V.B. Denisenko, A.N. Simpvanyan

Rostov State Medical University, Rostov-on-Don, Russian Federation

The dynamics of clinical and laboratory indicators in children with HIV infection, treated with different schemes of start highly active antiretroviral therapy: a randomized controlled research

Author affiliation:

Denisenko Valentine Borisovich, MD, Associate Professor of Department of Pediatric Infectious Diseases of RostSMU
Address: 29 Nahichevanskii per., Rostov-on-Don, 344022, ph.: +7 (863) 232-73-58, **e-mail**: dvalentinb@gmail.com

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Unfavorable course of HIV infection, the rapid development of immunosuppression, heavy opportunistic infections and malignant tumors, high mortality rate determine the importance of timely prescription of highly-active antiretroviral therapy (HAART). Herewith the start scheme of the therapy should remain effective and safe in long-term use. ***Objective****: to study the effectiveness and safety of different schemes of starting HAART in children with HIV infection.* ***Methods:*** *children from 1 to 3 years of life with HIV infection were included in a randomized controlled research. Clinical, immunological, and virological examinations were performed before and after 12 months of HAART. The start treatment scheme included 2 nucleoside inhibitors of HIV reverse transcriptase — zidovudine and lamivudine. Children were randomly assigned to intake of the third therapy component: lopinavir/ritonavir or nevirapine.* ***Results:*** *25 patients were randomly assigned in lopinavir/ritonavir group, 23 children — in the nevirapine group. After 12 months of treatment HIV replication inhibition (blood viral load <50 copies/ml) was achieved in 25 (100%) patients in the lopinavir/ritonavir group and in 16 (70%) — in the nevirapine group (p = 0.003). While using both schemes, HIV infection was not progressing clinically. When using lopinavir/ritonavir median number of CD4+CD3+ lymphocytes increased from 20.5% (12; 23) to 30% (27; 34) (p <0.001), in treatment with nevirapine — from 21.5% (17; 23) to 29% (27; 38) (p <0.001). Unwanted occurrences developed in 13 (27%) children. During lopinavir/ritonavir intake, 3 children had nausea, and 2 patients — regurgitation. During nevirapine intake, 1 patient had the allergic rashes appearance, drug-induced hepatitis developed in 1 patient.* ***Conclusion:*** *high effectiveness and safety of lopinavir/ritonavir allow recommending the drug as the third component for start HAART scheme for HIV-infected children.*
Keywords: *children, HIV infection, antiretroviral triple therapy, lopinavir/ritonavir, nevirapine, effectiveness, safety.*
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REASONING

The importance of the prescription of highly active antiretroviral therapy (HAART) is determined by unfavorable course of HIV infection in children, rapid development of immunosuppression, leading to attachment of serious opportunistic infections, malignant tumors and, ultimately, to death [1, 2]. HAART can suppress the replication of human immunodeficiency virus (HIV) infection, as can seen by reduction of viremia level (virus load blood, VLB) <50 copies/ml. This contributes to improving the immune status, primarily, to the increase of the main HIV target cells — T helper cells (CD4 +

CD3 + lymphocytes) — in blood, which leads to the positive dynamics of clinical symptoms, reduction of rate of disease development and mortality [3, 4]. At present, specialists of the Russian Ministry of Healthcare Scientific and Practical Center of providing assistance to pregnant women and children with HIV infection have developed indications for HAART, the principles of formulating schemes of antiretroviral therapy, effectiveness and safety of the treatment [5].
Undoubtedly, the start HAART scheme should be effective and safe in long-term use [6, 7]. HAART schemes include three drugs with different mechanisms of antiviral activity: 2 nucleoside reverse transcriptase inhibitors (NRTI) HIV combined with 1 non-nucleoside reverse transcriptase inhibitor (NNRTI) or virus protease inhibitor [5, 7]. The list of antiretroviral drugs used in children is limited because of age contraindications or absence of liquid dosage forms. The drug from group NNRTI nevirapine is allowed for children older than 3 months, lopinavir/ritonavir [5] — for children older than 6 months. In this connection, it is a matter of interest to compare the effectiveness of various HAART schemes (achievement of stable suppression of HIV replication, improvement in clinical and immunological indicators) as well as the safety of their use, which will contribute to the optimization of antiviral therapy in children with HIV infection.
The aim of our research was to investigate the effectiveness and safety of different start HAART schemes in children with HIV infection.

METHODS

RESEARCH DESIGN

A randomized controlled research has been carried out. The allocation of consecutive patients into groups was performed using the fixed simple randomization method with a random number table.

COMPATIBILITY CRITERIA

Inclusion criteria:
- age from 1 to 3 years;
- laboratory-confirmed diagnosis of HIV infection (double positive result of polymerase chain reaction to proviral DNA identification);
- the stage of secondary diseases 4B (according to the classification by V.I. Pokrovsky);
- the absence of indications for the previously taken HAART;
- informed consent of the parents.

Non-inclusion criteria:
- nevirapine intake by the mother and the child as means of prevention of HIV vertical transmission;
- presence of background of liver pathology.

CONDITIONS

The research was conducted at the Center for AIDs and Infectious Diseases Control and Prevention (Rostov-on-Don).

DURATION OF THE RESEARCH

The patients were involved in the research during April-December 2013. The researc was completed 12 months after the start of HAART.

MEDICAL INTERVENTION DESCRIPTION

Haart start scheme included 2 NRTIs (zidovudine and lamivudine) combined with the drug from the group of viral protease inhibitors or NNRTIs. Zidovudine was prescribed at a daily dose of 360-480 mg/m^2 of body surface in 3-4 ingestions, lamivudine — at a daily dose of 8 mg/kg body weight 2 ingestions. The third component for the HAART patients was lopinavir/ritonavir at a daily dose of 460/115 mg/m^2 in 2 ingestions or nevirapine at a daily dose of 4 mg/kg, 1 ingestion daily during 2 weeks, afterwards — at a daily dose of 7 mg/kg in 2 ingestions. The duration of start HAART scheme was 12 months.

THE OUTCOME OF THE RESEARCH

The primary outcome was considered the inhibition of HIV replication, as evidenced by the decline in HIV VLB to <50 copies/ml. Complementary outcome was the absence of HIV infection transition to the stage of secondary diseases 4B according to classification by V.I. Pokrovsky [8] and the absence of the increase of CD4+CD3+ lymphocytes relative content.

METHODS OF THE OUTCOME REGISTRATION

Laboratory researches were performed in the laboratory of the Center for AIDs and Infectious Diseases Control and Prevention in Rostov region.
Suppression of HIV replication was assessed by the results of the polymerase chain reaction (test systems "AmpliSens", "InterLabService", Russia), which was conducted on a thermal cycler Rotor Gene (Qiagen, Germany).The relative content of T helper cells (CD4+CD3+ lymphocytes) in blood was investigated in the reaction of indirect immunofluorescence using two-parameter monoclonal antibodies (Beckman Coulter, France). The results obtained were registered by the laser flowing cytofluorimeter Coulter Epics-XL (Beckman Coulter, France).
The dynamics of the clinical indicators was assessed by analyzing the data history, objective examination of a patient, laboratory and instrumental research methods (clinical analysis of blood, urine, biochemical blood analysis, myelogram, chest X-ray examination, ultrasound of internal organs computer and magnetic-resonance tomography). The criteria for the active form of opportunistic infection was considered the presence of clinical symptoms and laboratory indicators of infection activity. The method of enzyme immunoassay (test systems "Vector-Best", Russia) was used to determine the antibodies of class (Ig) M and G to cytomegalovirus, herpes simplex virus (HSV), Toxoplasma, IgM to VCA, IgG to EA — and the EBNA-antigens of Epstein-Barr virus (EVB). By polymerase chain reaction the content of cytomegalovirus DNA, HSV and EVB in blood were examined (test systems "AmpliSens", Russia). The detection of IgM and/or the growth of IgG titer in 4 or more times, for EVB — IgM to VCA, IgG to EA-antigens, the growth of IgG titer to EBNA-antigen of the virus in 4 or more times were considered as serological markers of the activity of cytomegalovirus, HSV and toxoplasma. Virus DNA detection in the blood were considered as molecular-genetic markers of the activity of cytomegalovirus, HSV, EVB. The diagnostics of bacterial infections and candidiasis was performed considering the clinical symptoms, ejection of pathogens using bacteriological and mycological method.

ETHICAL EXPERTISE

The research was approved by the Local Independent Ethics Committee of the Rostov State Medical University (report № 5, 14.03.2013).

STATISTICAL ANALYSIS

The selection size was not calculated preliminarily.
The analysis of the series of values of all the studied quantitative indicators illustrated their contradiction to the normal distribution law ($p < 0.05$, Shapiro-Wilk criterion). In this regard, the median indicators (25th, 75th percentiles) were used to characterize them. Statistical significance of differences in quantitative indicators was assessed using the Mann-Whitney criterion, discrete indicators — using the Fisher's exact test. Bilateral options for non-parametric tests were used. The results were processed using the «R» software package. Differences were considered statistically significant at $p < 0.05$.

RESULTS

THE PARTICIPANTS OF THE RESEARCH

56 patients were screened to find out whether they match the criteria of the inclusion in the research or not. 48 children were included in the research. 7 people were not included because of nevirapine

intake to prevent vertical transmission of HIV by the mother and the child, 1 patient — because of his parents' rejection of participating in the research. 25 patients were randomly assigned to lopinavir/ritonavir group, 23 — to the nevirapine group.
Clinical and laboratory characteristics of the patients in the two groups before the treatment were comparable. At the clinical examination prior to treatment HIV-associated symptoms and localized opportunistic infections were diagnosed in all patients (table). The most common HIV-associated symptoms were generalized lymphadenopathy, hepatomegaly, body mass deficits and anemia 10-20%, less common — splenomegaly, cardiomyopathy, fever (without any apparent reason) lasting more than 1 month, nephropathy, enteropathy, thrombocytopenia. Bacterial infections prevailed in the etiological structure of opportunistic infections, candidiasis, active forms of EBV, HSV and cytomegalovirus infection appeared to be less common.
Heavy immunosuppression appeared in all patients (the number of CD4+CD3+ lymphocytes <25%). The content of CD4+CD3+ lymphocytes in children in lopinavir/ritonavir group, was 20.5% (12; 23) and was not different from the values of the similar indicator in nevirapine group — 21.5% (17; 23) (p = 0.457). The values of HIV VLB in the two groups were also comparable: 315.3 (166.0; 768.3) and 288.1 thousand copies/ml (133.1; 698.9), respectively (p = 0.328).

Table. Clinical and laboratory indicators in children with HIV infection before the start of highly active antiretroviral therapy

Indicators	Lopinavir/ritonavir group (*n* =25), abs (%)	Nevirapine group (*n* =23), abs. (%)	*p*
Generalized lymphadenopathy	25 (100)	23 (100)	1,000
Hepatomegaly	25 (100)	23 (100)	1,000
Splenomegaly	12 (48)	10 (43,5)	0,779
Body mass deficits over 10%	21 (84)	18 (78,3)	0,711
Myocardiopathy	8 (32)	6 (26,1)	0,755
Nephropathy	1 (4)	3 (13)	0,338
Enteropathy	2 (8)	2 (8,7)	1,000
Anemia	20 (80)	16 (69,6)	0,511
Thrombocytopenia	2 (8)	1 (4,3)	1,000
Long-term fever without any apparent reason	4 (16)	4 (17,4)	1,000
Bacterial infections	24 (96)	21 (91,3)	0,601
Active form of HSV infection	8 (32)	8 (34,8)	1,000
Active form of cytomegalovirus infection	8 (32)	7 (30,4)	1,000
Active form of EBV infection	9 (36)	9 (39,1)	1,000
CD4+CD3+ lymphocytes <25%	25 (100)	23 (100)	1,000
Viral blood load >100 th. copies/ml	25 (100)	23 (100)	1,000

Note. EBV — Epstein-Barr virus, HSV — herpes simplex virus.

THE KEY RESULTS OF THE RESEARCH

After 12 months after the initiation of HAART there was VLB < 50 copies/ml detected in 100% of patients, treated with lopinavir/ritonavir as the third component of the therapy, and in 16 (70%) patients from the nevirapine group (p = 0.003). BHK median was 12.5 (1.3; 19.7) thousand copies/ml in children (n = 7) of nevirapine group with the determined level of viremia.

SUPPLEMENTARY RESULTS OF THE RESEARCH

In both groups during 12 months of HAART the disease did progress: there was no transition from stage 4B of secondary diseases to 4C. All the children studied had an increase in relative content of CD4+CD3+ lymphocytes detected, including the children in lopinavir/ritonavir group — from 20.5 (12; 23) to 30% (27; 34) (p <0.001), in nevirapine group — from 21.5 (17; 23) to 29% (27; 38) (p <0.001).

UNWANTED OCCURANCES

Unwanted occurrences were reported in 13 (27%) children. Some of them was associated with zidovudine intake, in 5 patients, vomiting — in 4 patients. Lopinavir/ritonavir intake in 3 children was accompanied by nausea, in 3 patients — by vomiting. During the treatment with nevirapine allergic rashes were observed in 1 patient, also drug-induced hepatitis developed in 1 patient.

DISCUSSION

SUMMARY OF THE KEY RESULTS

The comparison of the clinical and laboratory indicators illustrates the effectiveness of schemes 2 NRTI + lopinavir/ritonavir and 2 NRTI + nevirapine in terms of stabilization of clinical symptoms and the increase in relative content of CD4+CD3+ lymphocytes. Besides, during the application of scheme 2 NRTI + lopinavir/ritonavir, the suppression of HIV replication succeeded in all patients, whereas during the use of scheme 2 NRTI + nevirapine, VLB was over 50 copies/ml in 1/3 of HIV patients. It is noted that unwanted occurrences from taking lopinavir/ritonavir had a form of dyspeptic symptoms (nausea, vomiting), while the application of nevirapine developed more heavy side effects, such as allergic rashes and drug-induced hepatitis.

DISCUSSION OF THE MAIN RESULT OF THE RESEARCH

The results of the research illustrate the effectiveness of HAART schemes which have been applied and which have allowed achieving suppression of HIV reproduction in all patients receiving 2 NRTI + lopinavir/ritonavir and in the majority of patients receiving 2 NRTI + nevirapine, as evidenced by reduction in VLB HIV <50 copies/ml. The viral replication control achieved contributed to the restoration of immune system, which was confirmed by the increasing number of major target cells for the virus — CD4+CD3+ lymphocytes [5, 6]. As a result, there was a stabilization of clinical symptoms of HIV and there was no progression of HIV infection.

However, it should be mentioned that the effectiveness of HAART with lopinavir/ritonavir in terms of suppression of HIV replication was higher, which can be explained by the composition of the drug. Not only has lopinavir marked antiretroviral activity but also high genetic resistance threshold — 6-8 mutations are required to generate drug resistance [9, 10]. On the other hand, the drug contains ritonavir at a boosting dose, which inhibits liver cytochrome P450 that contributes to reducing of lopinavir transformation in hepatic cells, provides high and stable content of the drug in the blood [10, 11]. In addition, scheme 2 NRTI + lopinavir/ritonavir operates in 2 stages of HIV replicative cycle: synthesis of proviral DNA and virion maturation [12]. As a result, there is sustained suppression of HIV replication, which creates prerequisites for long-term use of this scheme.

Scheme 2 NRTI + nevirapine demonstrated lower effectiveness in suppressing HIV replication. This may be due to the low threshold of genetic resistance: 1 mutation is enough to develop drug resistance to nevirapine [13]. Meanwhile, cross-resistance to other NNRTI representatives occurs with the resistance to nevirapine [12]. Scheme 2 NRTI + nevirapine inhibits only one stage of HIV lifecycle — proviral DNA synthesis with the involvement of reverse transcriptase enzyme [10]. All of these factors create difficulties in long-term application of this scheme and makes the therapy selection of the second-line difficult.

The unfavourable aspect was the development of unwanted heavy occurrences such as allergic rash and hepatitis during the intake of nevirapine, whereas dyspeptic symptoms were the only to appear during the intake of lopinavir/ritonavir. However, considering the profile of the side effects of the drugs from inhibitors of viral proteases group, the development of lipodystrophy and lipid metabolism disorder during the intake of scheme 2 NRTI + lopinavir/ritonavir can be expected in the future [10, 11]. In this connection, further observation of the patients studied seems reasonable and it will allow evaluating the effectiveness and safety of long-term use of various HAART schemes in children with HIV infection.

THE RESEARCH LIMITATIONS

Some of the limitations of the research are a small number of groups, absence of infants and children older than 3 years, patients with other indications for HAART [5]. Children with the following clinical indications should be also included: "Acute HIV infection with secondary diseases 2B", "Stage of secondary diseases 4B ", "End-stage V»; with immunological indications "Evident immunodeficiency regardless of the stage of HIV infection" and "VLB HIV (CD4+CD3+ lymphocytes in infants less than 30%, from 1 to 3 years - less than 25%, from 3 to 5 years - less than 20%, over 5 years - less than 0.35×10^9/l) "; with virological indications "VLB > 100 thousand

copies/ml in children in latent stage 3 and in the stage of secondary diseases 4A at moderate immunodeficiency (CD4+CD3+ lymphocytes in infants 30-35%, from 1 to 3 years. 25-30% from 3 to 5 years - 20-25% over 5 years - 0,35-0,5 × 10^9/l)". All these factors limit the dissemination of research results to a broader category of patients with HIV infection.

SHORT PRACTICAL RECOMMENDATIONS

High clinical and laboratory effectiveness and safety of lopinavir/ritonavir allow recommending the widespread application of this drug in the schemes selection of HAART for children.

CONCLUSION

Searching for the most effective and safe schemes of start HAART for children is one of the most essential problems of HIV medicine. The randomized controlled research, which involved 48 children with HIV at the age from 1 to 3 years, demonstrated the effectiveness of start HAART schemes which included zidovudine and lamivudine combined with lopinavir/ritonavir or nevirapine. Regardless of the composition, these schemes allowed achieving suppression of HIV replication and reducing of the key outcome indicator VLB HIV to levels <50 copies/ml. During the application of both schemes, there was a positive dynamics of supplemental results of the research: there was no progression of the disease and there was an increase in the number of CD4+CD3+ lymphocytes. The comparison of clinical and laboratory indicators illustrated higher effectiveness of the scheme using lopinavir/ritonavir in terms of suppression of HIV replication and the absence of heavy side effects during its application, that allows recommending the drug as the third component to start HAART scheme in children with HIV infection.

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Conflict of interest

The authors of this article have confirmed the absence of conflict of interest worth reporting.

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