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Receptor for advanced glycation end products (*RAGE*) and *glyoxalase I* gene polymorphisms in pathological pregnancy

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ABSTRACT

Objectives: The aim of the study was to analyze polymorphisms of receptor for advanced glycation end products (RAGE) gene, and *glyoxalase I* gene and soluble RAGE, sRAGE, in physiological and pathological pregnancy.

Design and methods: Polymorphisms of *RAGE* gene (−429 T/C, −374 T/A, 557 G/A, 2184 A/G) and *glyoxalase I* gene (A419C) and sRAGE serum levels were determined in 284 women with pathological and physiological pregnancy.

Results: No differences in distribution of genotype and allelic frequencies of studied polymorphisms were found. GA genotype of *RAGE* 557 G/A polymorphism (known as Gly82Ser) is associated with lower sRAGE serum levels in healthy pregnant women compared to GG genotype (483 ± 104 vs. 692 ± 262 pg/mL, $p = 0.008$). sRAGE correlates negatively with ALT in patients with pregnancy intrahepatic cholestasis ($r = -0.536$, $p = 0.05$).

Conclusions: We did not show any association of *RAGE* and *glyoxalase I* gene polymorphisms with pathological pregnancy, however further studies are needed to confirm the results.

Introduction

Advanced glycation end products, AGEs, are characterized as a wide group of heterogeneous compounds, which can cause tissue damage directly by modifying biological structure and changing their physical and chemical properties or indirectly via interaction with their receptors, especially with receptor for advanced glycation end products, RAGE. AGEs are involved in the pathogenesis of many diseases such as diabetes mellitus, cardiovascular diseases, and cancer. Their pathological effect can be diminished by reduction of production of AGEs in the organism by eliminating their precursors enzymatically with glyoxalase I, or by soluble form of RAGE, sRAGE, which binds AGEs, but doesn't trigger a signaling pathway for RAGE.

The receptor for advanced glycation end products, RAGE, is involved in the pathogenesis of many diseases, such as diabetes mellitus and its complications [1], cardiovascular diseases [2], chronic renal diseases [3], chronic inflammatory diseases and cancer [4], as well as AGEs.

RAGE, a transmembrane receptor and a member of the immunoglobuline superfamily [5] was first described as a receptor for advanced glycation end products [6]. However, RAGE also binds other ligands like proinflammatory S100 proteins/calgranulins, High Mobility Group proteins including HMGB1, amyloid β peptide [7,8]. RAGE is expressed on cell surface of numerous cell types, e.g. macrophages, monocytes, endothelial cells, neurons, smooth muscles. RAGE–ligand interaction results in the activation of intracellular pathways of nuclear factor κB [5]. Stimulation of RAGE leads to generation of oxidative stress and triggering of inflammatory and proliferative processes followed by tissue injury [9].

Soluble receptor for advanced glycation end products, sRAGE, is the truncated form of RAGE, lacking transmembrane domain. Soluble RAGE has two variants: endogenously secreted RAGE (esRAGE),

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which is secreted from the cells and cleaved RAGE (cRAGE), which is formed by proteolytic cleavage from the cell surface by matrix metalloproteinases. sRAGE might act as a decoy by binding RAGE ligands and so diminishes the pathological effects mediated by RAGE [10].

The gene encoding *RAGE* is located on chromosome 6p21.3 and comprises 11 exons. Several polymorphisms of *RAGE* gene are known today. In the centre of interest are particularly *RAGE −429 T/C, RAGE −374 T/A, RAGE 2184A/G* and *RAGE 557 G/A* polymorphisms. *RAGE −429 T/C* and *−374 T/A* polymorphisms are located in the gene promoter and have effect on transcriptional activity. *RAGE 2184A/G* polymorphism is located on intron 8. sRAGE produced by alternative splicing of RAGE mRNA involves areas between introns 7 and 9, so the *RAGE 2184A/G* polymorphism is hypothetically located in the regulatory binding site and influences the production of sRAGE. The study confirms that 2184 GG genotype is associated with elevated sRAGE as well as esRAGE serum levels [3]. *RAGE 557 G/A* polymorphism, also known as *Gly82Ser* polymorphism, located in exon 3, appears to be important in the regulation of ligand binding.

Glyoxalase I, zinc metalloenzyme, metabolizes predominantly glyoxal and methylglyoxal, precursors of AGEs. Decreased glyoxalase I activity leads to enhanced formation of AGEs and thus tissue damage [11,12]. There is evidence that glyoxalase I activity is decreased by RAGE–S100A12 interaction [12].

The gene encoding *glyoxalase I* is located on chromosome 6p21.2 and comprises 6 exons. The most studied *GLO1* polymorphism *A419C* results in Ala111Glu change. According to the latest studies the presence of A− allele causes reduced activity of glyoxalase I enzyme [13].

Preterm labor, hypertensive disorders in pregnancy, especially preeclampsia, intrahepatic cholestasis in pregnancy and intrauterine growth restriction are leading causes of fetal and maternal morbidity as well as mortality. These pregnancy associated diseases are intensively studied, however mechanisms of pathogenesis of the diseases still remain unclear.

The role of RAGE and sRAGE in pathological pregnancy has already been studied. Several studies demonstrated altered sRAGE serum levels in women with preterm labor [14,15], preeclampsia [16] or with gestational diabetes mellitus [17]. Affected amniotic fluid sRAGE concentrations were showed in patients with chorioamnitis and preeclampsia as well [18,19]. Cooke et al. showed in their study an increased expression of RAGE in myometrium of women with preeclampsia [20]. A recent study of Santos et al. focused on *RAGE* −429 T/C and *RAGE* −374 T/A polymorphisms in patients with gestational diabetes, though they did not find any association [21]. The role of glyoxalase I in pathological pregnancy was demonstrated in one single study of Sankaralingam, who showed decreased activity of glyoxalase I in the vasculature of women with preeclampsia [22].

The aim of our study was to investigate the polymorphisms of *RAGE* and *glyoxalase I* gene and sRAGE serum levels in patients with pathological pregnancy trying to describe the genetic background of pathological pregnancy or to find a new biochemical marker (sRAGE) of these pathological states in pregnancy.

Subjects and methods

Study population

The studied group consisted of 284 Caucasian subjects: 120 healthy pregnant women, 99 patients with threatening preterm labor, 35 pregnant women with preeclampsia, 22 patients with intrauterine growth restriction and 14 patients with intrahepatic pregnancy cholestasis.

Healthy pregnant women (N = 120) were examined and followed up during pregnancy in the Department of Gynecology and Obstetrics of the General University Hospital in Prague. Pregnant women were in various weeks of pregnancy, 27 of them were in the 1st trimester, 25 women in the 2nd trimester and 68 women were in the 3rd trimester at the time of enrolling in the study. All patients finally delivered at term. The women did not suffer from any pregnancy induced diseases nor internal diseases. Blood for sRAGE examination and genetic analysis was taken just once during the pregnancy together with blood for other routine examinations.

Pregnant women (N = 99) with symptoms of threatening preterm labor were examined and enrolled in the study. Patients who presented with regular contractions every 15 min (N = 50), with cervical dilatation (N = 50), with PPROM (N = 36) or with vaginal bleeding (N = 15) between the 24th and 36th weeks of pregnancy were included to the study. Subjects with other pregnancy induced diseases were excluded from the study subgroup. 75 patients delivered within 24 h after blood collection for routine laboratory parameters and for sRAGE examination, the rest of them delivered after 24 h after blood collection. Characteristic of patients with threatening preterm labor is in Table 1.

Table 1
Basic and biochemical characteristics of pregnant women with pathological and physiological pregnancy.

Parameter	Preterm labor	Hypertensive disorder	IUGR	ICP	Controls	P
	N = 99	N = 35	N = 22	N = 14	N = 120	
Age (years)	31 ± 5.2	31 ± 4.1	33 ± 3.9	32 ± 3.5	30 ± 4	0.429
Week of pregnancy	29 ± 5	35 ± 3.7[a]	33 ± 3.9[a]	36 ± 2.5[a]	33.8 ± 6.7[a]	<0.05
BMI	25.2 ± 3.8	27.9 ± 3.7[b,a]	26.2 ± 3.7	27.7 ± 3.7[b, a]	24.8 ± 3.6	<0.05
Leu (109/L exponent)	12.6 ± 4.6[b]	9.6 ± 2.8[a]	10.4 ± 3.3	9.6 ± 2.8	10.4 ± 2.4	<0.05
Neu relat. (%)	77.3 ± 9.3[b]	66.2 ± 11.2[a]	68.6 ± 8.9[a]	70.8 ± 8.7	71.5 ± 8.5	<0.05
CRP (mg/L)	15.9 ± 25.1	8.6 ± 14.9	5.7 ± 4.8	5.6 ± 6.2	n.a.	<0.05
Urea (mmol/L)	2.2 ± 1.2	4.8 ± 4.6[a]	3.2 ± 0.9	3.1 ± 1.1	n.a.	<0.05
Creatinine (μmol/L)	51.1 ± 10.7	65.4 ± 13.4[a,c]	55.9 ± 14.2	56.8 ± 9.9	n.a.	<0.05
Uric acid (μmol/L)	214.8 ± 53.2[c]	390.3 ± 80.3[a,c,d]	308.2 ± 77.7	264.9 ± 72.0	n.a.	<0.05
Bilirubin (μmol/L)	7.9 ± 3.3	6.66 ± 3.99°	6.6 ± 2.7°	10.9 ± 4.1	n.a.	<0.05
ALT (μkat/L)	0.34 ± 0.18°	0.39 ± 0.38°	0.41 ± 0.91°	2.9 ± 1.9	n.a.	<0.05
AST (μkat/L)	0.36 ± 0.15°	0.50 ± 0.28°	0.43 ± 0.65°	1.6 ± 1.0	n.a.	<0.05
sRAGE	834.7 ± 610.4	1239.1 ± 991.8[b,a,c,d]	717.0 ± 268.7	663.3 ± 241.2	681.8 ± 262.0	<0.05

ICP—intrahepatic cholestasis of pregnancy, IUGR—intrauterine growth restriction.
CRP—C-reactive protein, ALT—alanine aminotransferase, AST—aspartate aminotransferase, sRAGE—soluble receptor for advanced glycation end products, n.a.—not assessed.
[a] $p<0.05$ vs. preterm labor.
[b] $p<0.05$ vs. controls.
[c] $p<0.05$ vs. IUGR.
[d] $p<0.05$ vs. ICP.