



# Dextrose concentration for prolotherapy: A study on human neuroblastoma cells

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## ABSTRACT

**Aims:** Prolotherapy is performed using a 15%-concentrated hypertonic dextrose solution that targets damaged soft tissues to induce a regenerative process. A novel technique pointing at perineural regions using 5%-concentrated dextrose is also gaining popularity in daily clinical practice. Animal and human studies have revealed the clinical and functional benefits of perineural prolotherapy; however, the cytological effects of dextrose solution are immature. This study compared the effects of dextrose solutions at different concentrations on neurons at the cytological level.

**Methods:** Cultures of human neuroblastoma cell lines that simulate neurons at the cytological level were prepared. The sample cultures consisted of the control group (CG), 5% dextrose-supplemented group (5DG), and 15% dextrose-supplemented group (15DG). The neuronal viability assay of the cultures before and 6 h after supplementation was compared between the groups.

**Results:** The pre-supplementation cell viability percentage of CG was presumed to be 100%. The post-supplementation cell viability percentages of CG, 5DG, and 15DG were 93.33%, 22.22%, and 0%, respectively. The decreases in cell viability in both dextrose groups were statistically significant when compared with CG ( $p < 0.001$ ). Moreover, the difference in cell viability between the 5DG and 15DG groups was also statistically significant ( $p < 0.001$ ).

**Conclusions:** Our findings suggest that dextrose solution has an acute, concentration-dependent toxic effect on cultured human neuroblastoma cells.

## Introduction

Prolotherapy is the injection method of an irritant solution into a joint space, ligament, or tendon insertion site, mainly targeting chronic musculoskeletal pain (1). Clinical benefits of prolotherapy have been reported for osteoarthritis, tendinopathies, joint pains, and chondromalacia (2,3); thus, prolotherapy is becoming popular among physicians who deal with musculoskeletal system pathologies.

Prolotherapy acts as a trigger of local inflammation by inducing a regenerative response (4). Hypertonic dextrose is the most commonly used injection agent (5). The dextrose solution potentially contributes to the healing process in two ways: 1)

by increasing the osmotic pressure of the extracellular space, thus causing the cells to lose water and destroy, consequently resulting in a temporary state of inflammation, and 2) by stimulating the proliferation of platelet-derived growth factor, epidermal growth factor, basic fibroblast growth factor, insulin-like growth factor, and connective tissue growth factor (4).

The most preferred concentration of dextrose for prolotherapy is 15% (6,7), which was first described by Hackett et al. (8) in the 1950s and subsequently modified by others not only for dextrose concentration but also for the injection sites (9). One of the modified versions is perineural injection therapy, which involves the injection of 5% dextrose solution into the peripheral



nerves and perineural location, resulting in considerable pain-relief effects (10). Perineural injection therapy was supported in 2005 by Maniquis-Smigel et al. (9,11) and declared to have therapeutic effects with neuromodulatory effects (4) on peripheral entrapment neuropathic (9) and neuropathic pain (11), probably by improving sensory and motor functions (9,12) and decreasing neurogenic inflammation (9), in comparison with 15% dextrose.

Less is known about the complications of dextrose prolotherapy (13). Except for general injection adverse reactions such as self-limited pain, bruising (14), inflammation, and hematoma (15), no significant side effects of prolotherapy, including either the concentration of hypertonic dextrose solution or the localization of injection, have been reported (14,15). Few clinical studies have revealed that 5% dextrose solution is a safe and effective choice and has no side effects on nerves, but the conclusions of these animal and human studies were based on ultrasonographic findings and electrophysiological parameters (9,10,12). To the best of our knowledge, the cellular effects of different concentrations of dextrose have been studied only on the cell cultures of human fibroblasts (16,17), which presented findings relatively in contrast. To the best of our knowledge, no cytological study has evaluated the “safe” or “side” effect of dextrose solution on neural cells (18). The cumulative effects of dextrose injection also remain unknown (9).

A variety of hypotheses have been stated about the mechanisms of action explaining the beneficial clinical effects of dextrose prolotherapy. Although the evaluation of the cytological changes may shed light on the mystery. Thus, we compared the effects of 5% and 15%-concentrated dextrose solutions on primary neuronal cultures *in vitro*, simulating the cytological level at the injection site. The obtained findings may also help to determine the optimal concentration of dextrose solution and the correct injection site for prolotherapy.

## Methods

### Neuroblastoma cell cultures

The human neuroblastoma cell line [SH-SY5YATCC® CRL-2266™], which mimics neurons in the cell culture, was obtained from the Cancer Research Center, Institute of Health Sciences, University of Health Sciences Türkiye. The SH-SY5Y neuroblastoma cell line has distinct functional characteristics with natural dopaminergic and adrenergic features (18,19). Cultures of the cell line were prepared in accordance with classical standards (19). The cell line was incubated in RPMI (Sigma Aldrich-R8758) medium containing 10% (v/v) foetal calf serum (Biochrom AG, Berlin, Germany) with 1% (v/v) penicillin and streptomycin (Biological Industries Ltd., Haemek, Israel) (37 °C, 5% CO<sub>2</sub>) (Heraeus incubator, Hanau, Germany) for 24 h (19).

A total of nine Petri dishes of cell culture were used for the experiments to form three comparison groups: control group (CG), 5% dextrose-supplemented group (5DG), and 15% dextrose-supplemented group (15DG). Each comparison group comprised three Petri dishes of cell culture. The RPMI (Sigma Aldrich-R8758) Petri dishes were CGs themselves. 2 mL of 5% dextrose solution (prepared by dissolving 27.8 mM of Sigma-Aldrich dextrose, D9434) was directly added to the three RPMI (Sigma Aldrich-R8758) Petri dishes of 5DG. Likewise, 2 mL of 15% dextrose solution (prepared by dissolving 83.4 mM of Sigma-Aldrich dextrose, D9434) was directly added to the three RPMI (Sigma Aldrich-R8758) Petri dishes of 15DG on the next day. All cultures were incubated for 24 h (at 37 °C and 5% CO<sub>2</sub>) and examined under a microscope (Zeiss Axio Vert. A1 inverted phase fluorescence microscope, Germany) immediately after the supplementation of dextrose solutions and every 6 h after that.

### Neuronal viability assay

The viability of neuroblastoma cells was determined by the fixation of cells with an ethanol-formaldehyde-acetic acid (7:2:1) mixture and staining with trypan blue (Sigma Aldrich Co. 302643), because this method is simple, cheap, and allows rapid counting of cells with ruptured membranes under the microscope (20). Trypan blue was diluted to 0.8 mM with phosphate-buffered saline. The survival rates of neuroblastoma cells were calculated by counting the intact nuclei on a hemocytometer in five regions of view (19,21). A scanning microplate reader was used to measure the absorbance of the dye solution at 450 nm. The baseline viability of CG cell cultures was presumed to be 100%, and both dextrose solution-supplemented groups were calculated according to this approach (19,21).

This study was approved by the Gülhane Military Medical Academy Local Ethical Committee (decision/date no: session 05/04.07.2012). Because this study was conducted on a commercial cell line, no verbal or written consent was obtained.

### Statistical Analysis

All data were recorded using nine different cell cultures from three independent experiments. The viable cell percentages of CG, 5DG, and 15DG were compared by one-way analysis of variance and Bonferroni post-hoc tests using Statistical Package for the Social Sciences (SPSS) Statistics for Windows, version 18.0 (SPSS Inc., Chicago, Ill., USA). P values of  $p < 0.05$  were considered statistically significant.

## Results

The neuronal viability assay was planned to be performed by the end of incubation (and at the same time 24 h after the supplementation of dextrose solutions). However, the death of numerous neuroblastoma cells was observed in the 15DG at the first control and photographing stop, 6 h after the

supplementation (post-supplementation). We terminated the incubation process and proceeded to the neuronal viability assay stage.

The post-supplementation neuronal viability assay revealed that both 5% and 15% dextrose solutions caused a significant decrease in viable neuroblastoma cells in 5DG and 15DG (Table 1). In the 5DG and 15DG, the post-supplementation viability of neuroblastoma cells was significantly lower than CG ( $p < 0.001$ ). Moreover, the difference in post-supplementation viability of neuroblastoma cells was statistically significant between the 5DG and 15DG ( $p < 0.001$ ). The percentages of viable neuroblastoma cells were 93.33%, 22.22%, and 0% in CG, 5DG, and 15DG, respectively.

## Discussion

This study aimed to compare the effects of 5% and 15% dextrose solutions on human neuroblastoma cell cultures and, thus, to foresee the real effects of perineural 5% dextrose injection therapy at the injection site. Both dextrose solutions were toxic to neuroblastomas in the first 6 h. Furthermore, 15% dextrose solution had a significantly more lethal effect than 5% dextrose. Our findings suggest that dextrose prolotherapy may have similar acute harmful effects on the perineural regions in a concentration-dependent manner.

Prolotherapy has been a widely used injection treatment; however, the mechanism of action of the therapeutic effects of prolotherapy is still barely known, and the limited knowledge prevents us from proposing a clear opinion. The suggested conclusions are the induction of inflammatory response, anabolic reactions (22), cellular proliferation, and vascular changes locally by the injection of dextrose solution (23).

The threshold concentration of dextrose is 10% to induce local inflammation. Concentrations of dextrose solution above 10%, especially the clinically preferred concentration of 15%, activate the inflammation cascade by increasing osmotic pressure (23). Some studies have revealed that the mechanism of action of dextrose solution on neuropathic pain might be the inhibition of transient receptor potential vanilloid receptor-1 (TRPV-1) (4,11,12,22), inactivating the secretion of substance P, calcitonin gene-related peptide (4,10), and nitric oxide (4), thereby alleviating neurogenic inflammation. In addition, Wu et al. (10) compared the effects of perineural injections of 5% dextrose with normal saline in patients with mild-to-moderate

carpal tunnel syndrome and concluded that the neurogenic anti-inflammatory effects of 5% dextrose were significantly higher than those of normal saline. Similarly, Chen et al. (12) evaluated the neuropathic pain-relief effect of perineural 5% dextrose injection in a patient with radial nerve palsy. In another study, an epidural injection of 5% dextrose was compared with 0.9% saline in patients with moderate-to-severe low back pain, and pain relief was significantly higher in the 5% dextrose group (11). In addition to these pathways, non-inflammatory concentrations of dextrose solution contribute to the proliferation of musculoskeletal cells and influence the transport of amino acids and protein synthesis with no cellular toxicity (23). However, our findings indicated that 5% dextrose solution had lethal effects on cell cultures of neuroblastomas, simulating neurons at the perineural injection sites.

The only study evaluating the effects of dextrose solution on SH-SY5Y human neuroblastoma cell cultures compared the findings of different concentrations ranging between 0 and 25 mM with and without the existence of tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) (18). Based on the hypothesis that dextrose would act like an anti-inflammatory molecule as a response to the TNF- $\alpha$ -induced inflammation, the authors concluded that a higher concentration of dextrose (i.e., 25 mM) decreased the neuronal impairment developed by inflammation and suggested this finding as a probable mechanism of the therapeutic effect of dextrose on neuropathic pain, in contrast with our observation. However, the studies of Güran et al. (16) and Woo et al. (17) have declared the final cytotoxic effect of dextrose solutions on fibroblasts despite the confusing findings concerning the available concentrations. Güran et al. (16) concluded that 15%, 20%, and 25%-concentrated dextrose solutions, but not 5% and 10%-concentrated dextrose solutions, led to significant fibroblast death, while Woo et al. (17) observed a significant decrease in fibroblast viability in 5% dextrose and complete death of almost all fibroblasts in 10% dextrose. The preferred concentrations of dextrose used in these two studies were in agreement with our method, which may be more conducive to clinical interpretation despite the difference in examined cells.

The pathophysiology of diabetic neuropathy shows similarities with the possible pathways that explain our findings. Hyperglycemia in diabetes mellitus contributes to neuropathy through increasing oxidative stress, generation of glycation end products, accumulation of polyols, and vasculopathy-

**Table 1. Baseline and post-supplementation viable neuroblastoma cells**

Groups	Baseline	Post-supplementation	p
Control, mean $\pm$ SD	45.11 $\pm$ 0.78	42.67 $\pm$ 1.15	0.073
5% dextrose, mean $\pm$ SD	44.66 $\pm$ 1.15	9.33 $\pm$ 0.58	<b>&lt;0.001</b>
15% dextrose, mean $\pm$ SD	44.66 $\pm$ 0.58	0	<b>&lt;0.001</b>

Bold data are statistically significant.  
SD: Standard deviation

related nerve ischemia (24-27). Mohammadi-Farani et al. (28) evaluated the effects of glucose concentration on the PC12 cell line culture medium and reported that hyperglycemia had an unfavorable influence on neuronal viability by reducing the expression of cannabinoid-1 receptor and activating TRPV-1. Moreover, higher glucose concentrations were associated with more toxic effects through these receptor responses, confirming the concentration-dependent cellular death observed in our study. The same study also suggested that increased TRPV-1 expression facilitates neuronal damage due to long-term exposure to dextrose in the culture medium (28). However, we observed the neurotoxic effects of dextrose solution in the acute period, which is worth comprehensive research. In cultured neurons, glucose uptake is not dependent on insulin because of the absence of an interstitial barrier (25). This may explain the mechanism of our findings in part, but the concentration of the dextrose solution seems to have some influence.

The results of the studies regarding the effect of dextrose solutions at different concentrations on the morphology and function of nerves are contradictory. Yoshii et al. (23) studied New Zealand white rabbits by injecting 10% dextrose into the subsynovial connective tissue of one forepaw and 0.9% saline into the same location in the contralateral forepaw. The authors compared the electrophysiological, histological, and mechanical findings between the 10% dextrose and 0.9% saline groups and observed acute alterations in motor function of the median nerve in the 10% dextrose group, with no significant morphological difference. In another study testing the same hypothesis of a probable dose-response effect of hypertonic dextrose solution to experimentally induce carpal tunnel syndrome (29), the same authors compared the effects of saline, 10% dextrose, and 20% dextrose solutions with different numbers of injection sessions. They reported the development of an experimentally induced carpal tunnel syndrome and morphological flattening of the median nerve in 10% and 20% dextrose groups compared with saline, but no malfunction. In 2014, the same authors tested the effects of multiple once-a-week injections of 10% dextrose and saline to induce experimental carpal tunnel syndrome and reported the thinning of the myelin sheath and Wallerian degeneration in the 10% dextrose group compared with that in the saline group (30). They concluded that there was an association between larger doses of dextrose and the severity of neuropathy (30). Despite the controversy, the current knowledge supports the concentration-dependent neurotoxic effect of dextrose solution, which we observed in the current study. However, all were animal studies of experimental carpal tunnel syndrome. To the best of our knowledge, our study is the first to evaluate the neurotoxicity of dextrose prolotherapy on human cell culture and has indicated an acute concentration-dependent toxic effect on cultured human neuroblastoma cells.

These findings led us to think that prolotherapy might be harmful rather than helpful, especially in perineural localizations.

### Study Limitations

Despite the remarkable findings, this study has a few limitations. First, *in vitro* neurological disease models commonly use SH-SY5Y neuroblastoma cell cultures; however, these cells retain the properties of cancer cells. Second, we have not performed the advanced assay of neuronal viability, such as spectrophotometric, fluorometric, or caspase activity determinations. Our findings may be considered evocative but not sufficient to conclude a precise agreement on the negative effect of hypertonic dextrose prolotherapy. Not only *in vitro* designs but also *in vivo* studies evaluating the changes in cytokine levels in the localization of prolotherapy may enable us to obtain more accurate findings and clearly conclude.

### Conclusion

This study revealed that 5% and 15% dextrose solutions have cytotoxic effects on neuroblastomas in the first 6 h. Thus, the concentration of dextrose and the injection site may need to be considered during prolotherapy indications. Whether these findings occurred due to a dextrose-induced mechanism of action or the dextrose itself remains unclear. Further studies evaluating these mechanisms and cytokine expression may explore the unknown aspects of the cellular effects of different dextrose solutions in prolotherapy.

### Ethics

**Ethics Committee Approval:** This study was approved by the Gülhane Military Medical Academy Local Ethical Committee (decision/date no: session 05/04.07.2012).

**Informed Consent:** Because this study was conducted on a commercial cell line, no verbal or written consent was obtained.

### Authorship Contributions

Surgical and Medical Practices: Ö.K., Concept: Ö.K., Z.D.Ç., Design: Ö.K., Z.D.Ç., Data Collection or Processing: Z.D.Ç., Analysis or Interpretation: Ö.K., Z.D.Ç., Literature Search: Ö.K., Writing: Ö.K.

**Conflict of Interest:** No conflict of interest was declared by the authors.

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