

Original papers

Monitoring of plasma concentration of pyrimethamine (PYR) in infants with congenital *Toxoplasma gondii* infection – own observations[1]

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ABSTRACT. The study objective was to determine plasma concentration of pyrimethamine in 24 infants aged 1–5 months, treated for congenital toxoplasmosis. Pyrimethamine was used in a single daily dose at an amount of 0.35–0.98 mg/kg daily, with sulfadiazine (50–100 mg/kg/day) in divided doses 2–3 times a day, and folinic acid given twice a week (7.5 mg). This regimen was continued for 2–6 months, then Fansidar® was administered. Pyrimethamine concentration in plasma was measured using high-performance liquid chromatography method (HPLC). A total of 70 tests were performed. Concentration of pyrimethamine ranged from 0.01 to 1.2 μg/ml. In 14 children (58 tests) the concentration of pyrimethamine achieved therapeutic value. In 7 patients (8 tests) the concentration was below therapeutic level, and in 3 patients (4 tests) above therapeutic level. In 11/24 (46%) children transient moderate neutropenia was observed. Modification of therapy was necessary in 12 patients. Monitoring of pyrimethamine concentration in plasma improves safety and effectiveness of the therapy and is useful in obtaining correct individual dose of the drug. Neutropenia is the most common side-effect of pyrimethamine observed even when using the recommended dose.

Key words: *Toxoplasma gondii* infection, congenital, treatment, pyrimethamine, plasma concentration

Introduction

Primary infection with protozoan *Toxoplasma gondii* during pregnancy may result in congenital disease of the fetus [1]. Fetuses infected in early pregnancy (5–6%) are much more likely to show clinical signs of infection than those infected in the third trimester (70–80%) [2]. Most (80–90%) infants congenitally infected are asymptomatic at birth, but many will present recurrent retinochoroiditis or neurological abnormalities later in life [3–5]. Both the symptomatic and asymptomatic infections are an indication for treatment as soon as the diagnosis is established. Most of therapeutic regimens include a combination of pyrimethamine and sulfonamide – the folic acid inhibitors, which block reproduction of DNA-strands during cell replication [6,7]. Both drugs have a synergistic, parasitostatic activity [8]. The most common side-effect of treatment, especially with pyrimethamine, is transient, reversible neutropenia. Other hematological disorders or toxic epidermal necrolysis are rarely observed [1]. Folinic acid is supplemented to prevent adverse effects. Currently, there is no consensus as regards the duration of postnatal treatment, but most authors prefer 12 months or longer depending on the child condition [9,10]. In Poland the treatment is continued throughout the period of infancy, or even longer [11]. Monitoring of serum levels of pyrimethamine, especially in the youngest children, increases the safety and effectiveness of the therapy [12].

[1]This study was presented during 50th Clinical Day of Medical Parasitology, 19–20 May 2011, Lodz, Poland

The aim was to evaluate plasma concentration of pyrimethamine in infants treated for congenital toxoplasmosis.

Material and methods

The study included 24 infants (10 girls, 14 boys) hospitalized in the Department of Infant Diseases, The Children's Memorial Health Institute in Warsaw in the years 2006–2010. At the time of diagnosis the age of the children ranged from 1 to 5 months, and the length of observation – from 8 months to 3 years. Pyrimethamine was used in a single daily dose oscillating around the recommended amount of 0.5–1.0 mg/kg daily with sulfadiazine (50–100 mg/kg/day) in divided doses 2–3 times a day. The treatment was continued for 2–6 months, then to the end of the first year of life or even longer, Fansidar® was supplied. Furthermore, during therapy folinic acid was given twice a week (7.5 mg).

After confirmation of congenital *Toxoplasma* infection, antiparasitic treatment was introduced [11]. The pyrimethamine concentration in plasma was measured using the high-performance liquid chromatography method. The therapeutic concentration was established at 0.08–0.6 μg/ml [7]. The concentration of PYR in 250 μl of plasma was measured before the 6th dose of the drug, then at 4–6 weeks intervals, and additionally after modification of the dose. Furthermore, the peripheral blood count, activity of transaminases, concentration of creatinine in serum and urinalysis were controlled. The study received the approval of the local Bioethics Committee.

Results

Results are summarized in Table 1. A total of 70 tests in 24 patients were performed. The dose of pyrimethamine ranged from 0.35 to 0.98 mg/kg/dose. In 10 patients the dose of the drug was below the recommended range of 0.5–1.0 mg/kg, owing to neutropenia (6 patients), bad compliance (one patient) and rapid weight gain (3 patients). The concentration of pyrimethamine in serum ranged from 0.01 to 1.2 μg/ml. In 14 children (58 tests) the concentration of PYR achieved therapeutic value; in this group the dose of the drug ranged from 0.35 to 0.98 mg/kg/dose. In 7 patients (8 tests) the concentration value was below the therapeutic level. In 2 of them (Nos. 2, 13 – test I) because of initial neutropenia, the starting dose of pyrimethamine was below 0.5 mg/kg/day; in patient No. 13 the concentration in consecutive tests normalized. In one patient (No. 14) we obtained too low level in the third test; the dose of the drug was below the recommended value (probably bad compliance). In patient No. 20, after a reduced dose because of neutropenia, in two subsequent tests (V,VI) the level of PYR was too low and it normalized after increasing the dose (VII,VIII tests). In last 3 patients from this group (Nos. 11, 12 – test I; No. 19 – test II), despite an appropriate dose of the drug, the concentration was too low and spontaneously increased in patients No. 11 and 12 (in patient No. 19 the control test was not performed). In 3 cases (4 tests) the concentration value was above therapeutic limits despite using the recommended dose. In the first child (No. 1) treated with a dose of 0.63 mg/kg every 2 days (owing to neutropenia), the concentration of pyrimethamine in the first test was 0.786 μg/ml. When the dose was reduced to 0.5 mg/kg every 3 days, in the second test the concentration was 0.141 μg/ml, but the third sample showed a therapeutic level of the drug and normalization of the neutrophil count. In the second child (No. 5) with a daily dose of 0.55 mg/kg we obtained a therapeutic value in the first test, but too high level (1.2 and 0.8 μg/ml) in the following tests II and III performed at an interval of 2 days. When the dose was reduced to 0.51 mg/kg every 2 days, in a control test we obtained the concentration of 0.126 μg/ml and simultaneously neutropenia appeared. The third child (No. 18) was treated with a dose of 0.76 mg/kg. The first level of the drug was correct. During the second test (a lower dose of 0.68 mg/kg and a higher level of 0.82 μg/ml), neutropenia appeared. After reducing the dose (0.77 mg/kg every 2 days) the concentration value was normalized, but moderate neutropenia was still observed. Neutropenia was detected in 14 patients: in 3 before and in 11 after drug administration (7 with normal, 3 with increased, and 4 with decreased level of drug). Modification of the therapy was necessary in 12 patients: owing to neutropenia in 10 (in one also hypertransaminasemia), and elevated level of pyrimethamine in 2. Daily dose reduction was applied in one case, a sequential supply every 2–3 days in 9, and periodic discontinuation of therapy in 2 cases.

Table 1. Values of the doses of pyrimethamine, drug concentrations, and neutrophil counts in subsequent tests in 24 patients with congenital toxoplasmosis

No	I TEST			II TEST			III TEST			IV TEST			V TEST			VI TEST			VII TEST			VIII TEST		
	Dose mg/kg	Level µg/ml	Neu /µL	Dose mg/kg	Level µg/ml	Neu /µL	Dose mg/kg	Level µg/ml	Neu /µL	Dose mg/kg	Level µg/ml	Neu /µL	Dose mg/kg	Level µg/ml	Neu /µL	Dose mg/kg	Level µg/ml	Neu /µL	Dose mg/kg	Level µg/ml	Neu /µL	Dose mg/kg	Level µg/ml	Neu /µL
1.	0.63*	0.786	1235	0.5*	0.141	810	0.57*	0.51	1536															
2.	0.17*	0.076	280																					
3.	0.75	0.116	375	0.74	0.249	1620	0.63	0.301	1078															
4.	0.41*	0.149	904	0.5*	0.166	1464																		
5.	0.57	0.301	1196	0.55	1.2	894	0.55	0.8	894	0.51*	0.126	836												
6.	0.53	0.15	2496	0.88	0.25	1827	0.77	0.13	2000	0.76	0.23	1152												
7.	0.9	0.28	3304	0.8	0.59	2442	0.73	0.3	1456	0.78	0.36	3168												
8.	0.4	0.15	2318																					
9.	0.52	0.24	1230	0.47	0.29	780																		
10.	0.98	0.134	1224	0.88	0.22	1660	0.85	0,169	707	0.76	0.409	1290												
11.	0.83	0.01	1168	0.81	0.41	1168	0.7*	0.256	2310	0.63*	0.214	2156												
12.	0.9	0.048	882	0.53*	0.08	931																		
13.	0.45	0.037	642	0.45	0.277	781	0.38*	0.135	1144	0.55*	0.123	820	0.41	0.242	702									
14.	0.59	0.146	2755	0.47	0.162	1071	0.37*	0.075	3286															
15.	0.5	0.084	844	0.45	0.378	1900																		
16.	0.48	0.2	1216																					
17.	0.58	0.246	1037	0.55	0.23	2727																		
18.	0.76	0.19	1144	0.68	0.82	649	0.77*	0.36	570															
19.	0.57	0.26	1365	0.53*	0.078	1921																		
20.	0.9	0.22	1178	0.89	0.24	1591	0.52	0.13	648	0.42*	0.22	828	0.37*	0.059	2040	0.47	0.072	2040	0.55	0.218	2208	0.53	0.53	1938
21.	0.71	0.21	872	0.61*	0.42	3116	0.72	0.35	2160	0.65	0.201	2244												
22.	0.72	0.24	740	0.65*	0.42	1185	0.35	0.30	1757															
23.	0.67	0.111	3808	0.51	0.151	2376																		
24.	0.65	0.16	6615																					

* dosage of pyrimethamine every 2 days

Discussion

Monitoring of blood concentration is particularly important for drugs with a narrow therapeutic index or showing significant side-effects. The pharmacokinetics of drugs in young children, especially in infants, may differ from adults with regard to drug accumulation, metabolism and excretion of the drug and its metabolites due to the lack of maturity of the metabolising enzyme systems and differences in drug disposition [12,13]. There has been progress concerning the HPLC survey methodology, but the fact remains that there is still a limited availability of pharmacokinetic studies in children [12]. Research conducted in infants with congenital toxoplasmosis is of great importance since long-term therapy is used. With regard to pyrimethamine, the important pharmacokinetic parameter affecting determination of the appropriate dose is the very long half-life depending on age and body weight, ranging from 4.0 to 5.2 days and a relatively long time period after which the drug reaches the maximum concentration [14]. Such a long half-life, protein binding of 80–90%, and the possibility of side-effects (especially bone marrow depression), make it important to monitor drug plasma concentration [12]. Peak plasma concentration is observed after 2–4 hours of supply [14]. Pyrimethamine is largely metabolized, but 30% is excreted by the kidneys for several weeks in an unchanged form. During prolonged use a stable state is reached after 12–20 days of regular supply, and plasma concentration is proportional to the dose. In the analyzed group we observed such a result, though in several cases the drug concentration was incorrect in spite of appropriate dose. Gene polymorphism may be responsible for individual reduction in half-life or excessive accumulation of the drug, which may lead to inadequate concentration in the serum observed in some of our patients (irregular drug supply). We associate too high concentration after the use of an appropriate dose and normalization after dose reduction with possible individual agents impairing metabolism. The importance of maintaining proper concentration of antiparasitic drugs is emphasized by Schmidt [8]. In the course of 3-month treatment new lesions appeared in some patients during follow-up, but there are no data supporting better outcomes after longer treatment. In our group treated for 12 months there were no recurrences of ocular changes. In the study performed by Corvaisier [7] in 33 children treated for 12 months the concentration of PYR was lower than in the group observed by Schmidt [8]. The difference was probably the result of various regimens used in both studies. In our patients total concentration of pyrimethamine ranged from 0.076 to 1.2 μg/ml. McLeod's [15] study assessed the *in vitro* minimum inhibitory level of pyrimethamine, resulting in approximately 90% inhibition of *T. gondii* replication, as 0.03 μg/ml. Johannessen [12] studying the concentration of pyrimethamine/sulfadiazine during a 3-month therapy in infants aged from 1 to 5 months observed a stable drug concentration in subsequent assays. In Trenque's [14] study (89 children aged from infancy to 14 years, treated with Fansidar®), the average of PYR concentration in serum was lower than in the Johannessen group [12], which is probably associated with the supply of the long-acting drug over an extended time interval. Toleration of the drug has generally been reported as good, which accords with our results. The main side-effect of the treatment was neutropenia, found in 11/24 (46%) of infants in our group ($<1.0\times10^9$/l). Schmidt [8] and colleagues found the reduction in neutrophils as less than 0.5×10^9/l in 13.8% of patients. Change of dose or discontinuation of treatment was necessary and all patients regained a neutrophil count within the normal range after treatment. Schmidt noticed that there is no uniform pattern of decreasing or increasing value of the neutrophil count during therapy. Some children, as in our group, were neutropenic even before treatment was initiated, others showed low levels of neutrophils during follow-up without a change in the drug schedule [8]. In the Chicago study, up to 58% of patients treated for 12 months developed neutropenia [5]. In our clinical material modification of the therapy was required in 12/24 (50%) patients, in 10 because of neutropenia and in 2 children owing to elevated level of pyrimethamine. Daily dose reduction was applied in one case, sequential supply every 2–3 days in 9, and periodic discontinuation of therapy in 2 cases. Because the individual variability of PYR concentration levels remains large despite the use of doses standardized to body weight, the population parameters cannot be used to calculate the starting dose and it seems necessary to work with an individual dosage adjustment. Consideration must be given to the wide individual variability in plasma PYR levels among patients receiving the same dose regimen [7]. The serum concentration of

pyrimethamine is not predictable even when the dose is standardized to body weight. In our group, despite the use of recommended and similar doses, the levels differed significantly between patients. These results indicate that pharmacological monitoring of pyrimethamine is necessary and allows to correct the dose of the drug. The optimal doses and target concentrations in serum still need to be established for antiparasitic drugs used in the treatment of congenital toxoplasmosis.

Conclusions

Monitoring of the plasma concentration of pyrimethamine improves the safety and effectiveness of therapy, and helps to achieve the correct individual dose of the drug.

Neutropenia is the most common side-effect of pyrimethamine observed even when the recommended dose is used.

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Monitorowanie stężenia pirymetaminy w surowicy krwi u niemowląt z wrodzonym zarażeniem *Toxoplasma gondii* – obserwacje własne

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Celem pracy była ocena stężenia pirymetaminy w surowicy krwi u 24 niemowląt w wieku 1–5 miesięcy, leczonych z powodu toksoplazmozy wrodzonej. Pirymetaminę podawano w jednej dawce dobowej w ilości 0,35–0,98 mg/kg, z sulfadiazyną (50–100 mg/kg/dobę) stosowaną w 2–3 dawkach podzielonych i kwasem folinowym (dwa razy w tygodniu po 7,5 mg). Leczenie kontynuowano przez 2–6 miesięcy, następnie do ukończenia 12 miesiąca życia lub dłużej stosowano Fansidar®. Stężenie pirymetaminy oznaczano przy użyciu wysokosprawnej chromatografii cieczowej (HPLC). Ogółem wykonano 70 oznaczeń. Stężenie pirymetaminy wahało się od 0,01 do 1,2 μg/ml. U 14 dzieci (58 oznaczeń) uzyskano stężenie leku mieszczące się w zakresie wartości terapeutycznych. U 7 dzieci (8 oznaczeń) stężenie leku było poniżej, zaś u 3 (4 oznaczenia) powyżej zakresu terapeutycznego. U 11/24 (46%) dzieci obserwowano łagodną, przejściową neutropenię. U 12 pacjentów konieczna była modyfikacja leczenia.

Monitorowanie stężenia pirymetaminy w surowicy krwi poprawia bezpieczeństwo i skuteczność leczenia i umożliwia dobór właściwej, indywidualnej dawki leku. Neutropenia jest najczęstszym działaniem niepożądanym pirymetaminy, występującym także przy stosowaniu rekomendowanej dawki leku.

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