# 50 - CONTINUOUS MODE OF HIGH INTENSITY ULTRASOUND THERAPY EFFECTS IN RATS SOFT TISSUES

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## INTRODUCTION

Ultrasound therapy is been used for years in the treatment of several injuries of both dermato-functional and musculo-squeletic systems. Nowadays, there is an increase in UST prescription due to its beneficial effects in the healing of varicose and pressure ulcers (FYFE and CHAHL, 1985; BYL and al, 1992); in the healing of soft tissues injuries (JACKSON and al, 1991); during bone consolidation development; soothing of chronic neurogenic pain (EVANS, 1990); remodeling of collagen fibers (FERNANDES, and al, 2002); during inflammatory processes, for instance (FYFE and CHAHL, 1992; LOW and REED, 2001).

UST has been indicated in tissues restoration for the past 50 years (TER HAAR and al, 1985), so two decades have past since treatments related to natural effects with scientific basis were suggested for its usage. Some studies have taken place to determine its therapeutic effects. However, there still exist doubts and uncertainties related to ultrasound stimulation in tissues. There's a need for a better definition of correct intensities to be used in the ultrasound stimulation, as already mentioned by authors who deal with UST biophysical effects (McDIARMID and BURNS, 1987; ROBERTSON and BAKER, 2001).

Today's evidences of UST efficiency are based upon individual clinical experiences, with the purpose of clarifying its action on organic structures being the most important (EVANS, 1990; BAKER and al, 2001).

Other works deal with UST undesirable and harmful effects when it's under high intensity and capable of harming tissues, free blood cells, or other biological structures (MAXWELL, 1992). These damages happen during a phenomenon known as temporary cavitation, transitory cavitation or unstable cavitation, by experts. The cavitation can happen in tissues when UST is applied with high intensities, or when ultrasound's electroacoustic transducer remains firm during stimulation. It's known that if a field of stationary waves was avoided, and lower intensities used during ultrasound therapy, then the risks of temporary cavitation could be minimized.

UST correct usage is important, not only to protect people against unnecessary applications, but to justify levels of exposition leading to significant biological effects, as well (OKUNO and al, 1982). Thus, this paper is about checking high intensity ultrasound stimulation in stationary and semi-stationary continuous mode based on light tissues morphological analysis in rats.

## MATERIALS AND METHODS

The descriptive experiment was made of 15 female Wistar rats, weighing 170g-230g. They were chosen randomly to form two groups, and kept in collective cages, five in each one, and fed with standard biotery diet and water. Group A, the controlled one, was composed of 5 animals free of ultrasound stimulation. Group B animals were divided into 2 subgroups ($B_3$ and $B_4$), with 10 in each. Animals in subgroups $B_3$ and $B_4$ received a 5 minutes continuous ultrasound under 2.0 W/cm$^2$ of intensity, in 3 (three) days. Subgroup $B_3$ ultrasound's electroacoustic transducer kept in stationary mode (E). Animals in subgroup $B_4$ were stimulated by ultrasound's electroacoustic transducer in semi-stationary mode (SE). Mode (E) performed with the electroacoustic transducer system stocked in the activated region, and the transducer applied with small circular and rhythmic movements in mode (SE).

The therapeutic ultrasound, Sonacel Dual (S.1015) of 1MHz by Bioset, was used in the experiment with a frequency of repetitive pulse of 100 Hz, and the pulse ratio at: 1:2 -50% (5.0 ms ON / 5.0 ms OFF). The transducer's area of effective radiation was 3.5 cm$^2$. The UST device was first taken to the Bioset Physical Therapy Equipments LTDA bio-engineering lab, in Rio Claro, S.P. where it was adjusted, then found regular under these norms: NBR IEC 601-1 and NBR IEC 601-2-5. The UST tests were as followed: protection adjustment, device functioning, dielectric capacity, leakage current and auxiliary currents through a patient.

UST was applied to anaesthetized animals, using 0.5 ml of Vetanarcol (5% Ketamine Chlorhydrate-König laboratory), 0.5% of Dorcipec (2% Xylazine Chlorhydrate - Vallée Laboratory), 0.5 ml of Compaz (Diazepam of 10 mg - Cristália Laboratory) diluted in 3.5 ml of saline solution, and applying almost 1.0 ml of it for each 200 g body weight. The intraperitoneal anaesthesia was used with syringes and needles for insulin. After, animals were placed on a plane surface where trichotomy was used and the assepsia back region stimulated. The ultrasound transducer remained stable in subgroup $B_3$, because here the application mode was of type (E). In subgroup $B_4$, it was continually running clockwise like for the application to be of type (SE). All the animals studied were put to death after inhaling sulfuric ether excessively. Next, a fragment of the stimulated area measuring about 5 cm$^2$ was removed. The fragments were put in a 10% formol solution, and processed according to the Hematoxylin-Eosin stain routine technique. Histologic cuts with 5$\mu$m of width from the material included in paraffin blocs were colored by the H/E technique, after a laboratory procedure of fragments.

## RESULTS

The morphologic analysis confirmed high intensity ultrasound's stimulation effects in rats´ subcutaneous tissues in $B_3$ and $B_4$ subgroups. The control group (group A) was analyzed for comparison purpose. The following characteristics in each study group were confirmed in the qualitative histological analysis: group A specimen showed a pavemented, stratified and keratinized epithelial tissue; a conjunctive fibrous tissue with sebaceous glands and hairy follicles, apart from muscular fibers and adipose tissue with normal morphologic characteristics (Figure 1-A/B). In subgroup $B_3$, a continuous UST usage was applied for 3 days on the specimen, and the examined fragments showed the absence of epithelial tissue, in almost all over their extension. The subjacent conjunctive tissue had basophilic aspect injuries suggesting a cellular necrosis (Figure 1-C), and a type of mononuclear inflammatory cells that were scarce. Also, leukocyte margination findings were confirmed, as well as hemorrhage and presence of lipid alterations of degenerative nature. Even a small number of cutaneous annexes were seen.

In subgroup $B_4$, specimens remained under continuous UST, semi-firm, for 3 days and, the studied fragments showed that the conjunctive tissue subjacent to epithelial covering had basophilic injuries compatible with necrosis aspect (Figure 1-D). Also, a moderate inflammatory mononuclear infiltrate was found in the weak conjunctive tissue, the adipose and between muscular fibers. Leukocyte margination findings were confirmed, as well as hemorrhage and lipid alterations of degenerative nature.

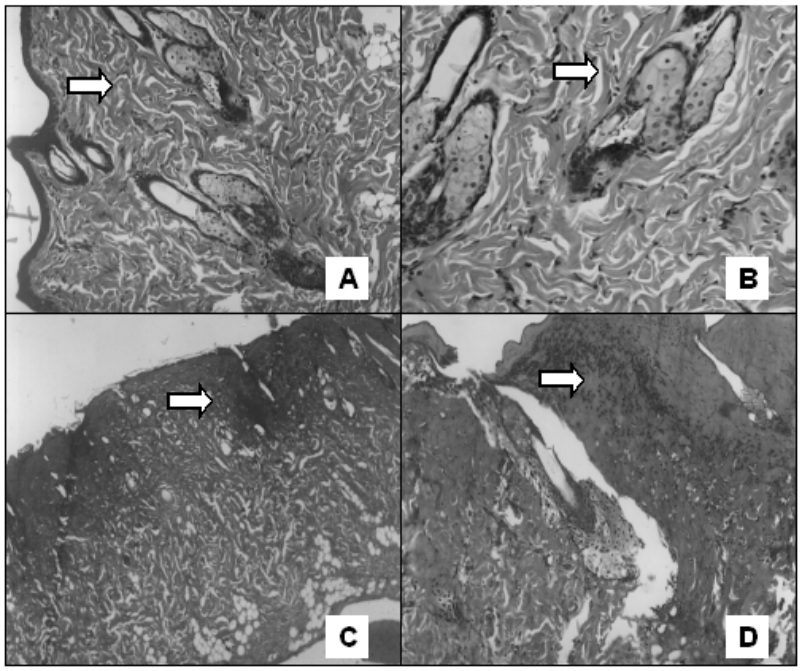


**Figure 1:** Microscopic aspects of specimen under experiment. **A/B** - Details of control group with normal morphological aspect (H/E-100x). **C.** Detail of subgroup $B_3$ showing basophilic degeneration of collagen meaning cellular necrosis (H/E-40x). **D.** Detail of subgroup $B_4$ with basophilic alterations (H/E-40x).

**DISCUSSION**

It's been proven highly impossible, in a review task about ultrasound therapeutic usage and its biophysical effects, that damage to the tissues' membrane could happen on intensities used within clinical practice, because they are generally used in small ultrasound stimulation doses. (BAKER and al, 2001)

The heating of tissues is intensified with UST in continuous mode (TER HAAR, 1997). UST higher doses can be harmful to stimulated tissues, while smaller aren't (ENWEMEKA and al, 1990). Generally, ultrasound non thermic effects are gotten from lower dosages, whereas higher ones produce more thermic effects (DYSON, 1992).

All specimens, here, went through 3 minutes ultrasound stimulation, knowing that the tissue's temperature must remain between 40ºC - 45ºC, to reach a therapeutic effect through heating. This is because temperatures above 45ºC are harmful to the tissues and enough to trigger thermic and non thermic effects in small areas (BARNETT, 1994).

In continuous mode, UST is frightening because of its thermic effects. The way that high-frequency waves are transmitted also has thermic effects, but with a significant reduction on their harmful capacity due to their own configuration as to the type of stimulation that creates activities cycles, followed by inactive periods which are small interruptions when transmitting a wave (FERREIRA and al, 1995; GUIRRO and GUIRRO, 1996).

Specimen of subgroup $B_3$ had no epithelial tissue. Other findings here, were injuries with basophilic aspects meaning cellular necrosis, leukocyte margination and hemorrhage; findings that show UST danger when used continually. Specimen of subgroup $B_4$, had a conjunctive tissue with moderate inflammatory mononuclear infiltrate, as well as leukocyte margination and hemorrhage.

These findings were similar to those from studies that mention UST action in biological tissues with higher doses; where findings of hyperemia, edema and necrosis were described suggesting being the transitory cavitation responsible for petequial hemorrhages formation. It's important to mention that a motionless ultrasound electroacoustic transducer (E) can cause a transitory cavitation. A transitory cavitation provoqued by UST can locally disintegrate tissues and generate a production of free radicals. Only UST stable cavitation has therapeutic value. UST transducer must be used perpendicularly to the area stimulated and continually moved in uniform circles, so as to shun stationary waves and their possible dangers (HEKKENBERG and al, 1986). The value of temperature is small when UST transducer is in motion (DOCKER, 1987). A pulsed UST can create a circulation separately from heating effect (HOGAN and al, 1987). However, this effect is stronger whenever the electroacoustic transducer is used in stationary mode (WELLS, 1997).

**CONCLUSION**

The results from this work morphological analysis showed that ultrasound stimulation by $2W/cm^2$ intensity can have dangerous effects in light tissues, with the device in continuous stationary-semi stationary mode. Animals from subgroups $B_3$ stimulated by UST electroacoustic transducer in stationary mode had injuries of higher degenerative nature, as opposed to those from subgroup $B_4$ under semi stationary mode. Thus, higher intensities are to be prohibited in acute inflammatory processes, as for a therapeutic exam, because they can have dangerous side effects. However, this doesn't prohibit the need for better inquiries about ultrasound effects in other dosages, modes and therapeutic frequencies.

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**CONTINUOUS MODE OF HIGH INTENSITY ULTRASOUND THERAPY EFFECTS IN RATS SOFT TISSUES**

**ABSTRACT**

INTRODUCTION: Today ultrasound therapy (UST) is being used in the treatment of both the musculo-squeletic and dermato-functional systems most diverse injuries based on its numerous positive effects. It has been two decades since treatments in relation to natural effects with scientific basis in the use of UST was suggested. OBJECTIVE: To investigate effects of high intensity ultrasound stimulation in continuous mode on rats morphological soft tissues. METHODS: The task was developed through experimental descriptive study with 15 female Wistar rats weighing between 170g -230g.The specimens were divided into a control group and 02 subgroups ($B_3$ and $B_4$), and submitted to a high intensity ultrasound stimulation in its back area in continuous mode with a power of 2W/cm$^2$ during 5 minutes. Parts of animals were processed for pigmentation using the hematoxiline-eosine method. As for the qualitative morphological analysis, optical microscopy was used. RESULTS: The control group show normal morphological characteristics. Parts of subgroups $B_3$ and $B_4$ used under ultrasound stimulation in continuous mode presented a basophilic aspect compatible with cellular necrosis. CONCLUSION: Our study showed that high intensity ultrasound stimulation has strong side effects in continuous mode. This suggests that ultrasound high dosages should not be used and the treatment of high inflammatory processes, because they can have dangerous side effects.

KEYWORDS: Ultrasound therapy. Side Effects. Tissues

**EFFETS DU TRAITEMENT ULTRASONORE Á FORTE INTENSITÉ EN MODE CONTINU SUR LES TISSUS MOUS DES SOURIS**

**RÉSUMÉ**

INTRODUCTION: Actuellement, l´ultrason thérapeuthique (UST) est de plus en plus utilisé dans le traitement des lésions les plus diverses du système musculosquelettique et dermato-fonctionnel, grâce à ses divers effets bénéfiques. Deux décennies déjà se sont écoulés, depuis la suggestion d´utiliser l´UST dans les traitements sur bases scientifiques, quant aux effets naturels. OBJECTIF : découvrir les effets de la stimulation ultrasonore de forte intensité en mode continu basée sur l´analyse morphologique des tissus mous des souris. MÉTHODES : Le travail est le fruit d´une étude descriptive et expérimentale faite sur 15 souris femelles de lignée Wistar, pesant entre 170g et 230g. Les specimen furent divisés entre un groupe de contrôle, et deux sous-groupes ($B_3$ et $B_4$), soumis à une stimulation ultrasonore à forte intensité sur le côté dorsal en mode continu, puis à une puissance de 2 W/cm$^2$ durant 5 minutes. La technique de coloration à l´hématoxyline-éosine fut utilisée sur des fragments des animaux étudiés. Quant à l´analyse morphologique et qualitative, l´on usa de la microscopie en lumière. RÉSULTATS: Le groupe de contrôle présenta des caractéristiques morphologiques normales. L´on observa que des fragments des sous-groupes $B_3$ et $B_4$ avec stimulation ultrasonore en mode continu présentèrent un aspect basophile compatible avec la nécrose tissulaire. CONCLUSION : Les découvertes de cette étude montrèrent que les effets secondaires de la stimulation ultasonore de forte intensité sont assez pervers en mode continu. Ceci suggère que les dosages ultrasonores élevés ne doivent pas être utilisés dans la conduite thérapeutique des processus inflammatoires aiguës, car ils peuvent provoquer des effets secondaires néfastes.

MOTS-CLES : Thérapie ultrasonore. Effets secondaires. Tissus

**EFECTOS DE LA TERAPIA ULTRASÓNICA DE ALTA INTENSIDAD EN MODO CONTINUO EN TEJIDOS SENSIBLES DE RATONES**

**RESUMEN**

INTRODUCCIÓN: Actualmente, el ultrasonido terapéutico (UST) viene a ser utilizado en el tratamiento de las más diversas lesiones del sistema musculoesquelético y dermato-fucional gracias a sus diversos efectos benéficos. Hace dos décadas desde que se sugerió los tratamientos de los efectos naturales con bases científicas para el uso del UST. OBJETIVO: Investigar los efectos de la estimulación ultrasónica de alta intensidad en el modo continuo baseada en el análisis morfológico de los tejidos sensibles de ratones. MÉTODOS: el trabajo fue desarollado a través de un estudio descriptivo experimental, realizado con 15 ratones hembras de linaje Wistar, de pesos diversos variando entre 170 g y 230 g. Los especímenes fueron divididos en un grupo de control y 02 subgrupos ($B_3$ y $B_4$), y sometidos a una estimulación ultrasónica de alta intensidad en su superficie dorsal a través del modo continuo, a una potencia de 2 W/cm$^2$ durante 5 minutos. Fragmentos de los animales fueron procesados para coloración usando la técnica de hematoxilina-eosina. Para el análisis morfológico qualitativo, fue utilizada la microscopía de luz. RESULTADOS: El grupo de control presentó características morfológicas normales. Se observó que fragmentos de los subgrupos $B_3$ y $B_4$, que habían sido estimulados por ultrason en modo continuo, presentaron un aspecto basofílico que estava compatible con necrosis de los tejidos. CONCLUSIÓN: Los descubrimientos de este estudio demostraron que los efectos adversos de la estimulación ultrasónica de alta intensidad son bastante agresivos en el modo continuo. Eso sugere que las altas dosificaciones del ultrason no deben ser utilizadas en la conducta terapeutica de los procesos inflamatorios agudos, pues pueden promover efectos adversos indeseables.

PALABRAS-LLAVES: Terapía por ultrason. Efectos adversos. Tejidos

**EFEITOS DA TERAPIA ULTRA-SÔNICA DE ALTA INTENSIDADE NO MODO CONTÍNUO EM TECIDOS MOLES DE RATOS**

**RESUMO**

INTRODUÇÃO: Atualmente o ultra-som terapêutico (UST) vem sendo utilizado no tratamento das mais diversas lesões do sistema músculo-esquelético e dermato-funcional devido aos seus diversos efeitos benéficos. Há duas décadas passadas, foi sugerido cuidados em relação aos efeitos naturais, com bases científicas no uso do UST. OBJETIVO: Investigar os efeitos da estimulação ultra-sônica de alta intensidade no modo contínuo baseada na análise morfológica dos tecidos moles de ratos. MÉTODOS: O trabalho foi desenvolvido através de um estudo descritivo experimental, realizado com 15 ratos fêmeas da linhagem Wistar, de pesos diversos variando entre 170 g e 230 g. Os espécimes foram divididos em um grupo de controle e 02 subgrupos ($B_3$ e $B_4$), e submetidos à estimulação ultra-sônica de alta intensidade em sua superfície dorsal através do modo contínuo, a uma potência de 2 W/cm$^2$ durante 5 minutos. Fragmentos dos animais foram processados para coloração usando a técnica de hematoxilina e eosina. Com relação à análise morfológica qualitativa, foi utilizada microscopia de luz. RESULTADOS: O grupo de controle apresentou características morfológicas normais. Observou-se que fragmentos dos subgrupos $B_3$ e $B_4$, que haviam sido estimulados por ultra-som em modo contínuo, apresentaram um aspecto basofílico que era compatível com necrose tecidual. CONCLUSÃO: As descobertas deste estudo demonstraram que os efeitos adversos da estimulação ultra-sônica de alta intensidade são bastante agressivos no modo contínuo. Isto sugere que as altas dosagens do ultra-som não devem ser utilizadas na conduta terapêutica de processos inflamatórios agudos, pois podem promover efeitos adversos indesejáveis.

PALAVRAS-CHAVE: Terapia por ultra-som. Efeitos adversos. Tecidos.