Savytskyi I. V., Tsypoviaz S. V., Lenik R. G., Bilash O. V., Zashchuk R. H., Miastkivska I. V., Zaiats L. M. Interdependence investigation of dynamic between the von Willebrand factor and erythrocyte and leukocyte intoxication indices in experimental peritonitis. Journal of Education, Health and Sport. 2019;9(6):572-583. eISSN 2391-8306. DOI http://dx.doi.org/10.5281/zenodo.3890396
http://ojs.ukw.edu.pl/index.php/johs/article/view/7774

The journal has had 7 points in Ministry of Science and Higher Education parametric evaluation. Part B item 1223 (26/01/2017).
1223 Journal of Education, Health and Sport eISSN 2391-8306 7

Received: 10.06.2019. Revised: 24.06.2019. Accepted: 28.06.2019.

UDC 616-092:616-036.8

Interdependence investigation of dynamic between the von Willebrand factor and erythrocyte and leukocyte intoxication indices in experimental peritonitis

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Abstract

The research was conducted on 175 white rats of reproductive age (3 months), weight of animals - 180-220 g. Animals were divided into 4 groups:

Group 1 - 20 intact animals.

Group 2 - 50 rats with simulated fecal peritonitis.

Group 3 - 50 rats with simulated fecal peritonitis with subsequent antibiotic correction and debridement by chlorhexidine solution.

Group 4 - 50 rats with simulated fecal peritonitis with subsequent antibiotic correction, chlorhexidine debridement and endothelial dysfunction correction with the use of a nitric oxide donor.

Fecal peritonitis was modeled using injection of 10% fecal suspension in a dose of 0.5 ml per 100 g of animal weight in the abdominal cavity of laboratory animals by puncture

method (Lazarenko V.A., et al., 2016, patent No. 233826).

The following results were obtained.

Endothelial dysfunction is the trigger mechanism of vascular catastrophes after experiencing experimental peritonitis.

Evidence of endothelial functional status impairment during experimental peritonitis is a significant increase in the level of Willebrand factor in the animals blood flow ($p < 0.001$).

It was revealed significant increase in erythrocyte intoxication index (EII).

It has been proved increase in leukocyte intoxication index (LII) in experimental animals during simulated peritonitis.

It has been confirmed effectiveness of nitric oxide donor use in the complex correction of peritonitis and endothelial dysfunction as its complication is confirmed.

Key word: peritonitis; experimental research; von Willebrand factor; erythrocyte intoxication index (EII); leukocyte intoxication index (LII); L-arginin.

Introduction. Nowadays, peritonitis is one of the most severe complications of acute inflammatory diseases of the abdominal cavity with a high mortality rate. [1-4]. According to various authors, it makes up from 18.3 to 62.8 % [5]. The highest mortality rate is observed during the postoperative peritonitis - from 45 to 92.3% [6, 7].

The cause of endothelial dysfunction is chronic endotoxin aggression. Endothelial dysfunction caused by peritonitis is not limited by vascular reactions of a single organ, and as a result leads to multiple organ failure. Therefore, the increase in the concentration of endotoxin in the blood plasma should be considered as the main trigger of endothelial dysfunction and its related diseases in the remote postoperative period.

After the intraabdominal infection the patient may has the systemic endotoxinemia, which affects the liver and causes severe metabolic disorders and endothelial dysfunction [8].

The study of the dismetabolic effects of peritonitis has showed that during surgical intra-abdominal sepsis, which accompanies almost all abdominal disasters, as well as during atherosclerosis, the initiating role belongs to the endotoxin of gram-negative microflora, which realizes its pathological potential through endothelial dysfunction. Endothelial dysfunction was identified by V.S. Saveliev (2009) as the main cause of cardiovascular diseases and death in patients after postponed peritonitis [8].

Materials and methods of research:

The research was conducted on 175 white rats of reproductive age (3 months), weight of animals - 180-220 g. Animals were divided into 4 groups:

Group 1 - 20 intact animals.

Group 2 - 50 rats with simulated fecal peritonitis.

Group 3 - 50 rats with simulated fecal peritonitis with subsequent antibiotic correction and debridement by chlorhexidine solution.

Group 4 - 50 rats with simulated fecal peritonitis with subsequent antibiotic correction, chlorhexidine debridement and endothelial dysfunction correction with the use of a nitric oxide donor.

Fecal peritonitis was modeled using injection of 10% fecal suspension in a dose of 0.5 ml per 100 g of animal weight in the abdominal cavity of laboratory animals by puncture method (Lazarenko V.A., et al., 2016, patent No. 233826).

Modeling technique: fecal peritonitis was simulated by introducing a fecal suspension into the abdominal cavity of experimental animals. The suspension was prepared by mixing isotonic solution and feces from the cecum of intact animals, followed by filtration through a double layer of gauze. The resulting suspension was administered to rats under puncture anesthesia no later than 20 minutes after preparation. To prevent damage to the internal organs, the introduction of fecal suspension into the abdominal cavity of animals was placed vertically, the caudal part of the torso up. The puncture was performed along the ventral wall, in the center of the midline of the abdomen, directing the end of the needle alternately in the right and left hypochondrium, right and left iliac regions, introducing a suspension [9].

Upper median laparotomy and abdominal revision were performed on anesthetized animals of groups №3 and №4. Inflammatory exudate was removed using an electroaspirator. Carried out the rehabilitation of the abdominal cavity to obtain clean wash water. Then the wound was drained and sutured in layers (Povylyaeva T.L., 2004). Animals were properly anesthetized with sodium thiopenta during the manipulations.

Antibiotic correction was performed as follows. Ceftriaxone dissolved in 0.2 ml of isotonic sodium chloride solution at a dose of 5 mg / 100 grams was injected intramuscularly into the right thigh (Popov PV et al., 2012).

Nitric oxide donor administration a solution of L-arginine (SIMESTA, made in China, quality standard USP32) was carried out by intragastric injection of L-arginine solution in 0.9% sodium chloride solution at a dose of 500 mg / kg (Pokrovsky M.V., Pokrovskaya T.G., Korchakov V.I., etc., 2008) through a syringe with a feeding tube [10]. The volume of the solution depended on the weight of the animal and did not exceed 1 ml. The drug was administered once a day before morning feeding, daily for 10 days [10].

Research was conducted in accordance with the "Rules for carrying out works using experimental animals", approved by the Order of the Ministry of Health of Ukraine No. 249 of 01.03.2012 and the Law of Ukraine No. 3447-IV "On the Protection of Animals from Cruel Treatment" (as amended on December 15, 2009, and 10/16/2012).

The erythrocyte intoxication index was determined by studying the sorption capacity of erythrocytes by their interaction with methylene blue, which under physiological conditions practically does not penetrate through their membrane [11].

Determination of leukocyte intoxication index (LII) was performed according to the method of Y.Y. Cal-Caliph [12] based on a general blood test using an automated hematology analyzer BC-2800Vet (PRC) using MINDRAY reagents (South Korea).

The level of Willebrand factor was determined by enzyme-linked immuno sorbent assay (ELISA) according to ristocetin time [13, 14].

Before using parametric, normality-based statistical distribution methods, it were used to test the series of quantitative data for normality using the Shapiro–Wilk test. Due to the normal distribution of digital data in the samples, was used Student's parametric criterion.

Research results and its discussion:

Research results of the erythrocyte intoxication index (EII) dynamics

Table 1 shows the value of erythrocyte intoxication index, which is a marker of endogenous intoxication development in the body at each experiment stages.

Table 1 - Erythrocyte intoxication index dynamics in animals, which simulated fecal peritonitis on the 1st, 3rd and 21st day of the experiment (M ± m)

Group /day	1st day beginning	1st day end	3rd day	21st day
Group 1	44,50±0,80	44,40±0,70	44,60±0,70	44,40±0,70
Group 2	81,00±0,60	88,60±1,20	94,90±0,50	Animals didn't survive
Group 3	80,8±0,9	65,0±0,9	53,3±0,7	45,1±0,5
Group 4	80,9±0,6	62,1±0,7	49,8±0,7	44,6±0,8

Consider in detail the changes in erythrocyte intoxication index in animals in which peritonitis was simulated. At the beginning of the first day, the second group had an increase of 82% compared to the control group ($p < 0.001$). At the end of the first day in group №2, there was a 99.5% increase in erythrocyte intoxication index, with differences in the significance level $p < 0.001$ compared not only with intact animals, but also in comparison with the results of the same group at the previous stage, which indicates a very pronounced

development of endogenous intoxication in uncorrected peritonitis. On the third day, the endogenous intoxication progression in the pathogenesis of experimental peritonitis was detected: an increase in EII by 112.8% ($p < 0.001$) was found in comparison with intact animals. The animals of the study group did not survive until the 21st day.

Analyze data of the third group, in which simulated fecal peritonitis was adjusted by antibiotic therapy and sanitation with chlorhexidine solution, at the beginning of the first day was established an increase of the investigated index by 81.6% in comparison with the data of intact animals ($p < 0.001$), and no differences were found in comparison with the data of group 2, which indicates their homogeneity. In the second stage in group 3 the expressed differences in comparison with the data of intact animals were revealed - EII is larger in the third group by 46,4%, but much smaller than in the group of animals in the same stage - by 26,6%.

When comparing the data of the same group with the results of the previous stage, a decrease in the index of endogenous intoxication was detected ($p < 0.001$). On the third day in group 3 there was a more pronounced tendency to normalization of this indicator: differences were found at the significance level $p < 0,001$ compared to the data of the previous stage, and a decrease in EII by 43.8% ($p < 0,001$) compared to the data of group 2, which did not correct the simulated peritonitis, which indicates the effectiveness of correcting the means. But at the same time the erythrocyte intoxication index remains elevated in comparison with the data of intact animals - its value differs by 19.5% ($p < 0.001$). On the 21st day, a significant improvement in the condition of experimental animals was detected, which was reflected significant decrease in endogenous intoxication, which is confirmed by a decrease in erythrocyte intoxication index at the level of statistical significance $p < 0.001$. Also, when compared with data from intact animals confirmed the normalization of EII - this figure is increased by only 1.6% compared with rats in the control group (no statistical significance).

In the dynamics research of EII in group 4, in which the pathological process was corrected by antibiotic correction, sanitation with chlorhexidine solution and endothelial dysfunction correction by using of nitric oxide donor, the following was established.

On the first stage was established an increase in EII by 81.8% compared to intact animals ($p < 0.001$), with statistical significance compared to group 2 and 3 in which peritonitis was also modeled, indicating their homogeneity and suitability for further comparisons. At the end of the first day was established decrease of this indicator by 23.2% ($p < 0.001$). Also, in favor of the proposed correction effectiveness, at the initial stage, the reduction of EII by 29.9% in comparison with the data of group 2, did not correct the pathological process ($p < 0.001$).

Also found greater efficiency of complex correction at the level of significance p <0,01 compared to the correction of pathological condition of group 3 animals. On the third day was found pronounced decrease in endogenous intoxication by 19.8% (p <0.001) compared with the data of the same group at the previous stage and by 47.5% (p <0.001) compared to the results of the 2nd group on this stage. Difference with the data of group 3 remains at the level of significance p <0.01. It is also noteworthy that the erythrocyte intoxication index for the third day in group 4 increased by 11.7% compared to intact animals. At the fourth stage - on the 21st day from the beginning of the pathological process development revealed an improvement of 10.4% (p <0.001) compared with the data of the same group in the previous stage. Also, at this stage, no statistical differences were found in comparison with data from intact animals, indicating that the manifestations of endogenous intoxication were eliminated at day 21 (only 0.5% difference from the control group). Differences from the results of group 3 also revealed no statistical differences. This indicates that both methods of correction are effective, and when analyzing the impact on EII, the data of group 4 are only slightly better than group 3.

Research results of the leukocyte intoxication index (LII) dynamics

Table 2 shows the leukocyte intoxication index value in intact animals of group 1, and rats, which were simulated peritonitis (group No. 2) at each stage of the experiment.

Table 2 - Erythrocyte intoxication index dynamics in animals, which simulated fecal peritonitis on the 1st, 3rd and 21st day of the experiment (M ± m)

Group /day	1st day beginning	1st day end	3rd day	21st day
Group 1	1,53±0,03	1,52±0,03	1,53±0,03	1,54±0,03
Group 2	4,55±0,02	5,12±0,04	4,94±0,05	Animals didn't survive
Group 3	4,56±0,02	4,02±0,03	3,02±0,03	1,55±0,03
Group 4	4,56±0,02	3,88±0,03	2,23±0,03	1,48±0,03

Analyze leukocyte intoxication index there is a unidirectional trend with the erythrocyte index dynamics - a significant increase in the marker against the background of modeled peritonitis development. It should be noted that LII proved to be a more sensitive indicator than the erythrocyte index to the pathological changes caused by experimental peritonitis. Thus, at the beginning of the first day, compared with the data of intact animals in

group 2, the LII was increased by 197.4% ($p < 0.001$), and by the end of the 1st day - by 236.8% ($p < 0.001$)). Significant negative dynamics were established in animals that did not correct the modeled peritonitis, which resulted in an increase in LII in the second stage at a significance level of $p < 0.001$ compared with the previous stage. On the third day, the increase in LII is slightly less pronounced compared to the dynamics at the end of the first day (differences between the 1st and 2nd group are found at the significance level $p < 0.01$), but its increase remains very significant - by 222.9% compared to group 1. The animals of the second group did not survive before stage 4.

Analyze results of animals of group 3, which simulated peritonitis were corrected by sanitation with chlorhexidine and antibiotic therapy in the first stage - the beginning of the first day was established an increase of this indicator by 198% ($p < 0.001$) compared to the control group. However, no statistical differences were found in comparison with group 2. In the second stage, under the influence of correction, was found decrease in leukocyte intoxication index compared with the data of the previous stage at the significance level $p < 0.001$. Also, the decrease in the endogenous intoxication development in the animals of this group indicates that the difference between this group and intact animals is 164.5% ($p < 0.001$). This is significantly less than the data of group 2 - by 21.5% ($p < 0.001$). In the third stage was detected an even more pronounced decrease in LII ($p < 0.001$ compared to the previous stage). Also, comparing the data of the 2nd and 3rd groups, more pronounced positive dynamics was found in the 3rd group - by 38.9% ($p < 0.001$). In this group, the difference between intact animals is less pronounced - the difference between group 3 and group 1 is 97.4% ($p < 0.001$). In the fourth stage, there is also a significant positive dynamics compared to the previous stage ($p < 0.001$). There are no statistically significant differences compared to intact animals and are only 0.6%, which indicates the effectiveness of standard correction for the treatment of peritonitis and its manifestation in the form of endogenous intoxication.

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<0.001). This is significantly less than the data of group 2 - by 21.5% (p <0.001). In the third stage was detected an even more pronounced decrease in LII (p <0.001 compared to the previous stage). Also, comparing the data of the 2nd and 3rd groups, more pronounced positive dynamics was found in the 3rd group - by 38.9% (p <0.001). In this group, the difference between intact animals is less pronounced - the difference between group 3 and group 1 is 97.4% (p <0.001). In the fourth stage, there is also a significant positive dynamics compared to the previous stage (p <0.001). There are no statistically significant differences compared to intact animals and are only 0.6%, which indicates the effectiveness of standard correction for the treatment of peritonitis and its manifestation in the form of endogenous intoxication.

Therefore, we can conclude that standard correction is also effective in combating endogenous intoxication. But the purpose of our work is to analyze the efficacy of peritonitis caused by endothelial dysfunction. To do this, we were investigated the following markers dynamics in this research.

Investigation of the von Willebrand factor (VWF) level dynamics in the blood of experimental animals simulated with peritonitis and its correction

Table 3 presents the research results of the von Willebrand factor level, which, like endothelin-1, is one of the common markers of endothelial dysfunction. Increasing the concentration of this indicator indicates damage to the structural and functional state of endothelial cells.

Table 3 - Level dynamics of the von Willebrand factor in animals, which simulated fecal peritonitis and analysis of its correction methods effectiveness on the 1st, 3rd and 21st day of the experiment.

Group /day	1st day beginning	1st day end	3rd day	21st day
Group 1	84,1±1,1	84,2±0,9	84,1±0,9	84,0±1,1
Group 2	88,4±0,5	99,1±0,6	108,2±0,7	Animals didn't survive
Group 3	88,5±0,9	96,2±0,3	102,4±0,7	96,1±0,7
Group 4	88,5±0,5	92,4±0,5	96,1±0,6	86,2±0,8

At the beginning of the 1st day, in all the groups in which the animals were modeled with bile peritonitis, there was an established increase in VWF in the blood of rats by 5.1-5.2% compared with intact animals. These differences were found at a significance level of p

<0.001, and no statistical differences between the groups in which peritonitis was modeled were found, indicating that they are suitable for further study in the analysis of this marker of endothelial dysfunction.

At the end of the first day in the second group, the level of VWF was increased by 17.7% compared to intact tissues (p <0.001). Compared with the previous stage in group 2, the level of VWF is significantly increased (at the level of significance p <0.001). In group 3, the dynamics compared to the previous stage were found at the same level of significance. But it is noteworthy that the increase in this group is less pronounced than in group 2 compared to intact animals - in the 3rd group the value of the Willebrand factor is 14.3% higher than in the control group. This indicates that the correction of the main links of the pathological process already in the initial stages has a positive effect on holding back the development of endothelial dysfunction, but does not fully correct it. The level of the marker under study is 2.9% in group 3 than in group 2. In the fourth group, the level of VWF increased by 4.4% compared to the previous stage. Already at this stage, a pronounced effect of endothelial dysfunction correction with the involvement of nitric oxide donor in corrective means - compared to intact animals, the level of VWF increased less pronounced than in group 3 - by 9.7% (and in group 3 - by 14.3%). Result of group 4 in the second stage was 6.8% better than in the group of rats 2 (p <0.001). Differences between data of groups 3 and 4 were found at the significance level p <0.001 in favor of the correction used in the fourth group.

On the third day the situation was as follows. In the group in which the modeled peritonitis wasn't corrected in laboratory rats, the development of endothelial damage progressed - at this stage the level of VWF increased by 28.7% (p <0.001), as evidenced by the increase of the test marker in group 2 at this stage in compared to the previous one at the significance level p <0.001. In the third group, the VWF level also increased (at a significance level p <0.001 compared to the previous stage), but 5.9% less pronounced than in the second group. Compared with the results of the control group, the level of endothelial dysfunction in group 3 increased by 21.8% (p <0.001), and in the fourth group the results are slightly better - compared to intact animals, the value of VWF increased by 14.3% (p < 0,001) (differences between group 3 and group 4 data are set at the statistical significance level p <0,001). Also, the result of group 4 was 11.7% (p <0.001) better than the data of group 2 in laboratory rats. The above indicates that at this stage, the involvement of L-arginine in the composition of the corrective means is effective. But at this stage, the level of the Willebrand factor has not yet normalized - it increased by 4.0% compared to the previous stage, which indicates the need

for longer use of a donor of nitric oxide to normalize the functional state of endothelium, which is disturbed by the endogenous intoxication caused by the development of the experiment peritonitis.

At the fourth stage (on the 21st day of the study) the following data were obtained. Animals of group 2 did not survive before this stage of the experiment. In the third group, was established significant decrease of the studied indicator compared to the previous stage - differences were found at the level of statistical significance p <0,001. But it should be noted that at this stage the level of the Willebrand factor by 14,4% (p <0,001) exceeds the value of this indicator in intact animals. This indicates that the correction of peritonitis itself and the endogenous intoxication caused by it already has a positive effect on the restoration of the functional state of the endothelium, but does not normalize it to a full extent, which confirms the need for additional remedies aimed at the treatment of endothelial dysfunction. Results of the nitric oxide donor involvement indicate the correctness of the chosen tactics: in the fourth group a decrease in the level of VWF by 10.3% compared to the previous stage (at the level of significance p <0.001) and also at a significance level of p <0.001, a decrease in this indicator was found when compared with the results of animals of group 3. Also, noteworthy is the lack of statistically significant differences when comparing the results of animals who corrected simulated peritonitis with the involvement of L-arginine to a standard correction scheme - the level of the Willebrand factor was only 2.6% higher than that of intact rats, indicating structural normalization -functional condition of the damaged endothelium.

Conclusions

1. Endothelial dysfunction is the trigger mechanism of vascular catastrophes after experiencing experimental peritonitis.

2. Evidence of endothelial functional status impairment during experimental peritonitis is a significant increase in the level of Willebrand factor in the animals blood flow (p <0.001).

3. I t was revealed significant increase in erythrocyte intoxication index (EII).

4. It has been proved increase in leukocyte intoxication index (LII) in experimental animals during simulated peritonitis.

5. It has been confirmed effectiveness of nitric oxide donor use in the complex correction of peritonitis and endothelial dysfunction as its complication is confirmed.

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