Effects of multiple injections of hypertonic dextrose in the rabbit carpal tunnel: a potential model of carpal tunnel syndrome development

Yuichi Yoshii · Chunfeng Zhao · James D. Schmelzer · Phillip A. Low · Kai-Nan An · Peter C. Amadio

Abstract

Background This study investigated the effects of a series of four hypertonic dextrose injections on the subsynovial connective tissue (SSCT) and median nerve within the carpal tunnel of a rabbit model.

Methods Twenty New Zealand white rabbits were used. One forepaw carpal tunnel was randomly injected with 0.1 ml of 10 % dextrose solution. The contralateral forepaw was injected with a similar amount of saline. This injection was made once per week for 4 weeks. The animals were killed at 16 weeks after the initial injection and were evaluated by electrophysiology (EP), SSCT mechanical testing, and histology.

Results Mechanical testing revealed significantly greater ultimate load and energy absorption in the dextrose injection group compared to the saline injection group ($P < 0.05$). Histological evaluation revealed SSCT fibrosis and thickening and edema in the median nerve bundles in the dextrose injection group. There was a prolongation in the latency of the EP test in the dextrose injection group ($P = 0.08$).

Conclusions Previous studies had shown that one or two injections of 10 % dextrose could induce moderate SSCT fibrosis and mild EP changes without nerve histology changes. In this study, we have shown that higher doses create more severe fibrosis and, most importantly, more severe neuropathy, suggesting a dose–response effect, and confirming this as a potentially useful animal model for researching the etiology and treatment of carpal tunnel syndrome.

Keywords Subsynovial connective tissue · Rabbit · Carpal tunnel syndrome · Hypertonic dextrose

Introduction

Idiopathic carpal tunnel syndrome (CTS) is known to be associated with pathological changes in the carpal tunnel subsynovial connective tissue (SSCT). Non-inflammatory fibrosis and synovial tissue thickening increase the volume of the SSCT and are associated with elevated carpal tunnel pressure [4, 13]. It has thus been hypothesized that the cause of nerve compression might be SSCT fibrosis [7]. Several investigators have reported on the pathophysiology of acute median nerve external compression [3, 8, 9] but reports on in vivo assessment of this chronic disease are sparse.

In previous studies, single or double injections of 10–20 % dextrose were observed to induce SSCT fibrosis in a rabbit model [11, 24, 25]. Double injections had a more pronounced effect than single injections of a higher dextrose concentration. Although those studies showed changes in SSCT morphology, material properties and median nerve electrophysiology, changes in nerve histology were not observed. Considering the context of these interesting preliminary observations, we hypothesized that additional dextrose injections might create more severe SSCT fibrosis and lead to median nerve histological changes. The purpose of this study was, therefore, to investigate the effects of multiple injections of hypertonic dextrose on rabbit carpal tunnel SSCT and on median nerve morphology.

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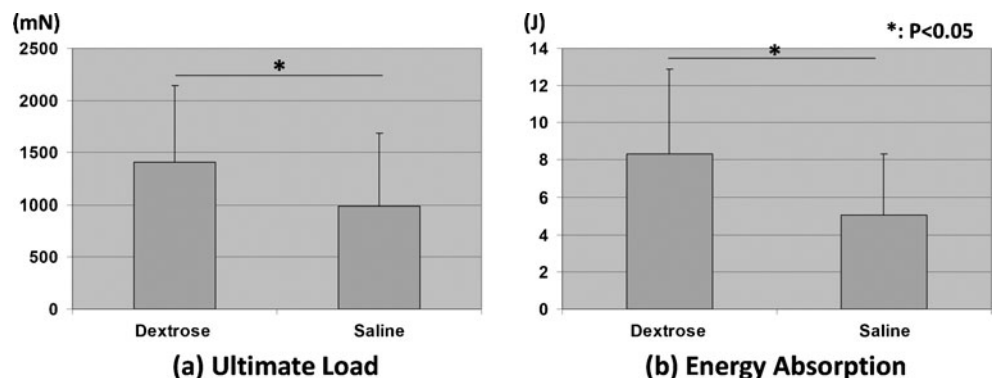
Methods

Surgical Procedure

All procedures were reviewed and approved by our Institutional Animal Care and Use Committee. All institutional and national guidelines for the care and use of laboratory animals were followed. Twenty 4.0–4.5 kg New Zealand white rabbits were used. The method has been described previously [24]. Briefly, a small incision was made proximal to the carpal tunnel. The synovium around the middle digit flexor digitorum superficialis (FDS) tendon was injected, by random allocation, with either 0.1 ml of 10 % dextrose solution (Hospira Inc., Illinois, USA) or 0.9 % saline solution. This treatment was repeated weekly for 4 weeks. At 16 weeks after the initial injection, the rabbits were anesthetized, electrophysiology (EP) was performed (n=12 for each group), and animals were killed. Following death, 12 paws were harvested, with intact carpal tunnel, for mechanical evaluation and either nerve or SSCT histology was performed on the remaining eight [25].

Evaluation of Subsynovial Connective Tissue

Mechanical Testing

Mechanical testing was performed using techniques described previously [23]. Briefly, the middle digit FDS tendon was resected proximal to the flexor retinaculum and mounted onto a custom-made microtester consisting of a linear servo motor (MX 80 Daedal, Irwin, Pennsylvania, USA) and a load cell (MDB-5, Transducer Techniques, Temecula, California, USA). The proximal end of the middle digit FDS tendon was joined to the load cell with 5-0 Vicryl suture. The connected tendon was then resected distal to the carpal tunnel. The tendon was pulled in the proximal direction through the carpal tunnel under a constant displacement rate of 0.5 mm/s. Specimens were moistened by a saline solution spray during testing. The ultimate tensile force and excursion energy (area under the load/displacement curve) were assessed.

Histological Analysis

The tissue for SSCT analysis was formalin-fixed and paraffin-embedded using a standard protocol. Five-micron sections of the carpal tunnel were made and stained with hematoxylin and eosin (H&E) using standard staining methods. Light microscopy analysis (BLX51, Olympus Co., Tokyo, Japan) was performed to evaluate cellularity, neovascularization, fibrosis, and inflammation. Image J software (National Institute of Mental Health, Bethesda, Maryland, USA) was used to measure the shortest distance between the middle finger FDS tendon and flexor digitorum profundus (FDP) tendon and the shortest distance between index and middle and middle and ring finger FDS tendons, at the mid-carpal tunnel level [25].

Evaluation of the Median Nerve

Electrophysiological Analysis

Under general anesthesia, EP testing was performed on the median nerve of both the treated and control forepaws. Compound muscle action potential (CMAP) of the thenar muscle was recorded while stimulating the median nerve at 3.0 cm proximal to the recording point. EP testing was performed at 12 weeks and immediately prior to killing, 4 weeks later. The distal motor latency and CMAP amplitude were measured.

Histological Analysis

Tissue harvested for median nerve histology was fixed in 4 % paraformaldehyde solution for 24 h, followed by immersion in a fixative solution of 10 % glutaraldehyde and 10 % paraformaldehyde for secondary fixation. Tissue harvested for transmission electron microscopy (TEM) was post-fixed in phosphate buffered 1 % osmium tetroxide, rinsed with distilled water, stained en bloc with 2 % uranyl acetate at 60 °C, and then embedded in plastic resin. The median nerve tissue

Fig. 1 Results of mechanical testing. **a** Ultimate load. **b** Energy absorption. *Asterisk* shows significant difference between each combination ($P<0.05$)

Fig. 2 Histological change of
SSCT. **a** SSCT histology: (*a*)
saline injection group and (*b*)
dextrose injection group. **b** SSCT
thickness: *1* thickness of SSCT
between FDS and FDP; *2*
thickness of SSCT between FDS
and FDS. *Asterisk* shows a
significant difference between the
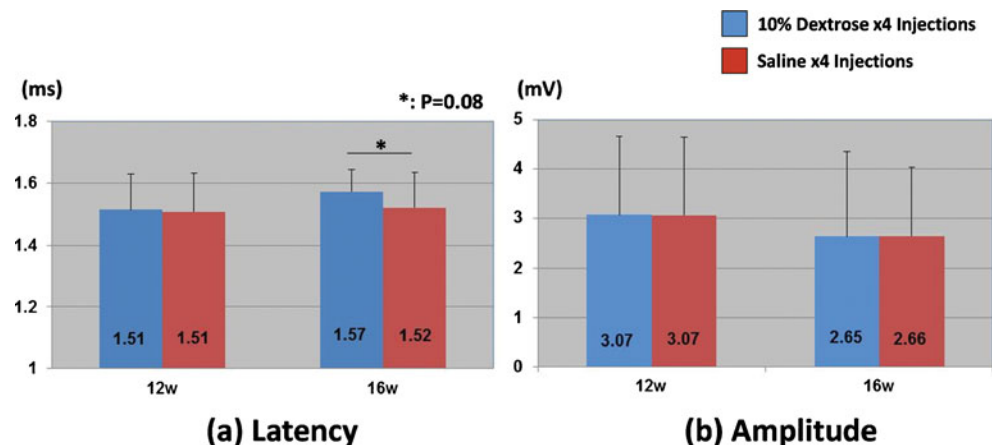dextrose injection group and the
saline injection group (*P*<0.05)
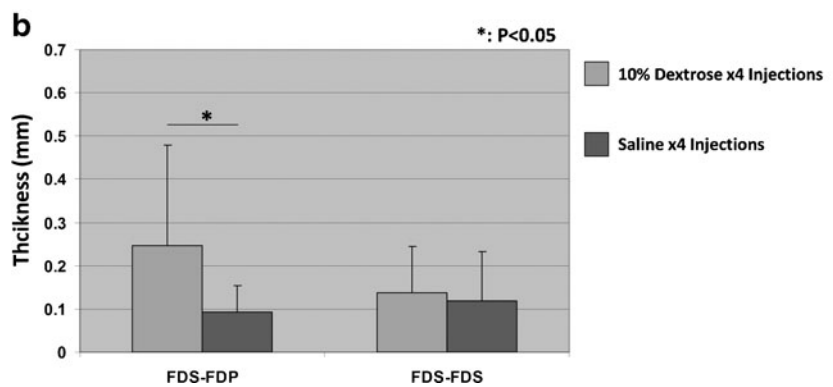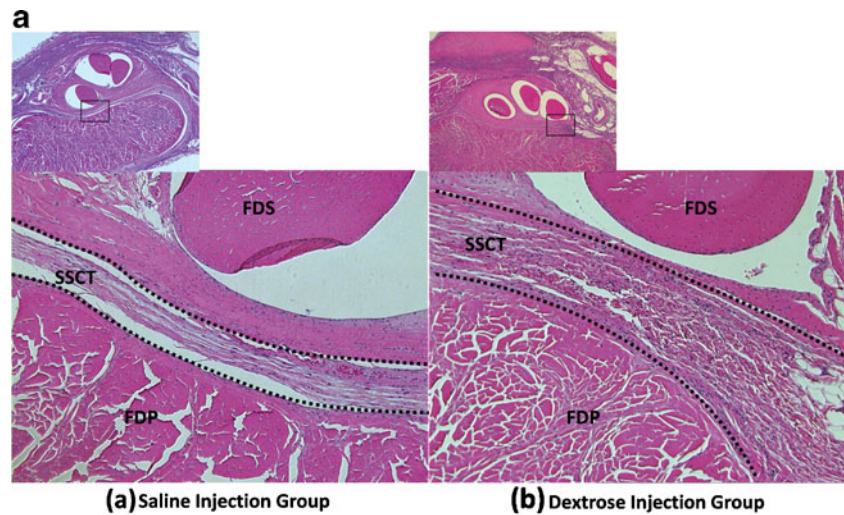
harvested from the carpal tunnel level was prepared for light
microscopy analysis by slicing into the 0.6-μm cross sections
and staining with toluidine blue. The sections were evaluated
qualitatively using a light microscope for fascicular demye-
lination and subperineurial edema. For the TEM analysis, thin
sections were mounted on copper grids and evaluated (JEOL
USA, Peabody, Massachusetts, USA).

Statistical Analysis

The means of selected parameters were compared using the
paired *t* test; *P*<0.05 was considered significant. The results
were expressed as mean ± standard deviation. All statistical
analyses were performed using the statistical package Statcel
QC for Excel (OMS Publishing Inc., Japan).

Fig. 3 Results of
electrophysiological test. **a**
Latency. **b** Amplitude. *Asterisk*
shows the difference between the
dextrose injection group and the
saline injection group (*P*=0.08)

Fig. 4 Nerve histology (light microscopy, toluidine blue stain). **a** Saline injection group. **b** Dextrose injection group

(a) Saline Injection Group   (b) Dextrose Injection Group
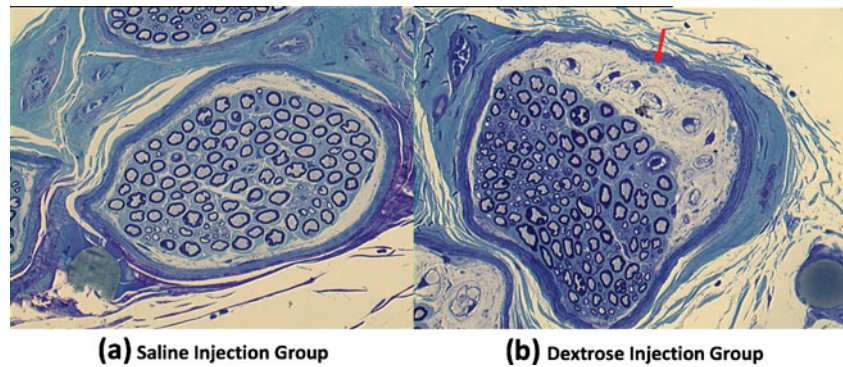
Results

Evaluation of the Subsynovial Connective Tissue

Mechanical testing showed that the ultimate load and energy absorption were significantly greater after dextrose injection than after saline injection ($P<0.05$) (Fig. 1).

In the saline injection group, the collagen fibers of the SSCT were distributed evenly. In the dextrose injection group, the SSCT structure was irregular and thickened (Fig. 2a). The vascular proliferation or vascular hypertrophy was not obvious. The thickness of the SSCT between the FDS and FDP tendons was significantly larger ($P<0.05$) in the dextrose-injected paws compared to the saline-injected paws (Fig. 2b).

Evaluation of the Median Nerve

In the electrophysiological testing, there was a relatively longer distal motor latency in the dextrose injection group 16 weeks after injection, but this difference was not significant ($P=0.08$) (Fig. 3). There was no obvious difference in the amplitude. Light microscopy showed edema in the median nerve bundles. The myelin sheath was thinner in the dextrose injection group compared to the saline injection group (Fig. 4). Under TEM, poorly myelinated nerve fibers were observed, with an increase in the axon/myelin ratio (Fig. 5). There was evidence of Wallerian degeneration as noted by the presence of stacked Schwann cells.

Discussion

In this study, we assessed the effect of a series of four hypertonic dextrose injections on the rabbit carpal tunnel SSCT. Multiple injections of dextrose intensified the mechanical property changes and SSCT fibrosis compared with previous studies, in which only one or two injections were given [24, 25]. We did not observe any edema or thickening of the tendons themselves.

The previous studies had also shown that injections without dextrose do not induce any change in either the nerve or SSCT, establishing that it is the dextrose, not the trauma of

Fig. 5 Nerve histology (TEM). **a** Saline injection group. **b** Dextrose injection group

(a) Saline Injection Group

(b) Dextrose Injection Group
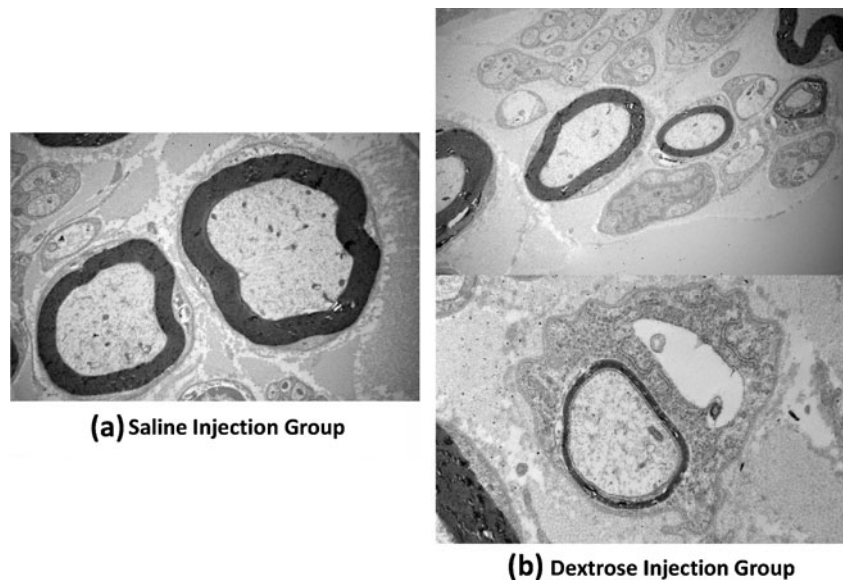
an injection, is responsible for the changes seen [24, 25]. In addition, the series of four injections created histologic changes in the median nerve, showing that a larger dose of dextrose induces a more severe neuropathy.

Hypertonic dextrose has been suggested as a stimulant to initiate proliferation of new cells or stimulate repair in existing cells. Many in vitro studies have shown that human cells produce various growth factors after exposure to hypertonic dextrose [12]. These growth factors include platelet-derived growth factor [2], transforming growth factor beta [10], epidermal growth factor [5], basic fibroblast growth factor [14], insulin-like growth factor [17], and connective tissue growth factor [10]. In addition, these growth factors have been previously identified as key for stimulating repair and growth of tendon, ligament, and cartilage tissues [22]. Recent studies have confirmed the clinical benefit of 10 % dextrose injections in the treatment of lateral epicondylitis [18], low back pain, and knee osteoarthritis [1, 19, 20]. In addition to the effect of dextrose on growth factors observed, injection of dextrose in concentrations greater than 10 % can briefly produce an inflammatory response. Because progressive fibrosis and overexpression of transforming growth factor and connective tissue growth factor have been observed in patients with CTS [4, 6, 15, 16], we thought that hypertonic dextrose might be a useful treatment to induce a similar phenomenon in an experimental animal [11, 24, 25].

As noted above, previous studies have shown that two injections of 10 % dextrose had a more pronounced impact on SSCT fibrosis than single injections [25]. The mechanical testing showed that both the ultimate load and energy absorption of the dextrose injection group were significantly greater than the saline injection group, whether for one or two injections (including previous results with one or two injections) [25]. In comparing our data for four injections, again, there was no significant difference when comparing one or two saline injections with four saline injections, suggesting again that it is not the number of injections, but rather the dextrose which is effecting the changes that we observed in the dextrose-injected animals.

While the single or double injections of 10 and 20 % dextrose were demonstrated to affect the SSCT, they had a lesser effect on nerve function in the previous studies. In contrast, in the four dextrose injection group of this study, edema was observed in the median nerve bundles with a thinner myelin sheath. These histologic changes are consistent with a more severe neuropathy in the four-injection group, suggesting a dose effect, with lower doses causing SSCT fibrosis and higher doses resulting in more neuropathy. This is consistent with a hypothesis for carpal tunnel syndrome etiology, in which progressive SSCT fibrosis results in eventual neuropathy [4, 13].

We believe that the animal model described in this work may be applied for several potential applications. As has already been noted in previous studies [24, 25], it can serve as a useful model of CTS. The effect of stimulating dose can be studied here, as well as the effect of drugs or other agents that might block the progression of fibrosis in the face of a known stimulatory dose.

There are several limitations in this study. First, we do not know if the nerve changes we observed were caused by a dextrose-induced chronic compression or by the dextrose itself. In addition, short-term effects were not investigated. However, since other data have shown that dextrose has a longer-term effect at 16 weeks, studying the short-term effects on nerve histology, as well as the mechanism of action, including cytokine expression, would be appropriate in this model. Second, the local concentrations of dextrose after the injections were not measured. An in vitro study of hyperglycemic effect revealed that pericellular glucose concentrations above 35 mM induced hyperglycemic stress and cell injury [21]. While the dose used in this study is larger, we cannot estimate the local pericellular concentration and results may have some variability due to the differences in local concentration of dextrose in vivo. Third, since only a single time point was included in this study, we have no data on the time course of the observed changes. EP showed slight changes from 12 to 16 weeks after injection. With the long-term effect confirmed, it will be important to consider shorter time points.

Conclusion

In conclusion, we observed that a series of four injections of hypertonic dextrose intensified fibrosis and material property changes of the rabbit carpal tunnel SSCT and induced significant changes in nerve histology. This may be a helpful model for studying the effects of synovial fibrosis on carpal tunnel syndrome.

Statement of Animal and Human Rights   All procedures were reviewed and approved by our Institutional Animal Care and Use Committee. All institutional and national guidelines for the care and use of laboratory animals were followed.

Statement of Informed Consent   Informed consent was not needed.

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