Quebec Cooperative Study
of Friedreich's Ataxia

Pyruvate Dehydrogenase, Lipoamide Dehydrogenase and Citrate Synthase Activity in Fibroblasts from Patients with Friedreich's and Charlevoix-Saguenay Ataxia

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SUMMARY: *The activity of lipoamide dehydrogenase and two closely related enzymes was studied simultaneously in early, mid, and late passage fibroblast cultures. Friedreich's ataxia fibroblasts tended to lose pyruvate dehydrogenase and citrate synthase activities, while lipoamide dehydrogenase activity remained constant with aging of the cells. Mean pyruvate dehydrogenase activity was lower over-all in fibroblasts from ataxics. Mean citrate synthase activity was higher in ataxic fibroblasts. Present tissue culture media do not represent the best conditions in which to reproduce cofactor binding defects such as those found in other genetic diseases with structural enzyme mutations.*

RÉSUMÉ: *L'activité de trois enzymes du carrefour "pyruvate-acetyl CoA" ne varie pas de façon significative au cours du vieillissement des cultures de fibroblastes cutanés provenant de patients ataxiques et témoins. L'activité moyenne des déshydrogénases du pyruvate et de la lipoamide, ainsi que de la citrate synthétase ne difère pas entre les trois groupes sur le plan statistique. Cependant, certains éléments essentiels contenus dans le milieu nutritif des cellules peuvent masquer des défauts de régulation dans l'activité de ces enzymes, défauts suggérés par des activités moyennes haussées de la citrate synthétase et réduites de la pyruvate déshydrogénase dans les fibroblastes provenant d'ataxiques.*

INTRODUCTION

Previous studies by our group (Melançon et al.,1977) and other investigators (Filla et al.,1978) disclosed low levels of lipoamide dehydrogenase (LAD) in serum of patients with Friedreich's ataxia. Repeated attempts to demonstrate a comparable reduction of LAD in platelets (Filla et al.,1978) and cultured skin fibroblasts (Melançon et al., 1978a,1978b) from ataxic patients have, however, not been successful. In order to assess the effect of prolonged culture conditions upon enzyme activity, we have measured the level of LAD and two closely related enzymes, PDH (pyruvate dehydrogenase) and CS (citrate synthase) in skin fibroblasts from Friedreich's and Charlevoix-Saguenay ataxias at different times of passages in culture.

MATERIALS AND METHODS

Skin biopsies obtained from 3 normal controls, 4 patients with Charlevoix-Saguenay ataxia (CSA), and 5 patients with Friedreich's ataxia (FA) were cultured as previously described (Melançon et al., 1972). Confluent monolayers were harvested after 7, 14, and 21 passages and assayed within a week.

LAD was determined as previously described (Melançon et al.,1978b). PDH was assayed according to Blass et al. (1972) and CS by the method of Schulman and Blass (1971). All assays were performed in duplicate, patients and control cell lysates being studied simultaneously each time. Cell protein was determined according to Lowry et al. (1951).

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TABLE I

Effect of Time in Culture on Enzyme Activity of Normal and Ataxia Fibroblasts

		Specific Activity* At Passage #7	#14	#21	Mean Activity
Lipoamide Dehydrogenase					
Control	(3)	68.3±13.1	86.8±17.0	88.5±11.5	81.2±15.6
Charlevoix	(4)	65.8±27.0	65.7±14.0	88.7±10.3	72.0±20.4
Friedreich	(5)	79.3±11.5	87.0± 6.4	74.3±13.9	80.6±11.2
Pyruvate Dehydrogenase		20.6± 8.7	40.3± 5.7	25.5± 6.9	29.2±11.2
Control	(3)				
Charlevoix	(4)	39.0±26.7	27.6±11.4	12.3±11.7	27.6±20.0
Friedreich	(5)	23.2±29.4	20.1±19.2	15.3± 6.6	20.2±20.0
Citrate Synthase					
Control	(3)	1.11± .31	0.86± .17	1.01± .18	.99± .23
Charlevoix	(4)	1.14± .47	1.03± .29	1.96±1.34	1.26± .66
Friedreich	(5)	1.44± .24	1.40± .26	1.07± .34	1.32± .37

* Specific activity (M ± SD) in μmol/min/mg protein; LAD × 10^{-3}, PDH × 10^{-4} and CS × 10^{-5}

RESULTS

Our results are summarized in Table I. LAD activity was not affected by aging of either patients or control fibroblast cultures. Although Charlevoix-Saguenay cells showed lower over-all LAD values, no significant difference could be demonstrated. PDH activity was higher in mid and late passage control fibroblasts and lower in ataxias fibroblasts as culture aged. These differences were not statistically different. CS activity followed the opposite trend, with increased levels in early passage FA cells and late passage CSA cells. Mean CS activity was higher over-all in fibroblasts from ataxic patients as compared with control values.

DISCUSSION

These data and our previous observations using skin fibroblasts cultured from patients with typical Friedreich's ataxia (Melançon et al.,1978a) do not favor a genetic defect in the lipoamide dehydrogenase apoenzyme as the basic mechanism for pyruvate accumulation in Friedreich's ataxia. We have investigated twenty-two fibroblast cultures from such patients over a two-year period and found no significant reduction of LAD in any of the cultures tested. Other investigators (Strump, D., personal communication) have also experienced results similar to ours.

The artificial nutrient mixture used in skin fibroblast cultures does not, however, reproduce *in vivo* conditions. Eagle MEM nutrient mixture, for instance, contains pharmacological levels of a number of vitamins not normally encountered in living tissues (thiamine 1mg/1, riboflavin 0.1mg/1, pyridoxine 1mg/1, and nicotinamide 1mg/1). A point mutation in coenzyme binding affinity would therefore be masked under such conditions. Blass and Gibson (1977) have recently uncovered a higher than normal apparent Km value of transketolase for thiamine in extracts of fibroblasts from patients with Wernicke-Korsakoff syndrome. In their study, the activity of transketolase in the presence of excess thiamine pyrophosphate was slightly higher in patients than in control fibroblasts. However, the Km for binding of thiamine pyrophosphate was 10 to 20 times higher in fibroblasts from the patients than the controls. If a comparable structural mutation existed in one of the three components of the PDH complex in Friedreich's ataxia fibroblasts, normal enzyme activities would remain meaningless under present tissue culture conditions.

ACKNOWLEGMENTS

These studies were supported by grants from l'Association Canadienne de l'Ataxie de Friedreich, the Quebec Network of Medical Genetics (Ministère des Affaires Sociales) and the Medical Research Council of Canada. We thank Jean-Pierre Bouchard, Richard Gagné, Bernard Lemieux, Guy Geoffroy and André Barbeau who provided us with the skin biopsies.

REFERENCES

BLASS, J. P., SCHULMAN, J. D. and YOUNG, D. S. (1972). An inherited defect affecting the tricarboxylic acid cycle in a patient with congenital lactic acidosis. J.Clin. Invest., 51, 1845-1851.

BLASS, J. P. and GIBSON, G. E. (1977). Abnormality of a thiamine-requiring enzyme in patients with Wernicke-Korsakoff syndrome. New Engl. J. Med., 297, 1367-1370.

FILLA, A., BUTTERWORTH, R. F., GEOFFROY, G., LEMIEUX, B. and BARBEAU, A. (1978). Serum and platelet lipoamide dehydrogenase in Friedreich's ataxia. Can. J. Neurol. Sci.,5, 111-114.

LOWRY, O. H., ROSEBROUGH, N. J., FARR, A. L. and RANDALL, R. J. (1951). Protein measurement with the folin phenol reagent. J. Biol. Chem., 93, 265-275.

MELANCON, S. B., TAYCO, J. and NADLER, H. L. (1972). The free amino acid pool of cultivated human skin fibroblasts. Proc. Soc. Exp. Biol. and Med., 141, 391-395.

MELANCON, S. B., POTIER, M., DALLAIRE, L., GEOFFROY, G., LEMIEUX, B. and BARBEAU, A. (1977). Serum lipoamide dehydrogenase in Friedreich's ataxia. Pediat. Res., 11, 460.

MELANCON, S. B., POTIER, M., DALLAIRE, L., FONTAINE, G. GRENIER, B., LEMIEUX, B., GEOFFROY, G. and BARBEAU, A. (1978a). Lipoamide dehydrogenase in Friedreich's ataxia fibroblasts. Can. J. Neurol. Sci. 5, 115-118.

MELANCON, S. B., DALLAIRE, L. and POTIER, M. (1978b). Lipoamide dehydrogenase in cultured human skin fibroblasts. Clin. Clim. Acta. 87, 29-34.

SCHULMAN, J. D. and BLASS, J. P. (1971). Measurement of citrate synthetase activity in human fibroblasts. Clin. Chim. Acta, 33, 467-468.