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Indices of oxidative stress in pregnancy with fetal growth restriction

AGATA KAROWICZ-BILINSKA[1], KORNELIA KĘDZIORA-KORNATOWSKA[2], & GRZEGORZ BARTOSZ[3,4]

[1]*High-Risk Pregnancy Unit, Medical University of Łódź, Lodz, Poland,* [2]*Department and Clinic of Geriatry, Medical College in Bydgoszcz, Mikolaj Kopernik University of Torun, Bydgoszcz, Poland,* [3]*Department of Molecular Biophysics, University of Lodz, Lodz, Poland, and* [4]*Department of Biochemistry and Cell Biology, University of Rzeszów, Rzeszow, Poland*

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Abstract
Intrauterine fetal growth restriction (IUGR), the main cause of premature delivery and fetal mortality, has been suggested to involve oxidative stress. We found elevated values of indices of oxidative stress in the blood serum of pregnant women with IUGR: increased levels of malondialdehyde and 4-hydroxyalkenals, decreased activity of α-1-antitrypsin and decreased total antioxidant capacity of the serum, with respect to healthy pregnancy. Twenty day treatment with 3 g of L-arginine and 75 mg of acetylsalicylic acid daily resulted in a decrease of the level of lipid peroxidation products and augmentation of α-1-antitrypsin activity. This study confirms the occurrence of oxidative stress in IUGR and demonstrates the beneficial effect of arginine/acetylsalicylic acid therapy in reducing oxidative stress in IUGR.

Keywords: *Pregnancy, fetal growth restriction, lipid peroxidation, total antioxidant capacity, α-1-antitrypsin, arginine*

Introduction

Intrauterine fetal growth restriction (IUGR) is defined as a birth of a newborn with a body mass less than the 10th centile with respect to the reference value for current pregnancy age. IUGR may occur from second trimester of the pregnancy and may be due to various causes. IUGR may be associated with a higher risk of perinatal morbidity and up to seven-fold risk of perinatal mortality [1]. The intrauterine assessment of IUGR is based on the ultrasound biometric evaluation of the fetus state which allows a relatively early and precise diagnosis [2,3].

Indices of oxidative stress have been reported for the placentum [4] and amniotic fluid [5] in IUGR. The aim of this study was to ascertain whether values of indices of oxidative stress are elevated in the blood plasma of pregnant women with IUGR.

Eighty percent of IUGR cases of unclear origin is ascribed to changes in utero-placental circulation and blood vessel function, so normalization of the circulatory function can be expected to have positive effects in IUGR. Treatment with arginine, a precursor of nitric oxide which has a strong diastolic effect on the vessels, has been proposed as a means of therapy of IUGR [3,6,7]. It has been demonstrated that in preeclampsia, one of the causes of IUGR, the capacity for cellular L-arginine transport is augmented [8,9] and that administration of L-arginine (3 g/day) improves fetal conditions and neonatal outcome in preeclampsia [10]. In our department, a routine treatment of IUGR involves administration of L-arginine and acetylsalicylic acid, an inhibitor of the thromboxane synthase, which decreases the synthesis of thromboxane, a factor exerting a systolic effect on the vessels. Thromboxane biosynthesis and action

Correspondence: A. Karowicz-Bilińska, High-Risk Pregnancy Unit, Medical University of Łódź, Lodz, Poland. Tel/Fax: 48 42 686 04 71. E-mail: agakar@interia.pl

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involves increased generation of reactive oxygen species. Therefore, the arginine/acetylsalicylic acid therapy can be expected to affect the prooxidant/antioxidant balance in the body. Acetylsalicylic acid has also a strong anti-aggregative action on blood platelets. Nitric oxide has an antioxidant action [11–13] although its reaction with superoxide can lead to formation of peroxynitrite, a strong oxidant [14,15]. Another aim of this study was, therefore, to examine whether the arginine/acetylsalicylic acid therapy affects the indices of oxidative stress, especially those concerning the level of lipid peroxidation, in IUGR.

Materials and methods

The study was conducted in the High-Risk Pregnancy Unit of the Medical University of Lodz during the years 2004–2005, among hospitalized pregnant women. The study group consisted of pregnant women with asymmetrical IUGR between the 9th and the 5th centile with respect to the current pregnancy age. IUGR was diagnosed by ultrasound measurement of fetal biometry. The measurement was done twice by two independent specialists to diminish the danger of incorrect diagnosis [3].

The study group comprised 34 pregnant women not suffering from hypertension or illnesses of circulatory system, liver or kidneys. The pregnancy age in this group was between the 34th and the 38th weeks. Pregnant women with abnormal results of cardiotocograms or abnormalities in the vascular flow measured by Doppler sonography were excluded from the study. The control group consisted of 42 healthy pregnant women with normal weight pregnancy, being in the same pregnancy age (36 weeks at the beginning of the study in both cases). The average age was 27.6 years in the patient group and 25.9 years in the control group.

The pregnant women with diagnosed IUGR were given 1 g of L-arginine in the form of tablets three times a day and Acard (75 mg of acetylsalicylic acid) in one daily dose for 20 days. Since, the effects of acetylsalicylic acid on blood platelets can be overcome by production of new platelets, which takes from 4 to 7 days, it seemed safe to terminate the treatment with acetylsalicylic acid at least 5 days before the expected delivery [3]. The study was approved by the Ethics Committee of the Medical University of Łódź and all written consent was obtained from all patients participating in the study.

Blood samples were taken from all the women during the first 24 h of hospitalization, before the application of the treatment, from the veins of the triangle of the elbow and then after 20 days of treatment in IUGR group. Blood was stored at room temperature for 20 min to allow for clotting. After clotting, the samples were centrifuged. The separated serum was aliquoted into Eppendorf tubes and frozen at −75°C until the measurement. Every measurement was done from a sample which was thawed only once.

Total antioxidant capacity (TAC) was measured using the Bioxytech® AOP-490™ (Oxis International) colorimetric assay based upon the reduction of Cu^{2+} to Cu^{+} by antioxidants present in the sample and complex formation by bathocuproine (2,9-dimethyl-4,7-diphenyl-1,10-phenantroline) with Cu^{+}. Malondialdehyde (MDA) and 4-hydroxynonenals were measured by the Bioxytech® LPO-586™ assay (Oxis International), based on the reaction of aldehydes with *N*-methyl-2-phenylindole at 45°C to form an intensely colored carbocyanine dye. α-1 Antitrypsin activity was estimated with the colorimetric Bioxytech® α1AP-410™ Assay (Oxis International), according to the instructions of the manufacturer.

Statistical significance of differences was estimated using and the non-parametric *U*-Mann Whitney test. The normality of distributions was verified by Shapiro–Wilk tests. Evaluation of the effect of treatment was done by the Student's *t*-test and the Wilcoxon tests. $P < 0.05$ was considered as the level of the statistical significance.

Results

Four indices of oxidative stress were evaluated in the blood of women with uncomplicated pregnancy and in women with IUGR. All of them pointed to the higher oxidative stress load in pregnant women with IUGR. Both indices of lipid peroxidation, the level of MDA and the level of MDA plus alkenals, were increased in pregnancy with IUGR. Increased lipid peroxidation was accompanied by decreased values of TAC of blood and decreased activity of α-1 antitrypsin (Table I). It is known that oxidative stress may lead to oxidation of methionine in the α-1 antitrypsin molecule which causes inactivation of the inhibitor and release of elastase activity which may induce vascular abnormalities [16]. Therefore, decrease of α-1 antitrypsin activity can be also an index of oxidative stress [17,18].

In the second part of this study the oxidative stress parameters were compared in the group of pregnancies with IUGR before and after 20-day treatment

Table I. Indices of oxidative stress in women with uncomplicated pregnancy and in pregnant women with IUGR (mean ± SD, number of cases in parentheses).

Parameter	Uncomplicated pregnancy	IUGR
TAC [μM]	0.91 ± 0.19 (42)	0.75 ± 0.26 (34)*
α1-Antitrypsin [A410]	15.64 ± 7.04 (42)	12.46 ± 7.82 (34)*
MDA [nM]	137 ± 62 (41)	217 ± 158 (33)*
MDA + alkenals [nM]	175 ± 68 (41)	370 ± 120 (33)*

Student's *t*-test; * $P < 0.01$.

Table II. Effect of L-arginine/salicylic acid treatment on the oxidative stress in pregnancy with IUGR (mean ± SD, $n = 24$).

Parameter	Before treatment	After treatment
TAC [μM]	0.72 ± 0.19	0.84 ± 0.17*
α1-Antitrypsin [A410]	12.80 ± 3.06	14.56 ± 3.34
MDA [nM]	245 ± 190	166 ± 54
MDA + alkenals [nM]	380 ± 91	277 ± 82†

Student's *t*-test; * $P < 0.05$; † $P < 0.01$.

with L-arginine plus acetylsalicylic acid. While changes in α-1-antitrypsin activity and MDA level were at the borderline of statistical significance ($0.05 < P < 0.07$), the significant increase of TAC and decrease of HNE level (Table II) clearly demonstrate that the therapy employed alleviated oxidative stress in the patients.

Discussion

Numerous studies have demonstrated that pregnancy is a situation of physiological oxidative stress for the mother [19,20]. The present results substantiate the view that IUGR is a condition in which this pregnancy-associated oxidative stress is yet aggravated [4,21,22]. The underlying reason may be a long-lasting malfunction of the vessels.

In our study two parameters of lipid peroxidation process were measured, viz. the level of MDA and the level of MDA + alkenals. Both parameters of lipid peroxidation were elevated in the group of women with IUGR when compared with the group of uncomplicated pregnancy. The results were similar to the results presented by other authors [23].

Reactive aldehydes are known to be second messengers of the harmful action of reactive oxygen species. They are known to modify all types of biomolecules, especially proteins [24–26]. Malondialdehyde is one of by-products formed in the process of lipid peroxidation of polyunsaturated fatty acid residues, and its level is treated as the indicator of intensity of free radicals process in the body. MDA is also a by-product of formation of inactive thromboxane B2 (TXB2) from its active form, TXA2 which is one of vasoconstrictive factors. The production of MDA in this process is equimolar to TXB2 formation so the MDA level can reflect the rate of inactivation of TXA2 and, in this way, the vessel relaxation capacity [23].

Atherosclerosis, observed also in placentas from IUGR pregnancies, is mediated by inflammatory and oxidative mechanisms including lipid peroxidation. HNE, another end-product of lipid peroxidation, induces proliferation of vascular smooth muscle cells which contributes to the mechanism of vessel injury [27]. High concentration of HNE in the group of IUGR can be harmful not only to the mother but also to the fetus.

Low value of TAC reflects deficiency of antioxidant defense in case of IUGR and is another index of augmented oxidative stress.

Alpha-1 antitrypsin (AAT) is one of the major serine proteinase inhibitors and a signaling protein for the expression of the different pro-inflammatory molecules [28]. As an inhibitor of elastase, AAT inhibits also the neointima formation in the small-diameter artheries [16]. AAT has anti-inflammatory effect, preventing inflammatory reactions, especially in soft tissues [29]. Lowered activities of AAT were found in the group of IUGR women may be connected with the incorrect reconstruction of small vessels wall in utero-placental circulation system during pregnancy. These changes can also cause decrease of utero-placental blood flow and initiate IUGR in the second trimester of pregnancy. We are not aware of any data demonstrating the role of AAT in the ethiopathogenesis of IUGR but it can be postulated that the deficiency of the activity of this inhibitor may result in impaired stabilization of small vessels and restricted blood circulation between mother and fetus.

Limitation of oxidative stress in IUGR seems, therefore, to be a reasonable goal of therapy. The present results confirm that the L-arginine/acetylsalicylic acid therapy is efficient in this respect. Undoubtedly, possible adverse effects of the treatment should be carefully taken into account. The suspected increased risk of congenital heart defects in infants by taking aspirin during pregnancy has been dismissed even for the first trimester of pregnancy [30]. An increased risk of miscarriage by the prenatal use of acetylsalicylic acid has been reported [31]. However, in our previous clinical trial we found no increased risk of pour pregnancy outcome in patient with IUGR (not complicated by preeclampsia) [3,32,33]. Moreover, we found that the arginine/acetylsalicylic acid therapy improves fetal growth and helps to decrease the number of Caesarean sections [3,33]. The present results demonstrate that it reduces also oxidative stress in IUGR.

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