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ALTERATIONS OF BILE SECRETION INDICATORS WITH BLUNT MULTIPLE ABDOMINAL INJURY AND UNDERLYING ISCHEMIA-REPERFUSION OF THE LIMBS

I. V. Kuzminskyi[1], V. K. Grodetskyi[2], H. Yu. Thymbalyuk[3]

[1]GO "Ukrainian Scientific and Practical Center Emergency and Disaster Medicine MOH of Ukraine"
[2]Bukovinian State Medical University
[3]Ternopil National Medical University named after I.Ya. Gorbachevskiy, Ternopol, Ukraine

Abstract

It is known that ischemia-reperfusion has a significant negative impact on combined trauma. It is clear that there is an impression and dysfunction of internal organs against the background of activation of systemic pathological processes, especially those responsible for detoxification processes. However, these processes are little studied in detail, in particular regarding the violation of bile secretion. There is no clear data, and evidence of exactly how ischemia-reperfusion affects liver function in the case of blunt combined trauma to the abdominal organs and how much bile production deteriorates. This is what has become the subject of research.

Objective of research: to study dynamic peculiarities of the liver bile secreting function in response to abdominal injury complicated by hypovolemic shock and ischemic-

reperfusion syndrome.

Materials and methods. 80 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 8 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 syndrome. Closed abdominal injury was simulated by means of delivering two dosed blows in the region of the abdominal cavity in the second research group; hypovolemic shock was simulated by means of cutting the femoral vessels and bloodletting from 20 to 22 % 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.

Bile secreting function of the liver was studied on the basis of identification of the content of total, conjugated/direct and unconjugated/indirect bilirubin in bile, and the volume of bilirubin conjugation degree in bile.

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. A considerable disorder of the bile secreting function of the liver was found in response to simulated injuries: bile secretion rate in the first research group in comparison with the control one achieves minimal values in 3 days and remains on the same level till the seventh day of the post-traumatic period. In the second group the parameter does not change during 1-3 days, though it decreases sharply in seven days. Unidirectional decrease was found in the third group till the seventh day of the experiment.

The data obtained are clearly indicative of a negative effect of ischemic-reperfusion syndrome on the bile-forming and bile secreting functions of the liver. Effect of the liver function was found both with isolated action and with multiple injuries of the abdominal organs, when bilateral compromised syndrome is observed.

Conclusions: The study proved that the combined blunt trauma of the abdominal organs negatively affects the process of bile formation. Also, in the presence of ischemia-reperfusion of the limb, pathological processes are characterized by a tendency to increase significantly.

Key words: bile formation; injury; bleeding; reperfusion syndrome; experiment.

Introduction. Reperfusion injury is defined as a pathological process when cellular damage in the organ caused by hypoxia paradoxically exacerbates after renewal of oxygen supply [1]. It is a dynamic process including two interrelated phases: local ischemic injury and affliction by reperfusion inflammatory reaction, that is, a systemic phenomenon [2]. In severe cases inflammatory reaction due to ischemic-reperfusion syndrome can result in occurrence of systemic inflammatory response or multiple organ failure syndromes [3]. Ischemic-reperfusion injuries of the liver are frequent and serious complication in clinical practice, which disturbs its function and makes post-operative and rehabilitation periods considerably longer, increases morbidity rate, and on the whole deteriorates the total result of treatment the victims [4]. The above is explained by the fact that the liver as an organ with high energy requirements depends substantially on oxygen supply. It is sensitive to hypoxic or anoxic conditions associated with multiple injury complicated by massive blood loss [5, 6].

A number of authors in their studies used the model of development of multiple organ dysfunction under conditions of severe experimental injury [7, 8]. Special attention was paid to the study of the liver functional state as a central organ of the body detoxification [9, 10]. Its specific bile-forming and bile-secreting functions are sensitive indicators of the development of hepatic failure. They are closely associated with deviation of the key markers of traumatic disease [2, 3, 6, 9]. Meanwhile, analysis of scientific literature showed that bile secreting function under conditions of abdominal injuries and ischemic-reperfusion syndrome available is not completely investigated.

Objective: to study dynamic peculiarities of the liver bile secreting function in response to abdominal injury complicated by hypovolemic shock and ischemic-reperfusion syndrome.

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 80 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 8 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 20 to 22 % 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.

Bile secreting function of the liver was examined in the animals from the research groups in 1, 3 and 7 days after injury. Under thiopental-sodium anesthesia (60 mg/kg^{-1}) the common bile duct was catheterized and bile was collected during 1 hour. The concentration of total, conjugated/direct and unconjugated/indirect bilirubin in bile was determined by means of H.Van den Bergh method modified by M.P.Skakun. Bilirubin conjugation degree was calculated on the basis of the data obtained by the following ratio: conjugated bilirubin×100/total bilirubin (%).

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 and discussion

Table 1 demonstrates that during the experiment the content of total bilirubin in the

blood ranges from high to low as compared to that of the control group. Though, the indicator higher than that of the control group was not statistically reliable (p>0,05).

The first day of observation over the groups showed the following differences from the control group: total bilirubin in the bile of the 1st group 3,4% increased, in the 2nd and 3rd groups it 3,5% 3,7% decreased respectively. The data of the first day are not statistically reliable in all the three groups (p>0,05).

The third day is indicative of decrease of the parameter in all the three groups: it was 2,7% lower in the 1st group, 4,1% - in the 2nd and 10,2% - in the 3rd compared to that of the control. The data appeared to be statistically reliable for the 2nd and 3rd groups only (p<0,05 and p<0,01 respectively).

For the seventh day of the experiment the parameter remains lower than that of the control: 1,2% lower in the 1st group, 3,5% - in the 2nd and 13,5% - in the 3rd one. The data are statistically reliable for the 3rd group only (p<0,001).

Table 1 – The content of total bilirubin in bile (mcmol/L^{-1}) in the research groups, Me (LQ;UQ) – median (lower and upper quartiles)

Research groups	Control	Day of the experiment		
		1st day	3rd day	7th day
1st group	106,3 (101,9;112,0) (n=8)	109,9 (103,7;111,6) (n=8)	103,4 (98,7;106,2) (n=8)	105,0 (101,7;107,9) (n=8)
2nd group		102,6 (99,9;111,2) (n=8)	101,9* (97,9; 102,6) (n=8)	102,6 (102,1;104,6) (n=8)
3rd group		102,4 (99,8;102,6) (n=8)	95,4* (94,1;98,5) (n=8)	91,9* (88,2;94,1) (n=8)
p_{1-2}		>0,05	>0,05	>0,05
p_{1-3}		>0,05	<0,05	<0,001
p_{2-3}		>0,05	>0,05	<0,01

Notes: here and in other tables:

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

2. p_{1-2} – reliability of differences between the groups of animals – 1st and 2nd; p_{1-3} – between the 1st and 3rd; p_{2-3} – between the 2nd and the 3rd.

After a tourniquet was applied on the two limbs of animals, the content of conjugated bilirubin in bile was examined. It was found to be statistically reliably lower (Table 2) in all the control points than that of the control group: in a day –20,8 % lower (p<0,001), in 3 days

–15,4 % lower (p<0,01), and in 7 days –10,8 % lower (p<0,05). Thus, conjugated bilirubin appeared to be the lowest on the 1st day of observation, and it increased gradually on the 3rd and 7th days, still remaining lower than that of the control group 7 days after.

Conjugated bilirubin content in the 2nd and 3rd groups decreases on the very 1st day – 11,3% (p<0,01) and 21,4% (p<0,05) respectively. It further decreases and becomes 23,7% (p<0,001) and 31,8% (p<0,001) lower on the 3rd day, reaching its maximum value on the 7th day – 26,1% (p<0,001) and 43,9% (p<0,001) respectively.

Comparison of the research groups by the terms of observation did not find any statistically significant differences between them on the 1st day (p>0,05). On the 3rd day the values in the 2nd and 3rd groups 9,8% (p_{1-2}>0,05) and 19,4% (p_{1-3}<0,001) decreased in comparison with the 1st one. The 7th day of observation is indicative of 17,2% (p_{1-3}<0,001) decrease in the 2nd group and 37,1% (p_{1-2}<0,001) decrease in the 3rd one compared to the 1st group. Therefore, the lowest values of conjugated bilirubin were registered in the 3rd group. They were minimal on the 7th day, while in the 1st group after decrease of conjugated bilirubin on the 1st day it increased gradually reaching the maximum value on the 7th day, but still it was lower than that of the control group.

Analysis of the results concerning the content of conjugated bilirubin in the patterns of injury enables to conclude that the liver function of bilirubin binding with glucuronic acid decreases with an increased severity of injury, and dysfunction increases even 7 days after getting injury.

Table 2 – The content of conjugated bilirubin in bile (mcmol/L^{-1}) in the research groups, Me (LQ;UQ) – median (lower and upper quartiles)

Research groups	Control	Day of the experiment		
		1st day	3rd day	7th day
1st group	68,3 (61,4;72,4) (n=8)	54,1* (51,2;58,3) (n=8)	57,8* (54,4;60,1) (n=8)	60,9* (54,1;63,5) (n=8)
2nd group		60,6* (59,4;65,9) (n=8)	52,1* (51,3; 55,5) (n=8)	50,5* (47,8;53,8) (n=8)
3rd group		53,7* (52,2;60,0) (n=8)	46,6* (41,2;51,2) (n=8)	38,3* (36,2;40,4) (n=8)
p_{1-2}		>0,05	>0,05	<0,001
p_{1-3}		>0,05	<0,001	<0,001
p_{2-3}		>0,05	<0,01	<0,001

Table 3 demonstrates that the content of unconjugated bilirubin during the experiment increased in all the research groups. It did not decrease to the control level in the points of observation, though in the majority of cases the results appeared to be statistically unreliable ($p>0,05$). During the 1st day the highest increase of unconjugated bilirubin was found in the 1st group. It was 36,6% higher than that of the control, and the data were statistically reliable ($p<0,05$). In the 2nd and 3rd groups this parameter 23,2% and 13,2% increased respectively ($p>0,05$). On the 3rd day of the experiment the parameter 12,2%, 9,9% and 32,1% increased in the 1st, 2nd and 3rd groups respectively ($p>0,05$). The 7th day was indicative of the values higher than that of the control in the 1st and 2nd groups –12,7% and 27,4% ($p>0,05$) respectively. The value was the highest in the 3rd group on the last day of the experiment – 53,9% in comparison with the control group, and it was statistically reliable ($p<0,001$).

Comparison of the groups on the 1st day showed the highest value of the parameter in the 1st group, which was 20,7 % higher than that of the 3rd group ($p_{1-3}<0,05$), and in the 2nd group the value was 8,8% higher than that of the 3rd one ($p_{2-3}>0,05$). On the 3rd day the value of the parameter was the lowest in the 2nd group, and the values of the 1st and 3rd groups were 2% ($p_{1-2}>0,05$) and 20,1% ($p_{2-3}>0,05$) higher respectively. On the 7th day the parameters in the 2nd and the 3rd groups were 13% ($p_{1-2}<0,01$) and 36,6% ($p_{1-3}<0,001$) higher respectively than that of the 1st group.

Table 3 – The content of unconjugated bilirubin in bile (mcmol/L^{-1}) in the research groups, Me (LQ;UQ) – median (lower and upper quartiles)

Research groups	Control	Day of the experiment		
		1st day	3rd day	7th day
1st group	40,2 (37,2;41,5) (n=8)	54,9* (49,2;56,9) (n=8)	45,1 (41,5;48,8) (n=8)	45,3 (41,9;48,4) (n=8)
2nd group		49,5 (45,6;55,6) (n=8)	44,2 (42,8; 49,8) (n=8)	51,2* (49,1;53,7) (n=8)
3rd group		45,5 (42,5;49,7) (n=8)	53,1 (42,8;54,4) (n=8)	61,9* (60,6;64,1) (n=8)
p_{1-2}		>0,05	>0,05	<0,01
p_{1-3}		<0,05	>0,05	<0,001
p_{2-3}		>0,05	>0,05	<0,05

In its turn, the value of conjugation degree under conditions of the injuries examined (Table 4) concerning the control group was the lowest in the 1st group during the 1st day of the

experiment – it was 19,5% ($p<0,01$) lower. It increased a little during the 3rd day remaining 8,2% lower and on the 7th day 9,7% lower than that of the control group of animals. The values of the 3rd and 7th days for the 1st group were not statistically reliable ($p>0,05$). A decreased degree of bilirubin conjugation in the points of observation was found in the 2nd group as well: 17,4% ($p<0,05$) lower on the 1st day, 12,2% ($p<0,01$) lower on the 3rd day and 25,9% ($p<0,001$) lower on the 7th day concerning the value in the control group. The following decreased degrees of bilirubin conjugation were found in the 3rd group: 13,4% ($p<0,05$) lower on the 1st day, 24,8% ($p<0,01$) lower on the 3rd day and 49,7% ($p<0,001$) lower on the 7th day concerning the value in the control group. Therefore, in the group with abdominal injury with underlying massive bleeding and tourniquets applied on the lower limbs, the degree of bilirubin conjugation on the 7th day decreased practically twice as much concerning the control group.

Comparison of the groups showed that on the 1st day bilirubin conjugation was the lowest in the 1st group. It was a little higher in the 2nd and 3rd groups: 2,6% and 7,6% higher than that of the 1st group. Comparison of the groups on the 1st day of the experiment is not statistically reliable ($p_{1-2}>0,05$, $p_{1-3}>0,05$, $p_{2-3}>0,05$).

On the 3rd day the value was the lowest in the 3rd group, and the value in the 1st group was 22% ($p_{1-3}<0,05$) higher, and in the 2nd group – 16,7% ($p_{2-3}>0,05$) higher in comparison with the 3rd one. On the 7th day the value in the 1st group was 79,8% ($p_{1-3}<0,001$), in the 2nd group – 47,4% ($p_{2-3}<0,001$) higher than that of the 3rd group.

Table 4 – Degree of bilirubin conjugation in bile (%) in the research groups, Me (LQ;UQ) – median (lower and upper quartiles)

Research groups	Control	Day of the experiment		
		1st day	3rd day	7th day
1st group	62,1 (59,3;66,1) (n=8)	50,0* (47,0;54,0) (n=8)	57,0 (53,7;58,7) (n=8)	56,1 (54,7;59,8) (n=8)
2nd group		51,3* (45,8;57,0) (n=8)	54,5* (51,2; 55,5) (n=8)	46,0* (44,6;50,5) (n=8)
3rd group		53,8 (51,5;59,4) (n=8)	46,7* (42,7;54,5) (n=8)	31,2* (26,2;33,6) (n=8)
p_{1-2}		>0,05	>0,05	<0,001
p_{1-3}		>0,05	<0,05	<0,001
p_{2-3}		>0,05	>0,05	<0,001

Conclusions

1. Multiple abdominal injuries produce a negative effect on the process of bile formation, and in case of ischemia-reperfusion pathological processes are characterized by a tendency to increase considerably.

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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