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Can the combination of localized ''proliferative therapy'' with ''minor ozonated autohemotherapy'' restore the natural healing process?

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Summary Regenerative injection therapy (RIT), also known as proliferative therapy, has been used for over 30 years in the USA in patients with spinal and peripheral joint and ligamentous pathologies. It involves the injection of mildly irritating medications onto ligaments and tendons, most commonly at origins and insertions. These injections cause a mild inflammatory response which ''turns on'' the normal healing process and results in the regeneration of these structures. At the same time they strengthen and become less sensitive to pain through a combination of neurolysis of small nerve fibers (C-fibers) and increased stability of the underlying structures.

Oxygen/ozone therapy is a well established complementary therapy practiced in many European countries. The ozone dissolves in body fluids and immediately reacts with biomolecules generating messengers responsible for biological and therapeutic activities. This results in an anti inflammatory response, which also results in a similar trophic reaction to that of RIT. It is logical to expect that combining these two modalities would result in enhanced healing and therefore improved clinical outcomes. Oxygen/ozone therapy, accomplished by autohemotherapy (AHT), is performed by either administering ozonated blood intravenously (Major AHT) or via intramuscular route (Minor AHT). These procedures result in stimulation of the immune and healing systems. Our concept is that the local injection of this activated blood injected directly to the ligamentous areas that are also being treated with RIT will act as a direct stimulation to the healing process. In addition, combining this with intravenous major AHT should stimulate the immune system to augment and support this process. RIT and oxygen/ozone therapy have been extensively studied separately. We propose a study of lumbosacral ligamentous pain to explore this therapeutic combination. We hope that this paper will stimulate general interest in this area of medicine and result in investigation of the ''interface'' between these treatment modalities.

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Introduction

Up to 80% of the world population is bound during their life-time to suffer from back pain. This is caused by a complex pathology characterized by ligamentous lumbosacral pain, degenerative disc disease, as well as degenerative arthritis which can affect almost any joint in the body. This incurs a huge social-economic cost from medical treatment, as well as work time lost.

There is a gradual and to some extent, natural degeneration of the moving parts of the body due to ongoing recurrent mechanical stresses. Failure of tissue repair with aging and inflammation. The ligaments, tendons, and cartilage become dehydrated and weaker. This results in laxity and secondarily, abnormal mechanical stress. Abnormal shearing and torsion forces gradually cause joint dysfunction. These cause a progressive separation of the ligaments from the periosteum. The space that this creates is filled with new bone formation. These bits of bone are the osteophytes that are a hallmark of degenerative arthritis. The increased joint play and abnormal forces also result in increased and abnormal wear of the cartilaginous joint surfaces, causing joint space narrowing, the other most common finding seen in degenerative arthritis of hips, knees, ankles, wrist and hand joints, and shoulders. The facet, sacroiliac joints, and the intervertebral disks and are also affected by this process. One would think that there would be an increased joint range due to laxity and loss of cartilage, but the opposite is true. The osteophytes and joint laxity result in a reduced range of motion (in a specific pattern for each joint), which causes abnormal strain on other contiguous structures, which themselves become increasingly dysfunctional, causing the process to spread to sometimes quite remote structures, which can cause further abnormal stresses.

At times ligamentous injury can start a rapid degenerative cascade with both local and remote sequelae. In others a subtle injury at young age can result in problems later in life, often in areas that are remote to the original injury. With aging, it is common to progressively undergo seemingly minor variations in gait and coordination which cause gradual dysfunctions. There are genetic differences as well, which can cause premature osteophytic changes in the metacarpal and phalangeal joints. Other common genetic predispositions cause ligamentous laxity of varying degrees, the best known and most severe of which are Ehler-Danlos and Marfan syndromes [1]. All of these factors can start the degenerative cascade and propensity to injury and pain.

Low back pain occurs frequently and is often caused, as commonly perceived, by nerve root compression from the intervertebral disc. The ligaments, however, are an under-appreciated and important cause of low back pain. Root pain is today widely considered to be due to a number of factors, including inflammation with release of biohumoral mediators, local venous stasis, edema, acidosis and to alteration of the perineural circulation. These are supported by the fact that some people have no back pain in spite of a significant disc herniation. The reverse is also true as there are many patients who have normal discs on MRI and have intense pain, often radiating in the sciatic distribution [2—12] In one double blinded study of regenerative injection therapy (RIT), MRI and CT scans were performed in all patients studied. There was no correlation between the radiographic findings and successful treatment of the ligamentous structures [13].

Injured structures often develop abnormal C-fibers, which can cause pain without appropriate painful stimulation. In addition there is increased neural firing by proprioceptive receptors, which can inappropriately cause painful firing of nociceptors. All of this stimulation to spinal cord structures causes ''wind up'', a type of remodeling and hypersensitization of the normal pain pathways that we see in chronic pain. The result is allodynia (pain from what is usually non painful stimulation) and increased sympathetic nervous system activity. This causes a decrease in blood supply and further diffuse pain tract stimulation that often crosses the midline and dermatomes [14—19].

The pathophysiology of back pain is therefore multifactorial. A common denominator may be represented by the chronic oxidative stress that perpetuates and cyclically aggravates the pathology [20]. Polymorphonuclear leukocytes and macrophages infiltrate the tissues and release noxious reactive oxygen species (ROS), proteinases (elastase, collagenase and matrix metalloproteinases, MMP), proinflammatory cytokines such as tumor necrosis factor-alpha (TNF alpha), interleukins 1, 6, 8, 12 and 15 which attract more leukocytes, thus accelerating tissue degeneration and destruction. Release of phospholipases (particularly PLA_2) and activation of cyclooygenase (COX II) allows the synthesis of prostaglandins that, together with factor P and bradykinin, cause pain and edema. Simultaneous activation of nitric oxide (iNOS) synthase, allows the release of NO, formation of peroxynitrite and further stimulation of eicosanoid production. This contrib-

utes to the chronic flux of cells into the inflamed tissues. Thus, it appears that even a small initial pathogenic event can initiate a vicious cycle that includes several biochemical and immunological pathways. There are also neurologic changes (wind up) that combine with these to perpetuate and progressively worsen the painful condition.

Orthodox medicine uses anti-inflammatory drugs (nonsteroidal anti-inflammatory drugs and glucocorticoids) that are able to block or slow down the release of eicosanoids and limit their cellular effects, opiate pain killers, and centrally acting medications such as anticonvulsants and antidepressants to modify pain processing and therefore perception. Interestingly, it has been observed that an intravenous infusion of infliximab (Remacaid), an antibody against TNF alpha, produced a rapid and dramatic improvement in leg pain among patients with severe sciatica [21].

Although surgical intervention is effective and at times imperative in a limited number of cases, such as large disc herniations, its efficacy in many situations is poor and the procedures, such as spinal fusion, can be debilitating with protracted disability during recuperation and rehabilitation [22—24]. Although these treatments can be beneficial by alleviating the worst symptoms, they neither eliminate the cause, nor markedly change the pathological histology and evolution of the underlying process. On the basis of our previous medical experience, we would like to put forward another approach.

A new hypothesis

As even the most sophisticated and costly conventional therapies do not modify the disease process and often cause side effects, we are proposing a new strategy that aims to reverse the inflammatory and degenerative process by using two safe approaches, namely ''proliferative therapy'' (prolotherapy) and ''ozonated autohemotherapy'' (AHT). The exciting aspects of this hypothesis is that although both approaches, particularly the former, stimulate an inflammatory reaction, they both eventually facilitate regeneration of weakened, painful tissue. Moreover, they have already been used in many patients, albeit not yet tested together. They are safe and easy to administer, with minimal side effects [25] and have yielded good results in controlled studies [13,26].

Proliferative therapy, also known as regenerative injection therapy (RIT), is the injection of mild inflammatory producing substances onto ligaments and/or tendons, most commonly at their origins and insertions. This causes a series of mechanical, biochemical and neurologically mediated processes culminating in a fibroblastic reaction akin to the natural healing process [27]. This, under the proper conditions, results in the creation of new, stronger, flexible, and less pain sensitive ligaments and/or tendons. RIT has been developed and practiced principally in the United States [28] although James Cyriax MD, the British ''Father of Orthopedic Medicine'' was a proponent of its use for the treatment of ligamentous laxity in the thoracic and lumbar spines [29,30].

Ozone therapy originated in Germany some 40 years ago and is being used in medicine in several European countries and in Asia. It is also currently practiced in several centers in the United States [31,32]. The fundamental concept is that a precise and appropriate ozone dosage added to a suitable volume of the patient's blood causes a transitory and calculated acute oxidative stress that activates several biochemical pathways in blood cells [33—35]. These then release many cyto- and immuno-active substances, which after reinjection into the donor, trigger a ''therapeutic shock'' able to reverse a pathological state. One of us has extensively described [33] the mechanisms of action of ozone in blood ex vivo, showing that, within a few minutes, the reactions between ozone and blood components are completed and the ozone disappears. This reaction generates well known chemical compounds such as hydrogen peroxide and lipid oxidation products (LOPs). As ozone and the blood reactants (plasma and cell antioxidants) are precisely calibrated, definitive biological effects without toxicity ensue. An interesting trait of ozone is that the activation of either erythrocytes or platelets enhances both delivery of oxygen and release of growth factors. When the ozonated blood is reinjected into an inflamed tissue, it leads to a normalization of metabolism, cell proliferation and synthesis of extracellular matrix.

We have therefore hypothesized that combining RIT with both major and minor AHT may produce a synergistic effect able to restore normal anatomy and function to ligaments and tendons, restoring function to the joints that they support.

Why do we think that the proposed hypothesis will be more effective than current conventional therapies? A simple analogy is provided by thinking how the hinges allow a door to work properly. If a hinge of a door is loose, the problem that we see may be with the door's opposite edge hitting the door frame. If one lifts the door back into place, it may be fine temporarily (for example, treating spinal joint dysfunction with manipulation), but

the next time it opens, the same problem will recur. Applying oil to either the hinge or the frame may lubricate and allow less stress for a while (for example using a local steroid injection), but this is also only a temporary solution. What we need to do is fix the hinges!

The state of the art and a preliminary evaluation

First of all let us examine how RIT is performed. In the USA, physicians practicing prolotherapy are currently using hyperosmotic glucose solutions (between 12.5% and 25.0%, while isoosmotic solution is 5.0%) with 0.5—1.0% lidocaine. Glycerine, also acting as a hyperosmotic, agent is frequently added. Another ingredient is phenol. It ensures sterility and is also neurolytic, destroying the bare C fibers exposed in pathologic connective tissue [36]. In two double blind controlled studies, the ''proliferant solution'', defined as P2G was composed of 12.5% glucose, 12.5% glycerin, 1.25% phenol and 0.5% lidocainecit [13,37]. One milliliter of sodium morrhuate 5%, a medication derived from fish oil and used for venous sclerotherapy, can be added to 9 ml of P2G solution to increase the inflammatory reaction in patients who do not adequately respond [38].

The injection of a proliferant mixture causes low level inflammation, which acts as a rejuvenating stimulus. Volumes of 0.1 ml are injected to multiple ligamentous attachments in the area to be treated. Twenty milliliters of solution is typically used to treat the lumbosacral spine.

This results in a process that is divided into three phases [27]. The early phase (Phase I) when granulocyte activity and release of inflammatory cellular contents prevail lasts about three days. Over the next 10—15 days macrophages predominate (Phase II). They release chemotactic factors, which attract fibroblasts and act as growth factors. Over the next several days collagen starts to form, giving strength to the tissue (Phase III). At first this is a soupy mixture without structure. Soon, however, a matrix forms, on which collagen is deposited. Macrophages are still active and they absorb some of this new material. Over time collagen predominates and there is a gradual dehydration of the matrix with more orderly collagen fibers. This eventually leads to new, stronger connective tissue. The whole process may take several months. During this time period, external forces affect the eventual outcome of the final tissue fibers as to length, strength, orientation and flexibility. This works much in the same way as the bone remodeling that occurs after fracture (Wolf's law). The elegance of this process is that the ''body decides'', depending on individual posture and stresses which ligaments to tighten, accommodating the physical needs of each individual. This is why it is important to advise RIT patients to stretch and stay active during therapy.

The current RIT protocol calls for a series of proliferant injections at least one week apart. By repeatedly starting and then restarting the inflammatory cascade with regularly scheduled injections, better and more effective healing takes place, the fibrous network is more extensive and the actual healing process is stronger and most likely prolonged for several months after the end of the injection series.

RIT has been evaluated by randomized trials and found to be effective for the treatment of low back pain, as well as many other musculoskeletal disorders [13,37,39,40]. A position paper committee of interventional pain physicians was formed and undertook a comprehensive review of pertinent literature. The committee reviewed 78 specific articles and nine textbooks, as well as 51 relevant articles and chapters from other text books. They concluded that ''RIT is a safe and effective treatment modality that is very useful in a significant number of pain syndromes arising from ligament and tendon diathesis, as well as other clearly delineated pain problems [41].'' Yelland et al. [42] published another meta analysis in 2004, which also showed the efficacy of this treatment.

By modifying the local milieu both on cellular, neurologic, and mechanical levels RIT can attack many of the abnormalities described above. RIT is effective for these conditions by effecting repair and tightening of the lax structures. In addition, RIT has a neurolytic effect ablating the aberrant C-fibers that are often present in painful structures. The result is decreased sympathetic tone allowing better local circulation and a more normal cytokine environment. The increased blood flow eliminates ischemia, acidosis and improves cell metabolism. With these changes comes increased range of motion and decreased pain.

Let us now examine the rationale for combining RIT with ozonetherapy: why and how minor or major ozonated AHT helps to normalize the variety of pathological states and positively influence the RIT procedure.

We propose to use a combination of both major and minor AHT. Major AHT will be carried out according an optimized and well standardized procedure in ozone-resistant glass bottles using 25 ml of sodium citrate (3.8%) and collecting 225 ml of blood by using a G19 butterfly needle. Two hund-

red and twenty-five milliliters of gas mixture (oxygen + ozone) with an ozone concentration of 30 mcg/ml, per ml of blood, will be added (total dose = 6750 mcg). After 10 min of gently rotating the bottle to completely exhaust the ozone dose, the ozonated blood will be reinfused into the donor during the next 15 min. The final 10 ml of blood will be collected in a 20 ml syringe just prefilled with 10 ml of gas (ozone concentration equal to 30 mcg/ml), rapidly mixed and then injected into the ligamentous sites used for prolotherapy. Thus, the blood that is injected is ozonated with a concentration of 60 mcg/ml.

What are the effects of major AHT? They include improved delivery of oxygen to all organs, a very mild immune activation [33] an upregulation of antioxidant enzymes with possible correction of a previous endogenous chronic oxidative stress, [35] and an hypothetical mobilization of staminal cells (cells with stem cell-like potential) from bone marrow or from tissue under RIT stimulation. If this happens, it will represent a great advantage for rebuilding a younger and healthy structure [35]. Last but not least, AHT induces gentle hormonal stimulation (likely resulting in increased secretion of HGH, ACTH, cortisol, DHEA) that can explain the feeling of wellness and euphoria reported by the majority of patients after AHT.

What are the purpose and effects of the minor AHT? Ozonated, hence primed, leukocytes may either infiltrate the tissue or/and may return via lymphatics into the blood pool or into other lymphoid microenvironments. Most of the erythrocytes will be slowly broken down locally and will provide substrates for rebuilding the extracellular matrix but, most importantly, heme will induce the synthesis of stress proteins, particularly heme oxygenase I (HO-I or HSP-32). This is a most protective enzyme [43] that, by enhancing the release of CO and bilirubin, facilitates the local circulation and neutralizes oxidant compounds. Finally, the twice ozonated platelets will be activated and release locally a wealth of growth factors (platelet-derived growth factor-PDGF, basic-fibroblast growth factor, b-FGF, transforming growth factor β1, TGF-β1), which will greatly help tissue reconstruction. The RIT local injections could be performed using autologous ozonated platelet-rich plasma (PRP), which could be more effective, but its preparation is time-consuming and not practical for a private physician. We discourage the use of a pooled PRP (even if it is well screened) due to the potential risk of a viral contamination.

PROS and CONS of the hypothesis

All the methodological aspects have already been thoroughly tested in thousands of patients. Using either RIT or ozone therapy alone yields excellent results. The cost of the material is very low but both procedures must be performed by an experienced physician. The RIT injections can cause transitory, tolerable pain and only rare serious adverse effects have been noted [25]. Actually, major AHT is frequently followed by a feeling of wellness. The proposed schedule: a RIT treatment every two weeks alternated with ozone therapy is biologically meaningful because we believe that a strategy of slow and progressive stimulation should yield the best results. The RIT will induce the necessary phase 1 stimulus and the AHT will promote phase 2 and 3 of the healing response, augmenting the proliferative effect.

Is there a need for additional nutritional or/and pharmacologic strategies aiming at modifying inflammation and stimulating repair? These include (1) reducing carbohydrates in the diets of those with insulin resistance, (2) consumption of highly purified fish oil (a source of critical omega-3 fatty acids), (3) use of glucosamine and chondroitin sulfate, (4) vitamin supplementation including vitamin C, various other micronutrients, amino acids (including lysine and proline) and minerals such as magnesium, and (5) careful correction of underlying hormone deficiencies (thyroid, sex hormone, and human growth hormone). While they are certainly not harmful and may be helpful in maintaining and sustaining optimized health therefore increasing one's healing ability, there is no objective proof that they will affect the outcome of this treatment.

Discussion

Care must be taken to be sure that the ligaments are the source of the problem, but based on a correct diagnosis, the proposed therapeutic regimen can be useful in several painful conditions that are caused by weakness and laxity of ligaments and tendons. Since this laxity is a major factor leading to degenerative joints there is a possibility that early treatment may prevent or postpone this condition as well. On the other hand, autoimmune diseases such as rheumatoid arthritis, systemic lupus erythematosus and arthropathic psoriasis should not be treated with the proposed therapy to avoid the risk of exacerbation. We believe that RIT plus ozonetherapy will yield far more advantageous and less toxic results than orthodox therapy based upon COX inhibitors (as was learned after the recent with-

drawal of Vioxx, which may have caused increased mortality in the united States of up to 55,000 lives) [44], glucocorticoids, opiates and surgery.

Both prolotherapy and ozone therapy are certainly far less known than traditional treatments. They are regarded with skepticism due to ignorance and interest in pharmaceuticals but, as we have seen excellent results in our treated patients, we feel compelled to pursue and encourage the use of our proposal.

To be complete we must add that the oxygen–ozone mixture (ozone represents only 2–3% of the gas mixture) as such, is already widely used for the direct treatment of herniated discs. This approach is performed under flouroscopic or CT guidance. A small volume of gas (3–10 ml) with an ozone concentration of 30–40 mcg/ml is injected directly into the intervertebral disc or into the epidural space through the neuroforamina. Thousands of patients have been treated with a success rate varying between 54% and 86% [45–51]. We have also injected the gas mixture (about 10 ml in each side) into the paravertebral muscles at the level of the affected herniated disk. We defined this procedure as ''chemical acupuncture'' because ozone elicits chemical reactions that lead to local nociceptors stimulation and consequent activation of the descending antinociceptive system, leading to significant reduction in pain [33].

Ozone has been also successfully injected intra-articularly for treatment of the knee and shoulder arthritis and/or injuries. Up to date the mechanisms of action of the direct injection of ozone as a gas remains conjectural [20].

One of the authors (V.B.) has established methodology to measure and evaluate the growth of several cell types in culture (fibroblasts, chondrocytes, synovial cells and keratinocytes). These cells can be tested after stimulation with P2G and/or ozone measuring the proliferation index and/or apoptosis, as well as the release of collagen, proteoglycans, both proinflammatory (IL-1 beta and TNF alpha) and immunosuppressive (TGF beta and IL-10) cytokines and, most important, the expression of antioxidant enzymes. We hope to demonstrate that RIT and ozone treatment can enhance the cellular anabolic activity and defense mechanisms, thus counteracting the effects of ligamentous laxity and chronic inflammation.

Animal studies can also be performed that would show the specific effects of varying doses and combinations of the experimental compounds. Injections of rabbit or rat ligaments have been used to study these effects in similar models both on histology and structural strength [38].

Finally, a pilot study of this treatment regimen is currently beginning. Patients are being treated with RIT injections alternating with ozone treated blood injections in the lumbosacral spine. In addition, they are being treated with systemic ozone major autohemotherapy. Clinical outcomes will be measured and attention paid to the types of patients that respond to this treatment regimen. This study, if positive, should be followed up with more comprehensive blinded studies of this process. For both ethical and practical reasons we cannot start with a randomized, blinded, controlled research protocol with the power to differentiate between the various groups that would be needed to completely study this complex process due to the fact that in previous studies of RIT the injection of lidocaine and even normal saline as placebo showed significant proliferant activity [13,40]. This shows that the injection itself provokes a proliferant response, but also makes creating a true double blind protocol very difficult. Finally, RIT itself is under intense study aiming at FDA approval for P2G. The animal studies are nearing completion and the human controlled trials are to start in the near future. The information that will result from these studies can further validate RIT/ozone therapy treatment and, hopefully, will eliminate prejudices.

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