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Aptoxin (bee venon therapy) delivered to ultrasound express change in joint diameter

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Abstract

The long time comes to apitoxin being used as a potent anti rheumatic. In this study, we sought to use the complex substance, enshrined in an experimental model of arthritis induced by the literature. The ultrasound was used as a vehicle for apitoxin in the arthritic joint. For this purpose, we used animals, Wistar rats, in a model where arthritis was induced by inoculation in the knee joint with zymosan. The animals were divided into groups: GI (positive control), GII (negative control), GIII (group treated with topical apitoxin), GIV (treated group with ultrasound) and GV (group treated with phonophoresis), established after induced arthritis. With 21 days of treatment the animals were anesthetized with ketamine and xylazine, their knees were radiographic images, the temperatures collected and euthanized. The RX films were processed by Image J software to collect intra-articular distance of the femur and tibia. Distance was compared to articulate and temperature of each animal. Group I of 0.15 mm and average temperature of 35.7 ° C, group II had an average space of 0.03 mm and intraarticular temperature of 38.2, III, a distance of 0.4 mm and average temperature of 37.8 °. Group IV the average distance to articulate and temperature were 0.6 mm and 36.7 ° C for the group V was observed average values of intraarticular space of 0.78 mm and temperature of 36.2 ° C. We conclude that ultrasound combined with apitoxin via phonophoresis, was efficient in tissue repair and subsequent regeneration of articular contours when compared only with the application of ultrasound or topical apitoxin

Keywords: arthritis induced; apitoxin phonophoresis, Rattus norvegicus

INTRODUCTION

Arthritis reusing used matóide (RA) is a chronic systemic disease, with a predominance of the joints characterized by chronic inflammation of the synovial membrane. It is a disease influenced by hormonal factors, environmental and genetic factors combined lead to the development of an autoimmune process where antibodies produced are directed to the body itself that visualizes joint targets. Often affecting the hands, wrists, elbows, shoulders,feet,ankles,knees. When the process takes a systemic nature, develop extra-articular manifestations such as subcutaneous rheumatoid nodules, vasculitis, pericarditis, pleuritis and lung fibrosis. The chronic inflammatory process results in changes in cellular composition

and gene expression in the synovium. Occurring in synovial tissue proliferation, with production of intraarticular pannus with expansion with consequent structural damage to the cartilage, bone and ligaments. It is a systemic disease of unknown etiology, as evidenced by a persistent polyarticular synovitis, symmetrically distributed with great potential deforming. Estimated to reach 1% of the population in Brazil, with a great burden for social security. The changes entail more severe deformities that cause disability in a predominant age group between 30 and 50 years. AR usually manifests itself after 30 years of age and was higher in women. Men and women over 65 seem to be affected by the same percentage. Despite the high prevalence of the etiology of RA remains unknown, is believed to be related to genetic factors. The patient with RA causes great burden on social security. The knowledge of AR in animals and appropriate modes of treatment can be very useful in patients with this disease. (Morsoleto2007). To study various animal models of RA have been established and employees are to be accepted and must provide information on the histopathological, and molecular genetics of autoimmunity. Should emphasize the following characteristics, (Oliver and Brahne. 1996) Clinical, pathological and radiological findings similar to those of RA with few systemic manifestations: a few findings of non-rheumatoid arthritis; induction in animals easy to handle with low operating cost, reliability of results in short experimental time. Response to therapeutic agents, measurably. The experimental arthritis can be induced by various agents. From this universe of inducing agents, there is the zymosan-induced arthritis. A polysaccharide derived from the wall of the yeast Saccharomyces cerevisiae, a substance inducing phenomena systemic inflammation in the joints of rats, mice and rabbits with persistent proliferation of the synovium and cartilage degradation, playing most of the findings of RA. Developing joint manifestations that is associated with a process-2-dependent prostaglandin (pain, swelling, fever) with increased aggression to the articular cartilage and production of IL-1 (Morsoleto et al 2007). Traditional therapies to treat arthritic patient, using gold salts, NSAIDs, drugs that modify the course of disease and biological agents. These therapies have long-term side effects are accentuated by the prolonged use of permanent treatment (Moreira et al, 2001). Alternative medicine use among agents to treat rheumatoid arthritis, bee venom Apis mellifera. The property has echoes of this poison in ancient Egypt, when many diseases treated with ointment made of bees. Hippocrates (450AC, Galen 130DC, Charlemagne received bee stings in the body for treatment of arthritic joints (MAIA, 2002). The first publications on clinical studies with applications of bee venom occurred in 1864 in Likomskiy, Tere in 1888 and Beck 1997. Bee venon (Been Venon therapy) has been traditionally used in oriental medicine and Eastern European countries to relieve pain and treat inflammatory diseases such as rheumatoid arthritis (RA). apitoxin is the use of Western medicine, prejudices regarding the risk of allergic reactions and toxic effects. apitoxin is a neurotoxin, composed of 88% water, amino acids, enzymes, peptides, bioactive amines, sugars, and other volatile components in small quantities. Below the lethal doses of these toxins may have physiological actions therapeutics. (Meyer, 1996) The apitoxin is composed of a mixture of nitrogen components, which make up about 90% of its dry weight.

Table 1. Are listed some of the major components (Maia 2002).

Molecular Weight (D)	Component	% dry basis	Observations
< 1000	Peptides	15	Chains of up 9 amino acids
	Monoamines	3	Histamine, dopamine, norepinephrine...
	Others	6	Isoleted amino acids, carbohydrates, phospholipids...
Polypeptides	Melittin	50	Anti-inflammatory
1000 a 10.000	Apamin	2	Anti-inflammatory
	Peptide MCD	2	Anti-inflammatory
	Others	3	Tertiapin, secapin
Enzymes	Phospholipase A2	12	Prime allergen
> 10.000	Hiauronidase	2	Secondary allergen
	Others	3	Acid phosphatase, glucosidase, esterase
Others substances		2	Adolapin, protease inhibitor

Source: Fractionation and characterization of apitoxin, Maia et al, 2002

According to Ter Haar (1987) ultrasound is a longitudinal mechanical wave is not audible, frequently above 20 KHz which transports energy through vibrations of the particles of the medium through which the wave is propagating, and do not propagate in vacuum, cause expansions and compressions of the environment every half wavelength energy that carries the wave decreases with distance traveled by the vibrating beam and this phenomenon is known as attenuation. The attenuation is the result of absorption of energy by the tissues and the dispersion of the wave out of the main beam. This phenomenon, attenuation, causes physical effects in tissues that can cause biological changes (Morsoleto, 2003). In 1990, phonophoresis WILLIANS defined as the movement of drugs through the skin into skin tissues under the influence of ultrasound. These drugs are pharmacodynamically active. It is an efficient alternative for transporting substances in addition to drug use oral or intradermal

injections. There are several advantages to using this type of treatment, among them the localized action of the drug with consequent absence of side effects due to systemic actions if the drug has this kind of action. Another advantage of this form of treatment is the sum of the inherent effects of ultrasound associated with the effects of the drug. Various types of drugs such as steroids and anti-inflammatory, has been administered via phonophoresis. The aim of this study was to induce rheumatoid arthritis in the knees of female rats and evaluate the performance of aptoxina via phonophoresis, used as drug coupler.

MATERIALS AND METHODS

The ethics committee of under number 876/2007 approved the methodology. We used 20 female Wistar rats weighing 170 g on average, 60 days, divided into 5 groups of 4 animals, which were maintained with balanced feed and water ad libitum, during periods of light / dark for 12 hours, heated to room temperature average of 25 ° C. Group I (control) was maintained without treatment. Groups II to V, were anesthetized with intraperitoneal injection of 0.3 mL of ketamine and xylazine 0.1 mL for each 200g of body weight of the animal and subjected to injection of zymosan (1 mg) diluted in 50µl of saline 0.9% in the right knee. The apitoxin was used Apitox cream (Apifarma) with the active ingredient apisinum, 21g (6mg/kg) homogenized water soluble gel was administered 1 ml in each animal in the topical treatment or phonophoresis. Group IV was treated with the same amount of carbopol gel.

After a period of 7 days the animals in group II received no treatment at all. The animals in Group III were treated with topical apitoxin through, and the animals of group IV were treated with the use of therapeutic ultrasound. The animals in group V were treated with carbopol gel apitoxin via phonophoresis through a circular motion, at an angle 90 ° and continuous pulse regime, with 1 MHz frequency for one minute, with a daily frequency and intensity 0.2 W / cm ^{2} for 21 consecutive days. The animals were euthanized according to the days of confinement, from the beginning of the experiment, that are 3, 7, 14 and 21. Radiographs were obtained by X-ray machine periapical dental film, following the protocol: Distance to the focal area of film 20 cm, exposure time of 0.1 seconds of play was, the film development time by 30 seconds. It was quickly washed for 10 seconds then remained in the liquid fixative por10 minutes and again washed for 10 minutes. Both the developer and fixative used were Kodak brand. The intra-articular spaces were measured in millimeter scale through a free software Image J image processor based on Java that displays, edits, analyzes, processes, saves and prints images of 8-bit, 16 - bit. Temperature was collected from animals in the groups during treatment.

RESULTS

Radiographs of the Group I revealed contours intact, preserved articular structures. Group II with 3 days, showed thickening of the synovial membrane; to 7 days, early bone demineralization, with patella femoral collapsed on the surface, at 14 days demineralization suffered the knee, the tibial plateau lost the morphological boundary, there was calcification of soft tissues intra-articular, at 21 days, radiographic image showed osteoporosis, visible trabeculae without space patella and femur. Group III presented with 3 days intra and periarticular edema decreased, the contours at 7 days started getting sharper, at 14 days

showed the contours are different in the joint region with signs of bone regeneration at 21 days and observed an increase the space between the patella and femur. Group IV had three days to a decrease in intra-and periarticular swelling. 7 days with the contours become sharper, while at 14 days showed signs of bone regeneration. With 21 days of the Group IV images revealed an increase in space between the patella patella and femur. Animals in Group V at 3 days showed disappearance of synovial thickening at 7 days and the contour of the distal femur began to emerge, at 14 days the images reveal the distal femur delineated, more mineralized and groove poplíleo more visible. At 21 days, was allowed to view the popliteal fossa were more clear with no signs of arthrosis or thickening. Measurements of the intraarticular space group control showed a mean of 0.15 mm and temperature of 35.7 ° C. After 21 days of inoculation with zymosan, in group II had an average space of 0.03 mm and intraarticular temperature of 38.2, III, a distance of 0.4 mm and average temperature of 37.8 °. Group IV the average distance to articulate and temperature were 0.6 mm and 36.7 ° C for the group V was observed average values of intraarticular space of 0.78 mm and 36.2 ° C temperature. Values considered for n equal to 10. As shown in Table (2)

Table 2 shows that as the temperature increases the joint value decreases intraarticular space between the groups when compared to negative control group I.

Groups	***intraarticular Distance (mm)***	***Temperature(C^0)***
Control (-) GI	*0,15*	*35,7*
Control (+) GII	*0,03*	*38,2*
Group APi GIII	*0,4*	*37,8*
Group US GIV	*0,6*	*36,7*
Group APi US GV	*0,78*	*36,2*
Groups	*intraarticular Distance (mm)*	*Temperature(C0)*
Control (-) GI	*0,15*	*35,7*
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Group APi GIII	*0,4*	*37,8*
Group US GIV	*0,6*	*36,7*
Group APi US GV	*0,78*	*36,2*

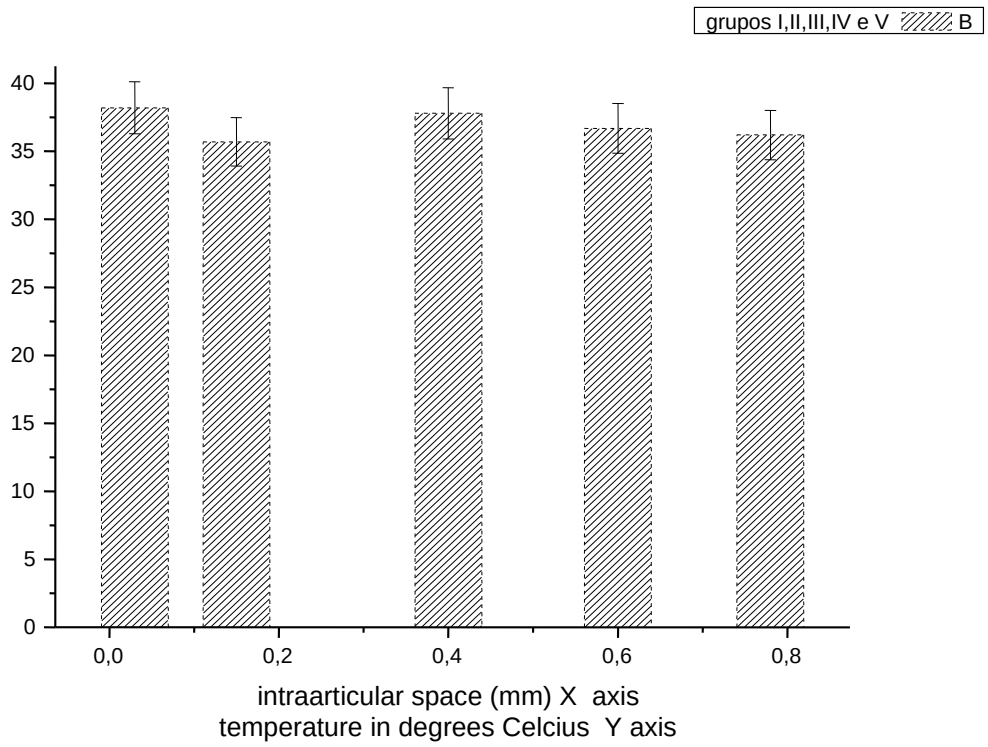

Figure 1a. It may be noted that a decrease in temperature articulate, when increasing the intraarticular space. p <0.05, paired Wilcoxon

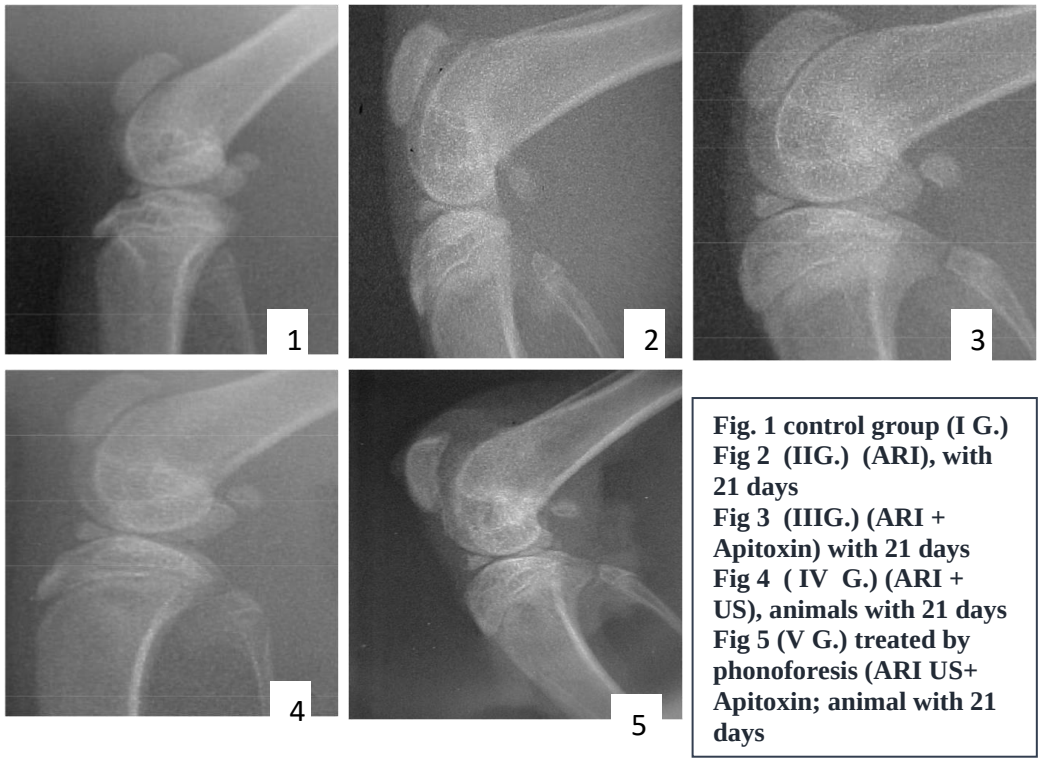

Fig. 1 control group (I G.)
Fig 2 (IIG.) (ARI), with 21 days
Fig 3 (IIIG.) (ARI + Apitoxin) with 21 days
Fig 4 (IV G.) (ARI + US), animals with 21 days
Fig 5 (V G.) treated by phonoforesis (ARI US+ Apitoxin; animal with 21 days

Figure 1b

Table 2 Summary of explanatory figure 1a et b

DAYS	*GROUP I*	*GROUP II*	*GROUP III*	*GROUP IV*	*GROUP V*
3 days	**Contours intact, preserved joint structures**	**Thickening of the synovial membrane**	**Intra-periarticular had decreased;**	**A decreased in intra-periarticular edema;**	**Had complete resolution of synovitis**
7 days		**Early demineralization with collapsed patella in the femural space**	**Contours become to get clearer;**	**Contours become sharper;**	**Contour of the distal fêmur began to take shape;**
14 days		**Tibial plateau loses contour, calcification of soft tissue intra-articular;**	**Diferent contours in the joint region with signs of bone regeneration**	**Showed signs of bone regeneration.;**	**Distal fêmur delineated, more mineralized**
21 days		**Osteoporosis image, visible trabeculae, without patella fêmur space** **(Figure 2).**	**Increasing space between patella and femur (Figura 3).**	**Increasing space between patella and femur (Figure 4).**	**No signs of arthrosis or thickening** **(Figure 5).**

DISCUSSION

In this study, we investigated the effects on arthritis induced apitoxin carried by the ultrasound. The association was evaluated using an experimental model of arthritis. After several trials, testing an ideal induction agent, we chose a model of arthritis induced Wistar rats that used for its ease of acquisition and management. And this demand, the authors became aware of experimental arthritis-inducing agent, zymosan. A polysaccharide derived from the yeast Saccharomyces cerevisiae, whose administration in tissues promotes an intense inflammatory reaction. This model is widely used to test the efficacy of drugs. (Di Carlo et al 1981)

One of the earliest signs of the appearance of a lesion caused by joint inflammation induced in ZyMos is depletion of PGs in cartilage matrix, which induces a progressive irreversible damage of the affected joint, confirmed by X-ray images (C Carvalho Moreira, 2001) . Researchers who worked with zymosan-induced arthritis observed (presence of osteophytes in the radiographic examination, and desquamation, ulceration and cartilaginous cleft, disorganization of chondrocyte columns and niches on histological examination were consistent with osteoarthritis or degenerative joint disease, as verified by Marshall (1969), Pond & Nuki (1973), Lipowitz et al. (1985), Altman et al. (1989a and b) and Biasi et al,

(2004). Radiologically manifestations of rheumatoid arthritis indicate thickening of soft tissues with compromised periarticular, osteopenia, osteoporosis in later stages, reduction of joint spaces, subchondral erosion, bone cyst represented by translucent subchondral areas and whether the invasion of the rheumatoid pannus (Moreira 2001).

The joint destruction and interference in the inflammatory process was accompanied by the authors (Keystone et al, 1997, Gegout et al, 1995, Lee, 2004) through the different mediators involved in these events.

In cartilage damage by zymosan, there are histopathologic features, immunohistochemical and biochemical changes that apply to the experimental study of RA and, in part, meet the minimum requirements proposed by Oliver and Brahne In 1996, for the study of human disease models animals.

The study of these mechanisms may contribute to an approach with physiotherapy resources and effective alternative way of ensuring success in the election of the treatment protocol. (Bradshaw, 1994)

In this study, when analyzing radiographic images the authors observed that the control group showed sharp and distinct outlines joint holding, the structures preserved. Radiographic imaging of the induced group (Azy), were found, erosion of the surfaces that suggest the destruction of cartilage followed by bone resorption.

Several authors have also worked with these models were able to demonstrate induction of these radiological findings in their studies (Bradshaw, JP et al, 1994, Biasi et al 2004, Morsoleto et al, 2007).

In view of these destructive inflammatory episodes, the researchers strive to study mechanisms that may influence the course of autoimmune diseases (Van den Berg WB, 2005).

Following the methodological design of this study, a group of animals was treated with doses of topical and apitoxin via phonophoresis (Williams, 1990), placement of the drug by ultrasound) (Morsoleto, 2003). Attempted to produce the joint primary or immediate effects, increasing cellular metabolism, synthesis of endorphins and decrease the release of nociceptive transmitters (bradykinin and serotonin). objective remission of the inflammatory state, thus reducing pain and job performance (Kwon Young-bae, 2001). At doses of apitoxin observed in this work, the rapid regression of inflammatory signs of inflammation, which are compatible with the present aspects of the control group, healthy.

In 1979, studies conducted by Y. Chang and M. Bliven, we found that bee venom reduced the inflammation in arthritic rats and in another Xperiment, prevented the development of arthritis when administered a single dose of a apitoxin subcutaneously 24 hours prior to the introduction of the substance inducing arthritis. Also in the study effected by Broadman, 1962, Lorenzetti, B. Fortenberry and E. Busby, 1972, Beck 1997, Lee et al., 2004, apitoxin suppressed the development of arthritis effectively.

Do not know all the mechanisms of therapeutic action of apitoxin, but has learned that the most important substances present in this composition

Such as histamine, lecithinase, hyaluronidase (Beck, 1997). These compounds have an action of non-specific protein that alters the sensitivity and immunological mechanisms of the body are also present melittin with high anti-inflammatory action. The peptides responsible for the degranulation of mast cells (immune cells of the body). The Apamina that blocks Ca2 + K during synaptic transmission channels in the nerves (Sokol et al., 1994, Wadsworth et al. 1994; & Xu) Hyaluronidase, adopamina a neurotransmitter that increases motor activity and Adolapina (neurotransmitter) with analgesic effect (Nelson, 1994, Leo and Simic M et al, 2005).

CONCLUSIONS

Zymosan caused an intra-articular inflammatory process observed by the reduction of intra-articular space and synovial thickening by means of radiographic images. Ultrasound combined with apitoxin via phonophoresis, was efficient in tissue repair and subsequent regeneration of articular contours when compared only with the application of ultrasound apitoxin topical

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