PECULIARITIES OF BILIARY FUNCTION OF THE LIVER IN THE DYNAMICS OF POLYTRAUMA IN THE EXPERIMENT

Kozak D.V. (https://orcid.org/0000-0002-5142-2057)
Horbachevsky Ternopil National Medical University, Ternopil, Ukraine
kozak@tdmu.edu.ua

Relevance. Severe trauma is accompanied by the development of multiple organ dysfunction and the insufficiency of internal organs. The dynamics of changes in the functional state of the liver didn’t investigate fully. It is the central organ of detoxification of the body, whose activity occurs in close integration with other organs and systems of the body.

Objective is to find out the features of the biliary function of the liver in the dynamics of the developed model of polytrauma.

Materials and methods. Polytrauma was performed using 62 nonlinear white male rats weighing 180-200 g under conditions of thiopental-sodium anesthesia (40 mg 1kg-1 intraperitoneally). In surviving animals, the biliary function of the liver was studied in 2 h, 1, 3, 7, 14, 21, and 28 days after injury. For this purpose, the common bile duct was catheterized, and bile was collected for 1 hour in animals under thiopental-sodium anesthesia (60 mg kg-1). The rate of bile excretion and the concentration of total bile acids, cholesterol, direct and indirect bilirubin in the selected portion of bile were determined. Based on these data, the rate of excretion of the studied components of bile was calculated. Euthanasia of rats throughout the experiment was performed by total bloodletting from the heart after previous thiopental-sodium anesthesia (60 mg kg-1 intraperitoneally). The obtained digital data were subject to statistical analysis.

Results. In the conditions of experimental polytrauma, there is a violation of the biliary function of the liver. It is manifested in the period of an acute reaction to the trauma first (after 2 hours) by a significant decrease, then (up to 1 day) development of polycholia - 1.52 times increased of bile secretion. Also increased excretion of the main components of bile, with their subsequent decrease to 7 days, development of the period of temporary improvement in 14 days with the repeated of exacerbation period in 21 days and approach to the norm - in 28 days.

The decrease in bile secretion corresponds to a period of shock, which is characterized by the centralization of blood circulation and reduced blood supply to the organs of the gastrointestinal tract. Increased bile secretion and excretion of main bile components in 1 day after a severe injury is associated with the increased biliary polarity of hepatocytes and unloading of the liver from endotoxins. It accumulates due to tissue damage, microcirculation, and hypoxia. Subsequently, the indicators of the biliary function of the liver changed by the identified patterns of lipoperoxidation deviations, antioxidant protection, cytolysis, and endogenous intoxication. The pathogenesis of biliary disorders is the damage of the endoplasmic reticulum membranes, where the synthesis of the main components of bile. As well as the development of edema of the organ, which prevents the outflow of bile.

Conclusion. The dynamics of the development of functional liver failure due to polytrauma coincides with the general pattern characteristic of the dynamics of other biochemical markers of traumatic disease. Namely: after 3 days of the post-traumatic period, there was a phase of maximum deepening of deviations of the studied indexes. After 7-14 days there was noted a phase of temporary improvement which is characterized by a change of indexes towards the norm. After 21 days there was a re-exacerbation of the pathological process. After 28 days the indexes changed towards the norm, but for most cases do not reach it.

This means that in a critical state of the body the organs and systems coupling is getting worse, which are remote from the site of injury. It can be considered as a factor of compensation and adaptation directed to the survival of the organism.

Keywords: rats, polytrauma, liver, bile

Relevance. In the structure of injuries in recent years, there has been a stable tendency to increase the frequency of combined injuries. It is 23.5-85.0% accompanied by the development of traumatic illness, and it is characterized by severe complications and high mortality [7]. Despite significant advances in the treatment of polytrauma victims, their effectiveness remains unsatisfactory. Therefore, many authors refer a comprehensive study of the pathogenetic mechanisms of the multiorgan dysfunction formation in conditions of polytrauma and traumatic illness to the main areas of modern theoretical and practical medicine [5].

In our previous works on the developed model of polytrauma [6], we showed that there is a pattern of the following indexes deviation. They are the dynamics of lipid peroxidation, antioxidant protection, cytolysis, and endogenous intoxication in the dynamics of the early and late manifestations of the traumatic disease. It consists of the fact that after 3 days of the post-traumatic period, there is a phase of maximum deepening of deviations of the studied indexes. After 7-14 days there is noted a phase of temporary improvement which is characterized by a change of indexes towards the norm. After 21 days there is a re-exacerbation of the pathological process. After 28 days the indexes change towards the norm, but for most cases do not reach it.

It is known that a set of systemic abnormalities on the background of severe trauma are accompanied by the development of multiple organ dysfunction and insufficiency. Therefore, to increase the informativeness of our model of polytrauma, there was a task of determine its impact on the functional state of internal organs. In several publications for this purpose use indexes of a functional condition of a liver as a central body of
detoxification of an organism which activity occurs in close integration with other bodies and systems of an organism. [2].

**Objective** is to find out the features of the biliary function of the liver in the dynamics of the developed model of polytrauma.

**MATERIALS AND METHODS**

The experiments were performed using 62 nonlinear white male rats weighing 180-200 g, which were kept on a standard diet into vivarium. All manipulations with experimental animals were carried out following generally accepted bioethical standards of humane treatment of laboratory animals of international and national regulations for animal experiments: «European Convention for the Protection of Vertebrate Animals for Research and Other Scientific Purposes» (Strasbourg, 1986); «General ethical principles of animal experiments» (Ukraine, 2001), the Law of Ukraine «On protection of animals from cruel treatment» № 3447-IV (Ukraine, 2006).

Polytrauma was performed by our methodology developed, under conditions of thiopental-sodium anesthesia (40 mg 1kg-1 intraperitoneally). In surviving animals, the biliary function of the liver was studied in 2, 3, 7, 14, 21, and 28 days after injury. For this purpose, the common bile duct was catheterized, and bile was collected for 1 hour in animals under thiopental-sodium anesthesia (60 mg kg-1). The rate of bile excretion and the concentration of total bile acids, cholesterol, direct and indirect bilirubin in the selected portion of bile were determined. Based on these data, the rate of excretion of the studied components of bile was calculated. Euthanasia of rats throughout the experiment was performed by total bloodletting from the heart after previous thiopental-sodium anesthesia (60 mg kg-1 intraperitoneally).

The obtained digital data were subject to statistical analysis. The significance of differences between experimental and control groups was evaluated using the program STATISTICA 10.0 (StatSoft, Inc., USA).

**RESULTS AND DISCUSSION**

The rate of bile excretion in 1 day of the post-traumatic period was significantly reduced by 37.9% compared to the control group by the influence of polytrauma (Table) (p<0,001).

However, in 3 days this index increased sharply by 51.5% relative to control (p<0,001) and more than 2 times relative to the previous observation period (p<0,001). After 3 and 7 days, the rate decreased again and was statistically significantly lower than in the control (respectively by 11.7 and 9.8%, p<0,05) and compared with 1 day of observation (respectively by 41.8 and 40, 5%, p<0,001). After 14 days, the rate increased slightly and reached the level of control (p<0,05). However, after 21 days there was a further decrease of 20.4% relative to the control group (p<0,01). The index remained at the

<table>
<thead>
<tr>
<th>Time</th>
<th>Control</th>
<th>2 hour</th>
<th>1 day</th>
<th>3 day</th>
<th>7 day</th>
<th>14 day</th>
<th>21 day</th>
<th>28 day</th>
</tr>
</thead>
<tbody>
<tr>
<td>(n=6)</td>
<td>(n=6)</td>
<td>(n=6)</td>
<td>(n=6)</td>
<td>(n=6)</td>
<td>(n=6)</td>
<td>(n=6)</td>
<td>(n=5)</td>
<td>(n=5)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rate of bile excretion</td>
<td>2,33±0,07</td>
<td>2,38±0,04</td>
<td>2,44±0,11</td>
<td>2,10±0,14</td>
<td>2,34±0,10</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>(5,67±0,20) mg h⁻¹.kg⁻¹</td>
<td>(5,67±0,20) mg h⁻¹.kg⁻¹</td>
<td>(5,67±0,20) mg h⁻¹.kg⁻¹</td>
<td>(5,67±0,20) mg h⁻¹.kg⁻¹</td>
<td>(5,67±0,20) mg h⁻¹.kg⁻¹</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rate of bile acid secretion</td>
<td>4,52±0,16</td>
<td>5,09±0,09</td>
<td>3,80±0,34</td>
<td>4,96±0,32</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>(1,13±0,07) mg h⁻¹.kg⁻¹</td>
<td>(1,13±0,07) mg h⁻¹.kg⁻¹</td>
<td>(1,13±0,07) mg h⁻¹.kg⁻¹</td>
<td>(1,13±0,07) mg h⁻¹.kg⁻¹</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cholesterol release rate</td>
<td>0,94±0,11</td>
<td>0,92±0,05</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>(264,4±15,6) mkmol h⁻¹.kg⁻¹</td>
<td>(264,4±15,6) mkmol h⁻¹.kg⁻¹</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>The rate of excretion of total bilirubin</td>
<td>220,5±13,3</td>
<td>238,0±16,0</td>
<td>228,3±7,9</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>(171,6±13,9) mkmol h⁻¹.kg⁻¹</td>
<td>(171,6±13,9) mkmol h⁻¹.kg⁻¹</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>The rate of release of direct bilirubin</td>
<td>145,9±9,9</td>
<td>98,8±7,9</td>
<td>128,1±8,1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>(92,8±6,2) mkmol h⁻¹.kg⁻¹</td>
<td>(92,8±6,2) mkmol h⁻¹.kg⁻¹</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>The rate of secretion of indirect bilirubin</td>
<td>109,8±6,5</td>
<td>103,7±6,3</td>
<td>100,2±9,6</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Note: *p – significance of differences concerning the control group (p<0,05); **p – p<0,01; ***p – p<0,001; p – p<0,10).
same level after 28 days and was 11.4% lower than in the control (p < 0.01). It is noteworthy that, starting from day 3 fluctuations in the value of the studied index in the dynamics of the post-traumatic period was insignificant (p > 0.05). It was statistically significantly different from the same, which was observed after 2 hours and 1 day after injury (p < 0.05).

The rate of excretion of bile components is responsible for the amplitude and vector at this rate of bile excretion. Thus, the rate of excretion of total bile acids (Table) in the acute period of injury (after 2 hours) decreases by 39.5% (p < 0.001). After 1 day, this index increased significantly in the control group (by 38.1%, p < 0.01), as well as the previous observation period (2.3 times, p < 0.001). After 3 days, the index was re-decreased and was created by 33.7% less of control (p < 0.001) and by 52.0% regarding the previous observation period (p < 0.001). Then this index was increasing to 14 days, and after 7 days it is significantly greater than after 3 days (20.2%, p < 0.05); after 14 days greater than after 7 days (by 12.6%, p < 0.05). In all these terms of observation, the studied index was significantly lower than in the control (after 7 days - by 20.3%, after 14 days - by 10.2%, p < 0.05). After 21 days, there was a new decrease in the rate of excretion of total bile acids with bile by 25.3% during the previous observation period (p < 0.01), which was 33.0% lower than in the control (p < 0.001). After 28 days, this index was increased and had a slight tendency to decrease according to the control (p < 0.10). It statistically significantly was above the same value after 21 days of the post-traumatic period (30.5%, p < 0.01).

The rate of excretion of cholesterol with bile after 2 hours significantly decreased relative to the control group (Table) by 52.8% (p < 0.001). After 1 day, the index increased significantly: concerning the previous observation period 3.1 times (p < 0.05), concerning control by 46.0% (p < 0.001). After 3 days, the rate decreased and reached the level of control (p > 0.05). After 7 days, it continuously decreased and its average became 33.6% lower than in the control (p < 0.001), and relative to the previous observation period (p < 0.05). After 14-21 days, the rate increased and did not differ statistically significantly from the control group (p > 0.05). After 28 days the phase of the decline came again. The index was 18.6% lower than the control (p < 0.05). It should be noted that its level after 7-28 day did not differ significantly despite significant fluctuations in control (p > 0.05).

Similar deviations were observed in the magnitude of the rate of excretion of total bilirubin (Table). In 2 hours there was a statistically significant decrease in the value of this index relative to the control group (by 39.6%, p < 0.001). In 1 hour the index increased (by 45.5% relative to the control, p < 0.001, and 2.4 times relative to the previous observation period (p < 0.001). After 3-7 days, the index decreased again and became lower than the control by 17.9 and 16.6% (p < 0.05) After 14 days, the index increased although this was not statistically significant compared to the previous observation period. But it reached the level of control (p > 0.05). After 21 days it was less by 23.4% than the control (p < 0.05), and after 28 days - by 13.7% (p > 0.10).

The rate of release of direct bilirubin (Table) after 2 hours decreased relative to control by 45.5% (p < 0.001). After 1 day, it increased and exceeded the control level by 21.5% (p < 0.10) and the previous observation period by 2.2 times (p < 0.001). After 3 and 7 days, the index again became less than the control (respectively by 35.7 and 32.0%, p < 0.01). After 14 days, the index increased reaching the control level (p > 0.05) and then decreasing after 21 days (by 47.6% relative to control, p < 0.01) and increasing after 28 days - by 29.7% relative to the previous observation period (p < 0.01). In the last observation period, the index was statistically significantly lower than in the control (25.9%, p < 0.05).

The rate of secretion of indirect bilirubin (Table) was similarly statistically significantly lower than in the control after 2 hours of the post-traumatic period (28.8%, p < 0.01). As in the previous terms, after 1 day the index increased and exceeded the control of 89.9% (p < 0.001), and the previous observation period in 2.7 times (p < 0.001). Then the rate decreased and, starting from 3 days did not differ from the control level (p < 0.05). After 3-28 days, the index also did not differ between the experimental groups (p > 0.05).

The obtained results indicate that the indexes of biliary function are characterized by a significant decrease after 2 hours of the post-traumatic period relative to the control group and a significant increase - after 1 day. It can be interpreted as a syndrome of "polycholia". The decrease of bile secretion corresponds to the period of shock, which is characterized by the centralization of blood circulation and reduced blood supply to the organs of the gastrointestinal tract [4]. Increased bile secretion and excretion of the main components of bile in 1 day after severe injury was observed in studies by other authors [2]. They have associated it with increased permeability of the biliary pole of hepatocytes and unloading of the liver from endotoxins accumulated due to tissue damage and microcirculation. Subsequently, the indexes of the biliary function of the liver changed according to the identified patterns of deviations of lipoperoxidation, antioxidant protection, cytolysis, and endogenous intoxication [6]. We can assume that in the pathogenesis of biliary disorders is the damage of the endoplasmic reticulum membranes, where the synthesis of the main components of bile [8], as well as the development of edema of the organ that prevents the outflow of bile [1].

Thus, in the conditions of the modeled polytrauma, there is a development of functional insufficiency of a liver. The formation of functional insufficiency of a liver obeys the general law which characteristic of deviation of other biochemical indicators (markers of a traumatic illness) from the third day. We can assume that in the critical state of the body the organs and systems coupling
is getting worse, which are remote from the site of injury. It can be considered as a factor of compensation and adaptation directed to the survival of the organism.

CONCLUSION

In the conditions of experimental polytrauma, there is a violation of the biliary function of the liver. It is manifested in the period of an acute reaction to trauma by the development of polycholia. Further decline of bile secretion and excretion of main bile components is shown up to 7 days. Their temporary improvement after 14 days with repeated reduction after 21 days, and approaching the norm in 28 days.

REFERENCES


6. Patent 63997 Ukraine, IPC G 09 B 23/28. [Method of polytrauma modeling]. Kozak D.V.; applicant and patent holder Gorbachevsky Ternopil State Medical University. No. u 201104110; statements 05.04.11; published 10/25/11, Bul. 20. [in Ukrainian].


ОСОБЕННОСТИ ЖЕЛЧЕВЫДАЮЩЕЙ ФУНКЦИИ ПЕЧЕНИ В ДИНАМИКЕ ПОЛИТРАВМЫ В ЭКСПЕРИМЕНТЕ

Козак Д.В. (https://orcid.org/0000-0002-5142-2057)
ГБУЗ “Тернопольский государственный медицинский университет имени И.Я. Горбачевского МОЗ Украины”, Тернополь, Украина
kozak@dmu.edu.ua

Актуальность. Тяжелая травма сопровождается развитием полиорганной дисфункции и недостаточности внутренних органов. Сегодня не до конца изучена динамика изменения функционального состояния печени – центрального органа детоксикации организма, деятельность которого происходит в тесной интеграции с другими органами и системами организма.

Цель: выявить особенности желчевыделяющей функции печени в динамике разработанной модели полиотравмы.

Материалы и методы. На 62 испытуемых белых крысах-самцах, массой 180-200 г, выполняли полиотравмы в условиях тяжелог-нативного наркоза (40 мкг/кг внутрибрюшинно). У выживших животных исследовали желчевыделяющую функцию печени через 2 часа, 1, 3, 7, 14, 21 и 28 суток после травмы. С этой целью под тяжелог-нативным наркозом (60 мг/кг) у животных катетеризировали общий желчный проток и собирали желчь в течение 1 ч. Определяли скорость желчеотделения и концентрацию в выделенной порции желчи суммарных желочных кислот, холестерина, общего, прямого и непрямого билирубина. На основе этих данных рассчитывали скорость экскреции изучаемых компонентов желчи. Эксперимент проводили путем тотального кровообращения из сердца после предъявления тяжелог-нативного наркоза (60 мг/кг внутрибрюшинно). Полученные цифровые данные подлежали статистическому анализу.

Результаты. В условиях экспериментальной полиотравмы возникает нарушение показателей желчевыделяющей функции печени, которое проявляется в период островой реакции на травму сначала (через 2 часа) существенным их снижением, затем (до 1 суток) развитием полилохии – в 1,52 раза увеличивается скорость желчеотделения, усиливается экскреция основных компонентов желчи, с последующим их снижением до 7 суток, развитием периода временного улучшения через 14 дней с повторным периодом обострения через 21 день и приближением к норме – через 28 суток.

Снижение желчеотделения соответствует периоду после, для которого характерна централизация кровообращения и снижение кровоснабжения органов желудочно-кишечного тракта. Повышение желчеотделения и экскреция основных компонентов желчи через 1 сутки после тяжелой травмы связано с увеличением проницаемости билиарного полоса гепатоцитов и с разгрузкой печени от эндотоксинов, которые накапливаются в результате повреждения тканей, нарушения микросфера и развития гипоксии. В дальнейшем показатели желчевыделяющей функции печени менялись, в соответствии с выявленной закономерностью отклонений показателей липопероксидации, антитоксикантной защиты, цитолиза и эндотоксинной интоксикации. В патогенезе нарушения желчеотделения лежит повреждение мембран эндотоксикогенного ретикулума, где происходит синтез основных компонентов желчи, а также развитие отека органа, препятствующего оттoku желчи.

Вывод. Динамика развития функциональной недостаточности печени, вследствие полиотравмы, совпадает с общей закономерностью, характерной для динамики других биохимических маркеров травматической болезни. А именно: через 3 суток после травматического периода наступает фаза максимального угнетения отклонений исследуемых показателей. Через 7-14 суток отмечается фаза временного благополучия, которая характеризуется изменением показателей в сторону нормы. Через 21 суток возникает повторное обострение патологического процесса. Через 28 суток показатели меняются в сторону нормы, однако в большинстве своем ее не достигают.

Это свидетельствует, что в условиях критического состояния организма углубляется спреженночность между органами и системами, удаленные от места повреждения, которую можно расценить как фактор компенсации и адаптации, направленный на выживание организма.

Ключевые слова: крысы, полиотравма, печень, желчеотделение.