Aqueous humour lactic dehydrogenase isoenzymes in retinoblastoma

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In 1971, Dias, Senthe Shanmuganathan, and Rajaratnam reported an association between retinoblastoma and an elevated total aqueous humour lactic dehydrogenase (LDH). These investigators found that the total aqueous humour LDH activity in 46 patients with non-neoplastic ocular conditions ranged from 0 to 350 units/100 ml and the total aqueous humour LDH activity in four patients with retinoblastoma ranged from 1800 to 3250 units/100 ml. (These units are roughly comparable to i.u./l.)

In 1973, Barber cited this investigation and suggested that 'determination of lactate dehydrogenase isoenzymes might be even more revealing'. To our knowledge there are no published reports concerning the LDH isoenzyme fractions in the human aqueous humour and the purpose of the present study, therefore, was to investigate the aqueous humour LDH, including the isoenzyme fractionation patterns, in retinoblastoma and other paediatric ophthalmological conditions.

Material and methods

The subjects were patients admitted to the Children's Memorial Hospital, Chicago, Illinois, for elective intra-ocular surgery or for diagnostic evaluation of suspected retinoblastoma. The aqueous humour samples, consisting of 150 μ l of primary aqueous, were obtained under general anaesthesia by anterior chamber paracentesis at the limbus using the Weck^(R) disposable paracentesis pipette. Simultaneous venous blood samples were obtained from most subjects.

The total LDH determinations were performed at 37°C using the LKB model 8600 kinetic enzyme analyser and Boehringer-Mannheim^(R) reagent kit. The isoenzyme fractionations were performed by rapid electrophoretic separation (Preston, Briere, and Batsakis, 1965) and the fractions were quantified colourmetrically (Raabo, 1963).

Results

The series consisted of ten patients; seven had non-

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neoplastic ocular conditions, and three had bilateral untreated retinoblastoma subsequently confirmed histologically. Both eyes were studied in one of the patients with retinoblastoma. None of the aqueous humour samples was contaminated with blood.

Table I identifies each patient's condition and shows the total LDH activity in the serum and aqueous. There was no correlation between the serum and aqueous humour LDH activity. The total aqueous humour LDH activity in the seven patients with non-neoplastic ocular conditions ranged from 0 to 99 i.u./l. The total aqueous humour LDH activity in the three patients with retinoblastoma ranged from 56 to 1832 i.u./l.

Table I Total LDH activity in serum and aqueous*

Diagnosis	Serum	Aqueous
Retinoblastoma	(not done)	1832
Retinoblastoma (right eye)	224	158
Retinoblastoma (left eye)	224	124
Retinoblastoma	413	56
Persistent hyperplastic primary vitreous	452	99
Congenital glaucoma	455	0
Traumatic cataract	(not done)	17
Traumatic cataract	388	0
Secondary membrane	399	43
Secondary membrane	224	9
Secondary membrane	421	0

* International units/litre

Table II shows the aqueous humour LDH isoenzyme fractions and the values for the ratio LDH₅:LDH₁. No isoenzyme patterns were obtained in six of the seven patients with non-neoplastic ocular conditions because LDH activity was either absent or too low to fractionate. Isoenzyme patterns were obtained in all three patients with retinoblastoma and in the patient with persistent hyperplastic primary vitreous. In the patients with retinoblastoma the ratio LDH₅:LDH₁ ranged from 5.1 to 17.0 and in the

Table II Aqueous humour LDH isoenzyme fractions*

Diagnosis	LDH _{Total}	LDH ₁	LDH ₂	LDH ₃	LDH ₄	LDH ₅	LDH ₅ /LDH ₁
Retinoblastoma	1832	97	300	469	474	492	5.1
Retinoblastoma (right eye)	158	3	7	39	61	48	16
Retinoblastoma (left eye)	124	6	9	25	44	40	6.7
Retinoblastoma	56	1	4	14	20	17	17
Persistent hyperplastic primary vitreous	99	22	14	18	15	30	1.4

* International units/litre.

patient with persistent hyperplastic primary vitreous the ratio LDH₅:LDH₁ was 1.4.

Comment

Serum and aqueous humour LDH activity showed no correlation. Wroblewski (1957) reported that the normal range of total LDH activity in the cerebrospinal (CSF) was 0 to 40 Wroblewski units/ml, (roughly comparable to i.u./l.), and that there was no correlation between the serum and the cerebrospinal fluid LDH. He attributed this to a function of the blood-brain barrier. The range of total aqueous humour LDH activity for the patients with non-neoplastic ocular conditions in the present study was comparable to that reported for the normal CSF. From the known similarities between the blood-brain barrier and the blood-aqueous barrier it is suggested that the lack of correlation between the serum and aqueous humour LDH may be a function of the blood-aqueous barrier.

The range of total aqueous humour LDH activity for the seven patients with non-neoplastic ocular conditions was lower than that reported for a similar group (Dias and others, 1971). The total aqueous humour LDH activity in one patient with retinoblastoma was elevated and fell within the range reported for retinoblastoma, but the total aqueous humour LDH activity in two patients with retinoblastoma was not elevated and fell within the range reported for non-neoplastic ocular conditions (Dias and others, 1971). However, the aqueous humour LDH isoenzyme fractionation patterns in all three patients with retinoblastoma were similar (LDH₅:LDH₁ greater than 5), and were different from the pattern obtained in the patient with persistent

hyperplastic primary vitreous (LDH₅:LDH₁ = 1.4).

Wroblewski (1957) postulated that neoplastic conditions are associated with an elevated LDH because neoplastic cells liberate increased quantities of LDH into the fluid medium which bathes them. The results obtained in the present study indicate that retinoblastoma is not invariably associated with an elevated total aqueous humour LDH, but suggest that retinoblastoma may be associated with a characteristic aqueous humour LDH isoenzyme fractionation pattern which is present before the total aqueous humour LDH becomes elevated. Consequently, the LDH isoenzymes may prove to be of greater diagnostic significance in retinoblastoma than the total enzyme concentration.

Summary

LDH activity was determined in aqueous humour samples from 11 eyes (of 10 children), four of which contained retinoblastoma. Simultaneous serum LDH levels were also determined in eight of the children.

There was no correlation between serum and aqueous humour LDH activity.

Total aqueous humour LDH activity ranged from 0 to 99 i.u./l. in the seven eyes with non-neoplastic conditions. It was 56, 124, 158, and 1832 i.u./l. respectively, in the four eyes with retinoblastoma. In all four eyes the ratio of isoenzymes LDH₅:LDH₁ was greater than 5.

The total aqueous humour LDH level in retinoblastoma was neither consistently elevated, nor related to the total serum LDH. There was a characteristic LDH isoenzyme fractionation pattern which, it is suggested, may be present before the total aqueous humour LDH becomes elevated.

References

- BARBER, G. W. (1973) *Arch. Ophthalmol.*, **89**, 236
 DIAS, P. L. R., SENTHE SHANMUGANATHAN, S., and RAJARATNAM, M. (1971) *Brit. J. Ophthalmol.*, **55**, 130
 PRESTON, J., BRIERE, R. O., and BATSAKIS, J. G. (1965) *Amer. J. clin. Pathol.*, **43**, 256
 RAABO, E. (1963) *Scand. J. clin. Lab. Invest.*, **15**, 405
 WROBLEWSKI, F. (1957) *Amer. J. med. Sci.*, **234**, 301