22 - STUDY OF THE ULTRASOUND EFFECT UNDER HUMORAL IMMUNE RESPONSE

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INTRODUCTION

The therapeutic ultrasound is a physiotherapeutic means applied in a great variety of conditions. Its use has been including rheumatoid diseases, chronicles arthritic, muscle spasms, cicatricial adherences and in the pain relief. (CAKMAK, 2005; BROSSEAU *et al*., 2002; GUROVICH *et al*., 2005; KAHN, 2001; LOW & REED, 2001).

The essential function of the immune system is defending the organism against infections. Therefore, an immune response is produced to the bacterial or viral infections and auto-immune diseases. So the organism has a prodigious range immune response because the immune response can be produced through several ways. (ROITT *et al*., 2003; BEUTLER *et al*., 2001; RANG *et al*., 2001).

Immune response depends directly to the cells and to their respective products of the immune system. The antibodies (immunoglobulin) are plasmatic proteins, that binding specifically to substances known as antigens. Immunoglobulines are produced by plasmatic cells in response to the infection or immunization, and binding to infectious agents. They can neutralize the infectious agents or prepare them for being destroyed by the phagocytic cells (JANEWAY *et al*., 2000).

The IgM protein is the first immunoglobulin produced in the humoral immune response, prevailing in the first phase of the immune response and later tending to be less abundant (PEAKMAN & VERGANI, 1999).

According to Horton *et al* (1995) the heating of a solution of proteins, as IgM, cause an increase of vibration energy and of rotation that can disturb the delicate balance of weak interactions that stabilize the functional reeled resignation.

For Low & Reed (2001) the therapeutic ultrasound increases the movement of the molecules causing more vibration and molecular collisions, what results in heat. Than the kinetic energy is converted in thermic energy while pass by the material. This can cause the denaturation and decrease of proteins as the IgM.

It is still described the following effects of the ultrasound: increase of the protein synthesis, increase of the mast cell secretion, increase of the cellular metabolism, increase of the calcium absorption by the second messengers and increase of the growth factors production by the macrophages (BAZIN & KITCHEN, 1998). All those effects could explain the acceleration in the immune responses and consequently a larger production of IgM.

As in the sanguine circulation there is great effects dissipation of the ultrasound and exactly where there is the largest concentration of IgM, this study make possible to generate information to answer the following question: Does the application of the ultrasound alter the plasmatic concentration of IgM in the immune response?

MATERIAL AND METHODS

Male SWISS mice were used with 12 weeks old and approximately 34 grams. The animals were divided in two groups with 18 mice each: 1) Experimental Group (EG): the ovoalbumin was inoculated and later the ultrasound was applied under frequently 3MHz, intensity of 1 w/cm2 for 50 seconds, for 60 consecutive days; 2) Control Group (CG): only the ovoalbumin was inoculated, there are not application of the ultrasound. The experimental procedures were executed in agreement with the items below.

1) *Inoculation of ovoalbumin*

The inoculation of ovoalbumin was accomplished to induce an immune response. This were done in 0, 7, 14 21 and 28 days of the experiment, in all the animals through intraperitonial (180 mg of ovoalbumin contained in 0,2 mL of physiologic solution).

2) *Application of the ultrasound*

The headstock with ondulatory movements of the ultrasound SONOPULSE III (IBRAMED) was applied in the abdominal area close the proximal epiphysis of the femur, with the specifications described together of each groups.

3) *Collection of blood*

The samples of blood (approximately 1ml of each mouse) were collected through puncture of the retro-orbital plexus in the 7, 14, 21 28 and 60 days of the experiment. After sanguine centrifugation, the serums were transferred for eppendorf and stored to -20ºC until the analysis of IgM concentration.

4) *Enzyme-linked immunosorbent assay (ELISA)*

Anti-OVA antibodies were meansured by ELISA as follows: Costar (Cambridge, MA, USA) E.I.A./R.I.A. flat bottom, high binding, 96-well plates were coated with 350 µl OVA (150 µg ml-l) coating solution in 0,05 M bicarbonate buffer, pH 9.2, incubated at 4°C and washed. This and subsequent washings were performed three times with 0,05 Tween 20 in phosphate-buffered saline (Tween PBS), pH 7,5. Blocking was carried out with 0,5% gelatin in Tween_PBS for 1h at 37°C and plates were washed again. If not used immediately, plates could be stored, covered, at 4°C, for least two_tree weeks. Mouse sera to be tested (300 l/well) were added in serial twofold dilutions, incubated for 2h at 37°C and the plates washed. For IgM assay, 250 l/well of horseradish peroxidase-conjugated sheep anti-mouse IgM (Cappel, Organon teknika Corp, Durham, NC, USA, 1 in 10000 in PBS) was used, and incubated for 1h at 37°C. After washing, 200 l/well of OPD solution was added and incubated for 30 min at room temperature. The reaction was stooped by adding 50 l/well of 1 M H_2SO_4. Absorbance was read at 4902 nm (TOMAI *et al*., 1999).

5) *Titles* The IgM titles were measure through the calculation of the means and standard-deviation of the densities

optical, being just considered the positive tests, that included those sample that were bigger than the standard-deviation of the negative serum multiplied by 16, to establishment of a cut factor.

6) *Statistical analysis*

The statistical analysis was made using the software Statistica 5.0 for Windows (StatSoft Inc), through the Breakdown test & one-ANOVA, for the analysis of kinetic variance, and applied the Duncan test for significant points ($p < 0,05$).

RESULTS

The means of the antibodies titles of each group, evaluated through the ELISA, is presented in the Table 1. In the IgM titles, the cut factor found by the standard-deviation was of 0,016.

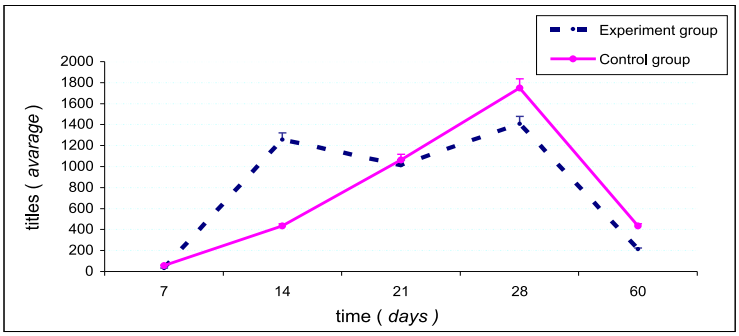
TABLE 1

Average of the titles of antibodies IgM-anti OVA in days 07, 14.21, 28 and 60 of the experiment

Groups	Days (time)				
	7	14	21	28	60
Control (CG)	56	434	1065	1749	434
Experiment (EG)	32	1259	989	1408	215

GRAPHIC 1

Average of the titles of antibodies IgM-anti OVA during the experiment.



The graphic 1 show that the means of the IgM titles of each group varies with the time. In the control group, as observed in the table, there was a lineal increase of the titles between 7 and 28 days, of 1:56 for 1:1749, and declined after 60 days for 1:434.

In the experiment group 01 there was a fast increase of the titles between 7 and 14 days, of 1:32 for 1:1259, and decline after 21 days for 1:1013. Between 21 and 28 days there is a fast increase of the titles, for 1:1408. Between 28 and 60 days there is a decrease until 60 days for 1:215. The IgM titles observed among 7 and 14 days demonstrated that there was an early humoral in the experimental group, and a subsequent decrease of this immune response when comparing with the control group.

DISCUSSION

The observed difference of means of titles of the immune response of EG and of the control group in the fourteenth day, according to Bazin & Kitchen (1998), can be explained because the therapeutic ultrasound alters the membrane permeability to the calcium. Like the calcium is an intracelular messenger, the increase of its transport through the cellular membranes help the degranulation and exercise effect in the cellular activity: as the increase of the synthesis and secretion of repair factors liberated by the macrophages. The performance of the macrophages, according to Sharon (2000), represents the beginning of the immune response by innate mechanisms of the immunity, because an antigen to unleash an immune response should be captured and processed by a macrophage or dendritic cell. According to Calich & Vaz (2001) after that event, a T-helper recognizes the fragments of this antigen. The T-helper activation will unleash a cellular and/or humoral immune response. The T-effectors, according to Janeway *et al*. (2000) lead the macrophages activation and the immunity mediated by cells B, promoting a humoral immune response with the production of antibodies. For Roitt *et al*. (2003), in the immunoglobulin synthesis of all classes, there is an early response of IgM. Than the graph is show the therapeutic ultrasound accelerates the production of IgM, because the titles of IgM are statistically bigger than the control on the 14th day of the experiment, anticipating the humoral immune response.

According to Calich & Vaz (2001), the macrophages activation induces the release of the products that act on the T-helper, modulating its activity and, mainly, influencing the pattern of the cytokines secreted for them. In this case, the interleucin-12, secreted by activated macrophages, is of fundamental importance because induces the interferon- secretion by the T-helper. For Janaway *et al*. (2000), T-helper 1 participates in the change of IgM for IgG, liberating IFN-. This could explain the decrease of the IgM titles in 14th and 21st days when compared with the control group until the 60th day of the experiment.

The increase of the IgM titles of the 21st to the 28th day can be explained for the ovoalbumin inoculation in the 28th day of the experiment, promoting maximum titles of the IgM.

CONCLUSION

Analyzing the results and the discussion of this research can be affirmed that the application of the therapeutic ultrasound alters the plasmatic concentration of IgM in an immune response.

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STUDY OF THE ULTRASOUND EFFECT UNDER HUMORAL IMMUNE RESPONSE

ABSTRACT
Introduction and Objective: the ultrasound enhances the movement of molecules causing: more vibrations, molecular collisions and increasing tha local temperature. The ultrasound therapy is applied to rheumatoidic diseases, helping in healing and reducing the pain. This work has the objective to study antibody response by IgM levels, using ovolbumin (OVA) as the immunogen, in mice with ultrasound therapy. Methods and Results: SWISS mice have been used for the studies. The animal have been dividied in 2 groups of 18 animals each. The first group (untreated) have been inoculated with 180g of OVA on 0,2 mL of PBS, on the 0, 7, 14, 21, 28 days. On the second we repeated the inoculation protocol to the OVA, as set before, and the groups fave received daily an ultrasound therapy in the abdominal region, far 50 seconds each, with intensity of 1w/cm2 (group two) under frequency of 3 MHz, both in continuous mode during 60 consecutives days. All inoculation have been done in the intraperitonial way. The sera of mice have been collected in 7,14,21,28 and 60 days for antibodies of the IgM class, which have been measured in assays by ELISA method. All animals, on the second group have survived until the end of experiment, showing higher titles (mean1259; sd 4,49) of IgM on the 14 than the ultreated group, however this titles (mean 989; sd 2,14) reduced 7 days after that. Conclusion: we concluded that ultrasound therapy anticipates the humoral response and induce down regulation by continued use.
Keywords: Humoral Immune Response, Ultrasound, IgM.

ÉTUDE DE L'EFFET DE L'ULTRASON SOUS L'IMMUNO-RÉPONSE HUMORALE

RÉSUMÉ
L'ultrason thérapeutique est un recours physiothérapeutique largement utilisé dans les maladies ostéo-musculaires, arthrites et processus inflammatoires.Son utilisation augmente le mouvement des molécules provoquant: plus de vibrations, collisions moléculaires et augmentation de la température locale. Ce travail a pour but d'étudier les niveaux de réponse immune à travers la IgM, utilisant l'ovo-albumine (OVA) comme antigène, dans les souris soumises à l'application de l'ultrason thérapeutique. Dans cette étude on a utilisé des souris SWISS. Les animaux ont été divisés en deux groupes de dix-huit animaux chacun. Le premier groupe (pas traité) a été inoculé avec 180 ug de OVA sur 0,2 ml de PBS, les jours 0, 7, 14, 21, 28 de l'éxpérience. Dans le second groupe (traité) on a répété le protocole d'inoculation de OVA, et le groupe a été traité

quotidiennement pendant 50 secondes dans la région abdominale avec l'ultrason thérapeutique de façon continue, fréquence de 3 MHZ, et intensités de 1 w/cm^2 .Les inoculations ont été réalisées voie intra-péritonéale. Le sérum des souris a été recueilli les jours 7, 14, 21, 28, et 60 pour la mesure de concentration d'anticorps de la classe IgM par la méthode ELISA. L'analyse plasmatique du groupe expérimental a révélé des titres élévés de IgM (présentant 1259) en comparaison avec le groupe contrôle le quatorzième jour, ces titres ont diminué 7 jours après (présentant 989). On a constaté dans cette étude que l'ultrason thérapeutique anticipe la réponse humorale et entraîne la diminution de la réponse subséquente.

Mots-Clés: Ultrason, Physiothérapie , IgM.

ESTUDIO DEL EFECTO DEL ULTRASONIDO BAJO INMUNORESPUESTA HUMORAL

RESUMEN

El ultrasonido terapéutico es un recurso fisioterapeutico usado extensamente en las enfermedades osteomusculares, artrites y procesos inflamatorios. Su uso aumenta el movimiento de las moléculas, causando: vibraciones, colisiones moleculares y un aumento de la temperatura local. Este trabajo tiene como objetivo estudiar niveles de la contestación inmune a través de la IgM, usando el ovoalbumina (OVA) como antígeno, en los ratones sometidos al uso del ultrasonido terapéutico. En este estudio habían sido usados los ratones SWISS. Los animales habían sido divididos en dos grupos con dieciocho animales cada uno. Inocularon al primer grupo (no tratado) con 180mg de OVA en 0.2 ml de PBS, días 0, 7, 14, 21, 28 del experimento. Repitieron al grupo tratado el protocolo de la inoculación de OVA, y trataron al grupo diariamente por 50 segundos en la región abdominal con ultrasonido terapéutico de la manera continua, frecuencias 3 MHz y intensidades de 1w/cm2. Las inoculaciones habían sido llevadas a través por intraperitonial. El suero de los ratones fue recogido en los días 7.14.21.28 y 60 para mensuração de la concentración de los anticuerpos IgM para el método ELISA (*Enzyme-linked immunosorbent assay*). Fue verificado en este estudio que el ultrasonido terapéutico anticipe la contestación humoral e induzca la reducción de la contestación subsecuente. Palabras llaves: Ultrasonido, Fisioterapia, IgM.

ESTUDO DOS EFEITOS DO ULTRASOM EM UMA RESPOSTA IMUNE HUMORAL

RESUMO

O ultra-som terapêutico é um recurso fisioterapêutico amplamente utilizado em doenças osteomusculares, artrites e processos inflamatórios. Sua utilização aumenta o movimento das moléculas causando: mais vibrações, colisões moleculares e aumento da temperatura local. Este trabalho tem como objetivo estudar níveis de resposta imune através da IgM, utilizando ovoalbumina (OVA) como antígeno, em camundongos submetidos à aplicação do ultra-som terapêutico. Nesse estudo foram utilizados camundongo SWISS. Os animais foram divididos em dois grupos com dezoito animais cada. O primeiro grupo (não tratado) foi inoculado com 180g de OVA em 0,2 mL de PBS, nos dias 0, 7, 14, 21, 28 do experimento. No segundo grupo (tratado) foi repetido o protocolo de inoculação de OVA, e o grupo foi tratado diariamente por 50 segundos na região abdominal com ultra-som terapêutico no modo contínuo, freqüência de 3 MHz, e intensidades de 1w/cm2. As inoculações foram realizadas via intraperitonial. O soro dos camundongos foi coletado nos dias 7,14,21,28 e 60 para mensuração da concentração de anticorpos da classe IgM pelo método ELISA (ensaio imunoenzimático). A análise plasmática do grupo experimental apresentou altos títulos de IgM (apresentando 1259) ao comparado com o grupo controle no décimo quarto dia, estes títulos reduziram 7 dias depois (apresentando 989). Verificou-se neste estudo que o ultra-som terapêutico antecipa a resposta humoral e induz a diminuição da resposta subseqüente.

Palavras Chaves: Ultra-som, Fisioterapia, IgM, eletroterapia, resposta imune humoral.