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LACTATE DEHYDROGENASE IN PSEUDOXANTHOMA ELASTICUM

TOTAL ACTIVITY AND ISOENZYME DISTRIBUTION IN THE SKIN*

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ABSTRACT

Total activity and isoenzyme distribution of lactate dehydrogenase (LDH) were assayed in the skin of patients with pseudoxanthoma elasticum (PXE). The values obtained from lesions of 9 patients with typical PXE were compared to those obtained from 8 age-matched controls.

In the skin, all LDH isoenzymes, with the exception of LDH_1, could be detected, LDH_5 being the predominant one.

The isoenzyme pattern in PXE was characterized by decreased LDH activity in isoenzyme fraction LDH_5 and increased in fraction LDH_2, as compared to the controls. In agreement with these changes, the relative proportion of polypeptide M, the sub-unit predominant in cathodic isoenzymes, was decreased in PXE. Total LDH activity was not changed in this disorder.

Pseudoxanthoma elasticum (PXE) is a relatively rare, genetically determined disease, particularly affecting skin, eyes and the cardiovascular system (1). In the skin, the disease is manifested by the appearance of small yellowish papules. Previous biochemical investigations have shown increased calcium content and decreased collagen concentrations in the lesions, but earlier studies have failed to reveal any definite abnormality in the metabolism of the diseased skin in PXE (2, 3).

Lactate dehydrogenase (LDH) is the enzyme catalyzing the conversion of pyruvate to lactate, *i.e.* the last step in anaerobic glycolysis. Its activity and isoenzyme composition in tissues are regulated by several factors, and changes in these parameters have been reported in various clinical disorders. Because the metabolism of the calcified lesions could be thought to be different from that in normal skin, in the present study we tried to characterize metabolic aspects in PXE by determining total activity and isoenzyme distribution of lactate dehydrogenase. The determinations were made using assay conditions specially controlled for their reproducibility and reliability for analyses of LDH in human skin (4). The values obtained from the lesions of 9 cases with typical PXE were compared with those in the normal skin of 8 age-matched controls.

MATERIAL AND METHODS

Patients and control subjects. The series included 9 patients with typical PXE skin lesions. A detailed description of the clinical findings is given in a previous paper; the present patients appear in that study as cases # one to nine (5). The values obtained from patients with PXE were compared to those of 8 age-matched controls. The control subjects were patients hospitalized due to venereal or local skin diseases. Neither their disease nor a possible medication could be thought to affect the assays made. An 8-mm full-thickness skin biopsy from an axillary lesion was taken from patients with PXE with a punch under local anesthesia. In the controls, a punch biopsy from the corresponding site was removed. All the biopsies were taken on the same day and by the same person (L.D.), and the skin specimens were immediately used for further analyses.

Preparation of tissue specimens. The samples were quickly and carefully freed of subcutaneous fat, weighed with a torsion balance, and homogenized in 1 ml of cold (0° C) physiological saline with an Ultra-Turrax homogenizer. The homogenate was centrifuged at 4° C for 30 minutes, and a small aliquot of the 15,000 × g supernatant was immediately used for determination of total activity and isoenzyme distribution of LDH. The rest of the supernatant and the precipitate were combined and used for determination of tissue DNA following the Schmidt-Thannhauser method (6) modified according to an earlier description (3).

Determination of total LDH activity. The activity of LDH was determined in 0.067 M phosphate buffer (pH 7.4) at 25° C using 10×10^{-4} pyruvate and 1.3×10^{-4} M NADH as substrates. The reaction rate was followed by measuring the oxidation of NADH with a Gilford spectrophotometer connected to a Honeywell strip chart recorder. The values for enzyme activity were expressed as International Units (I.U. = conversion of micromoles of substrate per minute) and further correlated either to the wet weight of skin (I.U./kg) or to the DNA in the corresponding tissue specimen (mI.U./ng DNA).

Determination of LDH isoenzyme distribution. The

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LDH isoenzymes were separated by using cellulose acetate electrophoresis, as described in detail elsewhere (4). The isoenzymes were visualized by staining the membranes with a Nitro-BT method (7), and the percent proportion of each isoenzyme was quantified by scanning the electropherograms with a Beckman Model R-110 densitometer. The subunit M percentage of LDH was calculated from the isoenzyme pattern by assuming that the isoenzymes from LDH_1 to LDH_5 contain 0, 25, 50, 75 and 100 percent of subunit M, respectively.

Presentation of the results. The results are presented as mean ± S.D., and the significance of the differences between the controls and the PXE group was tested using Student's *t*-test. The differences were regarded as significant at the level $p \leq 0.05$.

RESULTS

When total LDH activity was determined in the punch biopsy specimens of skin, no difference between the values in patients with PXE and those in the controls could be observed (Table I). However, when various isoenzymes of LDH were electrophoretically separated, the isoenzyme distribution in PXE was found to be different from that of the controls. The enzyme activity in isoenzyme fraction LDH_5 was significantly lower in PXE, and also the relative proportion of this isoenzyme out of the total activity was decreased, as compared to the controls (Table II). On the other hand, the relative distribution of LDH_2 was significantly increased in PXE, but the actual enzyme activity in the corresponding fraction did not differ significantly from the controls ($0.1 > p > 0.05$), probably due to considerable scatter in the latter values (Table II). Furthermore, the subunit M percentage of LDH, calculated from the isoenzyme pattern, was decreased in PXE (Table III). A typical LDH isoenzyme pattern in the skin from a patient with PXE and from a control is presented in the Figure.

The concentration of DNA in the skin was not changed in PXE. The values for DNA in PXE and the controls were 0.61 ± 0.06 and 0.60 ± 0.08 μg per mg wet weight of skin (mean ± S.D.), respectively. These values have been reported by us previously (3).

TABLE I

Total LDH activity in the skin of patients with pseudoxanthoma elasticum and of controls

Aliquots of the 15,000 × g supernatant of the skin homogenate were used for the determination of LDH activity, as described in Material and Methods.

Group	No. of patients	Lactate dehydrogenase activity*	
		I.U./kg wet weight of skin	mI.U./mg DNA
Control	8	12,623 ± 1,352	21,060 ± 3,000
PXE	9	11,746 ± 1,972	19,141 ± 3,280
p-values**		NS	NS

* The values are expressed as mean ± S.D.

** Calculated using Student's t-test; NS = statistically not significant.

TABLE III

The subunit M percentage of LDH in the skin of patients with pseudoxanthoma elasticum and of controls

The subunit M percentage was calculated from the isoenzyme pattern, as described under Material and Methods.

Group	No. of patients	Subunit M percentage* %
Control	8	85.8 ± 4.2
PXE	9	81.0 ± 2.7
p-value**		<0.01

* The values are expressed as mean ± S.D.

** Calculated using Student's t-test.

TABLE II

Lactate dehydrogenase activity and relative distribution of various isoenzyme fractions in the skin of patients with pseudoxanthoma elasticum and of controls

The activity of lactate dehydrogenase in various isoenzyme fractions was obtained by dividing the LDH total activity into fractions corresponding to the relative proportion of each isoenzyme. The isoenzyme distribution was determined by separating the isoenzymes electrophoretically, and, thereafter, scanning the stained membranes, as described in Material and Methods.

Group	No. of patients	LDH_1		LDH_2		LDH_3		LDH_4		LDH_5	
		Activity*	%**	Activity	%	Activity	%	Activity	%	Activity	%
Control	8	—	—	350 ± 440	2.6 ± 3.3	1,940 ± 602	15.0 ± 3.9	2,481 ± 315	19.4 ± 1.5	7,850 ± 1,230	63.1 ± 6.3
PXE	9	—	—	703 ± 298	6.0 ± 2.7	2.114 ± 384	18.2 ± 3.0	2,578 518	21.9 ± 1.8	6,351 ± 1,200	53.9 ± 4.2
p-values***		—	—	0.05 < p < 0.1	<0.05	NS	0.05 < p < 0.1	NS	NS	<0.02	<0.005

* The values are expressed as I.U./kg wet weight of skin (mean ± S.D.).

** The values are expressed as per cent of total activity in each individual case (mean ± S.D.).

*** Calculated using Student's t-test; NS = statistically not significant.

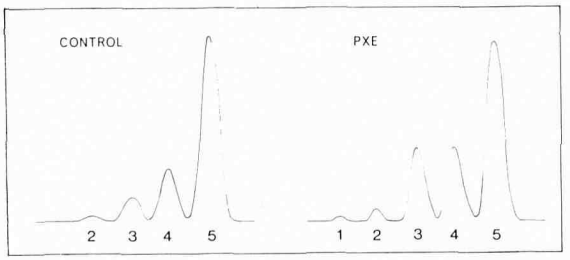

FIGURE. A typical LDH isoenzyme pattern in the skin of a patient with PXE and of a control. The isoenzymes were separated using cellulose acetate electrophoresis and the stained membranes were scanned with a densitometer. The numbers in the Figure refer to corresponding LDH isoenzymes.

DISCUSSION

The conversion of pyruvate to lactate, *i.e.* the last step in the anaerobic glycolysis, is catalyzed by the enzyme lactate dehydrogenase (LDH), with the systemic name L-lactate:NAD oxidoreductase (E.C. I.I.I.27). LDH exists in the form of five isoenzymes, assumed to be built of four polypeptide chains of two different kinds (8), called subunit H and M (9).

In the present study, LDH in human skin was found to be composed predominantly of cathodically migrating isoenzymes LDH_5 and LDH_4. This is in agreement with earlier publications on LDH in the skin (4, 10, 11, 12). The isoenzyme pattern in PXE was modified by a shift towards subunit H containing isoenzymes.

To explain the latter finding, the following theories of the factors regulating the LDH isoenzyme composition in tissues can be considered: i) change in ambient oxygen tension, ii) altered mitotic activity, iii) degradation of specific isoenzymes, and iv) change in cell composition. Changes in the ambient oxygen tension are able to modify the isoenzyme pattern. The synthesis of subunit M is favored by the hypoxic conditions (13–15), and consequently, the cathodic isoenzymes, i.e. those mainly containing subunit M, are predominant in tissues characterized by anaerobic metabolism (16). The possibility that the oxygen tension in the PXE skin is increased seems unlikely in the hardened skin, especially as no abnormalities in the small blood vessels have been found (17).

Increased mitotic activity has been suggested to be connected with the formation of subunit M independently of the oxygen level (18), but, on the other hand, this theory has been challenged (19). DNA concentration in PXE skin is normal. This seems to suggest that the cellular proliferation may not be impaired, thus making the latter possibility dubious.

The individual isoenzymes differ in their stability in many respects. The cathodic isoenzymes have lower thermal stability (20) and they are more susceptible to denaturation *e.g.* by urea than the anodic ones (21). It has previously been found that, despite unchanged synthesis, the concentrations of collagen and total proteins are markedly decreased in the skin in PXE (2, 3), thus suggesting enhanced protein degradation. Furthermore, it has been found that the degradation rate of LDH_5 (composed of four M subunits) is different in different tissues, which suggest environmental regulation of isoenzyme destruction (22). Based on these, it could be thought that the changes observed in PXE are due to specific degradation of subunit M peptides. However, the total LDH activity in the present work was unchanged in PXE skin. This suggests that only a shift in LDH isoenzyme pattern took place.

In addition, the change in epidermis/dermis ratio could be thought to contribute to the shift observed in LDH isoenzyme pattern, because these tissue layers differ considerably in their isoenzyme composition (4). However, no marked changes in the epidermis in PXE have been reported (17).

In conclusion, the LDH isoenzyme composition in diseased skin in PXE is changed to a pattern composed more of H subunits. No firm conclusion about the cause of this change can be found.

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