BIOPHYSICS AND BIOCHEMISTRY

Production of Vascular Endothelial Growth Factor and Endothelin in the Placenta and Umbilical Cord during Normal and Complicated Pregnancy

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The concentrations of vascular endothelial growth factor and endothelin in the placenta progressively increased during normal pregnancy. Production of vascular endothelial growth factor and endothelin in the placenta exceeded the normal during trimester I miscarriage and trimester III premature birth accompanied by intrauterine hypoxia. The concentration of these vasoactive substances during premature birth also increased in the umbilical cord. The compensatory decrease in the concentrations of vascular endothelial growth factor and endothelin in the placenta and umbilical cord was observed during full-term pregnancy with threatened abortion.

Key Words: *endothelin; vascular endothelial growth factor; placenta; umbilical cord*

Normal gestation and reproduction on the whole (ovulation, implantation, and placentation) depend on angiogenesis and production of angiogenic factors regulating growth, development, and regression of vessels and stimulating proliferation of cells and tissues [12]. Formation of the vascular network in the placenta, fetoplacental circulation, and uteroplacental blood flow determine the course of pregnancy and intrauterine fetal growth.

Vascular endothelial growth factor (VEGF) is one of the major activators of angiogenesis in the female reproductive system. VEGF induces angiogenesis and increases vascular permeability [8]. These processes also involve a variety of factors regulating vascular tone in the uteroplacental-fetal system. Endothelin is of particular importance in this respect. We previously studied production of vasodilating compounds in the placenta [2]. The role of the placenta in the maternal—placental—fetal system is obvious [6,7], but the role of the umbilical cord in the regulation of fetoplacental blood flow in the placental—fetal unit is poorly studied. It should be emphasized that the endothelium of umbilical vessels can serve as an additional source of vasoactive and angiogenic substances in fetal circulation. The imbalance between endogenous regulators of angiogenesis and compounds that modulate vascular tone in the placenta and umbilical cord can led to pregnancy complications.

Here we studied production of VEGF and endothelin in the placenta and umbilical cord during normal pregnancy and placental insufficiency (PI) accompanied by intrauterine hypoxia.

MATERIALS AND METHODS

Experiments were performed with homogenates of the early chorion, mature placenta, and umbilical cord from women of 22-29 years. Group 1 included 20 patients with medical abortion at 6-10 weeks'

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gestation. Group 2 consisted of 22 patients that carried normal pregnancy eventuated by term delivery at 39-40 weeks' gestation. Complicated pregnancy in group 3 (n=18) and 4 patients (n=20) resulted in miscarriage at 6-10 and 36-37 weeks' gestation, respectively. Full-term pregnancy in 34 women was complicated by PI (group 5). The diagnosis of PI was made after ultrasound examination of the fetoplacental system, hormonal studies, and measurement of activities of specific placental alkaline phosphatase and glutamate dehydrogenase isoenzymes [5]. The severity of hypoxia was estimated by gas composition, acid-base balance, and blood concentrations of xanthine and guanine [1]. The concentrations of endothelin-1 and VEGF in 10% homogenates of the placenta and umbilical cord were measured by enzyme immunoassay using commercial kits (R&D system).

The results were analyzed by Statistica 5.1 software (StatSoft. Inc.). Homogeneity of variances was estimated by Fischer's test. The significance of differences was determined by Student's test and Mann—Whitney test. The results were statistically significant at $p<0.05$.

RESULTS

During normal pregnancy the concentrations of VEGF and endothelin in mature placenta (39-40 weeks' gestation) were higher than in early chorion (by 2.2 and 1.7 times, respectively, $p<0.001$; Table 1). It was probably related to intensification of angiogenesis during placenta development. High endothelin level by the end of pregnancy contributes to uterine contraction during labor. The concentrations of VEGF and endothelin in the umbilical cord were lower than in the placenta (by 2.5 and 1.9 times, respectively, $p<0.001$), but much higher than in the blood and amniotic fluid [4]. Higher concentrations of VEGF and endothelin in the umbilical cord reflect production of these substances by umbilical vessels. It can be hypothesized that they produce a paracrine effect on fetoplacental circulation.

Hemodynamic disturbances in the maternal—placental—fetal system play a role in the pathogenesis of PI. This syndrome is one of the most serious complications of pregnancy accompanied by hypoxia and/or hypotrophy of the fetus. VEGF concentration in the chorion (6-10 weeks' gestation) obtained from patients with primary miscarriage and PI after spontaneous abortion (group 3) was 57% higher compared to that in group 1 patients (Table 1). It can be assumed that miscarriage at an early stage of pregnancy is associated with hemodynamic disturbances and insufficient cyto-

TABLE 1. Concentrations of VEGF and Endothelin in Chorion, Placenta, and Umbilical Cord during Normal Pregnancy and PI ($M\pm m$)

Parameter	Chorion	Placenta	Umbilical cord	Chorion	Placenta		Umbilical cord	
	group 1	group 2	group 2	group 3	group 4	group 5	group 4	group 5
VEGF, ng/g	1.05±0.09	2.31±0.18*	0.92±0.08	1.65±0.13*	3.73±0.31+	1.69±0.11+	1.29±0.09+	0.64±0.04+
Endothelin, pg/g	1.72±0.12	3.01±0.21*	1.58±0.13	4.12±0.32*	5.86±0.42+	1.65±0.14+	2.37±0.21+	1.06±0.08+

Note. $p<0.05$: *compared to group 1; +compared to group 2.

trophoblast invasion in spiral arteries of the placental bed, which results in intrauterine hypoxia.

Hypoxia stimulates production of VEGF, activates its receptors, and increases mRNA expression [10,11]. These changes contribute to the increase in placental VEGF level during spontaneous abortion. Apart from accumulation of VEGF exhibiting also vasoactive activity, the content of endothelin in the chorion of group 2 patients exceeded the normal by 2.4 times ($p<0.001$). Endothelin is one of the most potent vasoconstrictor agents [9,13]. The increase in endothelin concentration and autocrine influences of VEGF on placental vessels determine changes in placental blood flow and cause miscarriage.

VEGF concentration in the placenta obtained immediately after preterm labor (group 4) was 60% higher compared to that observed during normal full-term pregnancy (group 2, $p<0.01$). Endothelin concentration increased by 2 times ($p<0.01$). Probably, the critical level of the test parameters in these patients incompatible with pregnancy prolongation was achieved at a later stage. The concentrations of VEGF and endothelin in the umbilical cord increased by 40 and 50%, respectively ($p<0.01$). These changes were sufficient to cause vasospasm in the umbilical cord and decrease transport of oxygen and nutritive substances in the fetus.

In group 5 patients with full-term pregnancy associated with PI, production of VEGF and endothelin decreased in the placenta (by 27 and 45%, respectively) and umbilical cord (by 30%, $p<0.01$) compared to group 2 patients. These changes probably played a compensatory role and improved fetoplacental circulation due to a decrease in constrictor influences under conditions of vasoactive substance imbalance. This shift provided conditions for carrying full-term pregnancy with threatened abortion. Doppler study of maternal and fetal hemodynamics in pregnant women of this group revealed increased blood flow [3].

Our results and previous data on variations of nitric oxide production in the placenta of pregnant women with PI show that PI and miscarriage are associated with an imbalance between vasoactive and angiogenic substances. The decrease in vasoconstrictor influences in the placenta and umbilical cord improves hemodynamic parameters and prevents abortion.

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