GENETICS

Clustogenesis Level in Children with Gastroduodenal Diseases

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The mean count of cells with chromosome aberrations increased in a 72-h culture of peripheral blood lymphocytes of children with *Helicobacter pylori*-associated gastroduodenal diseases. After eradication therapy, intensification of clustogenesis was observed in the majority of children. Addition of vetoron to the treatment protocols reduced manifestations of clustogenesis.

Key Words: *peptic ulcer; chromosome aberrations; vetoron*

By prevalence and medical and social significance peptic ulcer occupies one of the central places among diseases of the digestive system. In about 60-70% adult patients, the formation of peptic ulcer and chronic gastroduodentis starts in childhood and adolescence [1].

Infections are associated with an increase in the level of chromosome aberrations (CA) in blood cells [5]. It was proven that some drugs are potentially mutagenic. For example, standard protocols for eradication of *Helicobacter pylori* (HP) include metronidazole, a potentially clustogenic drug [3,9]. Presumably, activities of potential mutagens in combined therapy with several drugs can be appreciably modified, potentiated (comutagenesis) or reduced (antimutagenesis) [2,4].

We evaluated CA levels in peripheral blood leukocyte cultures from children with gastroduodenal diseases before and after therapy with various drug complexes, including those with potential mutagens (metronidazole) and antimutagens (vetoron and dimephosphon).

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MATERIALS AND METHODS

A total of 98 children with HP-associated gastroduodenal diseases (42 boys and 56 girls, age 5-17 years), were examined during hospital treatment. *Helicobacter pylori* was detected using the histomorphological method, HELPIL test, and noninvasive respiratory test (Helic test).

Two protocols of antihelicobacter therapy were analyzed. Protocol 1 included omeprazole (1 mg/kg), de-nol (16 mg/kg), and amoxicillin (33 mg/kg). Protocol 2 included omeprazole (1 mg/kg), de-nol (16 mg/kg), amoxicillin (33 mg/kg), and metronidazole (17 mg/kg). The duration of a course was 7 days. The choice of drugs and doses conformed to the requirements suggested by the Russian Gastroenterological Association in accordance with the Maastricht Agreement [6]. Blood was collected from the ulnar vein twice in each patient: before and during the first 24 h after therapeutic course. The effects of the disease on hereditary structures of cells were evaluated by the results of cytogenetic analysis carried out before therapy. The results of analysis after therapy indicated its effects on the intactness of chromosomes in children with peptic ulcer. Cytogenetic studies 1 year after

therapy were carried out to evaluate the delayed effects of the disease and therapy.

In order to analyze CA, peripheral blood lymphocytes were cultured using a macromethod. The growth medium consisted of cattle serum (10%) and Eagle's medium (90%) with glutamine and medium 199 (1:1). Lymphocyte suspension culture included the leukocytic plasma and growth medium (1:9). Cell division was stimulated by PHA (Difco P). Cell material was fixed during the 72nd hour of culturing, after which the metaphase plates were analyzed. A total of 200 metaphase plates per patient were analyzed: 100 before and 100 after treatment.

The significance of the results was evaluated using Wilcoxon's and Student's tests.

RESULTS

The results of cytogenetic studies in children with HP-associated gastroduodenal disease showed a high mean level of CA, 4-fold higher than the mean percentage of CA in healthy children (Table 1). The percentage of cells with CA in patients varied from 0 to 10%. This indicated high variability of chromosome changeability in the patients infected by HP. Cells with chromosome aberrations were detected in the majority of patients; no CA were detected in only 31.2% children. The data differed significantly from the results of cytogenetic analysis of healthy children. Cytogenetic analysis revealed no more than 2% cells with CA in one-fourth of the examined healthy donors, while others had no CA.

Hence, cytogenetic analysis revealed a significant increase in the mean number of cells with CA in the patients compared to healthy children (2.1 *vs.* 0.5%). These results are in line with the data on elevation of 8-hydroxy-2'-dioxyguanosine level in biopsy specimens from the gastric antrum in patients with gastritis associated with HP infection [2,11].

Analysis of the results of cytogenetic studies in children after therapy showed individual drug sensitivity of patients' leukocytes. The level of clustogenesis decreased in some patients. However, the mean level of CA increased in the majority of children after eradication in comparison with the corresponding parameter before treatment (Table 1). The percentage of children who had no cells with CA after antihelicobacter therapy decreased 2-fold (according to the results of cytogenetic analysis) in comparison with the corresponding parameter before drug therapy.

Cytogenetic analysis of children during remission showed that the level of CA was higher than in healthy children, but lower than during the acute phase of the disease and directly after therapy (Table 1).

On the whole, the results of cytogenetic analysis in children with HP-associated gastroduodenal diseases are in line with the data on the mutagenic effects of metronidazole and higher incidence of mutations in HP infection [3,7-9].

In some children, therapy was supplemented by dimephosphon (50 mg/kg) or vetoron (0.25 ml/day). These drugs were added to the treatment protocols due to their known antimutagenic and immunomodulating effects and their capacity to stimulate tissue regenera-

TABLE 1. Mean Levels of CA in Peripheral Blood Lymphocytes of Patients before and after Therapy ($M \pm m$)

Group		*n*	Number of cells with multiple CA	Cells with CA, total	CA (per 100 cells)				Gaps
					solitary fragments	chromatide exchanges	paired fragments	chromosome exchanges	
Healthy children		10	0	0.5±0.2	0.30	0	0.10	0.10	5.0
Before therapy		98	0	2.1±0.2*	0	0.13	0.05	2.5	
After therapy	protocol 1	47	2	3.7±0.3*	1.79	0.02	0.95	0.91	2.1
	protocol 1+ dimephosphon	10	–	3.0±0.5	1.5	–	1.1	0.4	2.2
	protocol 2	31	0	4.3±0.4*	1.32	0	1.71	1.26	1.9
	protocol 2+ vetoron	10	–	3.0±0.5*	1.0	–	1.0	1.0	1.9
One year after therapy	protocol 1	10	0	2.5±0.5*	0.70	0	0.80	1.0	2.3
	protocol 2	10	0	3.0±0.5*	1.10	0	1.0	0.90	2.2

Note. "–": data not shown. *$p<0.001$ compared to the control.

tion and reduce (in a dose-dependent manner) the LPO intensity [8].

The number of cells with CA was minimum after therapy supplemented by vetoron or dimephosphon (Fig. 1). The mean level of cells with CA in patients receiving treatment according to protocol 2 with vetoron was lower than in those who received no antimutagen ($p<0.05$). In the dimephosphon group, the mean number of cells with CA decreased in comparison with the treatment without it, but the differences were negligible (Table 1).

Hence, the level of chromosome mutations surpassed the normal in children with HP-associated gastroduodenal diseases before therapy; the level of clustogenesis increased after therapy according to protocols including metronidazole. The results indicate the need in preventive antimutagenic therapy in this patient population and demonstrate the efficiency of vetoron for this purpose.

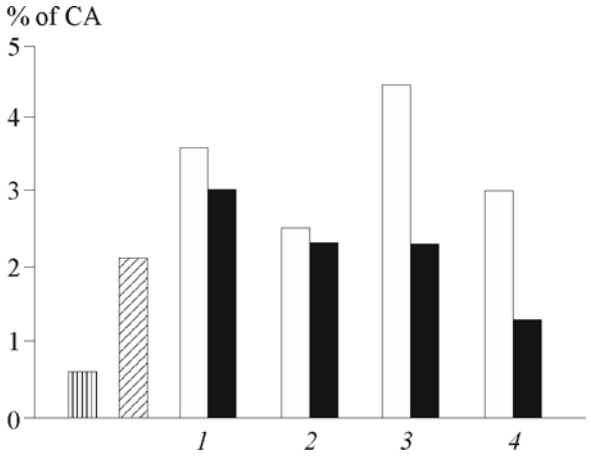

Fig. 1. Mean level of CA in lymphocyte culture from children whose treatment protocols were supplemented by antioxidant. Vertical hatching: control; oblique hatching: before therapy. Light bars: directly after therapy; dark bars: 1 year after therapy. *1*) protocol 1; *2*) protocol 1+dimephosphon; *3*) protocol 2; *4*) protocol 2+vetoron.

REFERENCES

1. T. Yu. Bandurina, *Ulcerative Diathesis* [in Russian], St. Petersburg (2004).
2. A. D. Durnev, N. O. Daugel'-Dauge, and S. B. Seredenin, *Eksp. Klin. Farmakol.*, **69**, No. 1, 56-59 (2006).
3. A. D. Durnev and S. B. Seredenin, *Mutagens: Screening and Drug Prevention of Effects* [in Russian], Moscow (1998).
4. A. D. Durnev and S. B. Seredenin, *Byull. Eksp. Biol. Med.*, **135**, No. 6, 604-612 (2003).
5. N. N. Ilyinskikh, A. G. Semenov, M. V. Romanova, and E. N. Ilyinskikh, *Aktual'n. Probl. Biol. Med. Ecol.*, **3**, No. 1, 218 (2004).
6. *Prevention and Therapy of Chronic Diseases of the Upper Gastrointestinal Tract*, Ed. V. T. Ivashkin [in Russian], Moscow (2002).
7. O. I. Aruoma and B. Halliwel, *Chem. Brit.*, **27**, 149-152 (1991).
8. S. C. Baik, H. S. Youn, M. H. Chung, *et al.*, *Cancer Res.*, **56**, No. 6, 1279-1282 (1996).
9. M. Chacko, M. Nagabhushan, S. Sarkar, *et al.*, *Ind. J. Exp. Biol.*, **25**, 243 (1994).