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Review Article

Dextrose Prolotherapy - Mechanism of Action

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Abstract

Introduction: Prolotherapy is an injection-based therapy used for multiple acute and chronic musculoskeletal pain drivers. The aim of this clinical discussion is to review the clinical considerations in the use of prolotherapy in sport and exercise medicine. We will also compare intervention options and provide valuable clinical pearls.

Methodology: The focus of this clinical discussion is on the application of prolotherapy in clinical practice.

Summary: it is hoped that this narrative review guides clinicians on possible uses of prolotherapy in clinical practice, based on the current evidence of efficacy, complications and risk profile. The integration of clinical experience, combined with a review of the best evidence in the field, may assist in clinical decision making.

Keywords: Dextrose; Prolotherapy; proliferant

Introduction

Proliferation therapy (prolotherapy) is an injection-based therapy for acute and chronic musculoskeletal 'pain drivers' and has a long history of clinical use [1]; the injectate (proliferant) is most commonly hypertonic dextrose, which is readily available, relatively cheap and has minimal adverse side effects. Dextrose is also considered to be an ideal proliferant because it is a normal component of blood chemistry, it can be injected safely into multiple areas and in large volumes and is water soluble [2]. Prolotherapy is considered a 'regenerative treatment' that induces growth of the connective tissue framework secondary to a local inflammatory response and it has been used clinically for multiple musculoskeletal disorders for many decades [2,3]. In general, there are two forms of dextrose prolotherapy: hypertonic (25-50%) dextrose and isotonic (5%) dextrose; both have different mechanisms of action and different clinical indications. There are considerable variations in delivery technique, use of dextrose with other agents (including sodium morrhuate, local anaesthetics, etc.) and volume of dextrose delivered.

Prolotherapy has grown in popularity and has received significant recent attention among sports medicine clinicians and others involved in the management of acute and chronic musculoskeletal disorders. There are myriad options for intervention in musculoskeletal medicine, from oral non-steroidal anti-inflammatory drugs (NSAIDS), corticosteroid injections, platelet rich plasma (PRP) and stem cells. We consider dextrose prolotherapy to be less of an 'alternative medicine' modality and more of a potential tool in sports medicine. The objective of this review is to examine the underlying biological plausibility of prolotherapy for the treatment of acute and musculoskeletal pain. Through judicious application and sound patient selection, good clinical outcomes may be expected for a host of musculoskeletal conditions, ranging from small and large joint

osteoarthritis, tendinopathy, partial ligament tears, painful entheses, tight fascia/myofascial pain syndrome and peripheral nerve entrapments.

A discussion with the patient about the risks, benefits, short and long-term clinical efficacy of the myriad treatment modalities is essential when deciding on any treatment pathway. In this context, given that dextrose prolotherapy has a very low risk profile and may result in cost savings in an increasingly strained healthcare sector, it is another useful tool in the sports physician's armamentarium.

Background

The rationale for the use of prolotherapy and its growing list of clinical indications first evolved 80 years ago as a method to treat various chronic musculoskeletal conditions [3], many of them seemingly unrelated. Prolotherapy, in its current form, was formalised by Dr. George Hackett, a general surgeon in the United States, in the 1950s [1-3]. Prolotherapy was, at that time, and continues to be, proposed to be a practical and effective therapeutic strategy to treat ligamentous laxity and related musculoskeletal and arthritic conditions [4]. Indeed, interest in prolotherapy has strengthened over the past 20 years among both clinicians working in musculoskeletal medicine and patients who are attracted to innovative or novel treatments. Other conditions amenable to prolotherapy include chronic low back pain, sacroiliac joint pain or instability or pain, refractory lateral epicondylosis, a host of overuse tendinopathies of the upper and lower limbs, and peripheral nerve entrapment syndromes (carpal tunnel syndrome, cubital tunnel syndrome, tarsal tunnel syndrome and sensory nerve entrapments) [4,5]. The clinical evidence for these myriad indications is mixed and difficult to synthesise without resorting to the rigour of a systematic review, which has been undertaken by this paper's authors and is pending peer review.

Biological plausibility of hypertonic dextrose

The effect of dextrose prolotherapy on human tendon mechanobiology and the induction of an inflammatory environment to promote soft tissue contraction, resulting in tissue 'stiffening', makes intuitive sense in relation to tendons and ligaments, but the biological plausibility of this mechanism is less convincing in relation to small and large joints [5]. An exploration will be made of the role of perineural injection therapies using dextrose in fascial planes and the role of hydrodissection and its mechanical effect of relieving local or regional sites of nerve compression or more widespread neural tension, especially in transition zones and between anatomical compartments bordered by fascial connections. In relation to this, there is increasing recognition of the importance of the body's fascial system, which constitutes a three-dimensional continuum of collagen-expressing, loose and dense fibrous connective tissue that envelopes muscles and organs, thereby allowing integration of function [6]. Fascial pathology has historically not received considerable attention in standard medical texts or research studies; however, it is increasingly recognised that injuries to the fascial system cause a significant loss of performance in both recreational exercise and high-performance sport [7,8]. Disorders of the fascia also have a potential role in the development or prolongation of musculoskeletal disorders, including lower back pain and limb myofascial syndromes [9].

Dextrose is considered to be an ideal proliferant because it is a normal constituent of blood, can be injected safely into myriad areas and in high volumes and it is water soluble [10,11]. However, the precise mechanism of effect in general is not well understood, and it is probably multifactorial. It is known that hyperosmolar dextrose dehydrates cells at the injection site until they lyse by creating a large osmotic gradient and starts an acute inflammatory process, followed by a healing response [12]. This cellularly-driven process relies upon granulocytes and macrophages, among other cell lines. The acute inflammation and the subsequent regenerative phase are intricately linked at a cellular and molecular level; the coupled dynamics of this process highlight the inherent healing capacity of injured soft tissue when placed under an inducible stress.

Specifically, inflammatory cytokines, including interleukins, prostaglandins, thromboxanes, and leukotrienes regulate the cellular environment that controls the reparative phase [13,14]. Conversely, when the inflammation is blunted via non-steroidal anti-inflammatory drugs (NSAIDs) or corticosteroids, the optimal milieu for true soft tissue healing is compromised, albeit at the cost of reduced pain and, perhaps, a transient clinical improvement. However, this incomplete tissue healing, may create the risk of increasing scar formation and injury recurrence. Aside from occasional local soft tissue irritation, the risk of infection is low and does not have a higher risk profile compared to the injection or local anaesthetics or corticosteroids [14].

The aforementioned granulocytes and macrophages from the initial stage of inflammation may release factors that attract and activate fibroblasts; these drivers of collagen deposition ensure that the regenerative process continues until tissue stability is restored. The deposited collagen eventually matures and contracts and may cause the tendon or ligament to tighten and/or strengthen. Along these lines, it has been theorised that periarticular injections or deposition of hypertonic dextrose into painful entheses decreases joint laxity and articular dysfunction, improves overall biomechanics and decreases pain on a number of objective measures [10-15]. Thus, by inducing the production of the growth factors and cytokines that aid in tissue healing at a local level, combined with the hypothesised direct analgesic effects of dextrose, particularly its lower concentration preparations (which may imply less of an inflammatory mechanism of action and more of a direct neurogenic effect at the nociceptor level), prolotherapy has a role to play in the active management of musculoskeletal injuries and 'pain drivers' [14,15].

The contraindications for dextrose prolotherapy include the presence of active infection, known allergy to dextrose (rare), previous hypersensitivity or other adverse local reactions or underlying medical conditions which compromise healing potential (significant immunosuppression). Rheumatoid arthritis and active gout are also contraindications, especially if the clinician targets an actively involved joint. Concurrent use of steroids or NSAIDS are also contraindicated when using dextrose prolotherapy, as these agents inhibit the inflammatory healing response.

The clinical effectiveness of dextrose prolotherapy is thought to secondary to its pro-inflammatory nature, especially in higher concentrations. The inflammatory process is the first phase of healing, followed by proliferative and remodelling phases; if the first phase is compromised, the subsequent processes are compromised, thereby reducing the healing response. The inflammatory response is driven by histamine release secondary to platelet activation. The effects of histamine are prolonged by mediators such as serotonin, bradykinins and prostaglandins, which increase capillary permeability, facilitate the migration of protein rich fluid to injured intercellular spaces, and attract inflammatory cells. Indeed, NSAIDs and corticosteroids reduce inflammation and subsequent symptoms by respectively inhibiting the enzyme cyclooxygenase, which in turn inhibits the formation of prostaglandins, or the enzyme phospholipase A2, which blocks the production of prostaglandins and leukotrienes.

Biological plausibility of isotonic dextrose and fascial dynamics

A delineation of the known fundamental biological mechanisms is required to understand the potential therapeutic value of dextrose prolotherapy. What is the biological plausibility of dextrose prolotherapy? Histologically, myofibroblasts have been consistently identified on detailed examination and are known to possess a host of contractile proteins, highlighting their intricate, quaternary structure [16,17]. Indeed, the density and local concentration of myofibroblasts varies significantly depending on the precise anatomical location studied and its proportion of connective tissue, including deep and superficial fascia; pathological states, including chronic fibrosis, also upregulates this highly reactive process and promotes the expression and enhanced function of myofibroblasts [18,19]. It has been shown experimentally that the density and local concentration of myofibroblasts is greater in the thoracolumbar fascia than in the tensor fascia lata (TFL); moreover, in cases of adhesive capsulitis, which is thought to be driven by glycosylation of basement membrane of the shoulder joint's synovial lining, the density of myofibroblasts is also increased, suggestive of a cellularly-driven inflammatory process [18-20]. Furthermore, it has previously been established that the density of myofibroblasts in cases of low back pain secondary to 'tight' lumbar fascia and adhesive capsulitis is similar [18-20].

Building on the idea that they act dynamically and synergistically within and between fascial planes, myofibroblasts have been observed to contract through video analysis and force output measurements [21,22]. Contraction is typically many orders of magnitude slower and weaker (in terms of absolute recorded force output) than that observed in striated muscle and occurs over minutes to hours. The density of myofibroblasts in different anatomical locations is hypothesised to be related to their role in maintaining stable posture and balance, with minimal expenditure of metabolic energy [18-21]. Key to this sustained force production role is stable cellular turnover. Furthermore, forces from the 'summed' contraction of facia-associated myofibroblasts have been quantified but have been determined to be insufficient for the generation of gross movement or the maintenance of core, truncal or limb stability, but may be related to tissue stiffness, generation of pain due to an im-

paired perineural blood flow and energy metabolism (myofascial trigger points), sensation/nociception and contribute to proprioception in the spine and limbs [22,23]. It appears that the cellular contractility is independent of direct synaptic signal transmission from the central nervous system (CNS) via acetylcholine and the catecholamines.

Local or regional myofibroblast contraction, acting somewhat like a 'functional syncytium', is influenced via the expression of various pro-inflammatory cytokines and other biologically active molecules arising from the ground substance constituting the 'foundations of the fascial plane'. Experimentally, in the form of histological analyses, *in vivo* studies on human cadavers and murine and other animal models, it has been determined that TGF-B1 increases contractility and correlates with a reduction in the tissue pH level (acidic pH increases the strength of contraction) [24-26]; furthermore, the local instillation of isotonic 5% dextrose creates a locally acidified environment. What is not fully understood is whether an increase or decrease in fascial contractility over time leads to improvements in terms of reduced pain and functional restoration and how this correlates with individual patients in the sports medicine context. In this sense, it is useful to compare different pharmacological agents, or chemicals more broadly, in terms of clinical efficacy. It has been noted that Botulinum toxin induces relaxation, whereas caffeine has no discernible effect at this level. Thromboxane A2 analogues and Mepyramine both increased the force of contraction, but do not prolong its duration [16].

Not all dextrose prolotherapy is the same

Work has been done to explore the mechanism of action of prolotherapy more broadly and the effects of different concentrations of dextrose, in particular [18-21]. The typical clinical use ranges of dextrose solutions are 5%-25%, although the use of higher concentrations has been described. Traditional prolotherapy is based on the theory of tendon and ligament regeneration by injecting > 10% (i.e., hypertonic) dextrose to stimulate local inflammation. The higher concentration preparations are associated with more deleterious effects on nerve form and function, i.e., it is directly neurotoxic [27]. At lower concentrations, it has been demonstrated that isotonic dextrose has the capacity for direct analgesic effects [12,13]. As previously mentioned, the most commonly accepted mechanism of action for hypertonic prolotherapy is the induction of a local osmotic cellular stressor, along a concentration gradient, which drives an augmented inflammatory response, necrosis and then, regeneration of soft tissue components. Others have postulated a direct effect on apoptosis or autophagy [28,29]. In relation to isotonic dextrose, however, the mechanism of action may be the modulation of neurogenic inflammation [27].

The induction of a sublethal insult, followed by a rapid cellular response appears to be pro-inflammatory in nature; this may be the key molecular step in the purported regenerative process of prolotherapy. The high extracellular concentration of glucose leads to a state of non-enzymatic glycosylation, whereby glucose binds to extracellular matrix components and attenuates the resorption of extracellular matrix (ECM) for tissue remodelling. These positive remodelling effects have been seen in both *in vivo* and human clinical studies in relation to increases in tendon and ligament strength and stiffness, i.e., an improved modulus of elasticity or Young's modulus (stress/strain relationship) in these soft tissues [30-32]. Furthermore, light microscopy and (electron microscopy) EM studies of human posterior sacroiliac ligaments three months following prolotherapy have shown an increase in the number of active fibroblasts and collagen 'waviness', with an average increase of collagen fiber diameter of more than 50% (from 0.055 ± .026microns to 0.088 ± .041 ($P < .001$) [33]

In addition to these effects on tissue healing and regeneration, there is an upregulation of inflammatory mediators, including COX-2, IL-1, IL-6, TNF alpha and VEGF after treatment with hypertonic dextrose [34]. In association with this, there is a strong decrease in TGF-beta bioactivity after proliferant treatment. Cell migration analysis has also revealed that prolotherapy decreases tenocyte migratory capabilities (capacity) and that the decreased cellular activity was seen at 24 hours, but that after this period, there is a tendency towards tissue regeneration as the acute inflammatory response becomes gradually attenuated over time [34,35]. Proliferant agents can be classified into three categories: namely, irritants, osmotic drivers and chemotaxis inducers. Each agent is named after the suggested mechanism of action for initiating the localised inflammatory response. Dextrose is considered an osmotic agent that functions through the dehydration and subsequent necrosis of cells after injection.

The lyftogt philosophy – hydrodissection and the perineural injection technique

Research groups have explored the utility of hypertonic dextrose solutions for inducing fibrosis and the mechanisms by which

this occurs [36,37]. Other agents have similar effects; for example, phenol works more as a direct tissue irritant, which subsequently oxidises into quinine groups, leading to cellular damage and finally, necrosis. It is now appreciated that treatment with both agents ultimately leads to localised cell death that is then followed by a regenerative, healing process, which intuitively correlates with the resolution of symptoms in patients [13,14,37]. Therefore, the implicated mechanism of action is direct cellular damage, subsequent necrosis, potentiation of the inflammatory milieu with interleukins and other cellular mediators of acute inflammation and then subsequent cellular-driven healing via tenocytes, myofibroblasts and other cell lines, involving signalling mechanisms that are not yet fully elucidated.

Aside from the direct biochemical and cellular effects of hypertonic dextrose, research has focused on the physical effects of local isotonic dextrose injections, especially when it is used adjacent to nerves, both superficial and deep [38-40]. The evidence base for this perineural injection philosophy is evolving, but the idea was first given a sound basis in 2005 by Dr. John Lyftogt [41-43]. It has been suggested that 5% dextrose is safe when applied in a perineural/interstitial fashion and has no known significant sequelae [41]; injection around nerves using higher concentrations of dextrose has been implicated in direct neurotoxic effects [41]. Perineural injection with buffered isotonic dextrose solutions represents an exciting clinical modality and can be performed under ultrasound guidance, for targeted therapy, or 'blind', that is, based on anatomical landmarks.

The scientific rationale for injecting dextrose around interstitial C-fibre nociceptors is derived from detailed neurophysiological experiments performed in the 1980s and 1990s by Professors Bruce MacIver and Darryl Tanelian from Stanford University [42]. Using a rat cornea nociceptor model (which is exclusively innervated by small fibre peptidergic nociceptors), they found that ischaemia activates C-fiber nociceptors causing pain; ischaemia then drives the observed hypoglycaemia, hypoxia and acidosis. Whilst lactic acid accumulation did not alter C-fiber discharge frequency, hypoxia increased C-fiber discharge frequency by more than 200%. The metabolically inactive L-glucose (as a way to simulate hypoglycaemia) increased the C-fiber firing rate by 652%. Combined hypoglycaemia and hypoxia did not produce a significantly greater increase in discharge frequency compared with hypoglycaemia alone. Subsequent glucose exposure normalised C-fiber discharge frequency within 30 min [42].

Dextrose prolotherapy in the management of myofascial pain

Despite research, it is still unclear what drives the clinical benefits of perineural prolotherapy. Is it the direct cellular effects outlined previously and supported in the literature? Are the benefits in association with 'nerve lesions' due to a direct mechanical effect of the dextrose injectate, that is, a neural hydrodissection effect, where there may be the added benefits of soft tissue decompression, freeing up the passage of a superficial nerve or the expansion/liberation of tight fascial bands which constrict a nerve's local blood supply (vaso nervosum)? These direct mechanical effects have been described in the literature with a host of other injectates and whether 5% dextrose adds additional clinical utility requires further exploration [43-45]. Furthermore, the effects of hydrodissection may not be isolated to small sensory or cutaneous nerves.

Given our limited understanding of dextrose perineural injection therapy, future well-designed trials are necessary to investigate the true effect of hydrodissection, especially at the nerve-fascial interface. Indeed, besides the mechanical effects, the possible biologic action of 5% dextrose is thought to be the reduction in neurogenic inflammation through the inhibition of transient receptor potential vanilloid receptor-1 (TRPV1) that is found in high concentrations in peripheral nerves [46,47]. The attenuation of TRPV1 may then block the release of neuropeptides that supply the inflammatory cascade that drives pain generation. One group demonstrated a significant reduction in the cross-sectional area of the median nerve compared with that in control groups, namely, perineural injection with normal saline; this observation suggests that there is an additional antineurogenic inflammation mechanism with 5% dextrose, although additional randomised clinical trials would be best placed to delineate this specific effect [48]. In relation to other nerve entrapment syndromes, high-quality clinical trials on perineural dextrose injections are still warranted. The direct, causal relationship between glucose and C-fibre activity in modulating neurogenic inflammation and stabilising the 'inflammatory perineural environment' remains incompletely understood. Nevertheless, as no major adverse outcomes have been described in the literature, perineural injection therapy using buffered isotonic dextrose appears to be a safer and equally effective alternative for injectables containing saline, local anaesthetics or corticosteroids for a host of musculo-

skeletal pain drivers [49,50].

Conclusion

Prolotherapy is an injection-based therapy for acute and chronic musculoskeletal pain and has a long history of clinical use. The two main forms of dextrose prolotherapy utilise isotonic or hypertonic preparations and are considered to work via different mechanisms. Hypertonic dextrose is the most commonly used injectate and has an excellent safety profile. Isotonic dextrose can provide reliable and reproducible analgesia, given the correct patient and indication. Indeed, the application of isotonic dextrose in a perineural injection technique fashion has proven benefit for myofascial disorders, including small and large nerve entrapment syndromes. As a therapeutic intervention, dextrose prolotherapy is a powerful tool in the sports medicine armamentarium. Further investigation with high-quality randomised controlled trials with non-injection control arms in studies specific to sports injuries is needed to determine the efficacy of prolotherapy in achieving good long-term outcomes.

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