The Effect of Exogenous Crosslinking in Restoring the Integrity of Porcine Lumbar Intervertebral Disc In Vivo after Stabbing Injury

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SUMMARY
Collagen crosslink plays a major role in regulating the mechanical properties of load-bearing tissues. The human intervertebral discs have high concentration of mature crosslinks than any other tissues which signals demanding mechanical requirements. In vitro, genipin, an exogenous crosslink, can increase spinal motion segment stability, improve mechanical properties of annulus fibrosus, and will recover integrity of disc after stabbing injury. However, the effect has not been investigated in vivo. The study was to test the effect of exogenous crosslinking in the integrity of in vivo porcine lumbar intervertebral disc by quantitative discomanometry and stabbing injury model. The results showed the disc integrity was better in the group with genipin treatment. The in vivo animal study provides information for the further clinical application of exogenous crosslinking in the treatment of degenerative disc disease.

INTRODUCTION
The intervertebral disc behaves like a sealed system [1]. The intradiscal hydrostatic pressure depends on the integrity of disc. It increases with injected fluid [2], decreases with degeneration or injury [3]. Collagen and proteoglycan are two major components of extracellular matrix in regulating mechanical properties of intervertebral disc. Collagen crosslinks are critical bonds for providing mechanical strength of load-supporting tissue [4]. There are high concentration of mature crosslinks in human disc which signals its mechanical demanding. In our laboratory, we had found in vitro by quantitative discomanometry technique that genipin could recover disc integrity in porcine stabbing injured intervertebral disc [5]. The quantitative discomanometry technique was used to quantify the disc dysfunction and degeneration by measuring the correlation between the volume of injected fluid and the developed hydrostatic pressure of the disc [5]. Therefore, the purpose of this research is to test the in vivo effect of genipin in the integrity of disc after stabbing injury by porcine animal experiment.

METHODS
There were four Lanyu Miniature Swine included in the experiment. The swine were 4-month-olds with average weight of 23.6±0.8kg (Taitung Animal Propagation Station, Livestock Research Institute, Taitung, Taiwan). The experiment has been approved by the Institutional Animal Care and Use Committee (IACUC) of National Defense Medical Center, Taipei, Taiwan. Before achievement of the surgical procedure, Zoletil® and Atropine sulfate for anesthetic induction were intramuscularly injected to the swine. Isoflurane as inhalation anesthesia was used with 3% and 1.5% concentration for anesthetic depth and maintenance, respectively. Ketoprofen was used for analgesia and cephalixin was used to prevent infection. Tranexamic acid was used as coagulant. During the swine was anesthetized deeply, a left paramedian incision was made and blunt dissection with elevator was done to separate the paraspinal muscle until the exposure of lumbar intervertebral discs. There were three lumbar intervertebral discs (L23, L34, L45) could be accessed in one swine with this exposure. They would be assigned into injury group (N=4) and crosslinking group (N=8) randomly. In the injury group, an 18 gauge spinal needle was placed into the center of the disc through the posterolateral approach to produce a stabbing injury of the disc. In vitro study suggested that the 18 gauge needle stabbing injury can sufficiently induce disc dysfunction [5]. In the discs of crosslinking group, same needle stabbing procedure was performed and 2 ml of 0.33% genipin solution (98% pure, Challenge Bioproducts Co., Taichung, Taiwan) was injected into the disc. After two weeks feeding, the swine were anesthetized and approached with the same surgical procedure. The in vivo quantitative discomanometry (QD) technique was performed (Figure 1). The custom-made QD apparatus consists of a 22 gauge spinal needle, a high pressure syringe, a high pressure tube (1200 psi), two high pressure three-way stopcocks (1200 psi), a programmable x-table that producing horizontal thrust, a pressure transducer for pressure measurement, a linear potentiometer for volume measurement, and a computer for data acquisition. The 22 gauge spinal needle was carefully placed in the center of the disc through the posterolateral approach. The QD apparatus allows injection of 2 ml fluid to the disc. The injection rate was set at 0.5 ml/min. The pressure-volume curve was recorded from once fluid injection. The saturation pressure of the disc was obtained from each pressure–volume curve. The saturation pressure was the maximum pressure during the injection. Mann–Whitney non-parametric test was used to analyze the significant difference of saturate pressure between injury and crosslinking group. The level of significant difference was set at p < 0.05.
RESULTS AND DISCUSSION
The pressure-volume data of the disc was obtained and displayed one specimen information in Figure 2. The result of average saturate pressure of the disc is $0.69 \pm 0.22$ MPa and $0.87 \pm 0.36$ MPa in the injury group and crosslinking group, respectively. The saturate pressure of disc was statistically significantly affected by genipin treatments ($p=0.05$). Higher saturate pressure represented better integrity of disc. The current in vivo animal study showed the injured disc after genipin treatment has better integrity. It signals that crosslink augmentation may have ability to successfully recover biomechanical dysfunctions of the degenerated intervertebral disc in vivo. The restoration of intradiscal pressure after stabbing injury by the crosslinking augmentation may imply that genipin has the effect to seal trauma induced by the needle puncture to disc. The mechanism of genipin reaction to the disc is not clearly understood. It was suggested that the amino acid groups may contribute the intramolecular, intermolecular, and intermicrofibrillar bonds, hence increasing the strength of the collagen fiber and realigning the extracellular matrix [5]. The current in vivo swine experiment validated that genipin may interact with the component of the annulus fibrosus, nucleus pulposus, or both of them although the existence of an annular puncture. However, the results only appeared the enhancement of intradiscal pressure of injured disc after genipin treatment in short-term, the continued long-term experiment is proceeding by our research group.

CONCLUSIONS
This study tests the integrity of injured lumbar intervertebral disc with or without exogenous crosslinking treatment using quantitative discomanometry technique by in vivo swine experiment. The current results revealed better integrity of the disc after genipin treatment in stabbing injured model. This study provides a preliminary reference for genipin treatment to injured or degenerative discs for future clinical application.

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