GENETICS

Clastogenesis and Aneugenesis in Children with Cerebral Palsy

D. D. Gainetdinova, G. K. Ziyatdinova, V. V. Semenov, I. A. Pakhalina, and E. V. Kolochkova

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The genetic system in children with cerebral palsy was studied by the method of registration of chromosome aberrations and micronuclei in peripheral blood lymphocytes and erythrocytes. A high level of chromosome aberrations and micronuclei in the peripheral blood cells was revealed. A significant reduction of the integral antioxidant capacity of the blood and plasma was detected by coulombometric titration with electrogenerated bromine in patients with all forms of cerebral palsy. Aneugenic and antianeugenic effects of glutamate were studied in experiments on mice. Biphasic effect of glutamate was revealed: it exhibited aneugenic effect in high doses and antianeugenic in low doses. Impairment of the genome stability in children with cerebral palsy is believed to be caused by increased generation of endomutagens under conditions of disease and reduction of the genome antimutagenic defense system.

Key Words: *cerebral palsy; micronuclei; chromosome aberrations; antioxidant capacity; mutagens*

Detection of the cause of Down's syndrome [3] triggered intense search for abnormal chromosomes in different diseases. These studies revealed two principal things. Chromosome mutations can be hereditary and cause the development of chromosome diseases. Chromosome aberrations can be an episode in human ontogeny and result from the effects of viruses or autoantibodies on the genome in respective diseases. Experimental findings confirmed the damaging effects of intermediate products of nitrogen metabolism (hydroxylamine and hyponitrites), deficit of adenine, vitamin B_{12}, folic acid, *etc.* [5], on chromosomes. It is hypothesized that metabolic disorders developing in patients with non-hereditary diseases can also contribute to development of chromosome aberrations. This was confirmed by the findings of cytogenetic studies in patients with asthma and chronic infections [4,5], which, in turn, gave grounds to characterize these aberrations as nonspecific and secondary to the disease. The triggering mechanisms inducing secondary chromosome aberrations are unknown. This determined the aim of this study: to evaluate the genome status of children with cerebral palsy (CP) by cytogenetic and physicochemical methods and mechanisms provoking the appearance of chromosome aberrations in these patients.

MATERIALS AND METHODS

A total of 98 children and adolescents with different CP forms and 40 healthy schoolchildren were examined. Peripheral blood erythrocytes with micronuclei (EM) were counted as described previously [6]. Blood smears were prepared on admission to hospital in patients and during planned prophylactic examinations in

Kazan State Medical University. ***Address for correspondence:*** anetdin@mi.ru. D. D. Gainetdinova

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healthy children. At least 20,000 erythrocytes in two and more smears from each child were examined. Chromosome aberrations (CA) were studied in 49 patients. Blood was cultured as described previously [6]. The culture was fixed for 48 h. Mutagenic and antimutagenic activity of glutamate metabolite was evaluated in mice (1/4 LD_{50}, 2 g/kg, orally). Subsequent doses were decreased by half until the final dose of 0.065 g/kg. Blood smears were prepared 24 h after drug administration. EM were detected from 2000 erythrocytes in preparations fixed and stained by Giemsa's method. Each experiment was carried out on 3 mice (25 g). For evaluation of the antimutagenic effect of glutamate it was injected in a non-mutagenic dose of 0.05 g/kg simultaneously and 2, 4, and 8 h before mutation inductor (40 mg/kg cyclophosphamide). Blood was collected after 24 h. Antimutagenic effect was estimated as the percentage of decrease in the level of aberrations in experimental animals in comparison with the number of aberrations induced by mutation inductor. The integral antioxidant capacity (AOC) of the blood and plasma was evaluated by coulombometric titration using electrogenerated bromine with biamperometric indication of the final point of titration [1].

The data were processed statistically using Statistica software. Variation analysis was used for processing the results of EM registration in the blood.

RESULTS

The level of chromosome aberrations and micronuclei in the peripheral blood cells of the majority of children with CP surpassed the control (Table 1). The highest percentage of aberrations was observed in patients with double hemiplegia (the most severe form of the disease in children).

Whole blood and plasma AOC decreased significantly in all patients irrespective of the disease form, this decrease was more pronounced in patients with mild forms of the disease (Table 2). AOC of whole blood decreased by 20.5% in children with spastic diplegia and by 50 and 31%, respectively, in patients with less severe right-side and left-side hemiparesis.

The study of aneugenic and antianeugenic activities of glutamate showed that in high doses (1, 2 g/kg) it appreciably stimulated the intensity of EM formation in mouse bone marrow (Table 3). The level of EM remained high on day 2 of the experiment. In lower doses glutamate exhibited no aneugenic activity.

Glutamate in a dose of 0.5 g/kg injected together with and 2, 4, and 8 h before cyclophosphamide inhibited cyclophosphamide-induced aneugenesis. The most pronounced protective effect of glutamate was observed, when it was injected together with the mutagen (65% antianeugenic effect). Simultaneous injection of glutamate (2 g/kg) with the inductor and 2 h before inductor produced minor protective effect (45%). In all other protocols the protective effect was undetectable, but the level of EM in the peripheral blood increased. This indirectly indicates that the development of CP can be associated with accumulation of glutamate, which can lead to inversion of its antimutagenic effect into mutagenic.

High level of clastogenesis is most often induced by two causes: increased effects of exo- or endomutagens on the genome and attenuation of the genome defense factors [5]. High generation of endomutagens in CP patients is also most likely associated with abnormal activation of clastogenesis metabolic cycles (CMC). These cycles contain intermediate products acting as endomutagens (*e. g.* AOF, some LPO products, *etc.*) or activate other CMC containing muta-

TABLE 1. Level of Chromosome Aberrations and Erythrocytes with Micronuclei in the Peripheral Blood of Children with Different Forms of CP

CP form	Chromosome aberrations			EM		
	number of children examined		$M\pm m$, %	number of children examined		$M\pm m$, %
	total	with high level of CA		total	with high level of CA	
Control	10		2.63±0.93	40		0.087±0.008*
SD	10	7	5.70±0.41*	23	23	0.209±0.006*
DH	9	7	7.63±0.51*+	23	23	0.213±0.007*
HK	10	7	5.80±0.43*	17	17	0.215±0.011*
LH	10	6	6.00±0.43*	15	15	0.193±0.007*
RH	10	6	5.53±0.42*	12	12	0.194±0.005*

Note. Here and in table 2: CA: chromosome aberration; SD: spastic diplegia; DH: double hemiplegia; HK: hyperkinetic form; LH: left-side hemiparesis; RH: right-side hemiparesis. $p<0.001$ compared to: *control, +other forms of disease.

TABLE 2. Integral Antioxidant Capacity of Whole Blood and Plasma in Children with Different CP Forms

Group	Number of children in group	Antioxidant capacity					
		whole blood			plasma		
		kCl/liter	%	S_r	kCl/liter	%	S_r
Healthy	5	42.0±3.5		0.07	18.2±0.6		0.02
SD	10	33.4±0.6	79.5	0.03			
DH	10	30.9±0.8	73.6	0.04	10.3±0.4	56.6	0.05
HK	9	24.5±0.9	58.3	0.05			
LH	11	29±3	69.0	0.1	13±2	71.4	0.2
RH	10	21±6	50.0	0.4	9±2	49.5	0.3

genic intermediate products. Normally endomutagens of metabolic cycles are not dangerous, but in disease their production sharply increases and their level surpasses the physiological potentialities of the defense systems. The cycle with participation of glutamate (one of the leading compounds in CP pathogenesis) is the most characteristic example of endomutagen (AOF) generation by the metabolic cycle in CP (Fig. 1).

AOF are characterized by high reaction capacity, and hence, are mainly concentrated in cells in sites of their generation. However they can be translocated through membranes into extracellular space and be released into circulation in complexes (superoxide anion radical in complex with Ca^{2+} and Zn^{2+}, NO in the form of S-nitrosothiol) [9]. Endomutagen MDA, an LPO product, whose blood level increased in CP patients,

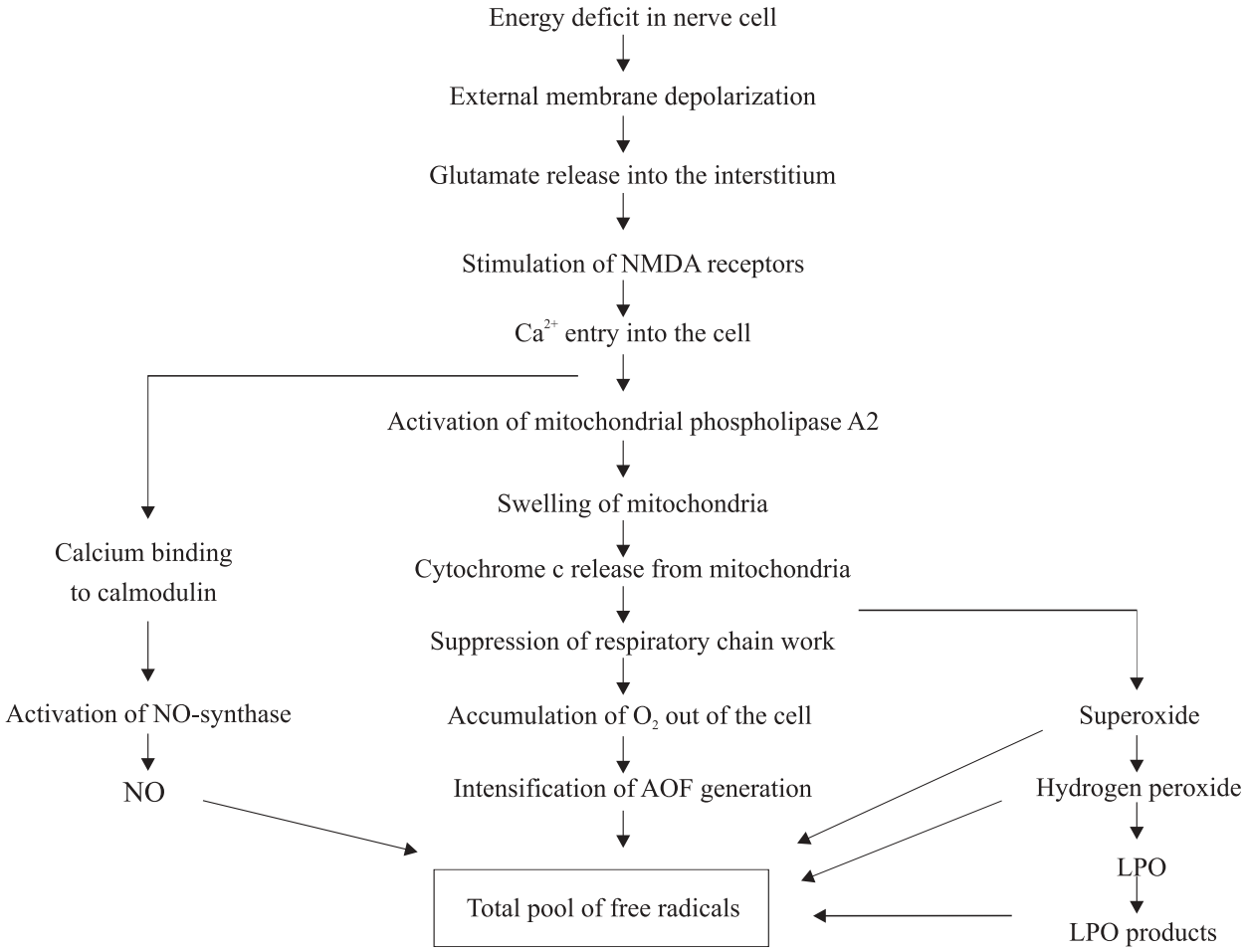

Fig. 1. Possible metabolic cycles of endomutagen (NO, AOF, LPO products) activation in children with cerebral palsy.

TABLE 3. Effect of Glutamate on EM Level in Mouse Peripheral Blood

Variant of experiment	Number of erythrocytes examined		EM			
			abs. count		*M*±*m*, %	
	24 h	48 h	24 h	48 h	24 h	48 h
Control	24,000	24,000	14	14	0.06±0.02	0.06±0.02
Glutamate, g/kg						
2.00	15,000	12,000	40	34	0.27±0.04*	0.27±0.05*
1.00	18,000	18,000	43	45	0.24±0.05*	0.25±0.05*
0.50	18,000	18,000	24	33	0.13±0.03	0.18±0.03*
0.25	18,000	18,000	31	35	0.17±0.03*	0.19±0.03*
0.13	18,000	18,000	22	18	0.12±0.04	0.10±0.04
0.06	18,000	18,000	15	11	0.08±0.02	0.06±0.02

Note. *$p<0.001$ *vs.* control.

is hazardous for the cell genome. This indicates that metabolic cycles of clastogenesis, triggered by glutamate in CP in organs (particularly in the brain) can be the sources of endomutagens. These CMC are not the only generators of endomutagens. Endomutagens in CP patients can be produced by hepatic cells (disorders in the biotransformation system, ceruloplasmin synthesis, and hence, decreased intensity of Fe^{2+} to Fe^{3+} oxidative reactions, *etc.*), blood cells (transmembrane transfer of some AOF from macrophages into erythrocytes and lymphocytes in sites of their formation), vascular epithelium cells, *etc.* [9,10].

Blood AOC decreases significantly in CP patients, but it remains appreciably higher in severe forms than in mild ones (Table 2). This is explained by several reasons. Metabolism of sulfur-containing amino acids, serving as targets for electrogenerated bromine, is impaired in severe forms of CP. Glutamate-induced cycles of clastogenesis result in the formation of hydrogen peroxide in the cell cytoplasm; it is inactivated by glutathione peroxidase. The deficit of the latter enzyme stimulates thiol-sulfide transition of glutathione in the cytoplasm proteins. The release of sulfur-containing amino acids and proteins enriched with disulfide form of glutathione into the blood under conditions of high destruction of tissues in severe CP can also be a cause of increased AOC of the blood, because of high reaction capacity of sulfur-containing compounds with electrogenerated bromine. In addition, albumin-globulin coefficient shifts towards globulins in CP. Some globulins possess pronounced antioxidant activity (α_2 and β_2 fractions). Blood AOF can also depend on changed rheological characteristics of the blood in CP patients [4]. Conformational changes in the blood proteins under these conditions can increase in the number of electrochemically active functional groups capable of reacting with electrogenerated bromine.

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