SHORT COMMUNICATIONS

Effect of Sodium Dichloroacetate on Human Pyruvate Metabolism

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Sodium dichloroacetate (DCA) was administered orally at doses of 12.5 to 50 mg/kg body weight twice or three times per day to a patient with mitochondrial encephalomyopathy associated with congenital lactic acidemia. During therapy, the rates of decarboxylation of (1-^{14}C) pyruvate and (3-^{14}C) pyruvate, which represent the activity of the pyruvate dehydrogenase (PDH) complex and the function of the TCA cycle, respectively, were markedly increased in the platelets and increases in the lactate levels in the blood and urine during exercise were markedly reduced. These results suggest that oral administration of DCA causes significant increases in the activities of the PDH complex and TCA cycle not only in the platelets but also in various tissues of humans, which is important as a pathway for production of energy, resulting in decreases in the lactate and pyruvate levels in the blood and cerebrospinal fluid. ***Key words: Dichloroacetate, lactate, pyruvate, mitochondrial myopathy.***

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Recently, sodium dichloroacetate (DCA) has been used in attempts to treat congenital lactic acidosis, because it reduces the lactate levels of the blood and cerebrospinal fluid [1, 2]. DCA is known to activate the pyruvate dehydrogenase (PDH) (EC 1.2.4.1) complex in various tissues of animals by inhibiting PDH kinase (EC 2.7.1.99) [3, 4]. However, the effects of DCA on the activity of the PDH complex and pyruvate metabolism in humans have not been investigated in detail. In the present study, to investigate the effects of DCA on human pyruvate metabolism, we examined the rate of pyruvate decarboxylation in platelets of a patient with mitochondrial encephalomyopathy associated with congenital lactic acidemia, and changes in the lactate levels of his blood and urine after exercise before and during DCA therapy.

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MATERIALS AND METHODS

Case report

A 15-year-old boy, born after an uncomplicated pregnancy, and weighing 3,080 g at birth. He developed normally until 10 years of age, when generalized clonic convulsions and myoclonic seizures of both hands developed. At 11 years of age, his IQ was 87 and single spikes, spike and wave complexes, and positive spikes were seen on his electroencephalogram. Thereafter, his neurological condition gradually deteriorated. At 14 years of age, his IQ was 43 and he was found to have an elevated blood lactate level of 4 mM. Moreover "ragged red" fibers were seen in a muscle biopsy specimen. He was diagnosed as having mitochondrial encephalomyopathy. Results of organic and amino acid screening were normal. Investigation of cultured skin fibroblasts revealed normal rates of decarboxylation of (1-^{14}C) pyruvate and (3-^{14}C) pyruvate and normal activities of the PDH complex, pyruvate carboxylase (EC 6.4.1.1), phosphoenolpyruvate carboxykinase (EC 4.1.1.49) and cytochrome c oxidase (EC 1.9.3.1). With informed consent from the parents, sodium DCA (Tokyo Kasei Kogyo, Japan) was administered orally at doses of 12.5 to 50 mg/kg body weight twice or three times per day for 8 months, while the lactate levels in the blood were monitored.

Preparation of platelets

Blood samples were taken from the peripheral vein of the patient and normal controls before DCA therapy and on the second, third and 20th days of therapy. Volumes of 10 ml of blood samples were mixed with 2.5 ml of hydroxyethyl starch (Hessol, Midorijuji, Japan) in a 20 ml syringe and stood for 1 hr at 4°C for platelet-enrichment. The platelets were collected from the supernatant by centrifugation at 4°C, washed twice with 10 ml of phosphate buffered saline (PBS, Nissui, Japan), and suspended in 0.35 ml of PBS.

Assay of rate of pyruvate decarboxylation

The rates of oxidative decarboxylation of (1-^{14}C) pyruvate and (3-^{14}C) pyruvate by platelets were measured by the method of Robinson et al [5]. Protein was assayed by the method of Lowry et al [6].

Assays of lactate and pyruvate

The concentrations of lactate and pyruvate in the blood, cerebrospinal fluid, and urine were measured by the enzymatic methods [6, 7].

Assay of DCA

The concentrations of DCA in the blood and cerebrospinal fluid were assayed by electron capture gas chromatography by the method of Wells et al [7].

RESULTS

The decarboxylation of (1-^{14}C) pyruvate by platelets from the patient was normal and increased markedly during DCA therapy (Table 1). The decarboxylation of (3-^{14}C) pyruvate by platelets from the patient was also normal and increased significantly during DCA therapy (Table 1). The lactate and pyruvate levels in the blood and cerebrospinal fluid decreased gradually when the decarboxylation rates of pyruvate by platelets increased (Table 2).

Table 1 *DCA concentrations in the blood and cerebrospinal fluid and the rates of pyruvate decarboxylation in platelets from the patient with mitochondrial encephalomyopathy and controls*

DCA therapy	Decarboxylation rate (1-^{14}C)pyruvate (nmol/h/mg protein)	Decarboxylation rate (3-^{14}C)pyruvate (nmol/h/mg protein)	DCA concentration Blood (μg/ml)	DCA concentration CSF (μg/ml)
Before (3)†	20.2 ± 0.8	11.1 ± 0.2	ND	–
During (4)†	41.0 ± 7.5*	18.0 ± 3.7**		
Day 2	32.4	16.0	ND	–
Day 3	35.4	24.2	6.2	9.2
Day 14	–	–	252.7	123.1
Day 20 (12h)	45.0	14.8	149.0	–
Day 20 (4h)	51.1	16.8	–	–
Day 24	–	–	28.3	31.3
Controls (9)††	26.4 ± 4.4	10.9 ± 2.2	ND	–

Mean ± SD, significance of difference from the value before therapy *p < 0.01, **p < 0.05, † number of assays, †† number of normal subjects, (12h): 12 hours after DCA administration, (4h): 4 hours after DCA administration, ND: not detectable.

Table 2 *Changes of lactate and pyruvate levels in the blood and cerebrospinal fluid from the patient with mitochondrial encephalomyopathy during DCA thierapy*

DCA therapy	Blood Lactate (mg/dl)	Blood Pyruvate (mg/dl)	CSF Lactate (mg/dl)	CSF Pyruvate (mg/dl)
Before	41.8 ± 13.3	1.58 ± 0.3	36.5	1.89
Day 2	15.4	0.53	–	–
Day 3	17.8	0.57	33.5	1.43
Day 14	6.7	0.26	23.5	0.53
Day 20	5.3	0.21	–	–
Day 24	11.3	0.54	24.3	0.86

Mean ± SD.

Before DCA therapy, the lactate levels in the blood and urine were markedly increased by physical exercise (going up and down stairs for 10 min) (Fig 1). During DCA therapy, the lactate levels in the blood and urine were maintained within the normal range and the increases of lactate levels in the blood and urine after physical exercise were markedly less than before therapy. His neurological deterioration was slowed down and no adverse effects associated with DCA therapy were observed.

DISCUSSION

Mitochondrial myopathies can be divided into three groups according to the area of mitochondrial metabolism affected: substrate utilization, oxidation and phosphorylation coupling, or the respiratory chain [8]. However, no underlying deficiency could be found in our patient with mitochondrial encephalomyopathy associated with congenital lactic acidemia. As shown in Table 1, the rates of oxidative decarboxylation of (1-^{14}C) pyruvate and (3-^{14}C) pyruvate which represent the activity of the PDH complex and the function of the TCA cycle, respectively, increased significantly in the platelets from our patient during DCA therapy. Furthermore, as shown in Fig 1, the increases in the lactate levels of the blood and urine after physical

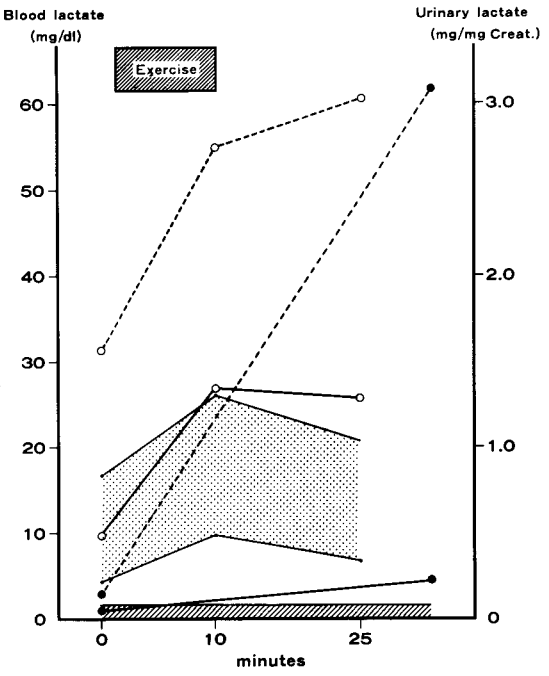

Fig 1 *Effects of physical exercise on the lactate levels in the blood and urine of the patient with mitochondrial encephalomyopathy. Open and closed circles with dotted lines indicate the lactate levels in the blood and urine, respectively, before DCA therapy. Open and closed circles with continuous lines indicate lactate levels in the blood and urine, respectively, during DCA therapy. Stippled and shaded areas represent the normal lactate levels in the blood and urine, respectively.*

exercise were markedly reduced during DCA therapy. The plasma half-life of DCA varies considerably in different species: in humans it is approximately 0.5 h, in rats it is approximately 3 h, and in dogs it is as long as 20 h [9]. However, our results suggest that DCA increases the activity of the PDH complex and pyruvate oxidation via the TCA cycle not only in the platelets but also in the liver, muscle, brain, and other tissues of humans, resulting in decreased lactate levels in the blood and cerebrospinal fluid.

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Chronic ACTH Treatment Increases Striatal Dopamine D-2 Receptor Binding in Developing Rat Brain

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ACTH has been reported to decrease elevated levels of dopamine metabolites in the CSF of patients with infantile spasms who respond clinically to ACTH therapy. To study the possible role of dopamine receptors in the effect of ACTH, we treated rat pups for thirty days with 40 IU/kg subcutaneously of porcine ACTH or with normal saline. Using 10 nM ^{3}H-spiperone and sulpiride to determine nonspecific binding, specific binding of D-2 receptors increased significantly (46%) in the striata of ACTH-treated rats when compared to controls. No significant difference in specific binding was found in the nucleus accumbens. Protein concentration was significantly decreased by ACTH treatment. Saturation studies will be necessary to determine if the increase in dopamine receptor binding induced by a high dose of ACTH represents a change in receptor density or affinity. The effect of lower clinical doses of ACTH on dopamine receptors warrants study. ***Key words: Dopamine (D-2) receptors, ACTH, striatum, infantile spasms.***

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ACTH has been reported to be an effective mode of therapy in infantile spasms [1]. The mechanism of anticonvulsant action of ACTH is not known. Changes in levels of homovanillic acid (HVA), a dopamine (DA) metabolite, have been observed in some epileptic patients [2-4]. Ito et al reported elevated HVA levels in the CSF of patients with infantile spasms which normalized following a clinical response to ACTH therapy [3]. However in other children with epilepsy, reduced HVA levels in CSF were found that did not correlate with anticonvulsant serum levels [4].

The DA-rich substantia nigra appears to modulate the expression of motor seizures in certain epileptic models [5, 6], but studies relating DA receptor abnormalities and seizures are lacking. The DA D-2 receptor has received the most attention, until recently, in neurological disorders such as Parkinson disease and chorea [7]. Age-depending increases in D-2 receptor density have been reported in mice, following early anticonvulsant therapy [8], but the effect of ACTH was not studied.

The present study was undertaken to evaluate the possible effect of ACTH on nigrostriatal and mesolimbic

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