

STATUS OF LIVER ENZYMES AS AN INDICATOR OF LOCAL TREATMENT OF BURN TRAUMA IN EXPERIMENT

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The energy and protein failure reducing adaptable compensation and regeneration potential of the body is an essential component in the pathogenesis of a burn disease.

The aim of the investigation was to study the activity of liver oxidoreductases of rats with heat injury in various options of local treatment.

Materials and Methods. The researches were carried out on 60 white rats of Wistar line. The activity of enzymes was determined in liver homogenates on day 3, 7 and 10 after the heat injury and local treatment of burn wounds.

Results. There was revealed the increase in lactate dehydrogenase activity in retroaction and significant decrease of the activity of detoxication enzymes in the liver of rats on 3, 7 and 10 days after a thermal trauma. The rate and extent of oxidoreductases restoration activity were found to determine the local burn treatment modality.

Key words: thermal trauma; oxidoreductases; liver enzymes; burn treatment.

Protein-energy malnutrition decreasing adaptive compensatory and regenerative potential of the organism is an important component of the burn disease pathogenesis [1]. At the same time the characteristics of malfunction of oxidoreductases in thermal trauma and also the possibilities of complete restoration of their activity in applying local and systemic treatment are still underinvestigated. From this point of view the assessment of changing of these characteristics in the use of different variants of local treatment of a burn wound allows to substantiate the rationality of choosing of medicine for accomplishing the combustiology tasks [2]

The aim of the investigation was to study the activity of liver oxidoreductases of rats with heat injury in various options of local treatment.

Materials and Methods. The researches were carried out on 60 white rats of Wistar line having the body mass 180–250 g. The animals being under ether anesthesia were burned with boiling water on 20% of body surface (the area had been epilated previously, exposition — 3 s). There were formed 4 main groups with 10 animals in every group: the rats of the group 1 were treated with “Levomocol” (Open Joint Stock Company “Nizhpharm”, Russia, standard therapy), group 2 — gel composition with “Tizol” (OLYMP, Russia) + ozonized oil “O’THREE-SUPEROZONIDE” (Medozons, Russia), group 3 — with “Tizol” + chlorhexidinum (1:200), group 4 — “Tizol” + “Levoxym” (ximedon composite — 8%,

succinic acid — 1% and Levomycetin — 1%). Control groups are presented by intact animals and the animals with thermal trauma (burn) without any treatment (n=10 in every one). The animals were taken out from the experiment on 3, 7 and 10 days after the trauma. The ethical principles approved the European Convention for the protection of vertebrate animals used for Experimental and other Scientific purposes (entered into force on 18 March 1986 in Strasbourg and confirmed on 15 June 2006 in Strasbourg) were rigorously adhered.

Enzyme activity was studied in rats’ liver homogenate on 3, 7 and 10 days after burning. The aldehyde dehydrogenase activity (AIDH) was determined by B.M. Kershengoltz and E.V. Serkina [3], lactate dehydrogenase (LDH) was determined by G.A. Kochetov [4] using lactic acid as a substrate for direct reaction (LDH_{direct}) and pyruvic acid for back reaction (LDH_{back}), alcohol dehydrogenase (ADH) — by E. Koivusalo et al. [4] with applying ethanol as a substrate for a direct reaction (ADH_{direct}) and acetaldehyde — for a back reaction (ADH_{back}).

The statistical analysis of the results was performed using the program Statistica 6.0.

Results and Discussion. It was ascertained that in thermal trauma of liver significant growth of LDH activity in back reaction — on 3, 7 and 10 day after the lesion of 30, 53 and 55% respectively in comparison with intact

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animals (Fig. 1). Herewith the activity of this enzyme in the direct reaction in burn is nearly unchangeable. It contributes lactate accumulation the latter being in high concentrations can serve as an indirect marker of the development of tissue hypoxia and acidosis [5, 6].

The increasing of LDH_{back} activity on day 3 after the trauma within the period of acute burn toxemia can be associated with the development of hypermetabolic syndrome including activation of the lipid peroxidation process and increasing of the level of molecules having an average molecule mass [5].

It was found a great decreasing of detoxication enzyme activity in the liver (AIDH and ADH; Fig. 2, 3) in scalding of the animals on every observation date, leading to the accumulation of high toxic aldehydes and ketones and also ethanol, its intermediate metabolic product is acetaldehyde. The latter, in turn, inhibites the activity of mane enzymes, destroy the structure and function of plasma membranes, activate lipid peroxidation process [3].

In the study of enzyme activity during the process of burn wound treatment it was found that applying compositions on the basis of "Tizol" doesn't influence greatly to the LDH_{direct} activity (Fig. 1, b). Meanwhile the animal treatment with "Levomecol" contributes decreasing LDH_{back} activity on day 3 after affection and leads to the significant decreasing of this enzyme activity on day 7 (for 24% in comparison with thermal trauma, $p < 0.05$), approaching the results of the control group of animals. The LDH_{back} activity decreasing under the influence of "Levomecol" can be explained by nonselective inhibitor activity of the antibiotic ("Levomycetin") being contained in it to the enzyme activity in back reaction [7]. However, further dynamics of LDH activity failed to backtrace due to the death of all animals by 10 day of the observation.

LDH_{back} activity after the trauma in applying the composition "Tizol" + chlorhexidinum in comparison with non-treated animals decreases greatly too on 7 and 10 days (for 10 and 54% respectively, $p < 0.05$). We can suggest that the mechanism of chlorhexidinum activity is double in this case — blocking of ione channel allosteric modulation of the active enzyme centre [8, 9].

The most strongly marked decreasing of LDH_{back} activity in the liver in thermal trauma is observed while using the composition "Tizol" + "Levoxym": on day 3 after the trauma the enzyme activity is decreased up to 62%; on day 7 — up to 38% and day 10 — up to 51% in comparison with similar in the burned rats without ant treatment, and these indexes are greatly less than the indexes of the intact animals on 3 and 10 days after the burn ($p < 0.05$; Fig. 1, a).

On the one hand, such marked positive influence of this composition to LDH_{back} activity can be specified by the properties of the components containing in "Levoxym": Levomycetin and succinic acid. Taking into account that succinic acid activity underlies the reparative effect of "Levoxym", the activity mechanism of this medicine was considered from the point of its molecular and cellular effects. We think the effect of succinic acid activity

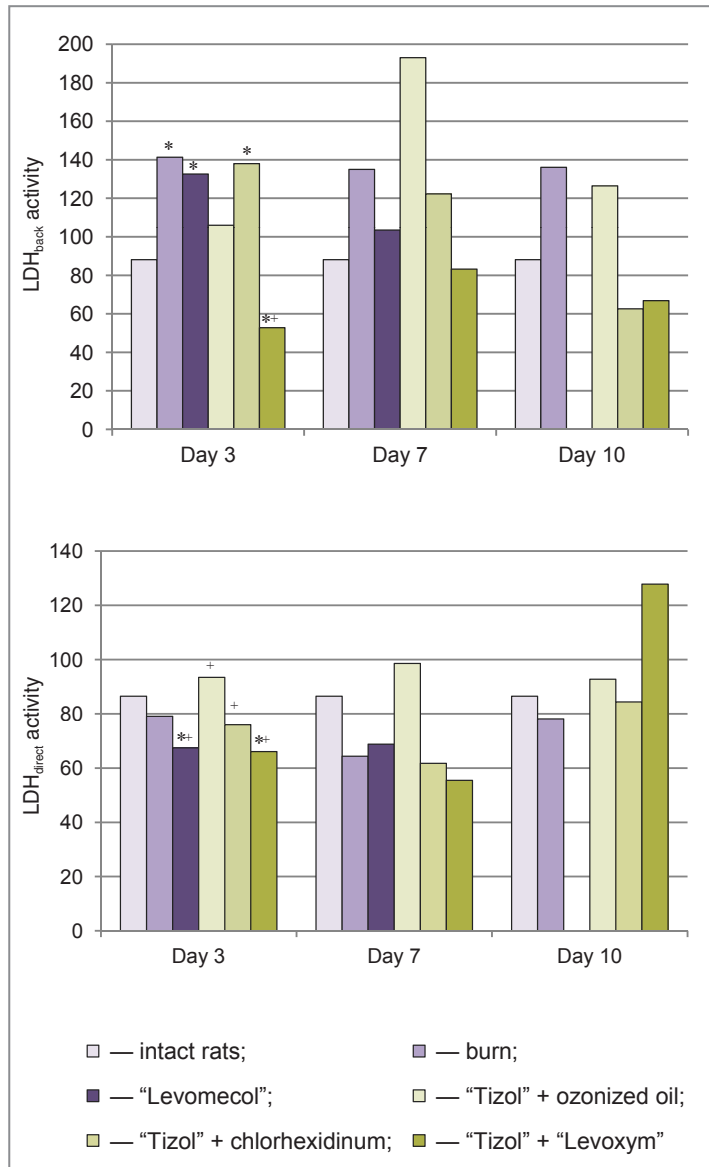


Fig. 1. Activity of rats' liver lactate dehydrogenase (nmole NADH/min) in the back (a) and direct reaction (b) in different variants of the local treatment of burn. * — difference is statistically significant in comparison with the intact animals ($p \leq 0.05$); + — in comparison with the burned animals ($p \leq 0.05$)

contained in "Levoxym" is mediated by the respiratory chain and normalization of energetic metabolism and implemented by its property to inhibit lipid peroxidation of cellular membranes. Succinic acid produce modulating effect to membrane associated antioxidant enzymes [10]. On the other hand, it is necessary to take into account the impact of the gel "Tizol" providing bioavailability growth of "Levoxym" components.

Composition "Tizol" + ozonized oil contributes statistically significant decreasing of LDH_{back} activity on day 3 after the trauma for 25% in comparison with the rats which burns were not treated.

It has been found that all applied variants of local treatment contribute the ADH_{direct} activity restoration having been decreased with thermal trauma, though the degree

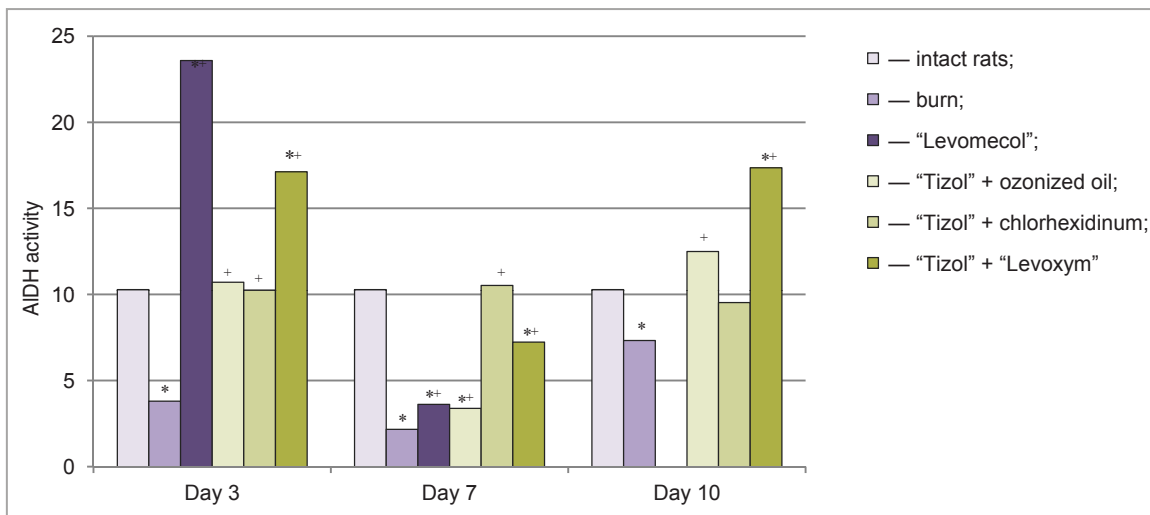


Fig. 2. Activity of rats' liver aldehydedehydrogenase (nmole NADH/min) in different variants of the local treatment of burn. * — difference is statistically significant in comparison with the intact animals ($p \leq 0.05$); + — in comparison with the burned animals ($p \leq 0.05$)

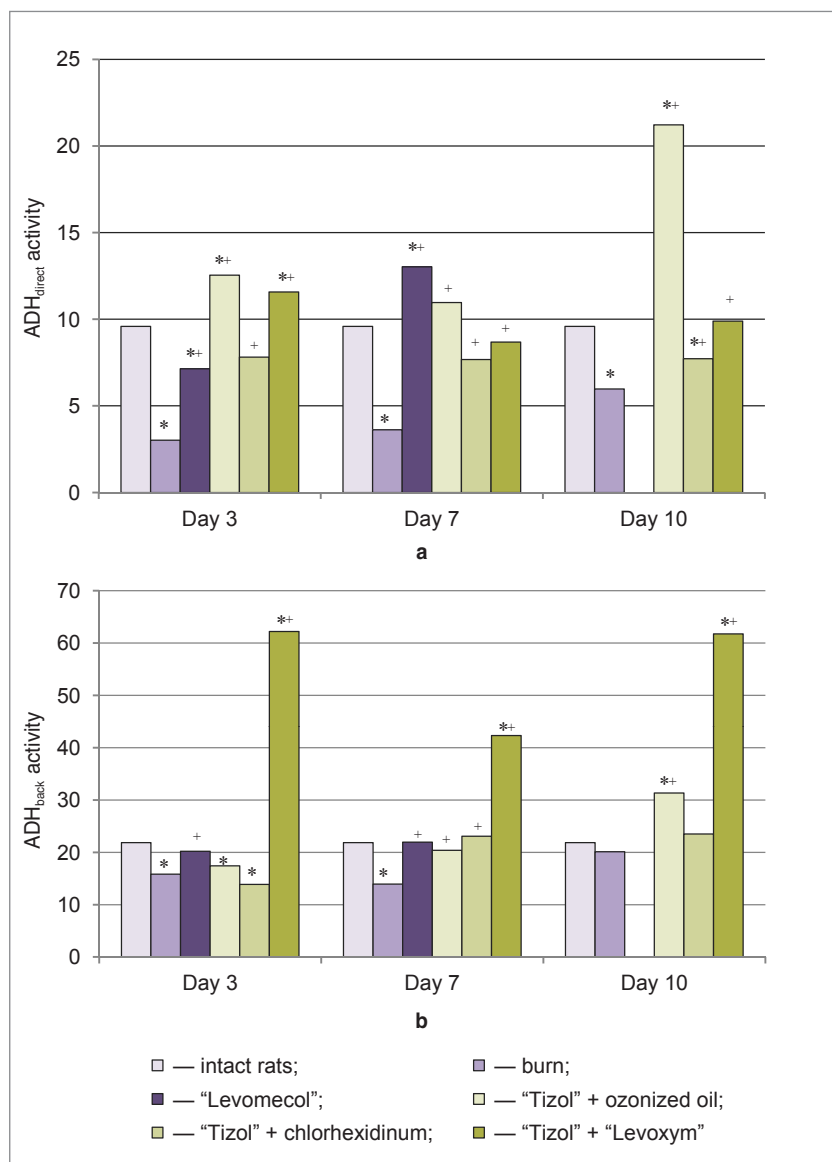


Fig. 3. Activity of rats' liver alcohol dehydrogenase (nmole NADH/min) in the direct (a) and back (b) reaction in different variants of the local treatment of burn. * — difference is statistically significant in comparison with the intact animals ($p \leq 0.05$); + — in comparison with the burned animals ($p \leq 0.05$)

of normalization of enzyme functioning depends on used medicines (Fig. 3, a). At that the activity index within the whole period of observation is higher than the physiological level when the scheme "Tizol" + ozonized oil is used only. The applied line of drugs following the significance level of the effect to the ADH_{direct} activity looks the following way: "Tizol" + ozonized oil > "Tizol" + "Levoxym" > "Tizol" + chlorhexidinum > "Levomocol" (Fig. 3, a).

The activity of AIDH and ADH in back reaction increases most effectively in thermal trauma under the influence of the composition "Tizol" + "Levoxym" (Fig. 2 and 3, b), AIDH activity significantly increases on day 3 after the affection by 350%, on day 7 — by 233%, on day 10 — by 137%; ADH_{back} activity statistically significantly increases on day 3 by 293%; on day 7— by 204%, on day 10— by 207% in comparison with non-treated rats ($p < 0.05$). The mechanism of succinate influence, "Levoxym" component to AIDH and ADH activity probably consists in restoration of liver oxidative ability with the consequent eliminating of ethanol and acetaldehyde by means of the stimulation of the Krebs cycle with this metabolite [10].

The AIDH activity increases less effectively under the influence of the composition "Tizol" + chlorhexidinum (on day 3 — by 170%, on day 7 — by 385% in comparison with non-treated rats; $p < 0.05$). The AIDH activity increases significantly under the influence of the composition "Tizol" + ozonized oil on day 3 — by 181%, on day 7 — by 56% in comparison with non-treated rats; $p < 0.05$).

Conclusion. It was found that the manifestation of enzyme activity of energy metabolism (lactate dehydrogenase) and the system of biotransformation (aldehyde dehydrogenase) of the animals liver in burn depends on a chosen variant of local treatment. So, the assessment of catalytic properties of these oxidoreductases can act as a metabolic marker of systemic action of the drugs for local treatment of thermal trauma and additional marker of its efficacy.

References

1. Ushakova T.A. *Adaptivnye reaktsii u tyazhelooobozhzhennykh v usloviyakh intensivnoy terapii*. Avtoref. dis. ... dokt. med. nauk

[Adaptive reactions in severe burn patients under intensive care conditions. Abstract for Dissertation for the degree of Doctor of Medical Science]. Moscow; 2008.

2. Smagina T.A., Moskvina L.M., Turkina Yu.V. *Opreделение skorosti vysvobozhdeniya lekarstvennykh veshchestv iz Tizolya*. V kn.: *Novye tekhnologii v meditsine i farmatsii. Tizol. Materialy mezhregion. nauchno-prakt. konferentsii* [Determination of drug release rate from Tisolum. In: New technologies in medicine and pharmacy. Tisolum. Proceedings of interregional research and practice conference]. Ekaterinburg; 2003; p. 49–56.

3. Kershengol'ts B.M., Serkina E.V. *Nekotorye metodicheskie podkhody k izucheniyu metabolizma etanola* [Some technical approaches to ethanol metabolism study]. *Laboratornoe delo — Laboratory Science* 1981; 2: 126.

4. Kochetov G.A. *Prakticheskoe rukovodstvo po enzimologii* [Practical guide on enzymology]. Moscow: Vysshaya shkola; 1980; 272 p.

5. Paramonov B.A., Porembskiy Ya.O., Yablonskiy V.G. *Ozhogi* [Burns]. Saint Petersburg: SpetsLit; 2000; 488 p.

6. Kuzin M.I. *Sindrom sistemnogo otveta na vospalenie* [Syndrome of systemic response to inflammation]. *Khirurgiya — Surgery* 2000; 2: 54–59.

7. Sopuev A.A., Mamatov N.N., Ovcharenko K.E., et al. *Otsenka effektivnosti mazi levomekol' pri intraoperatsionnoy profilaktike spaechnogo protsessa bryushnoy polosti* [The assessment of Levomekol efficiency in intraoperative prevention of an adhesive process of the abdominal cavity]. *Khirurgiya Kyrgyzstana — Kyrgyzstan Surgery* 2011; 2: 39–43.

8. Druzhinina O.S., Skorinkin A.I. *Issledovanie mekhanizmov deystviya khlorgeksidina i dimefosfona na vodnyy transport v eritrotsitakh* [The study of mechanisms of chlorhexidine and dimephosphon effect on water transport in erythrocytes]. *Struktura i dinamika molekulyarnykh sistem: elektronnyy zhurnal — Structure and Dynamics of Molecular Systems: Electronic Journal* 2009; 7A: 53–64.

9. Shaykhtudinova A.R. *Mekhanizmy modulyatsii raboty nikotinovogo retseptorno-kanal'nogo kompleksa khlorgeksidinom*. Avtoref. dis. ... kand. biol. nauk [Modulation mechanisms of nicotinic receptor-canal complex functioning by chlorhexidine. Abstract for Dissertation for the degree of Candidate of Biological Science]. Kazan; 2005.

10. Zarubina I.V., Lukk M.V., Shabanov P.D. *Antigipoksicheskie i antioksidantnye efekty ekzogennoy yantarnoy kisloty i aminotiolovykh suksinatsoderzhashchikh antigipoksantov* [Antihypoxic and antioxidant effects of exogenic amber acid and aminothiol succinate-containing antihypoxic agents]. *Bull Eksp Biol Med — Bulletin of Experimental Biology and Medicine* 2012; 153(3): 313–317.