

THE STIMULATING INFLUENCE OF ULTRASOUND ON THE PHAGOCYtic ACTIVITY OF NEUTROPHIL GRANULOCYTES IN EXPERIMENTS «IN VITRO»

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The effect of ultrasound on the organism as a whole and on the cellular structures is still insufficiently elucidated, despite the numerous studies carried out (1, 2, 3 a, 6, 11, 12, 13). It is a well-known fact that the biological action of ultrasound (US) on the tissues and cells is substantiated by several factors: mechanical, thermal and physico-chemical. The changes occurring are dependent upon the dose and time of ultrasound treatment. In lower doses, stimulation of the vital processes might be observed, and in higher doses — lesion and even death of the cells (1, 3 a, 4, 5, 6, 14, 15). The occurrence of reflectory changes is also proved (1, 3 a, 6, 16). With the present study we set out to investigate experimentally «in vitro» the influence of biological us doses on the opsono-phagocytic index (OPHI) of neutrophil granulocytes. This would allow the drawing of certain conclusions concerning the us influence on the mobile cellular protection within the organism which is a link of primary importance for the protective processes.

Method

The study was made on 10 healthy adult individuals — 5 males and 5 female — with age ranging from 28—39 years. Blood for ultrasound treatment was obtained from the cubital vein under aseptic conditions — 10 ml from each subject. Sterile 3.8% solution of sodium citrate in a 1:10 ratio with the blood was used as anticoagulant. Immediately thereafter the blood was distributed into 3 parts of 3 ml each in sterile test-tubes with a thin rubber bottom. The blood in one of the test-tubes was treated with ultrasound at dose 0.5 watts/cm², in the second test-tube — with dose 3 watts/cm², and the third served as a control. The duration of ultrasound treatment was 10 min., and the experimental setting — analogical to that of Giacomini (1, 13). The test-tubes with rubber bottom were immersed in a water medium immediately overneath the vibrator of the us generator, whilst the moving water medium was always with a starting temperature 33° C, so that during the ultrasound treatment, the temperature of the blood within the test-tubes did not exceed 37° C. Thus, the thermal effect upon the phagocytosis was avoided. As us source, the Hungarian apparatus Sonotherm was employed (800±20 KHz). Blood for OPHI investigation was obtained be-

fore ultrasound treatment, immediately after us application and 1 hour thereafter, preserving the test-tubes under conditions of sterility in a thermostat at 37 degrees C. Phagocytosis was studied after the method of Wright and Douglass, as modified by Valchanov (3), calculating the opsono-phagocytic index on the basis of the phagocytized staphylococci, counted in 100 intact granulocytes. Culture of killed *Staph. aureus* 209¹/at concentration 1000 millions per 1 ml was used.

Results and Discussion

It was established that us treatment of the blood stimulates the phagocytic activity of neutrophil granulocytes, manifested by OPHI increase. This increase is strongest in the lower dose — 0.5 watts/cm² — and does not occur immediately after the application of us, but within one hour. OPHI prior to us treatment with 0.5 watts/cm² amounts to 1.76 ± 0.38 ,

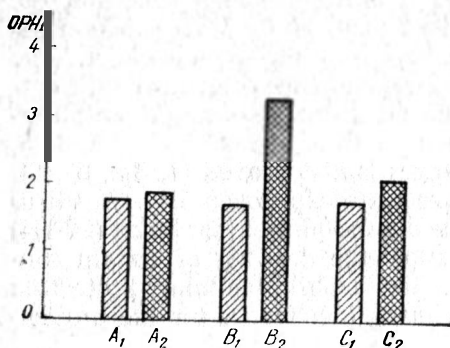


Fig. 1. Vertical — opsono-phagocytic index — mean values for all the experiments

A₁ — Opsono-phagocytic index before treatment with ultrasound (control); A₂ — Opsono-phagocytic index of blood not treated with us; 1 hour later (control); B₁ — OPHI of blood treated with us at 0.5 watts/cm² dose, immediately after the treatment; B₂ — OPHI of the same blood, 1 hour after us treatment; C₁ — OPHI of blood us treated with 3 watts/cm² immediately after us treatment; C₂ — OPHI of the same blood after 1 hour

immediately after the us treatment — 1.72 ± 0.95 and 1 hour thereafter — 3.33 ± 1.45 , i. e. an increase is present with 93%, with statistical reliability at $p < 0.01$. At us dose 3 watts/cm², OPHI immediately after the application of us is 1.78 ± 0.69 , and 1 hour later — 2.13 ± 0.72 . The increase in the latter case is smaller — with 14.04%. The comparison of OPHI of the us treated blood with the control blood shows that there is no difference between them prior to and immediately after the us treatment (Fig. 1). The modification of the index in the control group after 1 hour is insignificant — from 1.76 ± 0.38 it reaches 1.89 ± 0.40 , whereas the changes at us dose 0.5 watts/cm² are mostly pronounced. An increase with 76% in relation to the control is concerned, statistically reliable at $p < 0.01$. The increase of the OPHI at

ultrasound dose 3 watts/cm², within 1 hour after its application, in relation to the control after 1 hour is 12.69% and is not reliable in the series investigated by the authors.

We are inclined to explain the OPHI changes occurring under the effect of us, 1 hour after its application, with the stimulating effect of us, upon the biological structures in low and medium doses (1, 3a, 5, 6, 10, 11). In higher dose — 3 watts/cm² — the stimulation is weaker and accordingly the increase of OPHI in this instance, compared to the increase at dose 0.5

¹ We wish to express our gratitude to the Chair of Microbiology (Head — Prof. P. Panayotov) for the bacterial culture made available.

watts/cm² is substantially lower. In the experiments presented here, the thermic influence on the phagocytosis, manifested above the 37 degrees C level, is excluded (7). It is evident that time is needed for the manifestation of the stimulating effect of us on phagocytizing cells. So far as the intimate mechanism of this influence is concerned, a definite role is most probably played by the ultrasound induced displacements of subcellular structures and macromolecular complexes, brought about by microflows occurring within the cytoplasm, phenomena already described in various types of cells (4, 5, 8, 10, 11, 15). Moreover, it is known that the low us doses activate a number of enzymic systems not only within the cytoplasm, but in the cellular membranes also; new enzymic active centers are created and functionally active macromolecules are formed (8, 10, 11). The latter changes, in the opinion of the authors, might be assumed as the cause for a rather intense course of the basic cellular processes, underlying the phagocytosis. The weaker stimulating effect of the higher us dose is probably due to the occurrence of certain subcellular structures, parallel to the deeper changes already referred to, which reduce the positive effect of ultrasound upon the phagocytosis.

Along with the increased phagocytic activity, the presence of certain structural changes in some of the neutrophil granulocytes should be also emphasized, namely: obliteration of the cellular contours, disruption in varying degrees up to full dispersion of the cellular parts. The latter changes are more pronounced at us dose 3 watts/cm², and weaker at the low dose, but nevertheless existent, which is in compliance with literature data in terms of high doses (6, 9, 14).

In our opinion, the facts concerning the us action presented are not discordant with its stimulating effect on phagocytosis. We feel that the destroyed neutrophil granulocytes represent chiefly cells, damaged in advance or at the end of their vital cycle, which fail to resist the mechanical influence of ultrasound. Unlike the latter, the functionally active cells not only fail to show morphological changes, but manifest, as already pointed out, an increased phagocytic ability.

Inferences

1. The ultrasound treatment of blood with doses 0.5 and 3 watts/cm² increases OPHI of the neutrophil granulocytes in humans within 1 hour, and more substantially at 0.5 watts/cm² doses.-

2. The stimulation of the phagocytosis is not related to the temperature factor, but in all likelihood, to the us action upon the cytoplasm and cellular membranes: microdisplacements, activation of enzymes, formation of new active complexes.

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СТИМУЛИРУЮЩЕЕ ДЕЙСТВИЕ УЛЬТРАЗВУКА НА ФАГОЦИТАРНУЮ АКТИВНОСТЬ НЕЙТРОФИЛЬНЫХ ГРАНУЛОЦИТОВ В ОПЫТЕ «IN VITRO»

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РЕЗЮМЕ

Авторы прослеживают опсоно-фагоцитарный индекс (ОФИ) нейтрофильных гранулоцитов в крови человека после озвучения «in vitro». При этом обнаруживается стимулирующий эффект ультразвука на фагоцитоз. Он наблюдается не непосредственно после применения ультразвука, а спустя 1 час. ОФИ наиболее высокий при дозе ультразвука 0,5 ватт/см², где он на 93% выше индекса контрольных опытов и достоверно увеличен. Увеличение ОФИ после озвучения дозой 3 ватта/см² значительно слабее — 12,69% и является недостоверно статистическим. Описанный стимулирующий эффект объясняется действием ультразвука на цитоплазму и субклеточные структуры — микроразмещения, активирование энзимов и создание новых активных макромолекулярных комплексов.