PHYSIOLOGICAL STATE OF HORSE BLOOD CELLS IN CONTINUOUS AND PULSED ULTRASONIC FIELD
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Materials and Methods

The experimental work was carried out at the Department of Information Technology, Mathematics and Physics of Moscow State Academy of Veterinary Medicine and Biotechnology. All animals were adult and healthy. Groups of animals: 18 horses (an equal number of males and females) aged 5–8 years. Animals’ blood was exposed to US during a period from 15 seconds to 5 minutes. US therapy medical devices were: UST–1–01F, UST–5 and UST–1.02S, combined with thermostat U7c. We applied pulse mode – 10. The sonication technique has been specifically designed and tested in samples with the minimum volume. We adjusted the US exposure for each blood volume to receive the comparable results. Blood samples were sonicated under identical conditions (oscillator square, cooling fluid, circulative rate). The blood sonication was carried out in a temperature-controlled cuvette. Its walls were made of US conductive plastic. A coolant, distilled water, circulated continuously (so-called "flow-through cooling").
Morphological, biophysical and physiological studies on animal blood cells after exposure to ultrasound (US) were conducted. The US carrier frequency was 0.88 MHz or 2.64 MHz, therapeutic levels of intensity $I_{SATA}$ — average over space and time intensity — were from 0.05 W/cm$^2$ to 1.0 W/cm$^2$. The irradiated blood volume from dogs, cats and horses ranged from 5 to 10 ml. The exposure time was from 15 sec to 1 min, the 10 msec–impulse. No animal was harmed. The unused blood from the planned clinical and hematological studies in animal clinics and branches of the Academy was sonicated.
Methods of analysis

The untreated blood of the same animal (intact specimens) served as a control. Smears were made and stained per the DIFF-QUICK technique: smears were fixed in absolute methanol 15 sec, were heated in dye solutions for 10 seconds, were washed with buffered water, were dried and were examined. US effects on cells (control and after the US exposure) were observed under a light microscope (immersion, transmitted light microscope «Mikmed-5», optical objective–100х/1.25; ocular lens– 10х/18).
Destructive, cytolytic, nucleolytic, and some other effects were discovered. The change in the cell cytomorphology up to the completely destruction of blood cells and cell structures in horses’ tissues were found.
The impact of continuous ultrasound. Smear of horse blood. US-Intensity of 0.4 W/cm², exposure time 15 sec. Lymphocyte and, possibly, segmented neutrophil. Aggregation.
Smears of horse blood

0.4 W/cm², continuous ultrasound, 30 sec. Segmented neutrophil (top) and three lymphocytes with signs of cell lysis and destruction

0.7 W/cm², 18 seconds. Change in the nuclei of lymphocytes and segmented neutrophils, aggregation of platelets
Smears of horse blood

1.0 W/cm², continuous ultrasound, 30 sec. Cell’s aggregation. 1. Thrombocytes. 2. Lymphocyte in the environment of erythrocytes

1.0 W/cm², continuous ultrasound, 40 sec. Platelet groups and lysed leukocytes
Smears of horse blood

0.4 W/cm², 20 seconds. Destructive changes in neutrophils and eosinophils

0.4 W/cm², 30 seconds. Cytolysis of the segmented neutrophil: the cytoplasm is completely lysed and partially the nucleus. Chromatinolysis.
Smears of horse blood

0.4 W/cm², 60 seconds. Lymphocyte lysis

The chromatin concentration in the nuclei of neutrophils after 0.4 W/cm², 60 seconds
Smears of horse blood

0.7 W/cm², 25 seconds. WBC lysis, destructive changes in neutrophils

1.0 W/cm², 20 seconds. Irreversible changes in leukocytes. Foaming of the cytoplasm and karyorexis. Platelets’ aggregation.
Physiological effects of ultrasound

The following cytomorphological US effects in the red and white blood cells were detected: change of shape and of cell area, change in the volume of the cytoplasm, vacuolization of nuclei and cytosol, the formation of symmetric groups around the cell and red blood cell chains without cytolysis, the appearance of shadows cells, karyorexis, karyopicnosis, chromatinsis, karyokinesis, karyo-fragmentation. White blood cells changed before erythrocytes, after 12–20 sec sonication. The effect on granulocytes, led to the damage of cytoplasmic membrane and then the whole cell, began earlier than the same effect in agranulocytes. In small lymphocytes, degenerative and destructive changes recorded later, after 50–90 seconds. We recorded changes in platelet permeability, shape, deformation, rupture of the cytoplasmic membranes and trespassing. Effects named of continuous and pulse-modulated ultrasound generally correlated with the size of tissue cells of animal species. Changes in blood cells after sonication with pulsed US started to be recorded at larger ultrasound exposures due to a decrease in the thermal component at the time of the radiator.
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