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The interaction between liver oxidoreductases in experimental thermal injury

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Abstract: In this work the interaction between lactate dehydrogenase (LDH) and aldehyde dehydrogenase (ALDH) was studied in normal and thermal injury because thermal trauma is one of the central places in surgery and traumatology. The experiment was carried out on white Wistar rats. All animals under anesthesia (Zoletil (60 mg/kg) + XylaVET (6 mg/kg)) received flame burns on 20% of the skin surface. The catalytic and kinetic properties of aldehyde dehydrogenase and lactate dehydrogenase were determined in subcellular fractions of rat liver. In vitro we investigated the effect of Na lactate and Na pyruvate on the ALDH activity of intact rats and rats with thermal injury. It was established that during the burn the activity of ALDH and LDH in direct and reverse reactions decreased in the mitochondria. There was a high degree of positive correlation between the activities of LDH in direct reaction and ALDH ($r=0,995$; $p< 0.0001$) in thermal injury. In vitro it was revealed that pyruvate had a negative effect and lactate had positive effect on the activity of ALDH in the liver with intact rats and rats with burn.

Keywords: lactate dehydrogenase, aldehyde dehydrogenase, enzyme-enzyme interaction, burn.

INTRODUCTION

The subject of numerous studies is the problem of the existence and functioning of the complexes between the enzymes[1-6]. Some authors introduce the concept of enzymatic module, comprising at least two enzymes functionally interacting with each other on the basis of supramolecular regulation. There is direct transfer within enzymatic complexes from one enzyme to another in described model[7]. Experimental evidence was provided in the form of direct transfer of NADH between dehydrogenases[8,9]. It was also shown that lactate dehydrogenase (LDH; EC 1.1.1.27) forms a complex with glyceraldehyde-3-phosphate-dehydrogenase[7].

It was known about the functional and metabolic disorders of internal organs at burns[10, 11]. Developing burn disease accompanied by the appearance of tissue hypoxia and syndrome of endogenous intoxication, accumulation in blood of toxic substances and highly toxic aldehydes, a violation of the metabolism and the energy metabolism of cells, changes in the activity of enzyme systems[12]. In addition, thermal trauma has one of the central places in surgery and traumatology remaining relevant to this day[13].

In this regard, the aim of this study was to examine the nature of the interaction between lactate dehydrogenase and aldehyde dehydrogenase (ALDH; EC 1.2.1.3) in subcellular liver fractions in norm and in thermal injury.

MATERIALS AND METHODS

The study was reviewed and approved by the Institutional Review Board. The experiment was carried out on white Wistar rats weighing 180-200 g. All animals were kept in standard vivarium conditions in cages with free access to food and water. Conditions of working with animals corresponded with the rules of the European Convention ET/S 129, 1986 and the directives 86/609 ESC. After a 14 – day adaptation to the local vivarium and quarantine animals (n=10, experimental group) under anesthesia (Zoletil 100 (VIRBAC, France), (60 mg/kg) + Xyla VET (Pharmamagist Ltd., Hungary), (6 mg/kg)) received flame burns (III – IV degree) on the 20% of the body surface of the back, exposure – 45 sec. The activity of aldehyde dehydrogenase[14] and lactate dehydrogenase[15] was determined in homogenate and subcellular liver fractions (cytosol, M1 and M2-mitochondria) in one hour after the burn. We know about the heterogeneity of mitochondrial morphology, density, involvement in apoptosis and aging. There are "young" mitochondria with diameter less than 1 micrometer (M1) and "matured" mitochondria with diameter greater than 1 micrometer (M2) in an animal cell at the same time. Mitochondria of different sizes have similarity respiratory rate, the composition of dehydrogenases and proteins, phosphorylation of ADP. It indicates about close relationship between these organelles. At the same time mitochondria of different sizes differ in the NADH-dehydrogenases and phosphorylation activity, in the content of several proteins that reflects the ripening process of mitochondria[16].

M1 and M2-mitochondria were obtained by differential centrifugation in gradient of sucrose density[17]. A control group (n=21) included healthy intact animals not subjected to burn. Animals were removed from the experiment by decapitation with advanced transaction of carotid artery under anesthesia (Zoletil (60 mg/kg) + Xyla VET (6 mg/kg)). Protein concentration was determined by the method of Lowry in the modification[18]. In vitro we investigated the effect of Na lactate (0, 45 M) and Na pyruvate (0, 18 M) on the ALDH activity of intact rats and rats with thermal injury. These substances (0,1ml for 3 ml of sample) were introduced directly into the environment to determine the ALDH activity. Kinetic parameters of enzymatic reaction (Kt, Vmax, Vmax/Kt) were calculated with the use of mathematical method[19]. Statistical data processing was performed by the software (Statistica 6.0 (StatSoft Inc., USA)).

The significance of differences between groups was assessed using Student's t-test. The differences were considered statistically significant at p<0, 05.

RESULTS AND DISCUSSION

It was established that the specific activity of ALDH in the cytosol and mitochondria of rat liver during the burns significantly decreased to 2 times **(table 1).** The decrease of the ALDH activity in thermal injury had been accompanied by decrease the catalytic efficiency of ALDH in the cytosol to 2 times, in M1 and M2-mitochondria also to 2 times, in homogenate – to 1,4 times (**table 2).**

The main specific activity of ALDH was in "young" mitochondria (M1), the activity of which was greater the activity of ALDH «matured» mitochondria (M2) to 2 times. This was true for LDH **(table 1).** D.A. Shishmakov *et al.*[16] in their work also indicated a higher activity of small mitochondria and attributed this fact by the small diameter of the M1 mitochondria[16]. Therefore the total surface of membranes of M1 mitochondria was more than the M2-mitochondria.

Table 1: Activity of LDH and ALDH in the liver of rats in norm and at a burn (nmol NADH/min×mg of protein)

Fraction	Intact animals			Animals with burns		
	ALDH	LGH in direct reaction	LGH in reverse reaction	ALDH	LGH in direct reaction	LGH in reverse reaction
homogenate	65,47 ±7,07	669,15 ±35,23	1739,70 ±50,59	31,81±0,85 *	286,41 ±23,14 *	247,94 ±2,22 *
cytosol	76,70 ±4,81	500,69 ±22,36	2030,97 ±81,11	52,65±4,36 *	470,57 ±31,52	1312,68 ±65,07 *
M1	127,42 ±17,43	395,42 ±32,85	1060,77 ±61,68	53,95±8,99 *	356,06 ±44,86	1074,52 ±116,20
M2	47,51 ±2,35	119,02 ±4,99	391,26 ±27,18	25,98±3,28 *	41,62 ±9,09 *	226,63 ±32,36 *

Notes: *- differences are reliable in comparison with intact animals (p <0,05), (* - p< 0,01)

LDH has a significant influence on the redox potential of cells, regulating intracellular ratio of NAD/NADH, which depends on the activity of ALDH. The reversibility of the lactate dehydrogenase reaction and high activity of the enzyme allows the pair of substrates lactate-pyruvate plays an important role in the control of the attitude of the oxidized and reduced forms of NAD in the cell. Lactate dehydrogenase may be present in the cell in three states: free, in the form of a complex with other enzymes of glycolysis and associated with structural proteins and cell organelles[20]. In addition, natural products of lactate dehydrogenase reaction, lactate and pyruvate, in high concentrations are the components of molecules of medium molecular mass[21].

Therefore we investigated the activity of LDH. It was established LDH activity reduced in direct and reverse reactions in different fractions of hepatocytes during the burn **(Table 1).** The activity of LDH in

reverse reaction was significantly decreased in homogenate by 86%, in the cytosol – by 36%, in M2-mitochondria – by 43% in comparison with intact animals. Activity of LDH in direct reaction was significantly decreased in homogenate of 58%, in M2-mitochondria – by 66% in comparison with intact animals. The correlation analysis was performed between values of activity of the investigated oxidoreductases. In thermal injury a high degree of positive correlation between the activities of LDH in direct reaction and ALDH in M2-mitochondrial fraction was detected (r=0,995; p< 0, 0001).

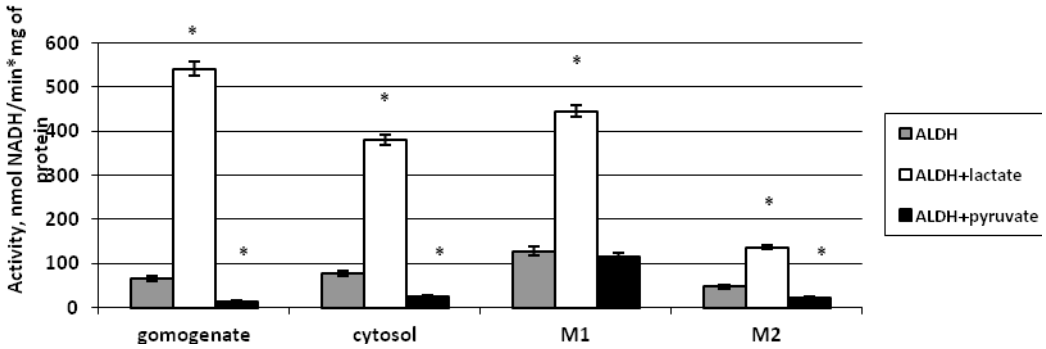
However the decrease of LDH activity in the direct and reverse reactions in thermal injury was accompanied by an increase in the relation of LDH activity in direct reaction to the activity of LDH in reverse reaction in the homogenate and cytosol of the liver. This led to increase in the content of pyruvate and the decrease in NAD used in direct lactate dehydrogenase reaction. The increase of pyruvate and decrease of NAD been a coenzyme for ALDH have a negative effect on activity ALDH. The decrease in LDH activity in thermal injury has been shown to decrease of the catalytic efficiency of the enzyme in all fractions of hepatocytes. The affinity of the enzyme LDH to substrate decreased in the M2-mitochondria to 3 times **(Table 2).**

Table 2: Kinetic indicators of ALDH and LDH in liver of rats in norm and at a burn

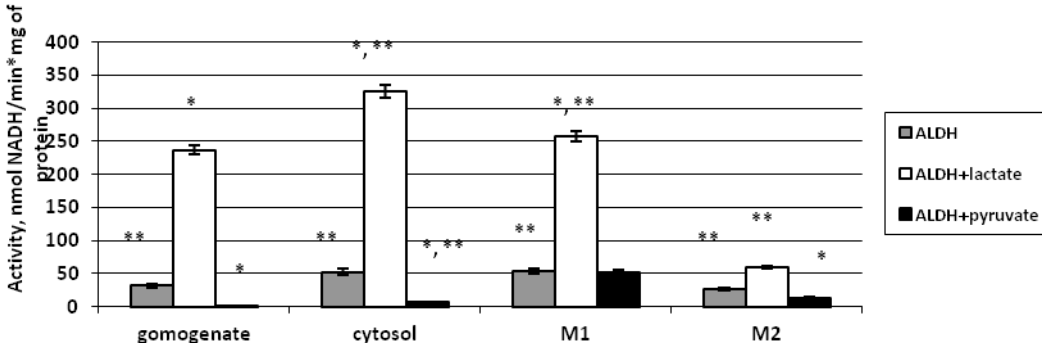
Fraction	Kinetic indicators	Intact animals			Animals with burns		
		ALDH	LGH in direct reaction	LGH in reverse reaction	ALDH	LGH in direct reaction	LGH in reverse reaction
homogenate	Kt	8,54 ±2,36	1,76 ±0,04	0,60 ±0,08	7,91 ±1,40 *	1,10 ±0,04*	0,18 ±0,02*
	Vmax	7,97 ±2,41	16,9 ±0,47	18,45 ±1,59	5,15 ±0,71	7,25 ±0,57*	3,04 ±0,04*
	Vm/Kt	0,92 ±0,08	9,61 ±0,13	34,91 ±4,18	0,68 ±0,03*	6,56 ±0,36*	17,20 ±1,10*
cytosol	Kt	3,16 ±0,66	0,65 ±0,02	19,11 ±5,37	2,58 ±0,01	0,66 ±0,03	2,58 ±1,57*
	Vmax	12,74 ±1,61	15,82 ±0,38	414,99 ±26,56	9,06 ±0,01	15,66 ±0,85	31,39 ±17,71*
	Vm/Kt	5,02 ±0,30	24,77 ±0,85	18,17 ±1,22	2,37 ±1,15*	24,05 ±1,99	13,67 ±1,72*
M1	Kt	5,79 ±1,12	4,30 ±1,42	6,16 ±1,58	13,66 ±4,77*	5,27 ±2,29	5,65 ±1,05
	Vmax	11,03 ±2,08	14,97 ±4,06	8,21 ±1,49	15,90 ±5,81	13,42 ±4,66	6,66 ±1,20
	Vm/Kt	2,00 ±0,15	4,41 ±0,28	1,56 ±0,11	1,10 ±0,11*	2,88 ±0,32*	1,32 ±0,31
M2	Kt	8,77 ±2,37	1,78 ±0,18	17,89 ±4,22	3,81 ±0,46*	5,30 ±0,45*	4,53 ±0,18*
	Vmax	25,74 ±8,77	10,61 ±0,50	36,05 ±9,06	6,00 ±1,36*	9,49 ±1,24	8,87 ±1,32*
	Vm/Kt	3,12 ±0,25	6,58 ±0,39	2,46 ±0,25	1,57 ±0,30*	2,66 ±0,67*	1,97 ±0,32

Notes: *- differences are reliable in comparison with intact animals (p <0,05), (* - p< 0,01)

In vitro it was revealed that pyruvate had a negative and lactate had positive effect on the ALDH activity in the liver of intact rats and rats with burn (**figure 1**). Under the influence of lactate activity of ALDH of intact rats significantly increased in the cytosol fraction by 80%, in M1-mitochondria – by 71%, in M2-mitochondria – by 65%. Under the influence of pyruvate the activity of ALDH significantly decreased in the cytosol fraction of the liver by 66%, in M2-mitochondria – by 50%.



A



B

Figure1: The influence of lactate and pyruvate on the activity of ALDH in the liver of rats with thermal injury in vitro. Notes: ** - differences are reliable in comparison with intact animals ($p < 0,05$); * - differences are reliable in comparison with the activity of ALDH ($p < 0,05$); A – intact animals; B – animals with burns.

Lactate caused a significant increase in activity of ALDH during the thermal injury in the homogenate and M2-mitochondria more than 100%. Thus, lactate had on the ALDH activity during the burns more positive effect than in intact rats. The indicators characterizing the activity of ALDH when adding lactate was significantly lower in comparison with intact animals (in the homogenate by 57%, in M1 mitochondria – by 42%, in M2-mitochondria – by 55%). Pyruvate caused a decrease in the activity of ALDH in animals with thermal injury in the homogenate at 99%, in the cytosol fraction – by 87%, in M2-mitochondrial fraction – by 49%.

Changes in the activity of ALDH under the influence of lactate and pyruvate accompanied by changes in the kinetic parameters of aldehyde dehydrogenase reactions of intact animals and animals with burns (**Table 3).**

Table 3: Kinetic indicators of ALDH in the liver in normal and burn in the presence of lactate and pyruvate

Fraction	Kinetic indicators	**ALDH+lactate**		**ALDH+pyruvate**	
		Intact animals	Animals with burns	Intact animals	Animals with burns
homogenate	Kt	1,63±0,01 **	0,81±0,04 */**	1,33±0,42**	0,82±0,14**
	Vmax	14,39±0,07**	5,31±0,59*	0,60±0,06**	0,16±0,01*/**
	Vmax/Kt	8,82±0,08**	6,45±0,36*/**	0,69±0,18**	0,15±0,01*/**
cytosol	Kt	0,58±0,01 **	0,66±0,04*/**	6,09±1,40**	1,80±0,52*
	Vmax	12,55±0,47	12,91±0,78	9,95±1,89	2,69±0,40*/**
	Vmax/Kt	21,58±0,69**	20,04±2,00**	2,33±0,27**	1,49±0,15
M1	Kt	2,03±0,18**	2,81±0,62**	8,87±1,44	3,74±1,17**
	Vmax	11,56±0,77	6,26±0,73*/**	15,32±2,13	4,40±0,70*/**
	Vmax/Kt	5,95±0,20**	2,66±0,02*/**	1,90±0,12	1,31±0,11*
M2	Kt	1,59±0,09**	3,04±1,01*	11,17±2,67	5,24±0,14*
	Vmax	12,96±0,57**	12,33±4,84	18,44±5,36	8,04±1,60*
	Vmax/Kt	8,49±0,46**	5,66±0,71**	1,80±0,14**	1,47±0,15

Notes: **- differences are reliable in comparison with ALDH (p <0,05), *- differences are reliable in comparison with intact animals (p <0,05; * - p< 0,01)

The lactate caused the increase the affinity of the enzyme to the substrate in the intact animals in the homogenate to 5,2 times, in cytosol – to 5,4 times, in M1 – 3 times, in M2 – 5,5 times in comparison with the original Kt of ALDH and increase of catalytic efficiency of ALDH in the cytosol fraction to 4,2 times, in M1–mitochondria – to 3 times, in M2-mitochondria – to 3 times. Under the influence of pyruvate the affinity of the enzyme to the substrate decreased in the cytosol fraction by 2 times, in the M1 – by 1,5 times and in M2–mitochondria – by 1,3 times and the catalytic efficiency of the ALDH decreased in the cytosol fraction – to 2 times, in M1 – to 1,3 times, in M2 – to 1,7 times.

It was shown that lactate increased the affinity of ALDH to acetaldehyde in animals with burns in the homogenate to 11 times, in M1-mitochondria - to 2 times, in the cytosol fraction – to 4,8 times and increased the catalytic efficiency of ALDH in all fractions of hepatocytes in comparison with the original Kt, and Vmax/Kt of aldehyde dehydrogenase reaction in rats with burns.

Under the influence of pyruvate the Kt decreased in the homogenate to 9,6 times, in M1-mitochondria – to 5 times and under the effect of pyruvate Vmax decreased in the homogenate to 32 times, in the cytosol - to 3,4 times, in M1-mitochondria – to 3,6 times in comparison with Kt and Vmax of aldehyde dehydrogenase of animals with burn.

The total activity of LDH in direct reaction and ALDH was higher the activity in the presence of lactate in all fractions of the liver. We can assume that there is a competition between ALDH and LDH in direct reaction for NAD. Probably, LDH works in tandem with ALDH. The activity of ALDH is dependent from the number of NAD and products of lactate dehydrogenase reaction. In protein-protein complex (ALDH+LDH) a competitive interaction for the coenzyme was observed.

CONCLUSION

Thus, by the example of the study LDH and ALlDH activity in the presence of lactate in the normal and in thermal injury it is possible to talk about the relationship between the dehydrogenase. Specific protein-protein interaction may be between the two dehydrogenase. Probably, the strength of the heterologous protein-protein contacts formed by dehydrogenase depends on the functional state of dehydrogenases and pH. This physical association between dehydrogenase may play a role in metabolic regulation.

REFERENCES

1. M. Cascante, A. Sorribas, E.I. Canela, Enzyme-enzyme interactions and metabolite channelling: alternative mechanisms and their evolutionary significance, *Biochem J.*, 1994, **298** (2), 313-320
2. J. Ovádi, P.A. Srere, Metabolic consequences of enzyme interactions, *Cell Biochem Funct*, 1996, **14** (4), 249-258
3. J. Massant, N. Glansdorff, Metabolic channelling of carbamoyl phosphate in the hyperthermophilic archaeon Pyrococcus furiosus: dynamic enzyme-enzyme interactions involved in the formation of the channelling complex, *Biochem Soc Trans*, 2004, **32** (2), 306-309
4. C. Huthmacher, C. Gille, H.G. Holzhütter, A computational analysis of protein interactions in metabolic networks reveals novel enzyme pairs potentially involved in metabolic channeling, *J Theor Biol.*, 2008, **252** (3), 456-464

5. T. Yamada, P. Bork, Evolution of biomolecular networks: lessons from metabolic and protein interactions, *Nat Rev Mol Cell Biol.*, 2009, **10** (11), 791-803
6. K. Selvakumar, L. Motiei, D. Margulies, Enzyme-Artificial Enzyme Interactions as a Means for Discriminating among Structurally Similar Isozymes, *J Am Chem Soc.*, 2015, **137** (15), 4892-4895
7. M.V. Sukhodolets, V.I. Muronetz, N.K. Nagradova, Interaction between glyceraldehyde-3-phosphate-dehydrogenase and lactate dehydrogenase, *Biochem Int.*, 1989, **19** (2), 379-384
8. F.S. Messiha, Effect of almond and anis oils on mouse liver alcohol dehydrogenase, aldehyde dehydrogenase and heart lactate dehydrogenase isoenzymes, *Toxicol Lett.*, 1990, **54** (2-3), 183-188
9. M.V. Sukhodolets, V.I. Muronetz, N.K. Nagradova, Interaction between D-glyceraldehyde-3-phosphate dehydrogenase and 3-phosphoglycerate kinase labeled by fluorescein-5'-isothiocyanate: evidence that the dye participates in the interaction, *Biochem Biophys Res Commun.*, 1989, **161** (1), 187-196
10. P. Xi, W. Kaifa, Z. Yong, Y. Hong, W. Chao, S. Lijuan, W. Hongyu, W. Dan, J. Hua, W. Shiliang, Establishment and assessment of new formulas for energy consumption estimation in adult burnpatients, *PLoS One*, 2014, **9** (10), 110409
11. S. Zhuang, J. Chai, H. Duan, Advances in the research on the relationship between brown adipose tissue and metabolism inburn and trauma, *Zhonghua Shao Shang Za Zhi*, 2014, **30** (3), 251-253
12. L. Zhi, X. Hu, J. Xu, C. Yu, H. Shao, X. Pan, H. Hu, C. Han, The characteristics and correlation between the ischemia-reperfusion and changes of redox status in the early stage of severe burns, *Am J Emerg Med.*, 2015, **33** (3), 338-343
13. B.A. Paramonov, Ya.O. Porembskiy, V.G.Yablonskiy, Burns, Spetslit Publ.: St. Petersburg, 2000
14. B.M. Kershengol'c, V.G. Alekseev, E.M. Gavrilova, N.G. Li, Nekotorye prichiny umen'shenija aktivnosti al'degiddegidrogenazy pecheni i krovi krys pri hronicheskoj alkogol'noj intoksikacii, *Voprosy med. Himii*, 1985, **31** (1), 47-51
15. G.A. Kochetov, Prakticheskoe rukovodstvo po jenzimologii, Vysshaja shkola: Moscow, 1980
16. D.A. Shishmakov, R.L. Anisimov, N.L. Vekshin, Nekotorye svojstva protomitohondrij, *Biologicheskie membrany*, 2004, **21** (5), 374-380
17. Dzh. Findlej, U. Jevanz, Biologicheskie membrany. Metody, Mir: Moscow, 1990
18. J.M. Dawson, P.L. Heatlic, Lowry method of protein quantification Evidence for Photosensitivity, *Anal. Biochem.*, 1984, **140** (2), 391-393
19. Ju.V. Zimin, A.G. Soloveva, A.A. Ulanova, Ocenka kineticheskih parametrov fermentov v geterogennoj nadmolekuljarnoj sisteme, *Fundamental'nye issledovanija*, 2013, **2**, 68-71
20. Ju.V. Zimin, A.G. Soloveva, Reguljatornaja rol' nadmolekuljarnogo kompleksa alkogol'degidrogenazy i laktatdegidrogenazy mitohondrij kletki, *Bjulleten' jeksperimental'noj biologii i mediciny*, 2009, **148** (12), 644-645

21. V.A. Korjachkin, V.I. Strashnov, V.N.Chufarov, Intensivnaja terapija ugrozhajushhih sostojanij, Saint-Petersburg Med. Publ.: St. Petersburg, 2002

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