CHARACTERISTICS OF THE SKIN LACTATE DEHYDROGENASE ISOZYME SPECTRUM AFTER THERMAL INJURIES

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Activity of lactate dehydrogenase (LD) and its isozymes in the injured zone and in adjacent areas of the skin was investigated at various times after infliction of burns in rats. On the 1st-8th day after burning total LD activity was reduced in the zone of the scab and in the underlying tissues by 70-80% and in the border and intact skin by 50%. These changes were accompanied by changes in the LD isozyme spectrum in the injured tissue: On the 1st day the activity of fraction 5 was sharply increased and activity of fractions 2 and 3 reduced; by the 8th day, on the other hand, some decrease in activity of fraction 5 and an increase in activity of fractions 2 and 3 were observed. By the 14th-22nd day after burning total LD activity remained low, the LD isozyme spectrum in the border and scab was largely restored to normal, whereas in the underlying tissue changes in the relative proportions of LD isozymes were still present (a decrease in activity of fractions 1-3 and an increase in activity of fraction 5).

KEY WORDS: *burns; skin; lactate dehydrogenase; isozymes.*

The primary mechanism in the pathogenesis of burns is injury to the skin. Its state largely determines the character and severity of the course of the burn, the onset of complications, and the rate of healing. Accordingly, the metabolism of the burn wound and, in particular, the state of its enzyme system are of interest to the investigator. Several studies [1, 6, 7, 10-12] have shown that the activity of oxidoreductases in the zone of injury is low. Meanwhile, in the serum of burned patients changes are found in the distribution of the lactate dehydrogenase (LD) isozyme spectrum, with an increase in cathode fractions [2, 3]. The writers have emphasized that the degree of change in LD activity in the serum and in its isozyme spectrum depends on the total area of the burn and they have suggested that disturbances of the LD isozyme composition in the blood serum in burns are connected with interruption of the respiratory function of the skin, and that they evidently take place not only in the blood but also in the skin itself.

The activity of LD and its isozymes in the zone of injury and in the adjacent areas of the skin was studied in animals in the early stages after burn injury.

EXPERIMENTAL METHOD

Experiments were carried out on male albino rats weighing 150-200 g. An open flame burn covering 20% of the body surface was produced. Intact rats served as the control. The animals were decapitated 1, 7, 14, or 22 days after burning. Burned skin (scab), intact skin taken at a distance from the burn (a strip of skin 0.5 cm wide) bordering the zone of the burn (border), and tissue directly beneath the scab of the experimental animals and beneath the dermis in the control (underlying tissue) were taken for investigation.

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TABLE 1. Changes in Activity of LD and Its Isozymes in Skin at Different Times after Burning (M ± m)

Skin region tested	Index	Control	Time of investigation after burning, days			
			1st	8th	14th	22nd
Intact skin	Total activity	2929,97±455,11	1261,00±129,12*	1039,00±109,91*	2984,00±246,00	2571,00±57,00*
	LD_1	5,60±1,10	7,36±1,36	4,25±0,16	5,20±1,70	3,03±0,25
	LD_2	13,83±1,05	15,80±1,27	12,92±2,60	13,40±0,30	8,2±0,80
	LD_3	18,36±0,96	21,86±1,02	16,55±2,67	15,00±2,50	14,10±2,40
	LD_4	24,19±0,85	25,82±1,01	25,32±0,73	24,60±1,50	25,50±3,10
	LD_5	37,30±2,66	27,94±1,92*	42,42±3,36	41,60±3,00	40,90±6,30
Scab	Total activity		784,25±131,93*	1108,00±76,16*	849,00±220,00*	772,33±83,90*
	LD_1		4,95±0,33	10,28±1,44*	6,80±0,45	3,86±0,28
	LD_2		8,07±0,62*	10,55±1,40*	8,20±1,70*	9,56±2,20*
	LD_3		14,72±0,70*	20,15±1,39	17,80±0,50	19,90±3,46
	LD_4		26,20±0,57	24,85±0,48	23,70±1,50	24,86±0,74
	LD_5		45,97±0,82	35,65±2,97	43,50±1,10	41,80±4,76
Border	Total activity		1478,25±9,76*	1922,33±251,03*	1305,00±244,0*	2035,00±21,70*
	LD_1		4,80±1,25	10,77±2,14*	7,63±3,43	3,26±0,49
	LD_2		8,45±1,26*	14,40±0,98	12,06±2,22	9,96±0,73
	LD_3		14,50±1,06*	21,13±2,43	17,10±1,08	19,70±1,80
	LD_4		25,27±1,04	22,40±2,34	28,50±1,65	27,70±0,75
	LD_5		46,95±4,08*	31,30±2,49	34,70±5,10	42,13±5,34
Underlying tissue	Total activity	5082,00±432,34	952,00±93,88*	926,00±181,68*	964,00±97,81*	1066,00±236,00*
	LD_1	10,80±0,98	4,43±1,28*	15,83±2,22*	4,87±1,11*	4,76±0,96*
	LD_2	17,40±0,86	8,60±0,77*	12,40±1,70*	7,67±0,77*	10,00±0,99*
	LD_3	20,0±1,02	10,97±0,41*	16,16±0,63*	15,60±1,47*	16,96±0,37*
	LD_4	21,6±0,76	22,22±1,31	20,96±3,86	25,65±1,34	23,80±0,72
	LD_5	24,6±1,20	52,60±2,15	34,63±0,78	47,70±3,54*	43,06±1,18*

*P < 0.05 relative to control.

LD activity was determined [13] in saline extracts from all samples. Isozymes were fractionated by electrophoresis on agar gel followed by staining with tetrazolium salts in the substrate medium and determined quantitatively [5].

EXPERIMENTAL RESULTS AND DISCUSSION

After burning considerable changes were observed in total LD activity in all regions of skin investigated (Table 1). The greatest changes were found in the zone of the scab and the underlying tissue, where the enzyme activity fell by 70-80%. LD activity in the border and intact skin fell on average by 50%. This decrease continued throughout the period of investigation in the region of the scab, in the underlying tissue, and in the border, whereas in the intact skin it was observed on the 1st and 8th days only.

The LD isozyme spectrum also showed substantial changes after burning. On the 1st day an increase in fraction 5 (by 232%) and a decrease in fractions 2 and 3 (by 41.3 and 20.2%, respectively) were observed in the zone of necrosis. Shifts in similar directions were found in the border and underlying tissue (an increase in fraction 5 by 25.9 and 118.3%, a decrease in fraction 2 by 38.8 and 50.6% and in fraction 3 by 21.1 and 50.6% respectively). A decrease in fraction 1 (by 59.0%) also was observed in the underlying tissue. In the intact skin, by contrast with the other regions studied, the cathode fraction was reduced (by 25.1%).

By the 8th day the changes in the LD isozyme spectrum of the skin in the scab and border were no longer similar: Fractions 2 and 3 were increased in the border up to the control level, whereas in the zone of necrosis only fraction 3 reached control figures and activity of fraction 2 remained low. In both regions activity of fraction 1 was considerably increased (by 92.3 and 83.6% respectively). In the underlying tissue the activity of fraction 5 remained 43.7% above normal, and that of fractions 2 and 3 was below normal (by 11.1 and 19.2% respectively). Activity of fraction 1 was 46.5% above normal. In the intact skin, the isozyme spectrum was restored to normal by this stage of the investigation.

In the zone of necrosis activity of LD fraction 2 remained lowered (by 41.0 and 30.8%) even on the 14th-22nd day after burning. In the region of the border by this time the normal LD isozyme composition was restored. In the underlying tissue the increase in activity of fraction 5 and decrease in fractions 2 and 3 of LD continued throughout the period of investigation; activity of fraction 1 fell again until the 14th day and was still below normal 3 weeks after burning.

In the early periods after burning marked changes in LD activity were thus observed in all regions of the skin studied, including intact skin, but the greatest changes occurred in the zone of burning and in the underlying tissue. Marked inhibition of LD activity in the burned skin and in the adjacent and underlying tissues continued throughout the period of investigation. This demonstrates the profound inhibition of respiratory function of the skin in burns. The LD isozyme spectrum also showed a characteristic reorganization, with decrease in fractions adapted for function under aerobic conditions and activation of fractions functioning under anaerobic conditions [9]. The oxygen uptake of the burned skin 5 min after burning has been shown to be reduced by 69.5%, and it still remains lowered by 19.6% 120 min after burning [8].

Normalization of the LD isozyme composition in the border and to some extent in the zone of the burn on the 2nd-3rd week after trauma was evidently due to active proliferation beginning at this time.

The changes discovered in the LD isozyme spectrum in the burned skin, border, and underlying tissue of the animals in the early stages after burning were analogous to changes in the LD spectrum in the blood serum of burned patients [3, 4]. This parallel may point to a role of the intact skin in the genesis of the isozyme reorganization in the blood.

However, in burns it is possible that the parallel reorganization of the LD isozyme spectrum in the blood serum and skin may be a manifestation of a general response of the body to thermal injury.

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PHOSPHOLIPIDS AND CHOLESTEROL OF THE BRAIN AND SPINAL CORD OF GUINEA PIGS POISONED WITH TRICRESYL PHOSPHATE

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The content of phospholipids and of total, free, and esterified cholesterol in the brain and spinal cord of guinea pigs with severe neuroparalytic signs of poisoning caused by intradermal injection of tricresyl phosphate (TCP) was investigated. No change was found in the content of phospholipids and total cholesterol in either the brain or the spinal cord, but accumulation of cholesterol esters — the characteristic degradation products of myelin sheaths — was observed.

KEY WORDS: *tricresyl phosphate; phospholipids; cholesterol; demyelination; spinal cord.*

Certain organophosphorus compounds (OPC) widely used in agriculture and industry have a marked neurotoxic action. In particular, tricresyl phosphate (TCP), in cases of chronic poisoning, leads to the development of permanent pareses and paralyses of the limbs and to disability [1, 4, 5]. Despite many clinical and histological investigations, the fundamental problems of the pathogenesis of demyelination produced by OPC still remain unsolved, possibly because changes in the content of the lipid components of the myelin sheaths have received little study.

The object of this investigation was to produce an experimental model of chronic TCP poisoning in mammals with the development of neuroparalytic signs and to investigate the content of phospholipids and cholesterol in the brain and spinal cord, the main site of myelin sheaths.

EXPERIMENTAL METHOD

Experiments were carried out on adult male guinea pigs weighing 350-450 g into which TCP (an industrial mixture containing 37% of the ortho isomer) was injected intradermally in a dose of 2.0-2.2 ml/kg body weight. The animals with severe neuroparalytic signs of TCP poisoning were killed 25-30 days later and the brain stem and lumbar region of the spinal cord were removed for biochemical analysis.

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