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## THE EFFECT OF PRP-THERAPY ON THE DEVELOPMENT OF IMPAIRED BILE EXCRETION UNDER THE INFLUENCE OF MECHANICAL DAMAGE TO THE SKIN, SKELETAL TRAUMA COMPLICATED BY ACUTE BLOOD LOSS, AND COMBINED TRAUMA

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### Abstract

**Introduction.** Traumatism is considered to be one of the most topical problems. Its structure is predominated by multiple and combined traumas, which lead to organ and tissue failure remote from the site of the initial trauma. Mechanical damage to the skin is frequently experienced by victims as the result of increase in the number of traffic accidents and combat injuries. However, the hepatic function in the presence of mechanical skin damage is insufficiently studied. There is a paucity of scientific evidence attesting to the efficacy of platelet-rich plasma grafts (PRP - therapy), which play an important role in promoting the healing processes of damaged body tissues.

**Objective:** to establish the effect of PRP-therapy on the development of impaired bile excretion under the influence of mechanical damage to the skin, skeletal trauma complicated by acute blood loss and combined trauma.

**Materials and methods.** The experimental studies were conducted on 126 nonlinear white male rats weighing 180-200 g, which were divided into five groups: the control and four experimental ones. The control group consisted solely of the intact animals exposed to general anesthesia. In the first experimental group, the animals were simulated a mechanical damage to the skin, the rats of the second experimental group were subjected to a skeletal trauma complicated by acute blood loss, the third experimental group had both these lesions combined. In the fourth experimental group, the PRP-therapy was applied using allogeneic platelet-rich plasma injections to the animals with combined trauma. After 3, 7, 14, 21 and 28 days of the posttraumatic period, the common bile duct was catheterized in the control and experimental groups, the bile was collected and the rate of bile excretion was determined.

**The results and discussion.** The conducted studies have evidenced that mechanical damage to the skin, skeletal trauma complicated by acute blood loss, and the combined trauma model were accompanied by a significant decrease in bile excretion rate, indicating the development of secondary liver failure and the deduced functional capacity of the liver. Consequently, the isolated damage to skin has systemic effects on the body and can aggravate impairments caused by skeletal trauma complicated by acute blood loss. The administration of the PRP-therapy injections to rats with combined trauma model resulted in less severe impairments of bile excretion compared to the animals without corrective medication, which was particularly noticeable in late manifestations of traumatic disease after 21 and 28 days. Hence, the PRP-therapy appears to be a promising medication in the presence of combined trauma in terms of decreasing the risk associated with the development of multiple organ failure, which substantiates the pertinence of further depth-in study.

**Conclusions.** Mechanical damage to the skin results in an impaired hepatic function in rats, which is manifested by a diminished bile flow. An additional infliction of mechanical damage to the skin in the presence of skeletal trauma complicated by acute blood loss exacerbates the impairment of bile excretion with a maximum of abnormalities occurred after 7 and 21 days of the posttraumatic period. The administration of the PRP-therapy injections in the background of combined trauma model is accompanied by less abnormal rate of bile excretion, which is statistically significant after 21 and 28 days of the experimental period, as compared to the animals without corrective medication.

**Key words: mechanical damage to the skin; skeletal trauma; blood loss; liver; bile excretion; PRP-therapy.**

**Introduction.** Traumatism is considered to be one of the most topical problems. Its structure is predominated by multiple and combined traumas, which are characterized by a considerable severity and high mortality rates. The leading cause of death among victims is the development of organ and tissue failure remote from the site of the initial trauma [1].

Mechanical damage to the skin is frequently experienced by victims as the result of increase in the number of traffic accidents and combat injuries [2]. However, its role in the development of systemic disorders associated with the presence of isolated and combined traumas is insufficiently studied.

One of the sensitive indicators of secondary liver failure in traumatic disease conditions is the assessment of the rate of bile excretion. [3, 4, 5]. However, the intensity of bile excretion under the influence of mechanical damage to the skin is insufficiently studied. There is a paucity of scientific evidence attesting to the efficacy of platelet-rich plasma grafts (PRP - therapy). Platelets are known to play a critical role in promoting the healing of damaged tissues [6]. Due to its great regenerative and reparative potentials, platelets can be used to restore the damaged tissues [7]. During platelet adhesion and degranulation,  $\alpha$ -granules release stored growth factors including platelet-derived growth factor (PDGF), fibroblast growth factor (FGF), transforming growth factor- $\beta$ 1 (TGF- $\beta$ 1), insulin-like growth factor (IGF-1), vascular endothelial growth factor (VEGF) and VGF nerve growth factor inducible VGF, which stimulate histogenesis, chemotaxis and cell differentiation [8]. As a result, a working hypothesis relevant for the possibility of treatment of systemic disorders, caused by skin damage, with the PRP therapy has emerged.

**Objective:** to establish the effect of PRP-therapy on the development of impaired bile excretion under the influence of mechanical damage to the skin, skeletal trauma complicated by acute blood loss and combined trauma.

**Materials and methods.** The experiments were conducted on 126 nonlinear white male rats weighing 180-200 g. All experimental studies on trauma were carried out under thiopental sodium anesthesia (40mg·kg of body weight). The animals were divided into five groups: control and four experimental ones. The control group consisted solely of the intact animals exposed to general anesthesia. In the first experimental group, the animals were subjected to a mechanical damage of skin: a skin flap with subcutaneous tissue of 2\*2 cm was cut on depilated back of the rat. In the second experimental group, skeletal trauma complicated with acute blood loss was simulated. Closed femoral fracture model was inflicted through a directed blow energy of 0,637 J hits the thighbone. Acute blood loss of 20% of circulating blood volume was inflicted by severing a femoral vein of the other thigh. The third

experimental group had both these lesions combined. In the fourth experimental group, the PRP-therapy was administered using allogeneic platelet-rich plasma injections to the animals with combined trauma. The PRP was prepared based on the method of Messori et al. (2011) [10]. The derived allogeneic platelet-rich plasma was subcutaneously injected at a standard depth in 0.1ml doses into the wound corners not more than 5 mm distance from the wound edge. The rats of the third experimental group were subcutaneously administered physiological saline in the same way. The wound was covered with a sterile dressing, and after 3 days was treated in an open manner.

After 3, 7, 14, 21 and 28 days of the posttraumatic period the rates of bile excretion were measured in experimental animals. The common bile duct was catheterized and the bile samples were collected within an hour from rats of control and experimental groups under thiopental sodium anesthesia ( $60 \text{ mg}\cdot\text{kg}^{-1}$ ). The rate of bile excretion was calculated in milliliters per hour per kilogram of body weight ( $\text{ml}/\text{h}/\text{kg}$  of body weight).

All experimental procedures fulfilled the international standards for the humane treatment of animals in compliance with the regulations of «The European

Convention for the protection of vertebrate animals used for experimental and other scientific purposes (European Convention, 1986)», General Ethical Principles of Animal Experimentation, approved by the First National Congress on Bioethics (Kyiv, 2001), The Law of Ukraine On the Protection of Animals from Cruelty (2006, № 3447-IV) and «Scientific and Practical Recommendations for the Care and Use of Laboratory Animals».

The differences between experimental groups were measured using the nonparametric Mann–Whitney U-test.

**The results and discussion.** The conducted studies have evidenced that mechanical damage to the skin (tab.1. fig.1) caused a statistically significant decrease in bile excretion rate starting from the 3<sup>rd</sup> day of the experiment. The parameter was 13,5% lower than the corresponding of the control group at this experimental point ( $p<0,05$ ). The parameter reached its minimum value up to the 7<sup>th</sup> day, and appeared to be decreased by 35.1% as compared to the control and by 17.2% than the value of studied parameter on the 3<sup>rd</sup> observation day ( $p<0,05$ ). Subsequently, the dynamics of the indicator fluctuated showing the increase after 14 days (by 19.6% as compared to the value on the 7<sup>th</sup> observation day,  $p<0,05$ ), the repeated decrease (by 11.8% as compared to the value reported on the 14<sup>th</sup> day,  $p<0,05$ ), and the repeated increase after 28 days (by 16, 9 %, as compared to the value reported on the 21<sup>st</sup> day). The parameter was significantly lower than the respective control after 14 and 21 days

(by 14.5% and 24.3%, respectively,  $p < 0.05$ ), and reached the control value at 28 day ( $p > 0.05$ ).

Table 1 – The rate of bile excretion ( $\text{ml} \cdot \text{h}^{-1} \cdot \text{kg}^{-1}$ ) after infliction of mechanical damage to the skin, skeletal trauma complicated by acute blood loss and combined trauma ((Me (LQ;UQ)) – median (lower and upper quartiles)

Experimental group	Observation period				
	3 days	7 days	14 days	21 days	28 days
Control group	2,29 (2,16; 2,45)				
Experimental group 1 Mechanical damage to the skin	1,97* (1,97; 2,00) (n=6)	1,63* (1,53; 1,76) (n=6)	1,95* (1,91; 2,03) (n=6)	1,72* (1,69; 1,75) (n=6)	2,01 (2,10; 2,18) (n=6)
Experimental group 2 Skeletal trauma+acute blood loss	1,95* (1,89; 2,06) (n=9)	1,71* (1,61; 1,79) (n=8)	1,92* (1,74; 2,09) (n=8)	1,69* (1,62; 1,77) (n=7)	1,90* (1,90; 1,95) (n=7)
Experimental group 3 Combined trauma	1,54* (1,64; 1,80) (n=8)	1,44* (1,39; 1,57) (n=7)	1,61* (1,50; 1,72) (n=6)	1,53* (1,46; 1,66) (n=6)	1,77* (1,66; 1,88) (n=6)
p <sub>1-2</sub>	>0,05	>0,05	>0,05	>0,05	<0,05
p <sub>1-3</sub>	<0,05	>0,05	<0,05	<0,05	<0,05
p <sub>2-3</sub>	<0,05	<0,05	<0,05	>0,05	<0,05
Note.:					
1. * – the differences concerning the control group are statistically significant ( $p < 0,05$ );					
2. p <sub>1-2</sub> – the probability of differences in the value of parameter between the 1 <sup>st</sup> and 2 <sup>nd</sup> experimental groups;					
3. p <sub>1-3</sub> – the probability of differences in the value of parameter between the 1 <sup>st</sup> and 3 <sup>rd</sup> experimental groups;					
4. p <sub>2-3</sub> – the probability of differences in the value of parameter 2 <sup>nd</sup> and 3 <sup>rd</sup> experimental groups.					

The bile excretion rate under the influence of skeletal trauma complicated by acute blood loss followed the same dynamics. The parameter reached its minimum value up to the 7<sup>th</sup> day, and appeared to be decreased by 28.4% as compared to the control and by 12.3% than the corresponding value observed on the 3<sup>rd</sup> day ( $p < 0.05$ ). After 14 days, the rate was found to be increased by up to 12.3% than the value reported on the 7<sup>th</sup> observation day, but remained 13, 7% higher than control ( $p < 0,05$ ). Subsequently, the parameter was repeatedly decreased in value (by 12.0% as compared to corresponding value reported on the 14<sup>th</sup> day) and was 25.8% less than in the control samples ( $p < 0.05$ ). The parameter showed the increase in value by 12, 4% after 28 days in contrast to the previous experimental time point (21 days). It should be

emphasized that the parameter did not reach the control level and remained 16,7% less in value ( $p<0,05$ ).

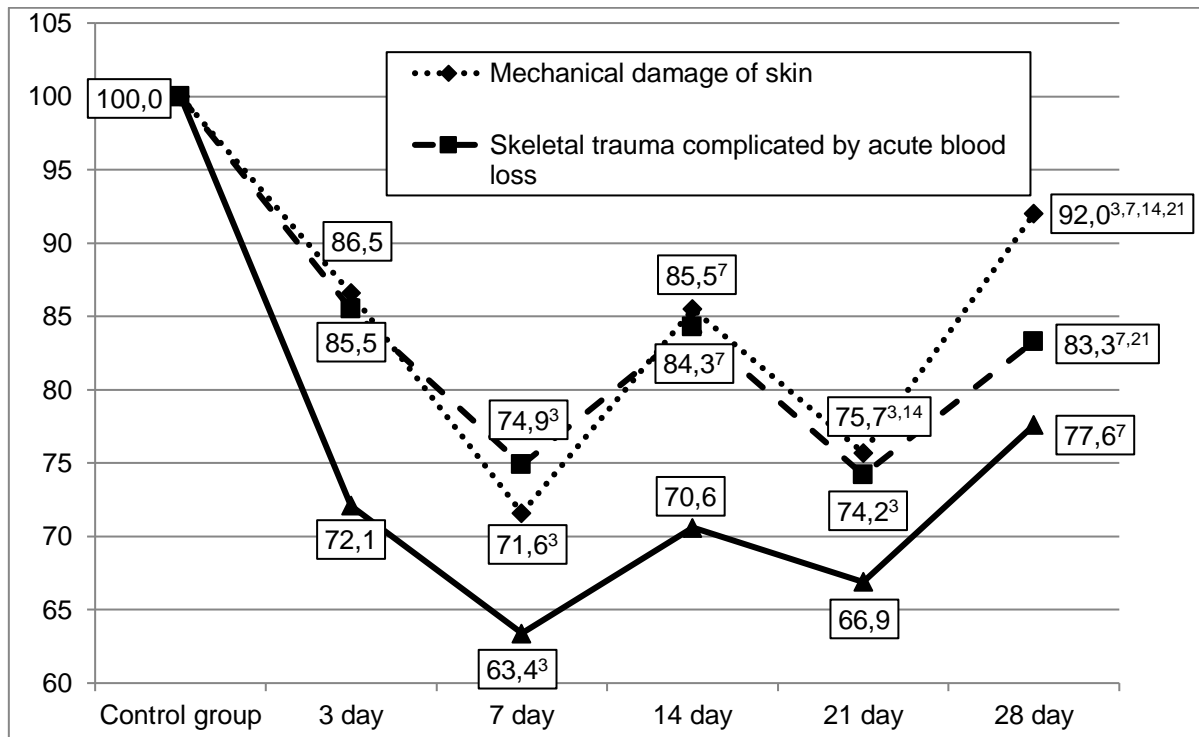


Figure 1 – The effect of combined trauma on the dynamics of bile excretion rate (percentage relative to the control). (Note. \* – the differences in relation to the control group are statistically significant,  $p<0,05$ ).

The bile excretion was impaired to the greatest extent under the combined trauma model. The parameter reached its minimum value up to the 7<sup>th</sup> day, and appeared to be decreased by 36,6 % compared to the control ( $p<0,05$ ). The bile excretion rate persisted at the same level for up to 21 days ( $p<0,05$ ) and further increased in value by 15,7% up to the 28<sup>th</sup> day, but was 22,4 % less than in the control group ( $p<0,05$ ).

The intergroup comparison demonstrated a statistically significantly lower value of bile excretion rate in the third experimental group than in rats of the 1<sup>st</sup> and 2<sup>nd</sup> experimental groups after 3 and 14 days of the post-traumatic period ( $p_{1-3}<0,05$ ,  $p_{2-3}<0,05$ ). The parameter was also significantly lower in rats of the third experimental group 3 after 7 days compared to other experimental groups, but reached the statistical significance only with the second experimental group (by 15,8 %,  $p_{2-3}<0,05$ ). The bile excretion was found to be statistically significantly decreased in rats with combined trauma model in contrast to rats subjected to either mechanical skin damage or skeletal trauma complicated by acute blood loss after 21

days, but the significance was seen only with the experimental group with mechanical skin damage model (by 11,0 %,  $p_{1-3}<0,05$ ). After 28 days, it was established that a more severe trauma severity led to the statistically significant decrease in bile excretion rate ( $p_{1-2}<0,05$ ,  $p_{1-3}<0,05$ ,  $p_{2-3}<0,05$ ).

The administration of the PRP-therapy to rats with combined trauma model contributed to the increase in bile excretion rate (Fig.2) compared to the non-treated animals exposed to the same trauma model. The result was statistically significant after 21 and 28 days of the experiment. The parameter was found to be increased by up to 20.9% and 14.2%, respectively ( $p<0.05$ ).

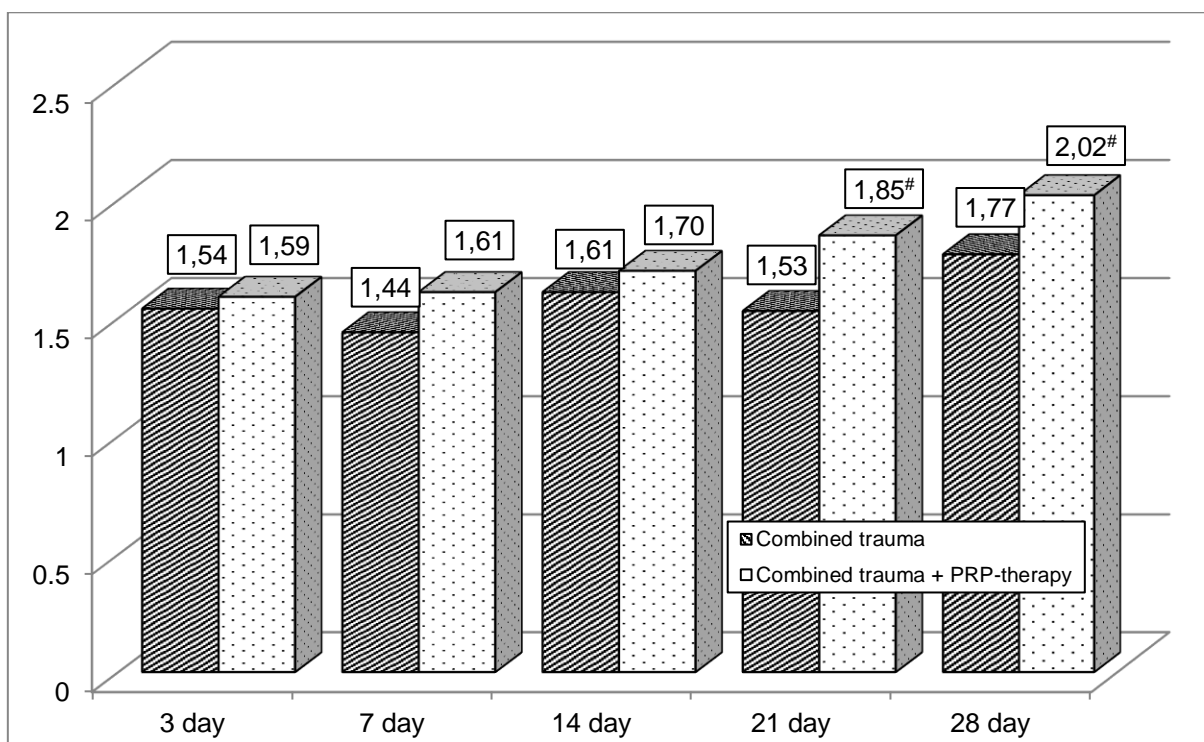


Figure 2 – The effect of PRP-therapy on the dynamics of bile excretion rate in the presence of combined trauma (%). (Note. # – the differences in relation to the group of animals subjected to combined trauma without corrective medication are statistically significant,  $p<0,05$ ).

The obtained results evidence that mechanical damage to the skin, skeletal trauma complicated by acute blood loss and the combination of mentioned traumas are accompanied by a significant decrease in the bile excretion rate. Taking into account the organ specificity of the bile excretion, it can be substantiated that the studied trauma models can cause the

development of secondary liver failure and the deduced liver functional capacity within the periods of early and late manifestations of the traumatic disease.

An impaired bile production and bile excretion functions, as described by some authors, are sensitive indicators of systemic disorders provoked by skeletal trauma and acute blood loss [3, 4, 5]. However, it has been first-time established that hepatic dysfunction occurs already in the presence of isolated damage to the skin. Inhibited synthesis of bile constituents, an impaired bile flow due to the development of swelling in the organ and biliary dyskinesia are, probably, the among the causes of detected impairments. [12] Therefore, an isolated damage to the skin has a marked systemic effect on the body. The latter can also be confirmed by the similar dynamics and amplitude of abnormal values in bile excretion rate within 3-21 days in rats with isolated skin damage and animals subjected to skeletal trauma complicated by acute blood loss. It is pertinent to emphasize that an additional infliction of damage to the skin in the background of skeletal trauma complicated by blood loss considerably exacerbates the abnormalities in bile flow rates, which confirms the fact of multiplicity of pathogenic mechanisms of trauma models and the considerable role of skin damage in the pathogenesis of combined trauma.

The administration of the PRP-therapy injections to rats with combined trauma model resulted in less severe impairments of bile excretion as compared to the animals without corrective medication, which was particularly noticeable in the period of late manifestations of traumatic disease after 21 and 28 days. It can be presumed that the obtained outcome is related to acceleration of the healing of mechanical defect of the skin and suppression of its pathogenic impact on the internal organs [6]. The systemic effect of released allogeneic platelet transfusion-related bioactive substances on the traumatized animals cannot be excluded either.

The obtained results prove that the PRP-therapy appears to be a promising medication in the presence of combined trauma including mechanical damage to the skin. The research findings are of a great importance for clinical practice in terms of decreasing the risk associated with the development of multiple organ failure, which substantiates the pertinence of further depth-in study.

### **Conclusions**

1. Mechanical damage to the skin results in an impaired hepatic function in experimental rats, which is manifested by a diminished bile flow. The dynamics of the abnormal values in studied indicators fluctuates with the first exacerbation phase after 7 days and the second one after 21 days.



2. An additional infliction of mechanical damage to the skin in the presence of skeletal trauma complicated by acute blood loss exacerbates the impairment of bile excretion with a maximum of abnormalities occurred after 7 and 21 days of the posttraumatic period.

3. The administration of the PRP-therapy injections in the background of combined trauma model is accompanied by less abnormal rate of bile excretion as compared to the non-treated animals exposed to the same trauma model, which is statistically significant after 21 and 28 days of the experiment..

**Perspectives.** The future research should be aimed at studying the mechanisms of systemic effect of the PRP-therapy under the influence of mechanical damage to the skin in the presence of skeletal trauma complicated by acute blood loss.

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