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DYNAMICS OF ANTIOXIDENT PTOTECTION INDICES IN ANSWER TO SKELETAL, CRANIAL-CEREBRAL TRAUMA AND COMBINED TRAUMAS IN THE PERIOD OF EARLY SIGNS OF TRAUMATIC DISEASE

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Abstract

In answer to skeletal, cranial-cerebral and combined traumas the significant abnormalities of liver tissue antioxidant protection is observed. There comes a significant reduction in the activity of superoxide dismutase, catalase, content of SH-groups compared to the control group. Against the background of cranial-cerebral trauma and combined trauma, the activity of superoxide dismutase and catalase gradually decreases from the 1st to the 7th days, and against the background of skeletal trauma itself it reaches the maximum decrease after 3 days and increase after 7 days without reaching the control level. Maximum abnormalities occur against the background of combined trauma after 7 days of post-traumatic period. The maximum infringement of SH-groups content in all the experimental groups occur up to the 3rd day and are larger in the group with combined trauma. Henceforth the index against the background of all the traumas increases up to the 7th day without reaching control dimensions. In the conditions of modeled traumas the abnormalities of ceruloplasmin content is observed. In all the experimental groups it grows from the 1st to the 7th day and is statistically reliable bigger than in the control group.

Key words: skeletal traumas, cranial-cerebral trauma, cranial-skeletal trauma, antioxidant protection, early period of the traumatic disease.

Introduction. In the pathogenesis of acute trauma the key position is given to the antioxidant protection system. As evidenced by many authors' data, traumatic disease development is accompanied by a set of mechanisms that contribute to strengthening of the formation of active oxygen forms and lipid peroxidation initiation. First of all this accumulation of catecholamines and their metabolites, stimulation in these circumstances hypermetabolism against the background of microcirculation disorder, further development of the traumatic shock and systemic hypoxia [4, 11]. Under the influence of inflammatory mediators, the syndrome of systemic body response to inflammation develops, which is accompanied by the activation of cells-phagocytes (neutrophils, monocytes and macrophages) that are capable of generating active oxygen forms. This is the most clearly seen in their bactericidal function, which is realized under the influence of active oxygen forms inside by the phagolysis, and cytotoxic effect in action on the objects located outside of phagocyte by release of active oxygen forms ($O^{\bullet}$) out of the cell [15]. In the experiment the dynamics of antioxidant protection indices is described by several authors in the early and late post-traumatic period [3, 5, 6]. The authors state the phase course of the pathological process with the extreme fluctuation of antioxidant protection. However, its dynamics peculiarities under the influence of mechanical trauma of various localization are studied insufficiently.

Research aim: to clear up the dynamics of the antioxidant protection indices in answer to skeletal, cranial-cerebral and combined traumas in the period of early indices of traumatic disease.

Materials and methods. The experiments were carried out on 90 nonlinear white male rats with the weight 180-200 gr, which were on the standard vivarium ration. The animals were divided into 4 groups: one control (6 intact rats) and three experimental. In the first experimental group we modeled a skeletal trauma by means of striking a dosed blow with obtaining a closed fracture of the both femurs [12], in the second one – we modeled a closed CCT of a medium type of complexity with the methods described in the research [3], in the third one – these traumas were combined. All the experiments were conducted in the conditions of Sodium-thiopental anesthesia (40 $mg \cdot kg^{-1}$ of the weight).

After 1, 3 and 7 days after striking the trauma in the conditions of Sodium-thiopental anesthesia, the animals were taken out the experiment by the method of a total phlebotomy of the heart. In the liver homogenate, the activity of superoxide dismutase (SOD), [13] and catalase [10] were measured and the contents of SH-groups [2] as well. The contents of ceruloplasmin (CP) [8] were measured in the blood serum.

Statistical analysis of the obtained results was conducted in the system statistical researches department of State High Education Establishment " I.Ya.Horbachevskyy Ternopil State Medical University, the Ministry of Healthcare of Ukraine" utilizing Mann — Whitney's criteria in the program packet STATISTICA 10.0 ("StatSoft, Inc.", USA).

Researches results and their discussion. As it can be seen from table 1, the activity of SOD in the liver tissue under the influence of skeletal trauma was decreasing. After 1 day the index was becoming lower than the control level by 26,6 % ($p<0,05$), after 3 days – by 28,2 % ($p<0,05$). After 7 days the index was on the control group level ($p>0,05$). After the CCT the index during all the terms of the observation was lower than the control group: accordingly by 19,0, 26,2 i 30,6 % ($p<0,05$). The analogous situation was registered after the combined trauma as well: accordingly by 19,8, 33,1 i 38,7 % ($p<0,05$).

Table 1 – SOD activity in the liver tissue (μkat ·kg^{-1}) after skeletal and cranial-cerebral traumas and their combination (M±m)

Trauma type	Control	Post-traumatic trauma duration		
		1 day	3 days	7 days
Skeletal	0,248± 0,013 (n=6)	0,182± 0,007* (n=8)	0,178± 0,005* (n=10)	0,209± 0,008 (n=10)
Cranial-cerebral		0,201± 0,008* (n=8)	0,183± 0,006* (n=10)	0,172± 0,005* (n=9)
Combined		0,199± 0,011* (n=8)	0,166± 0,008* (n=9)	0,152± 0,006* (n=8)
p_{1-2}		>0,05	>0,05	<0,05
p_{1-3}		>0,05	>0,05	<0,05
p_{2-3}		>0,05	>0,05	<0,05

Notes. Here and in the other tables:

1. * – differences in conformity with the control group are statistically reliable ($p<0,05$);

2. p_{1-2} – the reliability of the index differences among the animals' groups with skeletal and cranial-cerebral traumas; p_{1-3} – between skeletal and the combined traumas; p_{2-3} – between cranial-cerebral and combined traumas.

Comparing the experimental groups among themselves, it was cleared up that after 1 and 3 days statistically significant differences among the groups with different by the localization traumas ($p_{1-2}>0,05$, $p_{1-3}>0,05$, $p_{2-3}>0,05$) weren't noticed. However after 7 days the index in the group with the combined trauma turned out to be essentially slight than in the groups with the skeletal and cranial-cerebral traumas themselves (accordingly by 27,3 i 11,6 %, $p_{1-3}<0,05$, $p_{2-3}<0,05$). In its turn the index of the animal group with CCT was substantially lower, than in the

group of animals with the skeletal trauma itself (by 17,7 %, $p_{1-2}<0,05$).

Analyzing the dynamics of the investigated index, it was found out (image 1) that after the skeletal trauma itself it reached the minimum level after 1-3 days and henceforth up to the 7th day it was increasing significantly, which was found to be statistically trustworthy in comparison with the previous terms of the investigation ($p<0,05$). After CCT the index was gradually lowering from the 1st up to the 7th day. In these conditions after 7 days it was substantially lower, than after the 1st day ($p<0,05$). After the combined trauma the index got its minimum level after 3 days which turned out to be essentially lower, than after the 1st day ($p<0,05$). Henceforth it remained at the same level, substantially exceeding the 1st day ($p<0,05$) and practically wasn't different from the level of the 3rd ($p>0,05$).

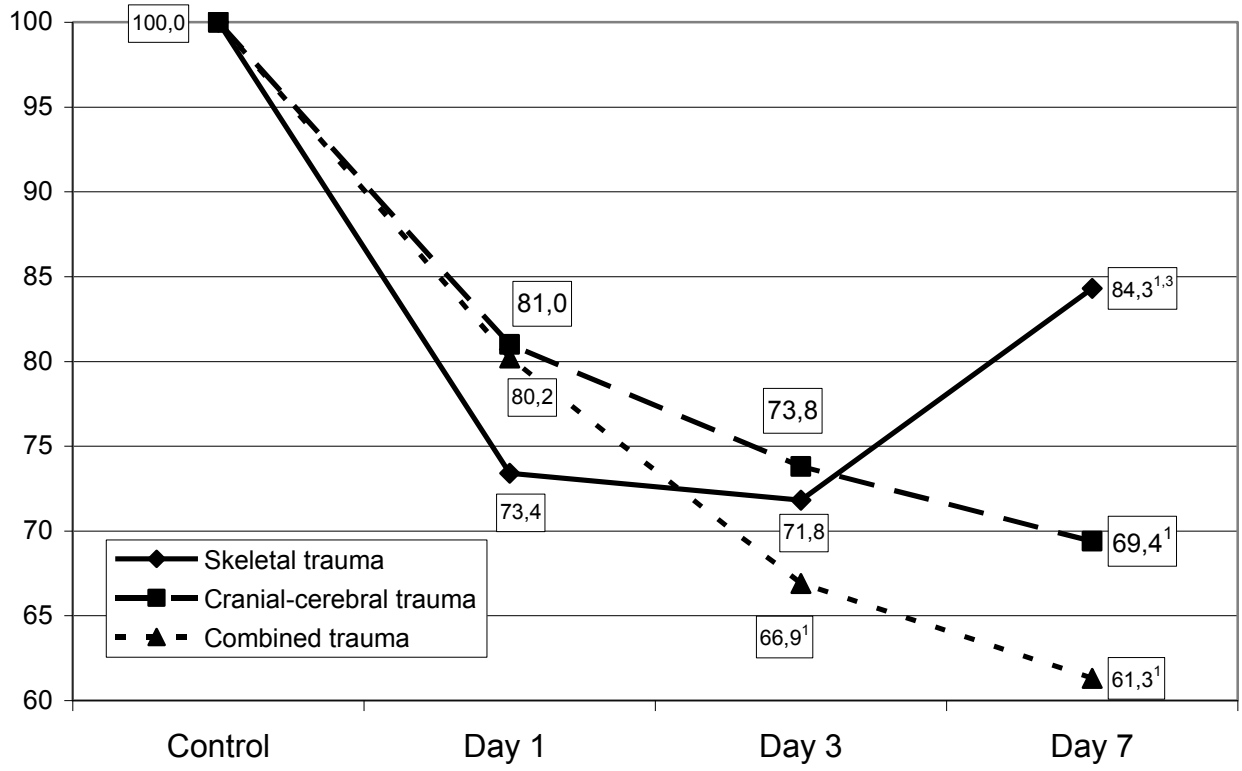

Image 1 – Dynamics of the SOD activity in the liver tissue (in the percentage to the control group level) after skeletal and cranial-cerebral traumas and their combination. Here and in the other images of the paragraph 3: [1,3] – the differences in conformity with the 1st and the 3rd days of the post-traumatic period are statistically reliable($p<0,05$)

As it can be seen from table 2, the catalase activity in the liver tissue under the influence of the modeled traumas was lowering substantially compared to the control group. After the skeletal trauma after 1 day the index was becoming lower by 27,2%, after 3 days – by на 41,5 %, after 7 days – by 32,5 % ($p<0,05$); after CCT – accordingly by 30,6, 36,7 and 46,9 % ($p<0,05$); after the combined trauma accordingly by 33,4, 42,0 and 51,3 % ($p<0,05$).

Comparing the experimental groups among themselves, it was found out that after the 1st and the 3rd days the index among the experimental groups practically wasn't different ($p>0,05$).

After 7 days against the background of the skeletal trauma it appeared to be statistically reliable bigger than after the CCT and the combined trauma (accordingly by 27,1 and 38,6%, $p<0,05$). The fact that attracts the attention is that after 7 days of the post-traumatic period the catalase activity in the tissue after the CCT and the combined trauma was the lowest and practically equal ($p<0,05$).

Table 2 – The catalase activity in the liver tissue (μkat ·kg^{-1}) after the skeletal and cranial-cerebral traumas and their combination (M±m)

Trauma type	Control	Post-traumatic period duration		
		1 day	3 days	7 days
Skeletal	0,431± 0,034 (n=6)	0,314± 0,011* (n=8)	0,252± 0,014* (n=10)	0,291± 0,010* (n=10)
Cranial-cerebral		0,299± 0,008* (n=8)	0,273± 0,013* (n=10)	0,229± 0,009* (n=9)
Combined		0,287± 0,010* (n=8)	0,250± 0,015* (n=9)	0,210± 0,008* (n=8)
p_{1-2}		>0,05	>0,05	<0,05
p_{1-3}		>0,05	>0,05	<0,05
p_{2-3}		>0,05	>0,05	>0,05

Analysis of the investigated index dynamics showed (image 2) that in the group of animals with the skeletal trauma the index was reaching the minimum level after 3 days of the trauma striking, that turned out to be statistically reliable less than after 1 day ($p<0,05$). After 7 days it was increasing and becoming substantially bigger than after 3 days ($p<0,05$). In the conditions of the CCT and combined traumas the catalose activity dynamics in the liver tissue was similar: the index was gradually lowering from the 1st to the 7th day and was becoming substantially lower than after the 1st and the 3rd days ($p<0,05$).

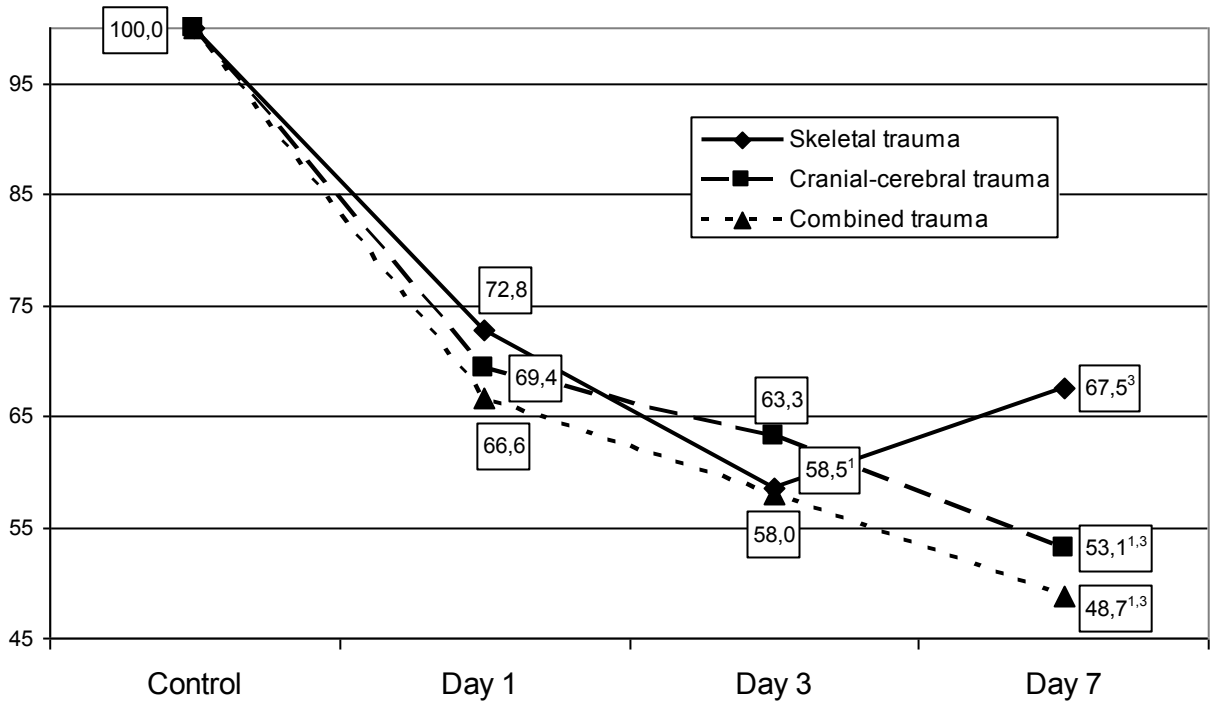

Image 2 – The catalose activity dynamics in the liver tissue (in the percentage to the control group level) after the skeletal and cranial cerebral traumas and their combination.

As it can be seen from table 3, under the influence of the skeletal trauma the contents in the liver tissue of SH-group in comparison to the control group was lowering: after 1 day by 17,9%, after 3 days – by 43,1 %, after 7 days by 15,9 %. The result was statistically reliable ($p<0,05$). The analogous situation was registered after the CCT and the combined trauma accordingly by 22,8, 34,4 and 22,8 % ($p<0,05$) and by 26,2, 40,7 and 29,7 % ($p<0,05$).

Comparing the experimental groups among themselves, it was found out, that after 1 and 7 days the statistically significant differences among the groups with the different trauma types weren't noticed ($p_{1-2}>0,05$; $p_{1-3}>0,05$; $p_{2-3}>0,05$). However after 3 days in the group of animals with the combined trauma the index turned out to be statistically reliable lower, than in the group of animals with the CCT (by 12,2 %, $p_{2-3}<0,05$). Its dimensions among the animals with the skeletal trauma itself wasn't substantially different (p1-2>0,05; p1-3>0,05).

Table 3 – SH-groups contents in the liver tissue (μkat ·kg^{-1}) after the skeletal and cranial-cerebral traumas and their combination (M±m)

Trauma type	Control	Post-traumatic period duration		
		1 day	3 days	7 days
Skeletal	1,45±0,07 (n=6)	1,19±0,05* (n=8)	0,97±0,04* (n=10)	1,22±0,08* (n=10)
Cranial-cerebral		1,12±0,03* (n=8)	0,98±0,04* (n=10)	1,12±0,06* (n=9)
Combined		1,07±0,03* (n=8)	0,86±0,04* (n=9)	1,02±0,07* (n=8)
p_{1-2}		>0,05	>0,05	>0,05
p_{1-3}		>0,05	>0,05	>0,05
p_{2-3}		>0,05	<0,05	>0,05

Analyzing the SH-group contents dynamics in the liver tissue (image 3), it was found out, that in all the experimental groups the index was decreasing substantially up to the 3rd day and was becoming statistically reliable lower, than after 1 day (p<0,05). After 7 days it was increasing in all the groups. After the skeletal trauma it was essentially exceeding its previous observation term (p<0,05), and after the CCT and the combined trauma it stayed at the same level.

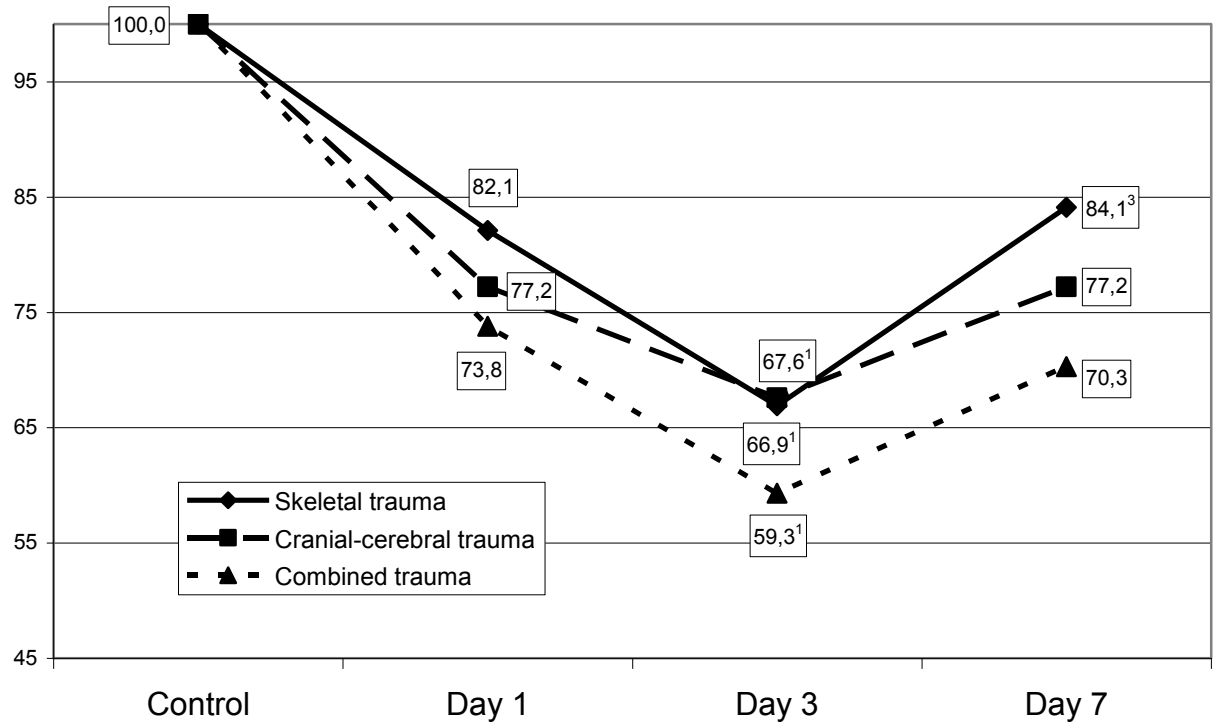

Image 3 – SH-groups contents dynamics in the liver tissue (in the percentage to the control group level) after the skeletal and cranial cerebral traumas and their combination.

As it can be seen from table 4, the CP contents in the blood serum after all types of traumas in the early period of TD were increasing substantially in comparison to the control

group. Thus, after the skeletal trauma itself after 1 day it was bigger by 33,4 %, after 3 days – by 69,3 %, after 7 days – by 121,1 % ($p<0,05$), after CCT – correspondingly by 42,9, 90,6 and 218,3 %, after the combined trauma – accordingly by 58,1, 64,7 and 206,3 % ($p<0,05$).

Comparing the experimental groups among themselves, it was found out the after 1 day in the group of animals with the combined trauma the index turned out to be statistically reliable bigger, than in the groups of animals with the skeletal and the CCT traumas themselves (accordingly by 18,6 and 10,6 %, $p1\text{-}3<0,05$, $p2\text{-}3<0,05$). In the two last groups it was practically identical ($p1\text{-}2>0,05$). After 3 days against the background of the skeletal and combined traumas themselves the index was becoming lower than after the CCT, moreover in comparison with the skeletal trauma it appeared statistically reliable ($p_{1-2}<0,05$). After 7 days the statistically significant differences weren't noticed among the experimental groups ($p_{1-2}>0,05$; $p_{1-3}>0,05$; $p_{2-3}>0,05$).

Table 4 – CP contents in the blood serum ($gr \cdot l^{-1}$) after the skeletal and cranial cerebral traumas and their combination (M±m)

Trauma type	Control	Post-traumatic period duration		
		1 day	3 days	7 days
Skeletal	7,43±0,25 (n=6)	9,91±0,32* (n=8)	12,58±0,39* (n=10)	16,43±0,42* (n=10)
Cranial-cerebral		10,62±0,38* (n=8)	14,16±0,37* (n=10)	16,22±0,60* (n=9)
Combined		11,75±0,23* (n=8)	12,24±0,51* (n=9)	15,33±0,44* (n=8)
p_{1-2}		>0,05	<0,05	>0,05
p_{1-3}		<0,05	>0,05	>0,05
p_{2-3}		<0,05	>0,05	>0,05

Analyzing the dynamics of the investigated index in the early period after the trauma (image 4), it turned out, that the index was increasing from the 1st up to the 7th day of the post-traumatic period in all the experimental groups. After 3 days in the groups of animals with skeletal and cranial-cerebral traumas the index was increasing and becoming substantially bigger, than after the 1st day ($p<0,05$). In the group of animals with the combined trauma after 3 days it turned out to be analogous to the 1st day ($p>0,05$). After 7 days the index was continuing to increase and was substantially bigger in all the groups than after the 1st and the 3rd days ($p<0,05$).

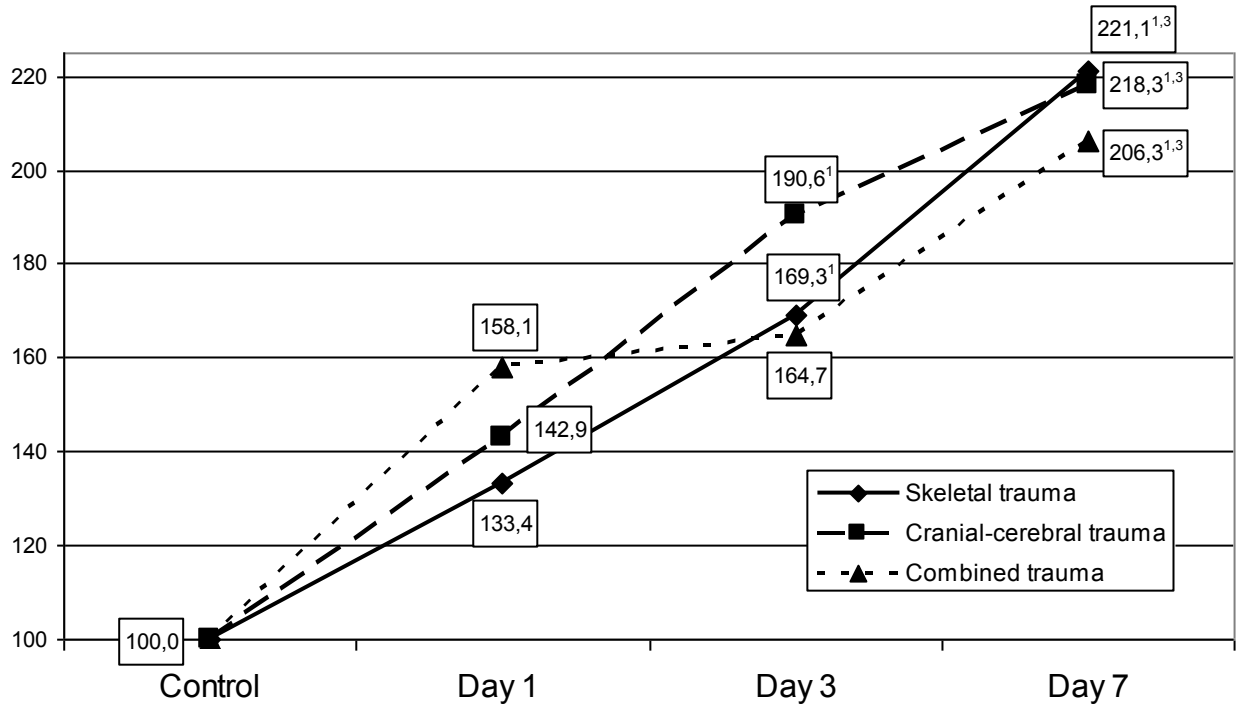

Image 4 – The dynamics of CP contents in the blood serum (in the percentage to the control group level) after the skeletal and cranial-cerebral traumas and their combination.

Hereby, as an answer to the skeletal, cranial-cerebral and combined traumas, the essential abnormality of the antioxidant protection of the liver tissue is noticed. Here comes the essential lowering of the SOD activity, catalase and SH-groups contents in comparison with the control group which affirms about the exhaustion the fermentative and non-fermentative links of the antioxidant protection in the liver tissue in the conditions of the modeled traumas. Therefore, in the pathogenesis of the acute skeletal trauma, the leading mechanism of the active oxygen forms, which bears the system character and is obviously the first link in the multiple organ dysfunction. The similar abnormalities were noticed in the researches of the other authors as well [1, 11].

Depending on the trauma localization there were some differences in the antioxidant protection dysfunction. Thus, against the backgrounds of the CCT and the combined trauma SOD and catalose activity was gradually lowering from the 1st up to the 7th days, and against the background of the skeletal trauma itself it was reaching the maximum lowering after 3 days, but after 7 days it was increasing, not reaching the control level. The maximum abnormalities appeared against the background of the combined trauma after 7 days of the post-traumatic period. These data show that both the soft tissues and long tubular bones lesions along with the crane and brain tissue lesions become the source of the active oxygen forms, which getting into the circulatory flow take the part of the pathogenesis factor of the other organs and systems

dysfunction which are remote from the immediate trauma place. The phenomenon of the mutual encumbrance in the conditions of the combined skeletal and cranial-cerebral traumas is clearly traced.

The maximum abnormalities of SH-groups contents which indicate indirectly on the renewed glutathione level, in all the experimental groups began up to the 3rd day and again were bigger in the group with the combined trauma. Henceforth the index against the background of all the trauma types was increasing, not reaching the control dimensions. Thus, in correspondence to SH-groups contents the exhaustion occurs faster – before the 3rd day. Probably, it is connected with the specific function of the renewed glutathione – to take part in the renewing of lipids peroxides and hydrogen peroxide under the influence of the glutathoineperoxidase, and to form the twin hydrophilic combinations with the xenobiotics, the products of their metabolism and endotoxins under the influence of the glutathointransferase [7, 14], which assists its faster exhaustion. Up to the 7th day, the strengthening factors of its renewal are obviously stimulated, because SH-groups contents increases. The similar compensatory changes in the conditions of the polytrauma were registered in the researches of the other authors [6].

In the conditions of the modeled traumas the abnormality of the contents of the main blood serum antioxidant – CP is noticed as well. In all the experimental groups it was growing from the 1st up to the 7th days and was statistically reliable more than in the control group. This result indicated the development of the organism system reply to the inflammation, as CP belongs to the acute phase proteins and takes part in the active oxygen forms neutralization, preventing the peroxide oxidation of the cells membranes lipids, and performs anti-inflammatory action as well, crushing the blood serum histaminase [9].

Thus, the mechanical trauma, in spite of the localization, is accompanied by the antioxidant protection abnormalities of the same type with the exhaustion of its main components, which should be considered when developing the strategy of the first aid dressing to the person affected by an acute trauma.

Conclusions. 1. In answer to the skeletal, cranial-cerebral and combined traumas a substantial dysfunction of the antioxidant protection of the liver tissue is observed. Here comes the essential lowering of the SOD activity, catalase and SH-groups contents in comparison with the control group. Against the background of the CCT and combined trauma, SOD and catalose activity is gradually decreasing from the 1st up to the 7th days (accordingly by 30,6 and 48,7 %, $p<0,05$ and by 46,9 and 51,3 %, $p<0,05$), and against the background of the skeletal trauma itself it reaches the maximum lowering after 3 days (accordingly by 28,2 and 47,5 %, $p<0,05$) and increases after 7 days not reaching the control level. Maximum abnormalities arise against the

background of the combined trauma after 7 days of the post-traumatic period.

2. Maximum abnormalities of SH-groups contents in all the experimental groups occur up to the 3rd day and are bigger in the group with the combined trauma. Henceforth the index against the background of all the trauma types increases up to the 7th day, not reaching the control dimensions.

3. In the conditions of the modeled traumas the dysfunction of the main antioxidant of the blood serum –CP, is registered. In all the experimental groups it increases from the 1st up to the 7th days and is statistically bigger than in the control group (bigger than twice, $p<0,05$).

Perspectives of the further researches. In prospect it is foreseen to perform the screening of the medicine with the antioxidant characteristics in order to choose the optimal one in the conditions of the early period of the traumatic disease.

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