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# FEATURES OF THE LIVER'S FUNCTIONAL STATE UNDER CONDITIONS OF CRANIO-SKELETAL INJURY COMBINED WITH BLUNT ABDOMINAL TRAUMA

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Blunt abdominal trauma and cranioskeletal trauma were simulated in mature male Wistar rats. In a separate group of rats, these injuries were combined. The control group consisted of intact animals. After 1, 3, 7, 14, 21 and 28 days, the rate of bile secretion was determined in the experimental animals. It was established that in the dynamics of the post-traumatic period, the additional infliction of blunt trauma to the abdomen significantly deepens the systemic impact of cranioskeletal trauma, which is manifested by the deepening of liver dysfunction, in particular, a significant decrease in the rate of bile secretion with a minimum of 14 days after inflicting the injury. By the 28th day of the experiment, the index increases, but does not reach the control level. Therefore, the complication of cranioskeletal trauma by abdominal trauma is accompanied by an increase in the systemic impact on the body with deepening liver dysfunction, which should be taken into account when developing measures to prevent and correct multiorgan dysfunction of traumatic origin.

**Key words:** abdominal and craniocerebral trauma, hip fracture, hepatic excretion.

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# ОСОБЛИВОСТІ ФУНКЦІОНАЛЬНОГО СТАНУ ПЕЧІНКИ ЗА УМОВ КРАНІОСКЕЛЕТНОЇ ТРАВМИ, ПОЄДНАНОЇ З ТУПОЮ ТРАВМОЮ ЖИВОТА

У статевозрілих щурів-самців лінії Вістар моделювали тупу травму живота та краніоскелетну травму. В окремій групі щурів ці травми поєднували. Контрольну групу склали інтактні тварини. Через 1, 3, 7, 14, 21 та 28 діб у піддослідних тварин визначали швидкість жовчовиділення. Встановлено, що в динаміці посттравматичного періоду додаткове нанесення тупої травми живота суттєво поглиблює системний вплив краніоскелетної травми, що в'являють поглибленням дисфункції печінки, зокрема суттєвим зниженням швидкості жовчовиділення з мінімумом через 14 діб після нанесення травми. До 28 доби експерименту показник зростає, проте не досягає рівня контролю. Отже, ускладнення краніоскелетної травми абдомінальною травмою супроводжується посиленням системного впливу на організм з поглибленням дисфункції печінки, що доцільно враховувати при розробці заходів профілактики і корекції поліорганної дисфункції травматичного походження.

**Ключові слова:** абдомінальна і черепно-мозкова травма, перелом стегна, печінкова екскреція.

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Traumatism is one of the urgent medical and social problems of today. Despite significant achievements in the diagnosis and treatment of the injured, even under the conditions of effective provision of emergency medical care, the level of mortality and lethality remains at a high level. In general, trauma is the fifth leading cause of death after coronary heart disease, lung cancer, stroke, and chronic obstructive pulmonary diseases [13].

The growth of traumatism in modern urbanized society is caused by a significant increase in a number of technogenic, natural, social and military emergencies. In the structure of modern peacetime trauma, the leading place is occupied by combined trauma, the prevalence of which, according to various authors, ranges from 23.5 to 85.0 %. Among such injuries, the combined trauma of the skull and bones of the skeleton (cranioskeleton trauma) stands out, which is characterized by a particularly severe course and high mortality [6].

Despite the change in the nature of combat operations and the use of protective equipment, such lesions are also characteristic of combat trauma. In its structure, injuries to the limbs and head occupy the first place and constitute 52.0 and 16.8 %, respectively [1]. Under these conditions, 24.0 % of the injured with gunshot wounds make up the lower leg [10], 32.4 % of the femur [11]. Among the latter, 36.0 injured people required highly specialized medical care [11].

A characteristic feature of cranioskeletal trauma is the secondary effect on internal organs with the development of multiple organ failure, which becomes the main cause of death after the victims are brought out of shock [3].

As a result of the impact of high-energy traumatizing factors, in the structure of combined trauma, 20 % of victims, in addition to other injuries, are diagnosed with abdominal trauma, mainly blunt abdominal trauma, which in 26.7–40.8 % of cases is accompanied by direct damage to parenchymal organs, primarily the liver (24 %) [5, 12]. In this regard, the study of the combined effect of cranioskeletal and blunt abdominal trauma is of considerable interest. Until now, the specifics of the multiorgan dysfunction formation due to the layering of pathogenic mechanisms of direct and indirect effects of these injuries on the state of parenchymal organs are unknown.

Recently, the biliary function of the liver has been studied as a model of multiple organ dysfunction in conditions of combined trauma in the experiment. In the works of a number of authors, it is shown that the intensity of bile secretion, due to its organ specificity, is a sensitive indicator of the liver failure development and is closely related to other disorders that occur under the conditions of combined trauma, in particular, the intensity of lipid peroxidation in the liver, a decrease in antioxidant protection, the severity of cytolytic processes and endogenous intoxication [9, 15].

However, the peculiarities of the liver dysfunction formation under the conditions of inflicting cranioskeletal and abdominal trauma in the experiment have practically not been studied until now, which makes it impossible to develop pathogenetically justified means of correction.

**The purpose** of the study was to find out the effect of cranioskeletal trauma, blunt abdominal trauma and their combination on the liver's biliary function in the experiment.

**Materials and methods.** Experiments were performed on 116 white male Wistar rats weighing 180─200 g, which were randomly selected and divided into 3 experimental groups (36 rats each) and 1 control group (8 rats). In the conditions of thiopental sodium anesthesia (40 mg·kg$^{1}$) in the first study group, blunt trauma to the abdomen was simulated by applying a dosed blow to the abdomen with a cylindrical impact device with the energy of 0.14 j·cm$^2$, which was borrowed from work [14]. In the second study group, a cranioskeletal injury was simulated: consecutively inflicted a closed fracture of the femur by a dosed impact with a device with a wedge-shaped nozzle with an energy of 0.637 J on the thigh [8] and a closed craniocerebral injury, which was caused by a dosed impact on the skull at a point 5 cm in front of the interaural line with an energy of 0.375 J [ 8]. In the third study group, combined trauma was simulated by sequentially inflicting blunt abdominal trauma, skeletal and craniocerebral trauma. The control group was a group of intact animals that had just been anesthetized.

After 1, 3, 7, 14, 21 and 28 days, the functional state of the liver was determined in the experimental animals. Under the conditions of sodium thiopental anesthesia (60 mg·kg-1 mass) in each experimental group, the biliary function of the liver was studied by catheterization of the common bile duct and the collection of bile for 1 hour [2]. The volume of bile secreted in 1 hour based on the animal's body weight was determined.

All the performed experiments were carried out in accordance with the general rules and provisions of the European Convention for the Protection of Vertebrate Animals Used for Research and Other Scientific Purposes (Strasbourg, 1986), the General Ethical Principles of Animal Experiments (Kyiv, 2001), the Law of Ukraine "On the Protection of Animals from of cruel behavior" (2006 appendix 4), "Scientific and practical recommendations for keeping laboratory animals and working with them", as well as the conclusion of the commission on bioethics of I. Horbachevsky Ternopil National Medical University, MOH of Ukraine No. 42 dated 04.09.2017.

The probability of differences between experimental groups was assessed using the non-parametric Mann-Whitney test. Differences were considered true when the probability of the null hypothesis was less than 5 % ($p<0.05$). All calculations were performed in the STATISTICA 12 (2013) software package.

**Results of the study and their discussion.** Studies have shown that under the influence of blunt abdominal trauma, cranioskeletal trauma, and their combination, there was a significant impairment of the liver's functional state, in particular, its biliary function. Thus, after inflicting a blunt injury to the abdomen (Table 1), the rate of bile secretion decreased compared to the control. If after 1 day of the experiment the index decreased compared to the control by only 12.3 % (2.00/2.28), which turned out to be statistically insignificant ($p=0.1735$), then starting from the 3rd day of the experiment, the result became statistically reliable.

Table 1

**Bile excretion rate (ml·h$^{1}$·kg$^{1}$) after simulation of cranioskeletal trauma combined with blunt abdominal trauma ((Me (LQ;UQ)) – median (lower and upper quartiles))**

| Model | Term after inflicting a trauma | | | | | |
|---|---|---|---|---|---|---|
| | Day 1 | Day 3 | Day 7 | Day 14 | Day 21 | Day 28 |
| Control | | | | | | |
| Group 1 blunt abdominal trauma | 2.00 (1.87; 2.22) | 1.72* (1.62; 1.78) | 1.94* (1.90; 1.99) | 1.66* (1.57; 1.76) | 1.49* (1.47; 1.52) | 2.00* (2.00; 2.04) |
| Group 2 cranioskeletal trauma | 1.92* (1.84; 2.08) | 1.69* (1.62; 1.76) | 1.60* (1.51; 1.65) | 1.42* (1.38; 1.49) | 1.53* (1.49; 1.58) | 1.86* (1.79; 1.89) |
| Group 3 blunt abdominal trauma + cranioskeletal trauma | 1.89* (1.88; 1.93) | 1.51* (1.44; 1.55) | 1.26* (1.25; 1.35) | 1.13* (1.03; 1.19) | 1.35* (1.34; 1.39) | 1.57* (1.55; 1.60) |
| $p_{1-2}$ | 0.6310 | 0.8728 | 0.0082 | 0.0104 | 0.5218 | 0.0051 |
| $p_{1-3}$ | 0.2980 | 0.0374 | 0.0051 | 0.0051 | 0.0104 | 0.0051 |
| $p_{2-3}$ | 0.7488 | 0.0374 | 0.0202 | 0.0051 | 0.0051 | 0.0051 |

Note. * – differences compared to the control group are statistically significant (p<0.05).

After 3 days, the index became lower than the control by 24.6 % (1.72/2.28) (p=0.0065), after 7 days – by 14.9 % (1.94/2.28) (p =0.0104), after 14 days – by 27.1 % (1.66/2.28) (p=0.0051), after 21 days – by 34.6 % (1.49/2.28) (p=0.0051), after 28 days – by 12.3 % (2.00/2.28) (p=0.0374).

In the dynamics (fig. 1), the rate of bile secretion under the conditions of blunt abdominal trauma changed in a wave-like manner with the first decrease after 3 days, which turned out to be statistically less reliable, compared to the result of the 1st day of the experiment (by 14.0% (1.72/2.00) , p=0.0306). Later, after 7 days, the index increased and by 12.8 % (1.94/1.72) exceeded the level of the 3rd day (p=0.0374), reaching the result of the 1st day of the experiment (p=0.5752).

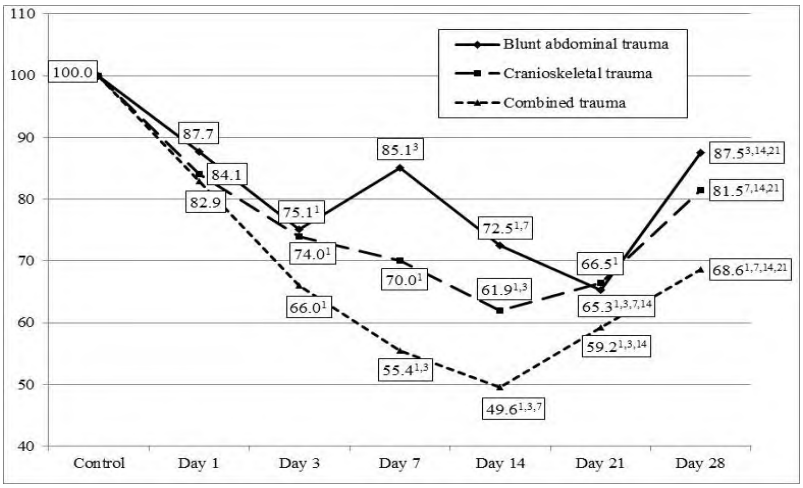


Fig. 1. Dynamics of the rate of bile secretion (as a percentage of the control level) after simulation of cranioskeletal trauma combined with blunt abdominal trauma. Note: 1, 3, 7, 14, 21 – differences in the results of 1, 3, 7, 14 and 21 days are statistically significant (p<0.05).

Starting from the 14th day, the index decreased again and became 14.4 % (1.66/1.94) less than after 7 days (p=0.0082) and 17.0 % (1.66/2, 00) compared to the result of 1 day (p=0.0131). During this period, the index reached the level of the 3rd day of the experiment (p=0.7488).

After 21 days of the experiment, the rate of bile secretion reached minimum values. In this period, it was by 25.5 % (1.49/2.00) less compared to the result of the 1st day of the experiment (p=0.0051), by 13.4 % (1.49/1.72) compared to the result of the 3rd day (p=0.0082), by 23.2 % (1.49/1.94) compared to the result of the 7th day (p=0.0051) and by 10.2 % (1.49/1, 66) compared to the result of the 14th day (p=0.0104).

After 28 days, the rate of bile secretion increased, which turned out to be statistically significantly higher, compared to the results of days 3, 14 and 21 (respectively by 16.3 % (2.00/1.72), p=0.0163, by 20.5 % (2.00/1.66), p=0.0051 and by 34.2 % (2.00/1.49), p=0.0051).

Cranioskeleton injury compared to the control caused a gradual decrease in the rate of bile secretion from 1 to 14 days. From the first day, the index became statistically significantly lower than in the control by 15.8 % (1.92/2.28), p=0.0250, after 3 days – by 25.9 % (1.69/2.28 ), p=0.0051, after 7 days - by 29.8 % (1.60/2.28), p=0.0051, and after 14 days – by 37.7 % (1.42/2.28) (p=0.0051). After 3 and 7 days, the index was significantly lower than after 1 day – respectively by 12.0 % (1.69/1.92), p=0.0051 and by 16.7 % (1.60/1.92), p=0.0131. After 14 days, the index turned out to be significantly lower than after 1 and 3 days of the experiment (respectively by 26.0 (1.42/1.92), p=0.0051 and 16.0 % (1.42/1.69), p=0.0082). Subsequently, the rate of bile secretion increased, but after 21 and 28 days of the experiment it did not reach the level of the control group and remained, respectively, by 32.9 % (1.53/2.28), p=0.0051 and by 18.4 % (1, 86/2.28) smaller (p=0.0051). After 21 days, the index remained at the level of 7 and 14 days (respectively p=0.3785, p=0.0656)

and was significantly lower than after 1 and 3 days of the experiment – by 20.3 % (1.53/1 .92), p=0.0051 and by 9.5 % (1.53/1.69), p=0.0306. After 28 days of the experiment, the index statistically significantly exceeded the results of 7, 14 and 21 days: respectively by 16.2 % (1.86/1.60), p=0.0250, by 31.0 % (1.86/1, 42), p=0.0051 and by 21.6 % (1.86/1.53), p=0.0051. During this period, the index reached the level of 1 and 3 days of the experiment (respectively, p=0.2980 and p=0.1735).

Under the conditions of combined trauma, violations of the bile secretion rate were even greater. The index compared to the control also gradually decreased from 1 to 14 days of the experiment. After 1 day, it was smaller than the control group by 17.1 % (1.89/2.28), p=0.0065, after 3 days – by 33.8 % (1.51/2.28), p =0.0051, after 7 days – by 44.7 % (1.26/2.28), p=0.0051, after 14 days – by 50.4 % (1.13/2.28), p =0.0051. It should be noted that in each subsequent period of observation, the index became significantly lower than in the previous one. Thus, after 3 days of the post-traumatic period, the rate of bile secretion was by 20.1 % (1.51/1.89) lower than after 1 day (p=0.0051), after 7 days it was lower compared to the results of 1 and 3 days : respectively by 33.3 % (1.26/1.89), p=0.0051 and by 16.6 % (1.26/1.51), p=0.0374, after 14 days – compared to by the results of 1, 3 and 7 days: respectively by 40.2 % (1.13/1.89), p=0.0051, by 25.2 % (1.13/1.51), p=0.0051 and by 10.3% (1.13/1.26), p=0.0051.

Subsequently, against the background of the combined trauma, the rate of bile secretion increased, but after 21 and 28 days of the experiment it did not reach the level of the control group and remained, respectively, by 40.8 % (1.35/2.28), p=0.0051 and by 31.1 % (1.57/2.28) less (p=0.0051). After 21 days, the index remained at the level of 7 days (p=0.5752), became significantly higher than after 14 days – by 19.5 % (1.35/1.13), p=0.0051, but remained statistically probably less than after 1 and 3 days of the experiment (respectively by 28.6 % (1.35/1.89), p=0.0051 and by 10.6 % (1.35/1.51), p= 0.0065). In turn, after 28 days of the experiment, the index reached the level of 3 days (p=0.4233) and was significantly higher than after 7, 14 and 21 days (respectively by 24.6 % (1.57/1.26), p=0.2020, by 38.9 % (1.57/1.13), p= 0.0051 and by 16.3 % (1.57/1.35), p=0.0131), however statistically probably less than after 1 day – by 16.9 % (1.57/1.89), p = 0.0051.

Comparison of the experimental groups among themselves showed that after 1 day of the post-traumatic period, no significant differences were observed in the rate of bile secretion ($p_{1-2}$=0.6310, $p_{1-3}$=0.2980, – $p_{2-3}$=0.7488). After 3 days of the experiment, the index in experimental group 3 became statistically significantly lower than in experimental groups 1 and 2 (respectively by 12.2 % (1.51/1.72), $p_{1-3}$=0.0374 and by 10.6 % (1.51/1.69), $p_{2-3}$=0.0374). Differences between research groups 1 and 2 were not statistically significant (p=0.8728).

Subsequently, after 7 and 14 days, as well as after 28 days, the rate of bile secretion was statistically significantly lower in experimental group 2 compared to experimental group 1 (by 17.5 % (1.60/1.94), $p_{1-2}$=0 .0082, by 14.4 % (1.42/1.66), $p_{1-2}$=0.0104 and by 7.0 % (1.86/2.00), $p_{1-2}$=0.0051). In experimental group 3, the value of the studied index was the smallest compared to experimental groups 1 and 2, starting from the 7th day of the experiment: after 7 days – respectively by 35.0 % (1.26/1.94), $p_{1-3}$=0.0051 and by 21.2 % (1.26/1.60), $p_{2-3}$=0.0202 ; after 14 days – respectively by 31.9 % (1.13/1.66), $p_{1-3}$=0.0051 and by 20.4 % (1.13/1.42), $p_{2-3}$=0.0051 ; after 21 days – by 9.3 % (1.35/1.49), $p_{1-3}$=0.0104 and by 11.7 % (1.35/1.53), $p_{2-3}$=0.0051, respectively ; after 28 days – by 21.5 % (1.57/2.00), p1-3=0.0051 and by 15.6 % (1.57/1.86), $p_{2-3}$=0.0051, respectively . It should be noted that after 21 days the differences between experimental groups 1 and 2 were not significant ($p_{1-2}$=0.5218).

The obtained results indicate that blunt abdominal trauma, cranioskeletal trauma and their combination lead to a significant violation of the liver's biliary function in the acute period, the period of early and late manifestations of the traumatic disease. The obtained result is obviously related to the decrease in the formation of bile components, in particular the synthesis of bile acids, cholesterol, conjugated bilirubin, which occurs in the membranes of the microsomal system of hepatocytes, which, as shown by the studies of a number of authors, are sufficiently sensitive to the influence of pathogenic factors of traumatic disease (hypoxia, excessive formation of active forms of oxygen, peroxidation of lipids and proteins of cell membranes, endotoxicosis, etc.) [4].

It is also impossible to exclude a mechanical influence on the outflow through the biliary tract, associated with the development of liver edema due to the development of reactive hepatitis [7].

The specifics of blunt abdominal injury are fluctuating bile flow disturbances with the first decrease after 3 days of the post-traumatic period, an increase that did not reach control – after 7 days, and a repeated decrease with a larger amplitude – after 21 days.

The obtained result is obviously related to the development of adaptive and compensatory reactions in response to trauma, which corresponds to the modern concept of the traumatic disease [6].

If a violation of the liver's functional state under conditions of blunt trauma to the abdomen is a logical consequence of a direct mechanical effect on the organs of the abdominal cavity, then the slowing down of bile secretion due to cranioskeletal trauma is secondary, which is caused by a pronounced systemic effect

of a combined injury of the skull, brain and bones of the skeleton, which corresponds to modern ideas about the course of craniocerebral and skeletal trauma [9, 15].

It is obviously based on the disruption of neurohormonal reactions inherent in brain injury [3], as well as the influence of free radicals, endotoxins, and inflammatory mediators that can enter the systemic bloodstream from the area of direct damage [6].

However, the combination of abdominal injury with cranioskeletal injury is accompanied by a significant deterioration of the biliary function of the liver. The rate of bile secretion, starting from the 3rd day of the post-traumatic period, is significantly lower than in other experimental groups. According to the severity of liver dysfunction, the simulated injuries can be divided as follows: blunt abdominal injury ← cranioskeletal injury ← combined injury. It has been suggested that the combination of blunt abdominal trauma and cranioskeletal trauma causes a syndrome of mutual aggravation, which is based on the summation of the pathogenic effect of each trauma in particular.

Thus, our studies further confirmed the statute that the biliary function of the liver is a sensitive indicator of the functional liver failure development under conditions of severe trauma. The obtained results should be taken into account in the analysis of systemic manifestations of traumatic disease caused by a combination of blunt abdominal trauma and cranioskeletal trauma, as well as in the development of measures to prevent multiorgan dysfunction.

## Conclusion

In the dynamics of the acute period, the period of early and late manifestations of the traumatic disease, the additional infliction of blunt trauma to the abdomen significantly aggravates the course of cranioskeletal trauma and exerts a pronounced systemic effect that negatively affects the biliary function of the liver, which in the group with combined trauma is manifested by a significantly greater decrease in the rate of bile secretion from 3 up to 28 days of the experiment (by 10.4, 21.2, 20.4, 11.8, 15.6 %, respectively) with reaching the minimum index compared to the control 14 days after injury (by 50.4 %), which on the 28th day of the experiment increases, but does not reach the control level and remains by 31.4 % lower.

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