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REVIEW ARTICLE

Use of Saline as a Placebo in Intra-articular Injections in Osteoarthritis: Potential Contributions to Nociceptive Pain Relief

David Bar-Or^{1,2,3,4,5,*}, Leonard T. Rael^{1,2,3,4,5} and Edward N. Brody⁶

¹Swedish Medical Center, Trauma Research, Englewood, CO 80133, USA

²St. Anthony Hospital, Lakewood, CO 80228, USA

³The Medical Center of Plano, Plano, TX 75075, USA

⁴Penrose Hospital, Colorado Springs, CO 80907, USA

⁵Ampio Pharmaceuticals Inc., Englewood, CO 80112, USA

⁶SomaLogic Inc., Boulder, CO 80301, USA

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Abstract:

Background:

Osteoarthritis of the knee (OAK) is a severe debilitating condition characterized by joint pain, stiffness, and resultant limited mobility. In recent years, intra-articular (IA) injections have been used to relieve symptoms and have succeeded to varying degrees either with sodium hyaluronate preparations or with a biologic.

Objective:

The objective of this review is to evaluate multiple studies that demonstrate some relief from the symptoms of OAK in the saline arm of various clinical trials.

Method:

A thorough literature search (PubMed) was performed assessing the pain efficacy of various compounds compared to saline injections in clinical trials. A total of 73 studies were identified in the literature search including a total of 5,816 patients. These clinical trials all involved the IA injection of a viscosupplement (hyaluronate, platelet rich plasma (PRP), *etc.*) or a biologic (the low molecular weight fraction (< 5kDa) of human serum albumin (LMWF-5A)). For all of these studies, the control arm was injection of sterile physiological saline that approximates the salt concentration and total solute concentration of blood and most tissues.

Results:

Based on our review of the current literature, the tested compounds performed with mixed results when compared to saline injections. Moreover, OAK is a variable disease, with severity measured on the Kellgren and Lawrence (KL) scale where various hyaluronate preparations have a therapeutic effect mostly on KL 2-3 patients while a biologic works best on KL 3-4 patients.

Conclusion:

Since the effect of saline injection is always greater than no treatment, the evaluations of these treatments can be confounded in clinical trials. Therefore, the question of whether there are known therapeutic effects of saline injections might explain these results.

Keywords: Biologicals, Clinical trial, Osteoarthritis, Neuropathic pain, Nociceptive pain, Pain relief.

* Address correspondence to this author at the Swedish Medical Center/Trauma Research Department, 501 E. Hampden Ave., Room 4-454, Englewood, CO 80113 USA; Tel: (303) 788-4089; Fax: (303) 788-4064; E-mail: dbaror@ampioharma.com

INTRODUCTION

Osteoarthritis of the knee (OAK) is a severe debilitating condition which is the leading cause of chronic disability in the U.S. It is characterized by joint pain, stiffness, and the resultant limited mobility. In recent years, intra-articular (IA) knee joint injections have been used to relieve symptoms of this condition. These have succeeded to varying degrees either with sodium hyaluronate preparations [1, 2], platelet-rich plasma (PRP) [3], or with a low molecular weight fraction (< 5kDa) of human serum albumin (LMWF-5A) [4, 5]. In all of these studies, the control arm was injection of the same volume of sterile physiological saline (*i.e.* 0.9% NaCl w/v or 286 mOsmol per kg). Physiological saline was developed to approximate the salt concentration and total solute concentration of blood and most tissues. Some of these studies involving the IA injection of viscosupplements for knee OA demonstrate a significant pain relief provided by saline only injection. In some cases, there are no significant differences for pain and/or function between viscosupplementation and saline only [2].

Variations in tissue osmolality are well known, and, since sodium concentration is often of physiological importance, these variations can have physiological and pathological importance. This is perhaps the crux of the problem which arises during these studies on IA knee injections. In every study, some relief from the symptoms of OAK was seen in the saline arm of the clinical trial. In many clinical trials, the compound tested performed with mixed results when compared to saline injection. Moreover, OAK is a variable disease, with severity measured on the Kellgren and Lawrence scale, on which KL 0 indicates no disease and KL 1 to 4 indicates arthritic changes of increasing severity. It is thought that the various hyaluronate preparations have a therapeutic effect mostly on KL 2-3 patients, whereas the LMWF-5A preparation works best on KL 3-4 patients [1, 4, 5]. Since the effect of saline injection is always greater than no treatment, evaluations of these treatments can be confounded in clinical trials. Therefore, the question arises of whether there are known effects of saline injections which might explain these results.

ANALGESIC EFFECTS OF SALINE

IA injection of saline has been widely used as a “placebo control” in multiple clinical trials studying the effects of various drugs compared to saline. Repeatedly, it has been demonstrated that saline has active analgesic effects compared to many drug regimens and as a stand-alone injection. A well-conducted study on the analgesic effects of IA injection of saline in 60 patients with moderate to severe pain after knee arthroscopy demonstrated that patients experienced good pain relief with either 10 or 1mL of saline injections [6]. In a previous study involving 57 patients following knee arthroscopy, the same group demonstrated equal efficacy in pain relief whether saline alone or saline with 2mg morphine were IA injected into the knee [7]. In a systematic review and meta-analysis of 74 randomized clinical trials for the treatment of OAK with hyaluronic acid, it was concluded that saline has a “large placebo effect” of approximately 30% pain reduction [8]. Other meta-analyses of randomized controlled trials have demonstrated that the administration of IA saline significantly improves short-term (≤ 3 months) and long-term (6-12 months) knee pain, improves function, and decreases stiffness [9, 10]. Finally, in a systematic review of the literature for IA therapy in OAK [11], several studies demonstrate efficacy of a single saline injection or saline lavage in patients with OAK [12, 13]. The analgesic effect of saline extends beyond just IA injections with pain relief observed in an intrathecally-pumped saline rat model [14].

OBSERVED EFFECTS OF SALINE ON OA BIOMARKERS

In both physiology and disease, hypertonic saline ($>0.9\%$ NaCl w/v) treatment has been used clinically for many years due to known beneficial effects. Hypertonic saline administration reduces lung leakage in both acute lung injury (ALI) and acute respiratory distress syndrome (ARDS) [15, 16]. Neutrophil activation is attenuated by hypertonic saline, and this attenuation is reversible when normal concentrations of saline are used [17]. This phenomenon became more interesting, and perhaps more pertinent to the subject treated here, when Wright et al recently published a paper on the anti-inflammatory effect of hypertonic saline in pulmonary epithelial cells [18]. Additionally, when inflammation was induced by TNF α , hyperosmolarity reduced 3 of the 6 pro-inflammatory cytokines (RANTES, MCP-1, and IP-10) which were initially induced more than 5-fold. When IL-1 β was used to induce inflammation, hyperosmolarity reduced 4 of 10 augmented pro-inflammatory cytokines (RANTES, IP-10, G-CSF, and MIP1b). Importantly, this hyperosmolar effect did not depend on a particular solute. Increasing osmolality using sorbitol gave the same results as hypertonic sodium chloride. Since joint cartilage has a large amount of proteoglycans, especially aggrecans [19], these can contribute significantly to the osmolality of synovial fluid. More importantly, these components are broken down to

variable degrees in different Kellgren Lawrence stage OAK patients adding to the complex osmolar control of inflammatory responses in arthritic knees.

Osmolar regulation of anti-inflammatory molecules is controlled, at least in part, by the transcription factor NFAT5 (TonEBP). NFAT5 was discovered as a factor responding to hypertonic saline solutions [20], but it is now known to respond to many situations in normotonic tissues [21]. NFAT5 is present in tissues from osteoarthritis (OA) and rheumatoid arthritis (RA) patients, although the levels found in synovial tissue were much higher in RA than in OA [22]. Also, the nuclear to cytoplasmic ratios were higher in RA than in OA, presumably reflecting greater transcriptional activity since NFAT5 is transported to the nucleus in order to facilitate transcription. With the focus switched to RA, hypertonic saline, TNF α , and IL-1 β all increased the synthesis of NFAT5 and implying that the inflammatory response was enhanced by having more NFAT5. This was supported by mouse experiments which showed that experimental RA was attenuated when NFAT5 siRNA was included. Nonetheless, it is still possible to reconcile these results with the pulmonary epithelial results if the increase in NFAT5 leads to an anti-inflammatory component in the total response. This will require new experiments with OAK tissue and models to see exactly what the relationship is in this disease with a primary focus on hypertonicity, hyperosmolarity, and NFAT5 activity.

In the OAK literature, there are scientific findings that address the effect of tonicity on the inflammatory response. A single stress applied to explanted bovine articular cartilage results in chondrocyte swelling and loss of viability. At low stress levels, only the most superficial layer of cartilage is affected, but with higher stress, significant damage extends into progressively deeper layers of cartilage [23]. Incubating post-impact cartilage in hypertonic saline protected chondrocytes with respect to swelling and viability. Conversely, incubation in hypotonic saline augmented chondrocyte swelling and death. Interestingly, chondrocyte swelling and cell death are seen clinically in OAK patients. The complexity of osmotic changes and possible effects on OAK extend beyond sodium chloride changes, since variable amounts of collagen degradation products and proteoglycan breakdown products (from increased aggrecanase activity) will contribute to overall osmolality. This effect will probably be more significant based on OA severity.

CONTRIBUTION OF SODIUM AND OSMOLALITY TO OA PATHOLOGY

Another facet of the role of sodium chloride in OAK was discovered using ^{23}Na Magnetic Resonance Imaging (MRI). Sodium ions are the main counter ion neutralizing the negative charges on aggrecans and other proteoglycans, mainly carboxylate and sulfate groups. When proteoglycans (mostly aggrecan) are broken down to various degrees in the different stages of OAK, sodium ions are released. This loss of proteoglycan in cartilage from OAK patients can be visualized using ^{23}Na MRI [24]. Using these MRI techniques on bovine cartilage, Na^+ ion concentrations were $\sim 200\text{mM}$ on the articular surface and in deep cartilage adjacent to sub-chondral bone, but $\sim 390\text{mM}$ in the middle layers of the cartilage. This results in an average Na^+ ion concentration of 320mM , a remarkable result bearing on the question in hand, but so far only indicated in a single study [25]. In fact, this technique has indicated that proteoglycan destruction as well as Na^+ ion concentration can be followed in knee cartilage as well as in other joints, but the results are dependent on a mathematically complex calculation based on derived sets of interpretations of the original NMR spectra [26]. It would be helpful to have confirmation of numbers derived from these NMR techniques by more straightforward physical chemical measurements.

All of these potential effects of sodium chloride on the progression and symptoms of OAK are confounded by the relative paucity of information on other measurements of osmolality of synovial fluid, both from normal joints and joints from OAK patients, especially as a function of Kellgren Lawrence scores. Using vapor phase depression measurements of osmolality, Shanfield and colleagues measured osmolality of synovial fluid from 15 RA patients and 15 KL stages III and IV OAK patients. Osmolality in RA synovial fluid was low (280 ± 7.7 mOsmol per kg) while the fluid from OA patients was somewhat higher (297 ± 16.9 mOsmol per kg) [27]. The problem with this study was that they could not get synovial fluid from normal knees at the same time and had to rely on measurements made only a short time earlier with the same personnel and same vapor pressure depression techniques. For synovial fluid samples from 13 normal volunteers, this yielded an average osmolality of 404 ± 57 mOsmol per kg [28]. This concentration for normal synovial fluid is much higher than the value for serum or plasma found in healthy volunteers, RA patients, or OAK patients, which were ~ 305 mOsmol per kg. If this value of 400 mOsmol per kg were correct for normal synovial fluid, then synovial fluid from OA as well as RA patients would be considered significantly lower. A thorough examination for confirmation or denial of this number is not in the literature. Using freezing point depression measurements, synovial fluid from traumatized or chondromalacic knee joints had osmolality values ranging from $292\text{-}310$ mOsmol per kg, not significantly different from values reported for OAK [29].

IMPORTANCE OF SODIUM CHANNELS IN OA PAIN RELIEF

Finally, there is one other aspect of sodium chloride metabolism which should be addressed, even though there is scarce experimental evidence that concentrations in the range discussed so far have any significant influence on function. This is based on the fact that inflammatory pain in OAK is primarily transmitted through voltage gated Na⁺ channels, which control action potentials of afferent fibers to the brain. There are a number of these voltage gated sodium channels in the knee joint, and one might well ask the question whether their relative utilization is in any way influenced by sodium chloride concentrations in the extracellular fluid. This is merely a question since the literature is mute on this subject. However, there are various sodium channel modulators that have reached clinical development for the treatment of pain [30].

NOCICEPTIVE AND NEUROPATHIC PAIN IN OA

Current evidence suggests that IA injected saline has an effect in relieving pain associated with OA. As a result, this may confuse results from clinical trials where physiological saline is the control arm. If this effect were uniform for all OA patients, a simple subtraction might be considered the appropriate manner of dealing with this confounding factor. This appears not to be the case, as demonstrated in the clinical trial testing IA injections of the low molecular weight fraction of human serum albumin for OA [4, 5]. In this study the amelioration of pain with physiological saline was much less pronounced in patients with the most severe OA (KL 4 patients). In fact, it was this decrease in the effect of saline that revealed the most pronounced therapeutic effect of the tested pharmaceutical. How can this be explained? A plausible answer is found in the literature and leads us to a discussion of the origin of this non-uniform pain associated with OA.

Since cartilage does not contain nerve endings, it has been a source of some debate where the pain in OA originates. Local pain is called nociceptive pain where inflamed tissue (in the case of OA this could be synovial, connective tissue, bone, or a combination) stimulates pain transmission that can be combated mostly by anti-inflammatory agents, and, to some degree, by physiological saline as outlined above.

In recent years, a second type of pain has been associated with OA, and seems to be most prominent in patients with severe OA (KL 4) [31, 32]. This is termed neuropathic pain and, although there is overlap with nociceptive pain, it has an extra dimension which renders it pharmacologically separate. Patients with neuropathic pain generally respond well to drugs such as tricyclic antidepressants, selective serotonin/norepinephrine reuptake inhibitors (SSRIs/SNRIs), or gabapentinoids since this type of pain is usually a direct consequence of a lesion or disease affecting the somatosensory system [33]. However, use of these drugs can result in severe side effects for the patient. Therefore, the discovery of safer analgesics is the focus of pain researchers worldwide.

With disease progression, nociceptor input from various pro-inflammatory mediators can induce both peripheral and central nerve sensitization contributing to the existence of neuropathic pain in late stage OA patients [31]. This central sensitization in OA is the result of massive and repetitive nociceptive input originating from peripheral joint nociceptors and transmitted to neurons located on the dorsal horn of the spinal cord [34]. As this migration to the central nervous system is reinforced, new symptoms come to characterize this type of “centralized” pain. These include multifocal pain, fatigue, insomnia, and mood disorders which are documented in OA patients [35]. In fact, it is possible that as much as one-third of OA pain is “centralized” in OA patients [36]. One could therefore make a plausible argument that the physiological saline control arm is not an appropriate control for OA therapeutic trials involving IA injection except perhaps in the most severe (KL 4) patients.

IA SALINE INJECTION IS NOT A TRUE PLACEBO IN OA

There is a clear unmet medical need for new therapies that are efficacious in treating the pain and inflammation of OA with limited side effects. Further complicating treatment is the lack of effective drugs that address both the nociceptive and neuropathic components of OA. Clearly, OA is a complex disease that involves bone, joint, and physical changes that are associated with symptom severity but are poorly understood. Treatment of OA should be on an individual basis since there are multiple studies that document various OA subgroups. One study found an association between classic neuropathic pain descriptors (burning, tingling, pins and needles, and numbness) and OA patients with higher pain intensity and severity and longer duration [37]. Treatment of neuropathic pain in severe OA has been attempted by intravenous infusion of the sodium channel blocker lignocaine resulting in a significant reduction in pain relief [38]. However, the intradermal injection of lignocaine in OA patients awaiting total knee replacement was

just as effective as saline [39]. This suggests that saline is effective at alleviating nociceptive pain calling into question whether it is a proper placebo in clinical trials that assess pain as the primary endpoint.

FUTURE PERSPECTIVES

In the meantime, it is probably prudent to measure the osmolality of synovial fluid from OAK patients enrolled in clinical trials and to reduce the variability of the sodium chloride concentrations for “isotonic saline” used in “control” injections. Two urgent matters which could be resolved rapidly are the thorough investigation of the osmolality of synovial fluid from non-arthritic knee joints (keeping in mind that this is the most difficult kind to collect because there is very little fluid in normal knee joints). If it is really 400 mOsmol per kg, then OAK synovial fluid is very hypo-osmotic, similar to RA synovial fluid. The other more difficult confirmation would be to determine if the gradient in human cartilage of Na⁺ ions also ranges from 200mM at the extremities to 390mM in the middle. The complex nature of pain in OA further adds to the placebo effect of saline with neuropathic pain becoming increasingly recognized in OA. It is quite possible that saline only affects nociceptive pain but is ineffective in neuropathic pain which is highly prevalent in severe OA patients. Therefore, a subset of OA patients that exhibit primarily neuropathic pain (*i.e.* KL 3 and KL 4 patients) might be a better choice to enroll in clinical trials in order to assess the true efficacy of potential OA treatments. To further complicate matters, the effect of simply inserting a needle could cause mechanical stimulation and bleeding of the degenerated tissue thereby potentially increasing the placebo effect [10, 40]. Perhaps clinical trials should be conducted using a different placebo control until these matters are resolved.

CONCLUSION

In our review, the plausible and potential beneficial effects of IA saline injections into knee joints of OAK patients have been addressed. There are quite a number of them, and it is thus not surprising that such injections are not ideal placebos for testing new compounds that reduce pain and inflammation in knee joints of OAK patients. More experimental evidence should be accumulated which could help understand the variability and extent of the saline effect, but this will require many years of controlled research.

LIST OF ABBREVIATIONS

OAK	=	Osteoarthritis of the knee
IA	=	Intra-articular
KL	=	Kellgren and Lawrence scale
LMWF-5A	=	Low molecular weight fraction (< 5kDa) of human serum albumin
OA	=	Osteoarthritis
RA	=	Rheumatoid arthritis
NaCl	=	Sodium chloride
TNF α	=	Tumor necrosis factor alpha
IL-1 β	=	Interleukin 1 beta

CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

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