A novel approach to the therapy of placental dysfunction using L-arginine

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The article presents the authors' own data on assessment of L-arginine as a therapy of placental dysfunction in pregnant women. According to the results of this study, Tivortin® was highly effective and safe at Week 24–27 and Week 32–34 of gestation. Furthermore, the incidence of preterm delivery has decreased 2.3 times, the incidence of foetoplacental dysfunction has decreased 4.6 times, the incidence of intrauterine growth restriction has decreased 4 times, and the incidence of foetal distress has decreased by 9.6%, whereas the blood flow in the maternal-placental-foetal circulation system has grown by 37.7%.

Key words: *placental dysfunction, L-arginine, intrauterine growth restriction, endothelium, therapy, Tivortin®.*

The placental dysfunction has been included in the International Statistical Classification of Diseases and Related Health Problems as a primary diagnosis of foetal and neonatal abnormalities. Virtually all complications of pregnancy are accompanied by the emergence and the build-up of placental dysfunction (PD). Thus, the incidence of the latter abnormality is 50-77% in habitual miscarriage, 32% in toxaemia of pregnancy and up to 45% in extragenital disease. The foetus developing under insufficient placental perfusion is considerably more susceptible to hypoxic damage of vital organs in course of in utero development and more prone to in labour injury [8].

In course of normal uneventful pregnancy, there is an increase in endothelium-dependent vasodilation, which returns to baseline after delivery. This process involves such mediators as nitric oxide (NO), prostacyclin and endothelium-derived hyperpolarizing factor [5]. The endothelium participates in regulation of vascular tone, vascular permeability, leukocyte and platelet adhesion, angiogenesis, thromboresistance, immune response, the synthesis of inflammatory mediators and their respective inhibitors and barrier functions [6]. The literature data [6] indicate that NO synthesis is an important function of the endothelium, since NO maintains baseline vascular tone and participates in vasodilation in response to various stimuli. When dysfunction occurs, the endothelium acquires atherogenic properties with a tendency to vasoconstrictor responses and thrombophilia; adhesion molecules and growth factors rush into the bloodstream; the inflammatory and oxidative activity of the serum increases.

Endothelial dysfunction is an imbalance between the mediators normally responsible for normal ratios between all endothelium-dependent processes. This primarily applies to production of vasodilators, angioprotectors and anti-proliferative substances, on the one hand, and the levels of vasoconstrictors and pro-thrombotic/proliferative factors, on the other hand [7, 12]. Endothelial dysfunction accompanies the complications of pregnancy and delivery, which are to different extents related to the placental factor and the impaired vascular adaptation in pregnancy [8].

Endothelial damage is facilitated by such factors as smoking, toxic exposures, hypertension, obesity, hyperhomocysteinaemia, dyslipidaemia, insulin resistance, activation of the cytokine cascade in systemic inflammation, oxidative stress, the effects of endogenous blockers of the endothelial NO-synthase, vasoactive peptides (angiotensin II, endothelin-1), accumulation of asymmetric dimethyl arginine, changes in uric acid levels, etc. [8]. The impact of the damaging factors in endothelial cells include destruction of the cells, accelerated apoptosis, reduced NO synthesis and reduced production of hyperpolarizing factors. Consequently, the increased functional activity of adhesion molecules and chemokines activates inflammatory responses and vasoconstriction and changes homeostatic parameters. Numerous studies have demonstrated the participation of NO in the maintenance of baseline vascular tone, stabilisation of blood rheology, prevention of aggregation of blood corpuscles, reduction of vascular wall permeability and elimination of the consequences of metabolic acidosis [2].

With every year there are more reports dedicated to practical obstetric use of various treatment protocols and correction of impaired utero-placental blood flow, the latter performed using several modalities. However, the pharmacological 'aggression' or obstetrical polypharmacy has increased the inci-

dence of both maternal and foetal adverse effects, has caused neonatal sensitisation, and delayed complications of drug therapy [8]. From this perspective, it is reasonable to employ pharmacological therapy with endogenous metabolites, which produce a multidimensional effect in several pathogenetic links of PD while exerting minimal adverse effects in the foetus.

Today, owing to the expanded diagnostic options to detect impaired placental functions, as well as in view of novel data on the mechanisms of regulation of placental blood flow in both physiological pregnancy and aggravated pregnancy, there is a new possibility to append the PD therapeutic protocols with NO donor substances (L-arginine). Tivortin®, being a gravidoprotector, fully meets all of the above expectations.

In a normally functioning endothelium, low levels of NO are continuously released to keep blood vessels dilated and provide for non-adhesion of blood corpuscles to the endothelium. When exposed to various damaging factors (mechanical, infectious, metabolic, related to immune complexes, etc), endothelial cells diminish their ability to release relaxing factors, whereas the formation of vasoconstriction factors is either intact or increased; this condition is defined as endothelial dysfunction [1, 3, 11].

It is known that in human body NO is synthesized from the L-arginine amino acid with NO-synthase (NOS) enzymes. Thus, this essential amino acid is a substrate for NO synthesis [12]; the use of L-arginine has a positive impact on the function of vascular endothelium and improves endothelium-dependent vasodilation. In Ukraine, L-arginine is authorised as a 4.2% solution for infusions, known under the Tivortin® brand (manufactured by Yuria-Pharm, Ukraine). This product provides the body with the building material for NO synthesis.

Much attention is given to the influence of the L-arginine/NO bioregulatory system on the utero-placental blood flow and to the intrauterine foetal development. Increased production and release of NO, mediated by L-arginine, may act as an antioxidant and facilitate improvements of endothelial function in pregnant women.

The aim of this research was to evaluate the efficacy of using L-arginine in the therapy of foetoplacental dysfunction in pregnant women.

MATERIALS AND METHODS

The study enrolled 79 pregnant women aged 18 to 39 years with foetoplacental dysfunction (FPD) at Weeks 16–40 of pregnancy. The main group (Group 1) consisted of 53 patients, which, in addition to standard therapy, received Tivortin® intravenously at the dose of 100 mL/day for 10 days. The reference group (Group 2) consisted of 26 women, which received standard background therapy for FPD, including vasoactive, metabolic and anti-platelet components. The control group included 26 pregnant women with uncomplicated obstetric and gynaecological history and no signs of FPD at Weeks 16–20. Oestriol levels were measured in venous blood at Week 36 of gestation by immunofluorescence assay using a commercially available test kit system.

Table 1

The peculiarities of the course of pregnancy in the assessed women after the treatment for FPD at Weeks 28–32

The complications of pregnancy	Group 1		Group 2		Control group	
	Absolute number	%	Absolute number	%	Absolute number	%
The risk for preterm delivery	12	22.6^	12	46.2*	3	11.5
Low placentation	4	7.6	1	3.8	1	3.8
Marginal placental presentation	1	1.9	1	3.8	-	-
Late pregnancy toxaemia	14	26.4*^	12	46.2*	2	7.7
Polyhydramnios	6	11.3	4	15.4	-	-
Oligohydramnios	3	5.7	2	7.7	-	-
Foetoplacental insufficiency	4	7.6^	9	34.6	1	3.8
Intrauterine growth restriction (IUGR)	3	5.7^	6	23.1	-	-
Late pregnancy anaemia	28	52.8	17	65.4	12	46.2
Asymptomatic bacteriuria	5	9.4	3	11.5	2	7.7
Acute respiratory disease	7	13.2	3	11.5	2	7.7
Colpitis	10	18.9	6	23.1	3	11.5

Notes: * $p < 0.05$ when compared to control group. ^ - $p < 0.05$ when compared to Group 2.

Table 2

The incidence of haemodynamic disturbances (%) in the maternal-placental-foetal circulation system before and after treatment in pregnant women of Group 1 and Group 2 (n=79)

Haemodynamic disturbances	Before treatment, Week 24-27		After treatment, Week 28-32	
	Group 1 (n=53)	Group 2 (n=26)	Group 1 (n=53)	Group 2 (n=26)
Uterine arteries only	9/17	5/19.2	3/5.7*	3/11.5
Umbilical artery	6/11.3	4/15.3	2/3.8	3/11.5
Middle cerebral artery	3/5.7	2/7.7	-	2/7.7
Umbilical artery and uterine arteries	7/13.2	3/11.5	2/3.8	2/7.7
Abnormalities in all circulatory links	2/3.8	1/3.8	-	1/3.8
Total	27/50.9	15/57.7	7/13.2*	11/42.3

Note: * p <0.05 when compared to the pre-treatment value

In ultrasound imaging, special attention was paid to potential signs of foetoplacental insufficiency, such as intrauterine growth restriction (IUGR), polyhydramnios, oligohydramnios, changes in placental structure and the degree of placental maturity. The Doppler vascular test, starting at Week 16, included assessment of utero-placental blood flow in the uterine arteries (when indicated); starting at Week 24, the test included assessment of foeto-placental blood flow (in umbilical artery and in middle cerebral artery). The indices of arterial vascular resistance were also determined, such as the pulse index (PI), resistance index (RI) and the systolic/diastolic ratio (SDR). The ultrasound tests were performed using the ESAOT diagnostic ultrasound imaging unit by 'Technos' (Italy), equipped with 3.5 MHz and 7.5 MHz probes and the Colour Doppler Imaging (CDI) mode. The following conditions were regarded as exclusion criteria: diabetes mellitus, hypertension and bronchial asthma.

Sequential therapy with Tivortin® was employed in patients with clinical manifestations of placental insufficiency (Stage I–II IUGR, signs of intrauterine foetal distress) and in patients with impaired blood flow in the utero-placental and foeto-placental compartments at the gestation age of 24–27 weeks. Tivortin® was administered via intravenous drip as a 4.2% solution, 100 mL per infusion; the treatment schedule included 10 infusions with subsequent conversion to oral presentation of the product (Tivortin® aspartate at 5 mL t.i.d. for 14 days). Treatment efficacy was checked every 10 days.

Statistical data processing was performed using the Statistica 6.0 statistical software package. The differences between the values was considered statistically significant at p <0.05.

RESULTS AND DISCUSSION

Clinical assessment of gestational process was performed in women of study groups with signs of FPD. Threatening miscarriages in the first half of pregnancy were documented in 43.4% women of the main group and in 53.8% women of the reference group, which were significant differences from the control group (11.5% cases, p<0.05). In pregnant subjects of Group 1 (those receiving a therapeutic/preventive regimen with Tivortin®, the risk of preterm delivery (most frequent at Week 28–30) has decreased 2-fold compared to conventional therapy (see Table 1). Quite a frequent complication in the second half of pregnancy in women with FPD was toxaemia of pregnancy. However, after therapy including L-arginine in women of Group 1, the incidence of this complication has decreased 1.8 times compared to conventional therapy, where every other subject was diagnosed with late toxaemia of pregnancy.

Thorough observation of pregnant women, timely treatment and prophylaxis of severe forms of late toxaemia of pregnancy have prevented conversion of toxaemia into severe forms in most women. In the control group, 2 patients (7.7%) were diagnosed with oedema gravidarum.

A convincing demonstration of the efficacy of pharmaceutical prophylaxis of severe complications in pregnancy (aimed at the full development of foetoplacental complex) are the data concerning the frequency of such pregnancy complication, as foetoplacental insufficiency (FPI) in our patients. Thus, in the group of women with FPD where Tivortin® was not used as a part of treatment, chronic FPD occurred 4.6 times more frequently than in Group 1 (34.6% vs. 7.6%, p<0.05). Besides that, in Group 1 gravidae (receiving L-arginine solution), the frequency of the above complication did not exceed that in the control group. One more clinical feature of gestation in study subjects was the high incidence of intrauterine growth restriction (IUGR) despite treatment (23.1%) and intrapartum foetal distress (11.5%) in Group 2 compared to the same parameters after Tivortin®-containing therapy in women of Group 1, where the incidence of IUGR was 4 times

lower and no cases of foetal distress were observed. In the control group, FPD was found only in one case (3.8%).

A reliable method to diagnose FPI is Doppler ultrasound. Doppler ultrasound assessment of blood flow in uterine arteries at Weeks 16–20 has demonstrated abnormal findings in 11 (20.8%) pregnant subjects of Group 1 and in 6 (23.1%) pregnant subjects of Group 2; such findings were absent in the control group. In further Doppler ultrasonography assessments at Weeks 24–27 of pregnancy, abnormalities of foeto-placental circulation were found as well. The haemodynamic disturbances of blood flow followed the pattern of distribution, presented in Table 2. It should be noted that pre-treatment Doppler ultrasounds in pregnant women of Group 1 and Group 2 did not reveal any significant intergroup differences concerning abnormalities of uteroplacental and foeto-placental blood flow. Blood flow in the uterine artery in the main and the reference groups at Weeks 24–27 of pregnancy was decreased by 17% and 19.2%, respectively; the decrease of blood flow in the umbilical artery was 11.3% and 15.3% in the respective groups ($p>0.05$). No disturbances of blood flow were documented in the control group.

The condition of blood flow in the maternal-placental-foetal circulation system largely depends on the physiological balance of vasoconstrictors and vasodilators in the mother. In turn, impaired blood flow in the uteroplacental and foetoplacental territories will inevitably affect the intrauterine well-being of the foetus. We have performed Doppler ultrasound assessments of circulation in uterine arteries, umbilical artery and middle cerebral artery in the foetus. The findings of these tests allowed detecting certain changes of qualitative indices (SDR and RI), manifested as upward trends in the group of non-Tivortin® pregnant women with FPD (Group 2). In Group 1, where the pregnant women had stepwise therapy with Tivortin® (including parenteral course over 10 days and oral course over 14 days), the values of vascular resistance indices were in 86.8% cases within the limits of standard values ($p<0.05$). The findings of Doppler assessments in foetal middle cerebral artery allow us to state that the circulatory changes in the vessel primarily indicate the problems of foetal circulation, that is, a more severe hypoxic impairment of the entire foetoplacental complex and depletion of compensatory reserves of this complex. Disturbances of blood flow in the maternal-placental-foetal circulation system were more frequent in the pregnant women of Group 2, who had combined anomalies of blood flow in the placental vessels, uterine arteries, umbilical artery and middle cerebral artery. The pattern of SDR changes with time in Group was similar to that in the control group; SDR was 2.5–3.2 in Group 1, 2.4–2.8 in the control group and 3.4–4.2 in Group 2. As it is known, higher SDR values are associated with more pronounced disturbances of foeto-placental blood flow, which was exactly the clinical situation diagnosed in Group 2.

Functional assessment methods were used to diagnose the problems with foetal well-being in utero, as well as to assess the responses of the foetus and the foetal compensatory/reserve capacities. In cardiomonitor assessment under a point-base scale according to W. Fisher, we have detected a decrease in overall points in Group 2 compared to Group 1. In the control group, foetal biophysical profile (BPP) score was 9 points in 80.8% of the patients, whereas the same score was found in 43.4% patients of the main group and only in 30.8% patients of the reference group ($p>0.05$). Pronounced signs of intrauterine foetal distress (5–6 points) were found in 11.5% patients in Group 2 and in just 1.9% women in the main group.

Concerning pregnancy outcomes, it is noteworthy that 17% of pregnancies in the main group and 38.5% of pregnancies in the reference group resulted in preterm delivery ($p<0.05$). All deliveries in the control group were in term. Unlike women in the control group, the labour in the main group and in the reference group was complicated by poor uterine contraction strength in 22 (41.5%) and in 8 (30.8%) patients, respectively ($p>0.05$). According to certain authors [10], chronic foetoplacental insufficiency may cause abnormal labour due to serious metabolic changes in the placenta, which was also confirmed in our study. Premature rupture of membranes occurred in 5 (9.4%) of women in Group 1, which was not different from the control value; however, this was considerably less frequent than in Group 2 (30.8%; $p<0.05$).

Taking into account the diversity of clinical presentations of FPD-related abnormalities of pregnancy, we have also studied the changes of oestriol at Weeks 28–32 of gestation. The latter hormone is one of the indices allowing reliable evaluation of the functions of the foetoplacental system. Oestrogens reflect both the placental function and the intrauterine condition of the foetus, since they are the product of a single foetoplacental structure. Most of subjects with foetoplacental problems had low oestrogen levels. Thus, at Week 36 (post-treatment) the levels of oestriol were 731±26.9 nmol/L in the main group, 325±17.8 nmol/L in the reference group ($p<0.05$) and 824±42.1 nmol/L in the control group.

In course of multidimensional gravida assessment and FPD treatment with Tivortin®, we have not detected a single case of perinatal death in the main group (compared to 3.8% perinatal death rate in Group 2). Preterm live births occurred in 17% and 34.6% cases in Group 1 and Group 2, respec-

tively. There were 2 (3.8%) children with signs of intrauterine failure to thrive in Group 1 and 5 children (19.2%) in Group 2 ($p<0.05$). Signs of intrauterine hypoxia were present in 4 children (7.5%) in Group 1 and in 7 children (26.9%) in Group 2 ($p<0.05$).

Taking into consideration literature reports [10, 14] and the direct linear relationship between serum arginine levels and foetal and neonatal well-being/ morphological characteristics of the placenta (as found by S.V. Khlybova et al. (2006) [9]), we define the following principal pathogenetic links of placental insufficiency in NO deficiency: vasoconstriction and decreased utero-placental-foetal blood flow, activation of oxidative stress (leading to disruption of haemodynamics and formation of endothelium-damaging reactive NO forms [peroxynitrite]), decreased glomerular filtration rate and renal blood flow, activation of the haemostatic system, reduced oxygen transport capacity of red blood cells and decreased functional capacity of the placenta. The study by E.V. Kazantseva (2004) has shown that NO levels in maternal blood and amniotic fluid were drastically reduced in foetal hypoxia or FGR or the combination of the two [4]. According to K. Rytlewski et al. (2006), L-arginine facilitates intrauterine foetal growth by increasing NO production and improving blood flow in the umbilical artery [13]. Therapy with L-arginine contributes to a substantial increase of the pulsatility index in the middle cerebral artery and the increase of cerebroplacental coefficient (these data were confirmed in our own research).

Therefore, the results of this study, as well as a number of recent publications, have demonstrated the clinical and pathogenetic relevance of therapeutic angiogenesis and support the feasibility, efficacy and safety of stepwise therapy with L-arginine as an active donor substance of nitric oxide in obstetric practice.

CONCLUSIONS

1. In terms of perinatal complications, the patients with FPD must be viewed as a high-risk population, as demonstrated by the results of this study.

2. The diversity of clinical presentations of abnormal gestational process in pregnant women with FPD is related to the functional state of the maternal-placental-foetal circulation system.

3. Patients with FPD may require interventions to prevent preterm delivery, abnormalities of labour, intrauterine failure to thrive and acute foetal distress.

4. Delayed clinical manifestations and lack of effective therapeutic options in the already manifest FPI necessitates effective correction of FPD and prevention of functional disorders in the maternal-placental-foetal circulation system.

5. Using the solution of L-arginine as a stepwise therapy is a novel approach in the management of FPD and prevention of perinatal complications in pregnant women. This approach allowed achieving a 4.6-fold decrease in FPD, a 4-fold decrease in IUGR, a 2.3-fold decrease in preterm delivery rates, a 9.6% decrease in foetal distress and a 37.7% improvement of utero-placental-foetal blood flow post-treatment.

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