The Efficacy Of Intrasound Therapy On The Acute Tendon Injury

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Citation

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Abstract

Objective: The study investigated the effects of the low and high intensity Intrasound therapy (IRT) on tenocyte proliferation and oxidative stress in healing tendon.Methodology: Twenty male rats were divided randomly into four groups. Group 1; intact animals with no induced injury and no treatment, Group 2; had induced crush injury without IRT treatment, Group 3; had crush injury with low intensity IRT and Group 4; had crush injury with high intensity IRT. Treatment commenced 24 hours post-injury on alternate days for 10 days. On the 11th day post injury, the animals were sacrificed and the tendons excised and processed for histological study and Malondialdehyde (MDA) evaluation. Statistical analysis of tenocyte and MDA counts was done using t-test and analysis of variance (ANOVA).Results: Results show a statistical significant difference in the tenocyte and MDA counts among the four groups. Analysis of the mean tenocyte counts and MDA between the low intensity IRT (group 3) and high intensity IRT (group 4) reveals a significance difference ($p<.05$) for MDA but no significance difference ($P>0.05$) for tenocyte counts.Conclusion: The study does suggest that IRT may be a treatment option to be considered in the treatment of acute tendon injuries considering its beneficial effects on healing and a reduction in the oxidative stress in healing tendon. Also this modality is an area waiting to be explored, especially the molecular effects on healing as well as the biological mechanisms of action.

INTRODUCTION

Tendon injury is a major problem in sports and occupational medicine [1]. It result in a high morbidity and disability and account for loss of several man-hours [23]. The achilles tendon is particularly prone to injury due to its limited blood supply and a combination of forces to which it is subjected to during activities of daily living, particularly movement and weight bearing.

About 90-95% of the cellular matrix of tendons is made up of specialized cells (fibroblasts) known as tenocytes and tenoblasts [4]. These specialized cells synthesize collagen molecules in response to injury. Conversely, failed acute inflammation following injury or micro trauma results in tendon degeneration known as tendinosis. Inadequate healing of acute tendon injuries may precipitate tendinosis, which is characterized by tenocyte death, decreased matrix and collagen production and increased risk of re-injury [5]. Tendinosis may also occur secondary to apoptosis induced by oxidative stress [6].

Non-Steroidal Anti-inflammatory drugs (NSAIDs) are currently among the most widely prescribed drugs for acute tendon injury [7] However, there are several documented adverse effects associated with these drugs [891011]. Pertinent here is the inhibition of fibroblast proliferation and increase in leukotriene production [10]. It is reported that any treatment that hampers fibroblast proliferation will be detrimental to healing [11]. It is important for acute tendon injuries to go through the normal acute inflammatory process in order to heal properly as chronic inflammatory reactions, can be a source of oxidative damage and stress [1213]. Malondialdehyde (MDA) is an established marker commonly used to determine tissue oxidative stress [14].

The main goal of treatment in tendon injuries is to return patients to the pre-injury level of function in the shortest possible time without compromising tissue healing [15]. It has therefore become imperative to search for other treatment options aside NSAID for acute tendon injury management that is non invasive and promotes tissue healing with minimal or no side effect.

Furthermore, mankind has for long recognized the inherent

ability of sound to heal. Sound waves are mechanical energy transmitted from one molecule to the other [16]. They are categorized into three types depending on the frequency: Infrasound; below 16 Hertz (Hz), Intrasound (audible sound); between 16-20,000 Hz and Ultrasound above 20,000Hz. These sound waves have been known to interact with biological tissue [17]. The efficacy of therapeutic ultrasound on tissue healing has been previously reported [181920]. But Intrasound therapy that appears to be gaining grounds lately has very scanty scientific evaluation of its acclaimed therapeutic benefit. However, it has been reported to be effective in diagnosing acute scaphoid and malleolar fracture [2122].

This study is therefore designed to investigate the effect of low and high intensity IRT on healing following acute tendon injury in sprague-dawley albino rats.

MATERIALS AND METHODS

Twenty, 8 weeks-old male Sprague-Dawley albino rats weighing 229-280g were used for the study. They were accommodated in the animal room of the department of Anatomy, College of Medicine, University of Lagos, Nigeria where the study was done. They were kept under standard conditions of 12- hour light and 12-hour darkness photoperiodicity. The rats were fed on commercial rat chow and water ad libitum.

Ethical approval of the study was obtained from Ethical Committee of College of Medicine, University of Lagos, Nigeria.

The animals were randomly assigned into 4 groups as follows:

Group 1(Normal control): The rats did not undergo a crush injury and had no IRT
Group 2 (Experimental control): The rats underwent a crush injury to both hind limbs but no IRT
Group 3 (Low Intensity IRT): The rats underwent a crush injury to both hind limbs and were treated with low intensity IRT.
Group 4 (High Intensity IRT): The rats had a crush injury to both hind limbs and were treated with high intensity IRT.

INSTRUMENTATION

The IRT machine used was the Novasonic- Novafon Intrasonic device manufactured by NOVASONIC MEDICAL House of Novasonic-Novafon, Denmark. Sterile K-Y jelly was used as a coupling medium [23].

The Intrasound machine was factory calibrated into either low or high intensity.

INJURY PROCEDURE

The achilles tendon of both hind limbs (right and left) was clamped with Artery forceps to the forceps maximum closure for 30 seconds to elicit a crush injury, adopting the method of Paulo et al [24]. The pre-injury circumference of both hind limbs for each rat was taken 0.2cm above the calcaneal insertion of the achilles tendon by means of a flexible inextensible marked cord. Twenty-four hours post injury, the post-injury circumference of the hind limbs was taken again at the same marked point to ascertain if there was tissue eodema, as an indication of inflammation.

INTRASOUND THERAPY (IRT)

The achilles tendons of both limbs of the rats in groups 3 and 4 were treated with low and high intensity IRT respectively. The hind limbs of each rat were treated alternatively using the small treatment head applied over the achilles tendon for 5 minutes. Treatment was given on alternate days for the first 10 days post-injury adopting the protocol of Leung et al [25].

ANIMAL SACRIFICE AND TENDON HARVEST PROCESS

On the 11th day post-injury, the animals were sacrificed and the achilles tendons excised and fixed in 10% Formol saline. The achilles tendon specimens were dehydrated in increasing concentrations of ethanol and thereafter embedded in paraffin wax. Serial vertical sections 10 microns thick were made and every 5th section selected for morphometric analysis using the technique of stereology described by Young and Dyson [26]. These were stained with Eosin, an acid dye, and thereafter counterstained with the basic dye Heamatoxylin after being mounted on plain glass slides in the routine E & H preparation.

STEREOLOGICAL ANALYSIS

The slides were observed under the light microscope fitted with an Ocular Test Grid at a magnification of x 400. The numerical density (NA) of tenocytes is the number of tenocytes per unit area of field [27]. The fibroblast profile identified was the nucleus. This is estimated as the profile number of tenocytes (N) within the frame of the test grid (A). N was determined by counting all the fibroblasts profile partially or totally within the frame area that did not intersect the forbidden lines, which are the top and left margins of the test grid [2728]. 50 random (10 per animal, with 5 rats/group)

values were obtained for each group.

TENDON MALONDIALDEHYDE (MDA) CONCENTRATION

Tendon samples were homogenized in 2cm³ of normal saline. The homogenate was centrifuged at 1000 revolutions/minute in a Uniscope laboratory centrifuge and the supernatant collected. Malondialdehyde (MDA) in the tissue supernatant was determined using the method of Buege and Aust [14] and calculated using 1.56105M□1cm□1 as the molar absorbity coefficient. MDA is expressed as micromole/mg-protein (□Mol/mg protein) [14].

STATISTICAL ANALYSIS

Data were presented as mean (X) ± Standard Deviation (SD). Statistical analysis was done using SPSS 10.0 software. Student's t-test and analysis of variance (ANOVA) were done to test for statistical significance.

Significance level was put at P<0.05

RESULTS

Comparison of the mean tenocyte counts and MDA value among the 4 groups reveals a significance difference (P<0.05) (Table 1). The result showed tenocyte proliferation in the treatment groups (Figures 1-3). This was inferred from the higher count recorded in the experimental groups (Groups 3 & 4) compared with the controls groups (Table 1).

There was an increase in the MDA (a measure of oxidative stress) count from the normal control (group 1) to the experimental control (group 2), suggesting increase in oxidative stress post injury. The difference was significance (Table 2). Also the MDA counts in the treatment groups (groups 3 & 4) were significantly reduced compared with the experimental control (group 2), suggesting reduction in oxidative stress following IRT treatment.

Comparison of the mean tenocyte counts and MDA between the low intensity IRT (group 3) and high intensity IRT (group 4) reveals a significance difference (P<0.05) for MDA but no significance difference for tenocyte counts (Table 5).

Figure 1

Table 1: Comparison of the mean tenocyte counts and MDA among the 4 groups

Mean	Group 1	Group 2	Group 3	Group 4
Tenocyte count	1.6 ± 1.2	6.6 ± 5.7	10.0 ± 12.0	9.9 ± 4.1
F statistic = 16.27, p-value = 0.00				
MDA (µMol/mg-protein)	0.20 ± 0.01	0.67 ± 0.01	0.50 ± 0.01	0.41 ± 0.01
F statistic = 293.00, p-value = 0.00				

Keys

- Group 1(Normal control): The rats did not undergo a crush injury and had no IRT
- Group 2 (Experimental control): The rats underwent a crush injury to both hind limbs but no IRT
- Group 3 (Low Intensity IRT): The rats underwent a crush injury to both hind limbs and were treated with low intensity IRT.
- Group 4 (High Intensity IRT): The rats had a crush injury to both hind limbs and were treated with high intensity IRT.

Figure 2

Table 2: Comparison of the mean tenocyte counts and MDA between group 1 and 2

Mean	Group 1	Group 2
Tenocyte count	1.6 ± 1.2	6.6 ± 5.7
Student's t statistic = 6.13, p-value = 0.00		
MDA (µMol/mg-protein)	0.20 ± 0.01	0.67 ± 0.01
Student's t statistic = 32.65, p-value = 0.001		

Figure 3

Table 3: Comparison of the mean tenocyte counts and MDA between group 2 and 3

Mean	Group 2	Group 3
Tenocyte count	6.6 ± 5.7	10.0 ± 12.0
Student's t statistic = 1.85, p-value = 0.07		
MDA (µMol/mg-protein)	0.67 ± 0.01	0.50 ± 0.01
Student's t statistic = 14.76, p-value = 0.01		

Figure 4

Table 4: Comparison of the mean tenocyte counts and MDA between group 2 and 4

Mean	Group 2	Group 4
Tenocyte count	6.6 ± 5.7	9.9 ± 4.1
Student's t statistic = 3.34, p-value = 0.001		
MDA (µMol/mg-protein)	0.67 ± 0.01	0.41 ± 0.01
Student's t statistic = 22.81, p-value = 0.002		

Figure 5

Table 5: Comparison of the mean tenocyte counts and MDA between group 3 and 4

Mean	Group 3	Group 4
Tenocyte count	10.0 ± 12.0	9.9 ± 4.1
Student's t statistic = 0.09, p-value = 0.93		
MDA (µMol/mg-protein)	0.50 ± 0.01	0.41 ± 0.01
Student's t statistic = 6.36, p-value = 0.02		

Figure 6

Figure 1: Photomicrograph of the L/S of Tendon in the Experimental control (Group 2). X 400

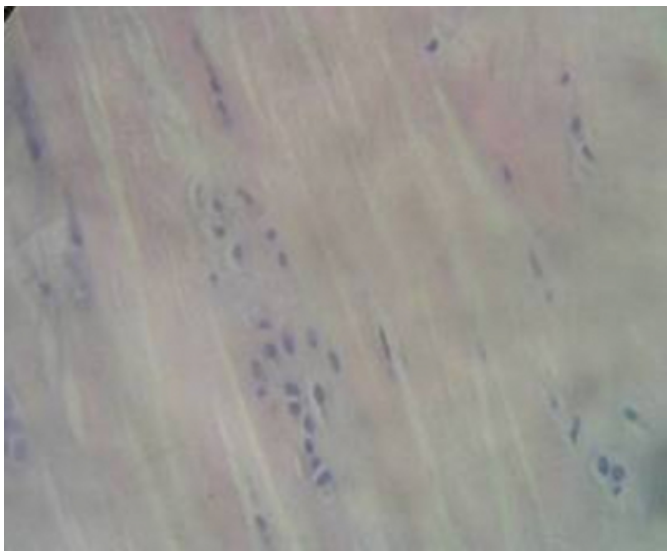


Figure 7

Figure 2: Photomicrograph of the L/S of Tendon treated with low intensity IRT (Group 3). X 400

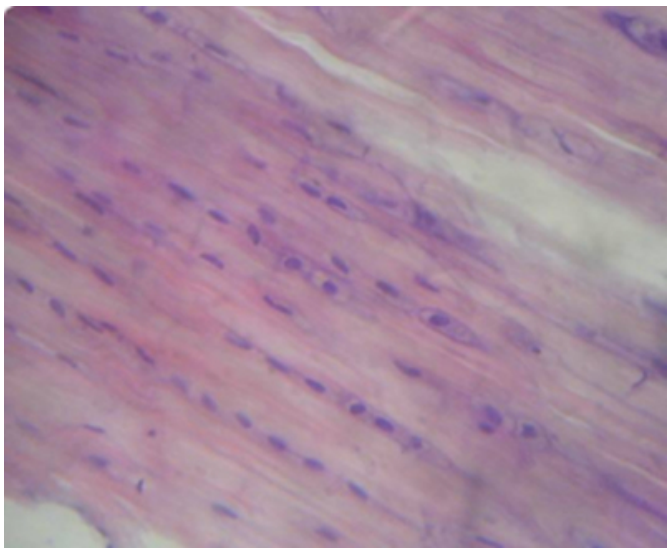
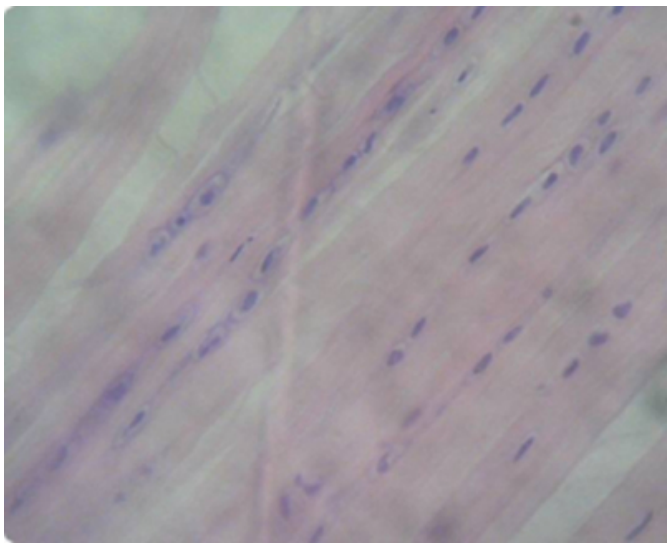


Figure 8

Figure 3: Photomicrograph of the L/S of Tendon treated with high intensity IRT (Group 4). X 400



DISCUSSION

The crush injury protocol adopted in this study is similar to the earlier reported study [24] and, arguably resulted in tissue injury. This inference is based on inflammation elicited in all the injured limbs following the crushed injury. Indicators of inflammation due to the induced injury were swelling and redness as evidenced by increase in the circumference of the injured areas of the limbs and tenderness evidenced by the animals' reaction to touch and evidence of loss of function in the injured limbs.

The tenocyte counts in the injured tendons were higher in the experimental groups as compared to the normal control (Table 1). Additionally, the increase in tenocyte population in the experimental animals (groups 3 & 4) compared with the experimental control (group 2) were statistically significant (Tables 3 & 4).

The use of fibroblast (tenocyte) count as a reliable measure of healing in structures such as tendons has been previously reported [181920]. The increase in tenocyte proliferation is suggestive of an increase in healing as proliferation is part of the normal healing process [420]. The low intensity mode was more effective in enhancing proliferation of tenocyte than the high intensity as indicated by a higher mean tenocyte count in the low intensity group.

The statistically significant difference in the means MDA levels of groups 1 and 2 is explained by the presence of inflammation in the injured tendon. Inflammation has been reported to be a source of oxidative stress in soft tissue

injuries [1213]. The experimental groups treated with low and high intensity IRT recorded statistically significant lower levels of MDA compared with the experimental control group suggesting reduced oxidative stress on the tendons, though the high intensity appears to be more effective in this regard. This may suggest that low and high intensity IRT does lower the oxidative stress in healing tendons in addition to promoting healing.

In view of the reported adverse effects of NSAIDs on the inflammatory process in injured tissue, [89101115] it is imperative to search for an alternative treatment that will promote healing and be non-invasive. This study was therefore carried out to address this issue. Though previous studies have reported the efficacy of therapeutic ultrasound on tendon healing [181920]. This study therefore suggest that IRT can be an alternative physical agent to therapeutic ultrasound in managing acute tendon injury.

CONCLUSION

Findings from this study suggest that IRT may be a treatment option to be considered in the management of acute tendon injuries, considering its beneficial effects on healing and reduction in the oxidative stress in healing tendon.

The present study was carried out with one session of IRT treatment on alternate days. Further studies could be done with twice or three times a day treatment protocol. Further studies may be conducted on the molecular effects of the modality on healing as well as its biological mechanisms of action. Also, further studies may compare chronic & acute injury in human studies.

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