Effects of Low-Intensity Continuous Ultrasound on Hematological Parameters of Rats

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ABSTRACT

Background: Low intensity ultrasound (US) has some well-known bio-effects which are of great importance to be considered.

Objective: We conducted the present study to investigate the effects of low intensity continuous ultrasound on blood cells count in rat.

Methods: Rats were anesthetized and blood samples were collected before US exposure. Then, they were exposed to US with nominal intensity of 0.2 W/cm2 at frequency of 3 MHz for a period of 10 minutes and this protocol was repeated for 7 days. Twenty four hours after the last US exposure, secondary blood samples were collected and the changes in blood parameters were evaluated.

Results: Analysis revealed that platelets, hematocrit (HCT) and hemoglobin (HGB) were significantly different between experimental and sham groups but no difference between sham and control groups was observed. The results show that HCT and HGB of exposed rats were significantly reduced.

Conclusion: This study shows that low intensity US may lead to side effects for hematological parameters such as reduction in the levels of HGB and HCT.

Keywords

Low Intensity Ultrasound, Continuous Wave, Blood Cells, Hematological Test, Biological Effects

Introduction

Ultrasound (US) has been shown to have greatly considerable applications in bio-medicine including surgery, medical imaging, physical therapy and cancer therapy [1-4]. In practice, US is administered in different types of wave (continuous or pulsed), intensity (0.1 to 3 W/cm^2), exposure time and frequency (1 to 3 MHz) [5-7].

Low-intensity US has some well-known bio-effects such as being effective on tissue regeneration, bone fracture heeling and bone growth [8, 9]. There are two components in the mechanisms of action of US producing bio-effects such as (1) heating and (2) cavitation. The levels of thermal and mechanical effects are not sole indicators responsible for US exposure, but the amount of these effects are currently accepted as potential risk estimators [10]. The literature revealed that US can affect cell membrane (cell adherence, cell membrane permeability and cell proliferation) and activates the signal-transmission pathways resulting in gene expression [5, 6, 11-13].

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The expanding use of US in medicine has led to increasing demand for more research works on the bio-effects of US interaction with living cells and tissues. We came up with the question whether or not low-intensity US is effective on bone marrow hematopoietic cells and hematologic parameters. In the literature, there are relatively few studies reporting the hematopoietic effects of low-intensity US. For the treatment of acute muscle injuries, Plentz et al. (2008) have concluded that continuous US promotes reductions in erythrocytes and increases segmented neutrophils and eosinophils [14]. Signori et al. (2011) have shown that low-intensity pulsed US reduces total leukocyte count and has no effect on the number of erythrocytes after acute incisional muscular lesion in rats [15]. Moreover, Singnori et al. (2014) have reported the effects of continuous US (1 MHz, 0.4 W/cm^{-2}) and pulsed US (mode 20%) on hematological dynamics and plasma fibrinogen during the inflammatory phase of muscle injury in rats [16]. They have shown pulsed US waves present an anti-inflammatory effect due to plasma fibrinogen reduction and continuous US waves favor hemorrhage due to the reduction of erythrocytes when applied in the first 72 hours after muscle injury. In another study, Zaiki et al. (2013) investigated prenatal ultrasound heating impacts on fluctuations in hematological parameters of Oryctolagus cuniculus [10]. Their research involved in-vivo experimental model by using 3rd trimester pregnant Oryctolagus cuniculus and exposing them to US exposures (0.13 to 0.19 W/cm^2) for various periods of time. They demonstrated that significant differences are detected in red blood cell count, hemoglobin concentration and also platelet count in newborn of Oryctolagus cuniculus.

Considering the clinical importance of bone marrow hematopoietic and peripheral circulatory cells and, on the other hand, easy access and frequent usage of US in clinic, we experimentally investigated the effects of low-intensity continuous US on hematological changes in rats.

Material and Methods

This experimental study was conducted at Cellular and Molecular Research Center, Iran University of Medical Sciences (IUMS) in accordance with the rules established for experimental research in animal laboratory by the University. Ethical approval was provided by the Ethics and Research Committee at IUMS.

Animals

A sample of 15 male Wistar rats weighting approximately 300±50 grams were randomized into three groups: 1) Group 1 (n=5) underwent anesthetic procedures and US exposure, called experimental group; 2) Group 2 (n=5) underwent anesthetic and all other stress-related procedures but not US exposure, called sham group; 3) Group 3 (n=5) underwent no any procedure, called control group.

Procedures

A primary one cubic centimeter of blood collection was performed for all subjects two days before US exposure in order to stimulate the erythrogenesis process. The collections were assembled from the right atrium of the rats' hearts.

To prevent any movement of animals during US exposure, both experimental and sham groups underwent anesthetic procedures by injecting 75mg/kg Ketamin and 14mg/kg Xylazine to each rat. Injections caused an anesthesia of about 20 minutes for each subject and were applied five minutes before US exposure. The right femoral zones of both experimental and sham animals were shaved and ultrasonic coupling agent was applied. The animals were fixed at especially customized apparatus and exposed to ultrasound in the last axial maximum (LAM) of circular therapeutic transducer with 4 cm^2 area operating at 3 MHZ in continuous wave mode. Nominal intensity of 0.2 W/cm^2 with intensity ratio of $I_{peak}/I_{average}$ = 3.5 and sonication period of 10 minutes was

chosen to avoid local temperature rise of the exposed area beyond 2° centigrade. The procedures were applied once a day for seven subsequent days.

Data Collection and Statistical Methods

Twenty four hours after the last US exposure, all subjects underwent the secondary and final blood sample collection to evaluate the hematologic parameters. Samples referred to the laboratory of hematology and Red Blood Cells (RBC), White Blood Cells (WBC), platelets (PLT), hemoglobin (HGB), hematocrit (HCT), Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH) and Reticulocyte counts were measured. The data are presented as mean ± standard deviation (SD).

For data analysis, ANOVA test was used for the comparison of WBC, HGB, HCT, MCV, MCH and reticulocyte counts across these groups. Because of lack of homogeneity of variances, Kruskal-Wallis test was performed for RBC and PLT. Least Significant Difference (LSD) and Games-Howell tests were also used as post-test analysis.

Results

Table 1 shows the effects of low intensity US on hematological variables. RBC had a reduction of approximately 11% (P value = 0.013) for experimental rats which were exposed to US, but this parameter did not change for sham group. The US exposure significantly decreased (P value = 0.032) the level of PLT in experimental group in comparison with sham and control groups (~ 35 % reduction). HGB showed a reduction of 9% (P value = 0.043) in the experimental group while the sham group has the same HGB as control group. Additionally, there was another significant reduction (~ 10%) for hematologic variables of experimental group in comparison with control and sham groups and it was HCT (P value = 0.035).

During our experiments, changes were not observed in MCV (P value = 0.894), MCH concentration (P value = 0.452), WBC (P value = 0.894) and in the reticulocyte (P value = 0. 385).

Additionally, post-test analysis revealed that HCT (P value = 0.033) and HGB (P value = 0.025) amounts differ significantly across sham and experimental groups (table 2). Though, RBC and PLT counts showed significant difference among three groups, in post-test analysis none of two-group comparisons were significantly different.

Discussions

There are many studies indicating the bio-effects of US in the literature. For instance, studies have indicated that treatment with US can promote satellite cell proliferation, increase the differentiation of muscle lineage cells, achieve collagen supra-molecular myoregeneration phase and reduce oxidative stress [17-19]. On the other hand, the hematological changes due to bone marrow US exposure are not well understood according to literature review. In fact, there are a few reports indicating the effects of US on hematological parameters, but the effects of a combination of factors including the type of examined tissue, the US application (continuous or pulsed), intensity and frequency of treatment must be investigated [16, 20]. Here, we presented some original findings obtained due to low intensity continuous US exposure (3 MHz, 0.2 W/cm^2) to right femoral zone of rats.

Signori et al. (2014) have studied the effects of US (1 MHz, 0.4 W/cm^2) in continuous (exposure time 3 minutes) and pulsed (20%; 2 ms on and 8 ms off) forms on hematological dynamics and plasma fibrinogen during the inflammatory phase of muscle injury in rats [16]. The data from this study suggested that muscle injury treated with US in the pulsed form promotes a reduction in plasma fibrinogen in the first 24 and 48 hours after muscle injury. However, the continuous form of US induced a reduction in erythrocytes at the 48thh.

Table 1: Effects of Low Intensity Ultrasound on Hematological Variables.

Hematological parameters	Groups	Mean ±SD	Unit	Sig.
RBC	Experimental	6.88E6±6.38E5	Count/µl	0.013*
	Sham	7.55E6±1.78E5		
	Control	7.73E6±2.31E5		
PLT	Experimental	5.80E5±3.24E5	Count/µl	0.032*
	Sham	9.24E5±1.31E5		
	Control	9.01E5±0.95E5		
HGB	Experimental	12.56±1.14	g/dL	0.043*
	Sham	13.86±.62		
	Control	13.74±.52		
HCT	Experimental	35±3.5	%	0.035*
	Sham	39±1.2		
	Control	39±3.9		
MCV	Experimental	51.75±3.38	fl	0.894
	Sham	51.86±2.49		
	Control	51.14 ±1.18		
MCH	Experimental	18.25±.84	pg	0.452
	Sham	18.36±.93		
	Control	17.76±.47		
WBC	Experimental	6833.33±4049.03	Count/µl	0.337
	Sham	10080.00±2793.20		
	Control	9520.00±4258.75		
Reticulocyte	Experimental	3.71E4±1.74E4	%	0.337
	Sham	3.04E4±1.61E4		
	Control	4.88E4±2.69E4		

Moreover, the results of this study showed no alterations to WBC. It may be stated that these observations and the results of our study that evaluated the effects of continuous form of US on hematological parameters of rats are in good agreement.

In another study published by Signori et al. in 2011, hematological effects of low-intensity pulsed US (LIPUS) in acute muscular inflammations have been assessed. In this controlled laboratory study, 18 male Wistar rats underwent surgical incision in the biceps femoris muscle. Then, 1 MHz US was applied for LIPUS group in the pulsed mode for three minutes at 1, 8 and 24 hours post-surgery. Although, no difference was detected between RBC measures, the results showed a significant reduction in total leukocyte count on the first day after iatrogenic muscle lesion. LIPUS also reduced the number of segmented neutrophils at 1 hour post-surgery. The investigators concluded that LIPUS reduced aspects of the inflammatory process following an acute incisional muscular lesion [15].

Plentz et al. (2008) have shown the continuous US exposure following acute iatrogenic muscle injury in rats can produce hematological effects described as a reduction in erythrocytes and an increase in segmented neutrophils and eosinophils [14]. Thermal effect of contin-

Table 2: Results of Post-test Analysis on Hematologic Variables of Three Groups Considered in the Study.

	Groups		P value
TEST: LSD			
HGB	Experimental	Sham	0.025
		Control	0.039
	Sham	Experimental	0.025
		Control	0.826
HCT	Experimental	Sham	0.033
		Control	0.020
	Sham	Experimental	0.033
		Control	0.801
TEST: Games-Howell			
RBC	Experimental	Sham	0.110
		Control	0.048
	Sham	Experimental	0.110
		Control	0.391
PLT	Experimental	Sham	0.111
		Control	0.130
	Sham	Experimental	0.111
		Control	0.948

uous US was suggested as a possible cause of erythrocyte reduction in their study in addition to other hemorrhagic effects of US such as nitric oxide release, fibrinolysis and thrombolysis. The thermal effect of US was eliminated in present study by its design; as a result, the hematocrit and erythrocyte reduction in our study cannot be attributed to thermal effect. In addition, in our work the continuous US exposure was not on the site of initial blood collection; therefore, the hemorrhagic effect of US is not an acceptable explanation. Another possible mechanism for justifying their results is the probable effect of US exposure on the bone marrow of the femor which was under the incised biceps femoris muscle.

Hemoglobin amounts were also assessed alongside other hematological factors in the present study. Hemoglobin reduction in experimental group and unchanged MCV and MCH among groups might suggest a hypoplastic anemia in experimental subjects. According to unchanged reticulocyte counts across these groups, a failing in RBC production might be triggered by continuous US exposure and the bone marrow seems to be suppressed. More histological investigations sound to be needed to assess the microscopic alteration of exposed bone marrow. Lessened PLT count may also propose a rebound reaction as a result of being suppressed by exposing to continuous US. Furthermore, additional experimental studies are required to clarify the PLT count and WBC count changes due to continuous US exposure.

In conclusion, the findings of this research, presenting the hematological harmful effects of continuous US, may change our point of view in the frequent incautious use of diagnostic ultrasound in clinics. On the other hand, the new aspects of therapeutic usage of US are suggested to be considered.

Acknowledgment

This work was supported by a grant (No. 15026) from the School of Medicine at Iran University of Medical Sciences (IUMS).

Conflict of Interest

Authors declare that they have no conflict of interest.

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