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PECULIARITIES OF CYTOLYTIC SYNDROME DEVELOPMENT WITH ASSOCIATED INJURY OF THE ABDOMINAL ORGANS AND MASSIVE BLOOD LOSS IN THE EXPERIMENT

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Abstract

The function of the organs responsible for detoxification processes, in particular the liver, deserves a special role in the conditions of combined trauma. It is proved that ischemia-reperfusion of the limb caused by the imposition of a tourniquet, is the cause of significant toxemia. Under such conditions, it becomes clear that the detoxification function may be impaired, especially against the background of blunt combined trauma to the abdominal organs complicated by massive bleeding. Changes in the detoxification function of the liver are indicated by markers of the cytolytic syndrome. However, there is no clear understanding of how ischemia - limb reperfusion affects the development of cytolytic syndrome.

Objective of research: to study the effect of massive blood loss associated with ischemia-reperfusion of the limb on the cytolytic syndrome indicators in case of injury of the abdominal organs.

Materials and methods. 100 mature albino male Wistar rats with the body weight of 190-220 grams kept on standard vivarium forage were used in the experiment.

The animals were divided into the control and 3 research groups (containing 10 animals each). Arresting bleeding tourniquet was applied proximally on the lower limbs of rats from the first research group for 120 minutes under thiopental-sodium anesthesia (40 mg/kg^{-1}), which caused development of ischemic-reperfusion. Closed abdominal injury was simulated by means of delivering two dosed blows in the region of the abdominal cavity in the second research group; massive blood loss was simulated by means of cutting the femoral vessels and bloodletting from 25 to 30 % of the circulating blood volume in the group. Injuries from the first two groups were combined in the third group. The control group of animals received anesthesia without formation of any injuries.

With the aim to determine alterations of cytolytic syndrome the activity of enzymes – markers of cytolytic syndrome ALT and AST were determined by means of a unified method.

The animals of the research groups were removed from the experiment under thiopental-sodium anesthesia by means of the total bloodletting from the heart.

Results. Analysis of the results of the study found that cytolytic syndrome develops irrespective of the kind of injuries simulated. Thus, even during a day of post-traumatic period ALT and AST activity increases which is clearly registered in all the three research groups. Further destruction of cellular membranes increases till the 3rd day, which is manifested by a considerable increase of ALT and AST activity in the blood serum. This process continues till the 7th day. A tendency to ALT and AST decrease is observed only in the 1st group on the 7th day. Comparison of the 2nd and 3rd research groups within the time interval enables to understand that application of tourniquets on the limbs of animals with closed abdominal injury and massive bleeding deteriorates cytolytic processes.

Conclusions: Simulated injuries in the research groups were associated with the occurrence of cytolytic processes possessing a tendency to develop in the period of early signs of traumatic disease. Ischemic-reperfusion syndrome of the limbs accelerates considerably the development of systemic changes with abdominal injuries and hypovolemic shock, which was manifested by a considerable increase of ALT and AST parameters in the group with abdominal trauma and massive blood loss and becoming higher than that of the control in end of the experiment.

Key words: reperfusion; experiment; tourniquet; cytolytic syndrome.

Introduction. In recent years the number of emergency situations of a natural and anthropogenic character has increased both in Ukraine and the whole world. Terroristic acts and the use of explosive devices in everyday life both on purpose and by accident take a special position among emergency situations [1]. Peculiarities of such situations are occurrence of injuries caused by high energy associated with massive bleeding, damage of a considerable part of the soft tissues [3]. Such cases require special attention of clinical workers since ischemic-reperfusion syndrome of the limbs due to application of arresting bleeding tourniquets is also involved to unfavorable factors of such victims [4]. Its action is not practically studied in case of multiple or associated injuries.

Functional liver disorders play a special role in case of associated injury concerning development of systemic changes in the body. It is stipulated by the fact that one of the causing agents of associated injury and ischemic-reperfusion syndrome is toxemia, and the liver experiences a greater part of stress performing detoxification function in particular [2,5,6]. Meanwhile, investigations studying the effect of ischemic-reperfusion syndrome on the liver function, and development of cytolytic syndrome with closed abdominal injury complicated by hypovolemic shock in particular are not sufficient.

Objective: to study the effect of massive blood loss associated with ischemia-reperfusion of the limb on the cytolytic syndrome indicators in case of injury of the abdominal organs.

Materials and methods. A working hypothesis of the experimental study is a suggestion that under conditions of a safe application of the arresting bleeding tourniquet from the point of view of the period of its use, reperfusion of ischemic tissues results in excessive formation of reactive oxygen species (ROS), activation of neutrophils and macrophages, hyperproduction of toxic metabolites, signal molecules of the cytokine line and other mediators of inflammation producing their systemic effect on the body with disturbance of vital activity of the internal organs in case of multiple injuries of the abdominal organs with underlying hypovolemic shock.

To realize the aim the experimental study was carried out on 100 mature albino male Wistar rats with the body weight of 190-220 grams keeping to the requirements of «European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes» (European Convention, 1984). The animals were divided into the control and 3 research groups (containing 10 animals each). Arresting bleeding tourniquet was applied

proximally on the lower limbs of rats from the research groups for 120 minutes under thiopental-sodium anesthesia (40 mg/kg⁻¹). An elastic stripe of «SWAT-T» tourniquet (USA) 10 mm wide was applied which corresponds to the width of a tourniquet applied on the femur of an adult. The tourniquet was tightened according to effective pressure indicator marked on it. The animals of the research groups were removed from the experiment under thiopental-sodium anesthesia (60 mg/kg⁻¹) by means of the total bloodletting from the heart in 1, 3, and 7 days after reperfusion. The control group of animals received anesthesia using an equivalent dose of thiopental-sodium. They were removed from the experiment 2 hours later.

Combined abdominal injury associated with ischemic-reperfusion syndrome was simulated in the research groups in the following way: after introduction of thiopental-sodium anesthesia two dosed blows were delivered in the region of the abdominal cavity by means of a special device; blood loss was achieved by means of surgical access and cutting the femoral vessels in the amount from 25 to 30 % of the circulating blood volume during 1 minute (acute blood loss); ischemic-reperfusion syndrome was stipulated by means of application of arresting bleeding tourniquets on both lower limbs of animals for two hours. The animals were divided into three research groups: in the 1st group reperfusion syndrome of the limb without bleeding and abdominal injuries was simulated; in the 2nd group severe injury of the abdominal organs and massive blood loss was simulated; in the 3rd group severe abdominal injury, massive blood loss and reperfusion syndrome were simulated. The data obtained were compared with the control group.

With the aim to determine cytolytic syndrome changes the activity of enzymes – markers of cytolytic syndrome ALT and AST were determined by means of a unified method for the biochemical analyzer Humalyzer 2000 and were expressed in the units per liter (un/L⁻¹).

The data obtained were statistically processed. Probability of differences between the research and control groups was assessed by means of the software STATISTICA (“StatSoft, Inc.”, USA) on the basis of non-parametric Mann-Whitney criterion.

Results. Table 1 demonstrates that after application of tourniquets on both posterior limbs (research group 1) ALT activity in a day statistically reliably 18,9 % increased in comparison with the control group (p<0,001). This indicator continued to increase till the 3rd day and became 21,3% higher than that of the control group. It decreased a little till the 7th day, but still it remained 5,3 % higher compared to the control group. In the 2nd research group where blunt abdominal injury was simulated with massive external bleeding, ALT activity in a day became 22,4% statistically higher in comparison with the group of animals

without injury ($p < 0,01$). It continued to increase on the 3rd and 7th days – 47,1% and 49,9% respectively in comparison with the control group ($p < 0,001$). The highest increase of ALT activity in the blood serum was found in the 3rd research group. In this group the value 42,9 % increased even on the 1st day. On the 3rd day the indicator 90,7% increased, and on the 7th day it became 131,4 % higher than that of the control. The data of the 3rd research group are highly statistically reliable ($p < 0,001$). Therefore, increasing ALT activity during the control points reflects intensification of cytolytic syndrome, when existing closed abdominal injury is associated with massive blood loss and application of tourniquets on both posterior limbs.

Table 1 – ALT activity in the blood plasma (un/L^{-1}) in the research groups, Me (LQ;UQ) – median (lower and upper quartile)

Research groups	Control	Day of the experiment		
		1 st day	3 rd day	7 th day
1 st group	191,5 (188,1;193,9) (n=10)	215,1* (210,3;219,3) (n=10)	219,5* (215,3;222,9) (n=10)	199,5* (194,7;207,6) (n=10)
2 nd group		220,3* (205,8;228,9) (n=10)	265,2* (254,8;287,7) (n=9)	268,7* (261,7;305,9) (n=8)
3 rd group		253,6* (239,5;266,0) (n=8)	341,4* (321,1;371,7) (n=5)	403,9* (392,5;427,1) (n=6)
p ₁₋₂		>0,05	<0,001	<0,001
p ₁₋₃		<0,001	<0,001	<0,001
p ₂₋₃		<0,01	<0,001	<0,001

Notes: here and in other tables:

1. * reliability of differences concerning the control group (* – $p < 0,05$; ** – $p < 0,01$; *** – $p < 0,001$).

2. p₁₋₂ – reliability of differences between the groups of animals – 1st and 2nd; p₁₋₃ – between the 1st and 3rd; p₂₋₃ – between the 2nd and the 3rd.

Comparison of the research groups found that in a day after getting injury ALT activity in the blood serum became higher together with increasing of injuries. 3 and 7 days after it became considerably higher with associated abdominal injury and massive blood loss. It became still higher when the above impairments associated with application of tourniquets on the limbs as compared to the control group. On the 7th day the value of this parameter in the 1st research group decreased gradually compared to the 1st and 3rd days. The maximum was registered on the 3rd day. All the data obtained on the 3rd and 7th days are highly statistically reliable ($p_{1-2} < 0,001$, $p_{1-3} < 0,001$, $p_{2-3} < 0,001$).

In its turn, AST activity in the blood serum (Table 2) under conditions of simulated

injuries was statistically higher than that of the control in all the terms of the experiment. After tourniquets were applied and additional injuries were not caused, the value of the indicator in a day became 24,1 % higher than that of the control, 3 days after –40,6 %, and 7 days after –33,5 %. In the 2nd research group it 39,5, 79,2 and 93,1 % increased and in the 3rd one –72,2, 133,0 and 162,6 % ($p < 0,001$) respectively concerning the day of the experiment.

Table 2 – AST activity in the blood plasma (un/L^{-1}) in the research groups, Me (LQ;UQ) – median (lower and upper quartile)

Research groups	Control	Day of the experiment		
		1 st day	3 rd day	7 th day
1 st group	110,2 (107,2;114,9) (n=10)	134,2* (123,6;138,4) (n=10)	159,5* (151,2;166,6) (n=10)	152,7* (146,8;164,3) (n=10)
2 nd group		151,0* (145,9;155,4) (n=10)	191,9* (164,4;197,6) (n=9)	200,1* (196,2;202,9) (n=8)
3 rd group		178,6* (175,3;197,9) (n=8)	246,1* (219,7;252,8) (n=5)	276,1* (225,6;289,2) (n=6)
p ₁₋₂		<0,05	<0,01	<0,001
p ₁₋₃		<0,001	<0,001	<0,001
p ₂₋₃		<0,001	<0,001	<0,001

Comparison of the research groups found that on the 1st day of the post-traumatic period AST activity in the blood serum was considerably higher after abdominal injury with associated massive bleeding than application of tourniquets on both posterior limbs. It was still higher when the injury associated with tourniquet application (12,5 % higher, $p_{1-2} < 0,05$ and 33% higher, $p_{1-3} < 0$, respectively). A tendency to the indicator increase from the 1st research group to the 3rd one respectively continued on the 3rd day ($p_{1-2} < 0,01$, $p_{1-3} < 0,001$, $p_{2-3} < 0,001$) and the 7th day ($p_{1-2} < 0,001$, $p_{1-3} < 0,001$, $p_{2-3} < 0,001$). Statistical reliability is indicated respectively.

Therefore, under conditions of simulated injuries irrespective of their origin cytolytic processes possessing a systemic character develop. Even on the 1st day of the post-traumatic period ALT and AST activity increases, and it clearly registered not only in the 2nd and 3rd research groups, but in the 1st one as well. The process of cellular membrane destruction further increases till the 3rd day which is manifested by a considerable increase of ALT and AST activity in the blood serum and continues till the 7th day. A tendency to decrease of ALT and AST levels is registered on the 7th day in the 1st research group only. Comparison of the

2nd and 3rd research groups within the time interval enables to understand that application of tourniquets on the limbs of animals with closed abdominal injury and massive bleeding deteriorates cytolytic processes.

The conducted experimental research and the obtained results indicate that ischemia-reperfusion of the limb, which occurs after the imposition of a tourniquet for two hours, causes the development of changes that are systemic in nature. This fact is confirmed by studies of other authors, who found that the development of irreversible changes in soft tissues distal to the site of application of the tourniquet occurs after 6 or more hours of complete ischemia [7].

Important is the fact that only the imposition of a tourniquet causes changes in the indicators from the first day of injury simulation. It is clear that these changes are compensatory in nature and by the seventh day of observation are actually residual. However, this proves that ischemia-reperfusion causes systemic changes that have an extremely negative impact on the function of internal organs. This fact is proved by other authors who studied the ischemic-reperfusion syndrome in the context of systemic changes and the body's ability to compensate [8].

Hepatocyte damage in victims of combined trauma is indicated by an increase in the activity of cytolysis enzymes in the serum (ALT, AST) in the early post-traumatic period. Hyperenzymemia in this case is considered as a non-specific reaction of the body to the influence of the compression factor of excessive intensity with impaired permeability of biomembranes and ischemia under traumatic injury, which indicates an increase in liver failure. It is believed that the main mechanisms of cytolytic hyperenzymemia are direct cell damage, as well as the result of systemic membranopathy with increased permeability of cell membranes due to hypoxia and activation of free radical processes [3,4].

Our studies, on the one hand, confirmed the above and proved that ischemia - reperfusion should be considered as a separate component of combined trauma to the abdominal organs.

Conclusions

1. Simulated injuries in the research groups were associated with the occurrence of cytolytic processes possessing a tendency to develop in the period of early signs of traumatic disease.

2. Ischemic-reperfusion syndrome of the limbs accelerates considerably the development of systemic changes with abdominal injuries and hypovolemic shock, which was

manifested by a considerable increase of ALT and AST parameters in the 3rd research group becoming higher than that of the control in 7 days of the experiment.

Prospects of further studies. In future the study should be targeted on more detailed examination of ischemic-reperfusion syndrome effect on the liver function and development of better ways of its correction.

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