results revealed no significant treatment effect when compared with the control group (Group 1). This contrasts with the previous findings that the herbal composite has a significant therapeutic effect for MCL injury in rats (Fung and Ng 2004, 2005). However, there are two major differences between this study with the previous ones which may explain the different results. First, we tested the specimens at two weeks after injury whereas in the previous studies, the earliest testing time was 3 weeks after injury. The difference in timing could explain the non-significant result in this study. Second, we used a tensile loading injury in the present study whereas in the previous studies, the injury in MCL was induced by cutting with a scalpel. Despite the cut injury model is highly reproducible, it does not simulate the normal tensile injury mechanism that happens in the clinical, in which the collagen fibers may be disrupted along their courses. The subsequent repair process of a tensile injured ligament may therefore be slower.

The present finding of PN extract to have a significantly better treatment effect than herbal composite is interesting. Since the herbal composite also contained PN, our findings suggest at least two possibilities: (1) dependence on the PN concentration in the application; and (2) interactions between the ingredients in the herbal composite.

In the herbal composite remedy, PN only constituted 8% by weight of the entire medication, whereas in the PN extract group (Group 3), its percentage proportion would be much higher. The concentration of PN could have a significant influence on the outcome of treatment as shown in this study. According to high performance liquid chromatography analyses, ginsenoside Rb1 in the herbal composite remedy (Tang 2000) and bFGF–like molecules in PN (Takei *et al*. 1996) were identified. These are believed to be the active ingredients for ligament healing.

The other possibility is that the ingredients in the composite herbal remedy may interact with one another because some of the ingredients such as *Dryobalanops aromatica*, *Capsicum frutescens*, *Eugenia caryphyllata* and *Cinnamomum cassia* were found to have anti-inflammatory effects at the initial stage of injury (Akira 1987; Kubo *et al*. 1996; Han *et al*. 2001), which affect the healing process. With too much inflammation, there may be over scarring but with too little inflammation, healing will be slow and sluggish (Oakes 1994). If the composite herbal formula does not have an optimal control on the inflammatory response, it could affect the treatment outcome. Therefore, further studies are needed to investigate the effects of different herbs in the herbal composite.

The present study has shown that externally applied PN extract could enhance the biomechanical strength of repairing MCLs at 2 weeks after injury. This is an important finding because at this early timing of the repair process, an improvement in strength of the repairing ligament will be vital to the progress of the rehabilitation program, thus the outcome of the joint. However, caution must be taken that improvement in strength does not necessarily manifest itself in functional improvement and there is no reported action of PN in pain control. These are important considerations for the holistic management of ligament injury, which need to be examined with further studies.

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.

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