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Lactate Dehydrogenase and its Isoenzymes in the Marrow and Peripheral Blood from Haematologically Normal Subjects

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Summary

Lactate dehydrogenase (LDH) activity and its isoenzymatic fractions were measured in bone marrow blood and in peripheral venous blood from 16 haematologically normal subjects. Total LDH activity was significantly higher in marrow than in venous blood (428.8±98.4 vs 260.1±40.2 mU/l, $p<0.0001$). The same was true for the absolute values of its isoenzymatic fractions. The percentage fractions LDH 1 and LDH 5 were similar in the two regions, while LDH 3 and LDH 4 were higher in medullary blood ($p<0.05$) and LDH 2 was higher in peripheral blood ($p<0.05$). The Spearman test showed a limited correlation between marrow and peripheral total LDH activity values ($p<0.05$). This seems to be at least in part sustained by the highly significant correlations existing in LDH 3 and LDH 4 values, reported to be pre-eminent isoforms in maturing haematopoietic cells ($p<0.005$ and $p<0.001$, respectively). These findings could be attributed to an apoptotic regulation of marrow cell production.

Key words
Lactate dehydrogenase – Isoenzymes – Marrow blood – Venous blood

Introduction

Anaerobic glycolysis plays an important role in the energy metabolism of haematopoietic cells. The last step of the pathway, the pyruvate-lactate reversible conversion, is catalyzed by the cytoplasmic enzyme lactate dehydrogenase (LDH), that transfers H^+ with the concurrent oxidation of NADH. Thus, in various haematologic disorders accompanied by enhanced cell destruction serum levels of LDH activity are increased in peripheral blood. Produced by the action of two genes, two different kinds of polypeptidic chains, H and M, comprise together five tetrameric active fractions of LDH : two electrophoretic extremes, LDH 1 (H_4) and LDH 5 (M_4), and three intermediate hybrid forms, LDH 2 (H_3M), LDH 3 (H_2M_2), LDH 4 (HM_3). Thus, different LDH isoenzyme profiles have been reported in haemolysis, leukaemias and thrombocytaemia (Cawley 1974, Carcamo *et al.* 1993, Patel *et al.* 1994). On the other hand, previous reports have suggested a bone marrow source of the higher LDH peripheral levels in megaloblastic anaemia as a consequence of severely ineffective erythropoiesis (Heller *et al.* 1960, Elliot and Fleming 1965).

Since previous studies were carried out mostly on pathological individuals and on peripheral blood, the aim of the present study was to perform measurement in haematologically normal subjects and to compare the total activity and isoenzymatic fractions of LDH in bone marrow blood and in peripheral venous blood.

Patients and Methods

The study was carried out, after informed consent, on patients undergoing bone marrow aspiration for diagnostic purposes, showing normal blood cell counts and normal serum LDH activity in routine analysis. Exclusion criteria were haematologic and coagulative disorders, coronary artery disease, obstructive peripheral arteriopathy, cerebral ischaemic

disease, impaired hepatic and renal function, neoplastic diseases.

After gentle aspiration of marrow samples from the iliac crest or sternum peripheral vein blood samples were collected shortly after. In 16 patients, who had no haematological disorders after a diagnostic examination, 10 men and 6 women, 54.5±9.8 years old, LDH activity was measured in their marrow and peripheral serum using a photometrical routine method assessing the reduced form of nicotinamide-adenine dinucleotide, that accompanies the LDH-catalyzed conversion of pyruvate to lactate (LD-P Reagent, Synchron CX System, Beckman Instruments, CA, USA). The normal reference interval for adults in venous blood serum expressed in mU/l was 150–400. LDH isoenzymes were separated by electrophoresis on agarose gel and quantified using the fluorescence mode as percentages of the total LDH activity (REP LD-2 STAT Isoenzyme Procedure, Helena Labs, Beaumont, TX, USA). The reference intervals for venous serum LDH isoenzyme percentages in normal adults were: 14.8–25.4 % for LDH 1, 31.7–41.4 % for LDH 2, 18.1–25.90% for LDH 3, 7.2–13.6 % for LDH 4, 5.3–16.5 % for LDH 5. The absolute values of the isoenzymatic activities were calculated from the percentages of the total LDH activity and expressed in mU/l.

The Mann-Whitney U-test and the Spearman correlation test were used for statistical analysis.

Table 1. Total and isoenzyme LDH activities (±S.D.) and isoenzyme percentage fractions in the bone marrow and venous blood in haematologically normal subjects

		M	V	p <*	Spearman r	p <
Total LDH	(mU/l)	428.88±98.43 (293–605)	260.13±40.24 (202–334)	0.0001	0.53	0.05
LDH 1	(%)	21.41±6.15 (10.52–27.90)	22.98±6.35 (14.31–28.92)	N.S.	0.10	N.S.
	(mU/l)	90.04±22.46 (47.12–118.65)	57.15±14.72 (40.73–73.15)	0.001	0.25	N.S.
LDH 2	(%)	30.57±3.39 (23.69–32.78)	34.48±4.55 (28.57–37.74)	0.05	−0.28	N.S.
	(mU/l)	130.91±28.11 (104.31–139.64)	91.35±16.83 (57.81–106.70)	0.001	0.17	N.S.
LDH 3	(%)	22.22±3.43 (18.89–28.85)	19.22±4.60 (13.18–27.44)	0.05	0.28	N.S.
	(mU/l)	95.92±23.88 (67.05–129.24)	55.60±21.15 (26.62–91.64)	0.001	0.72	0.005
LDH 4	(%)	13.31±3.47 (8.96–19.73)	10.68±3.26 (7.35–15.35)	0.05	0.42	N.S.
	(mU/l)	53.21±18.35 (35.47–88.39)	27.95±12.96 (18.03–51.26)	0.001	0.78	0.001
LDH 5	(%)	13.48±3.51 (7.82–18.09)	12.70±3.90 (8.45–19.80)	N.S.	0.21	N.S.
	(mU/l)	59.06±21.26 (28.85–88.09)	33.67±10.44 (23.66–51.16)	0.01	0.32	N.S.

*Data are means±S.D., range of values is given in parentheses. *Mann-Whitney U test and Spearman r statistical significance, n = 16.*

Results

Results are given in Table 1. We found the total LDH and absolute values of its isoenzymatic fractions to be significantly higher in medullary blood than in peripheral venous blood. The percentage distribution of LDH isoenzymes showed a significant difference of the two moieties in LDH 3 and LDH 4, which were higher in medullary blood ($p<0.05$), and in LDH 2, that was higher in peripheral blood ($p<0.05$), while no significant differences were found in LDH 1 and LDH 5 when expressed in percentages.

A significant ($p<0.05$) Spearman rank correlation was found between medullary and peripheral total LDH values. No significant correlation was found, however, between medullary and peripheral isoenzymatic percentage fractions and between LDH 1, LDH 2 and LDH 5 absolute values, while a highly significant correlation was found between the medullary and peripheral values of LDH 3 ($p<0.005$) and LDH 4 ($p<0.001$).

Discussion

The present study shows that in haematologically normal subjects there are significant differences between peripheral venous and bone marrow blood in total and fractionated LDH activities. Since previous studies showed that there are no differences between these regions in the corpuscular : plasmatic ratio and in the overall composition (Mazzuoli *et al.* 1992, Aliberti *et al.* 1996, 1997), the differences observed do not seem to be attributable to spurious determination of enzymatic activities in the marrow blood. The total LDH activity observed in marrow blood was significantly higher compared to that observed in peripheral venous blood, since a certain proportion of enzymatic activities originating from marrow cells will appear in the perfusing blood. The lower LDH activity found in peripheral venous blood can easily be explained by the dilution of marrow blood in the general circulation.

In fact, it is well known from erythrokinetic studies that a small proportion of ineffective erythropoiesis is detectable in normal subjects (London *et al.* 1950). However, the medullary LDH may also arise, although to a limited extent, from the extrusion of nuclei and of a rim of cytoplasm accumulated near the nuclear border (Skutelsky and Danon 1967, Tavassoli and Crosby 1973). Moreover, a contribution may be expected from physiologically ineffective leukopoiesis. It is of particular interest in this regard that a "myelocyte sink" in marrow flow of cells has been observed during granulocytopoiesis. The difference between myelocyte production and metamyelocyte input seems to support the concept of death-controlled marrow production, in relation to decreased or enhanced peripheral use (Cronkite 1964). Ineffective thrombopoiesis has been demonstrated in man only in some pathological situations (Harker and Finch 1969). However, a lower number of megakaryocytes than would be expected from the number of megakaryocytes progenitors (CFU-M) was reported in mice (Williams and Jackson 1978). Furthermore, the CFU-M compartment was found to have a limited capacity for self-renewal (Williams *et al.* 1978). Therefore, the extensive spectrum of higher LDH activity levels observed in bone marrow blood may be interpreted as an expression of this apoptotic medullary modulation of cell production. Moreover, the limited significance of the correlation between the LDH values found in the marrow and peripheral blood seems to indicate that the LDH activity found in bone marrow blood is largely an expression of local physiological behaviour, since it seems to be pre-eminently due to the significant correlation between the absolute values of medullary and peripheral LDH 3 and LDH 4 activities, while no correlation was found in LDH 1, LDH 2 and LDH 5 values and/or in their percentage representation.

Although consisting of the five types, the isoenzymatic content of haemopoietic precursors has been shown, in fact, to vary in their profile with the degree of cellular maturation. Thus, for the erythropoietic series, a high content of LDH 3 and LDH 4 has been found in proerythroblasts, erythroblasts and late normoblasts (Starkweather *et al.* 1966). This has also been reported in granuloblastic and lymphoblastic precursors, while in granulocytes and lymphocytes LDH 5 was the pre-eminent (Starkweather *et al.* 1966) or main isoenzymatic fraction (Blatt *et al.* 1982, Xue and Yeung 1996). Probably, for these reasons, large differences were found between marrow and peripheral blood in LDH 3 and LDH 4 values, with highly significant correlations between the two compartments. In contrast, no such correlation was found for LDH 1 and LDH 2 present in mature red cells and derived into peripheral blood from large reservoirs, including muscles, heart and overall cell turnover. Their peripheral percentages were, in fact, higher than the respective marrow percentages, although the difference was not significant for LDH 1. Moreover, the LDH 5 isoform, being largely present in mature granulocytes, liver and mature lymphocytes showed the lowest difference between the two moieties.

In conclusion, the total LDH activity in normal subjects is higher in the bone marrow than in peripheral blood. This seems to be an expression of apoptotic regulation of cell production. The isoenzymatic fractions exhibited a significant correlation between the two regions in LDH 3 and LDH 4 activities, the content of which is predominant in haematological precursors.

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