Acute Bloodloss and Cardiological Surgery [in Russian], Omsk (1981), pp. 25-30.
5. V. D. Konvai, A. V. Lukoshkin, and V. S. Pospelov, *Pat. Fiziol.*, **34**, No. 5, 57-59 (1987).
6. V. D. Konvai, A. V. Lukoshkin, and V. B. Smirnova, *Ibid.*, **28**, No. 4, 42-46 (1982).
7. Yu. E. Rashba, L. G. Nagler, L. S. Vartanyan, *et al.*, *Byull. Eksp. Biol. Med.*, **109**, No. 6, 548-550 (1990).
8. N. V. Shim, in: *Pathogenesis and Experimental Therapy of Terminal States* [in Russian], Omsk (1979), pp. 57-62.
9. M. R. Buhl, *Dan. Med. Bull.*, **29**, No. 1, 1-26 (1982).
10. E. Dela Corte and F. Stirpe, *Biochem. J.*, **117**, No. 1, 97-100 (1970).
11. A. Giacomello, *Ann. Nutr. Metab.*, **33**, No. 4, 194-195 (1989).
12. A. Hershko, E. Wind, A. Razin, and J. Mager, *Biochim. Biophys. Acta*, **71**, No. 3, 609-620 (1963).
13. S. Jorgensen, in: *Metoden der Enzymatischen Analyse.* Vol. 3, Berlin (1970), pp. 1874-1878.
14. M.-F. Vincent, G. Van der Berghe, and H. G. Hers, *Biochem. J.*, **222**, 145-155 (1984).

Free Radical Oxidation and Antioxidant Activity in Placental Tissue in Preterm Labor

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Effect of specific scavengers of reactive oxygen species on free-radical oxidation is studied in central and peripheral zones of the placenta from preterm delivering women (32-36 weeks) using the chemiluminescence method. A lower contribution of hypochlorite into free-radical processes and a reduced content of nonprotein SH-groups in the placenta are observed, superoxide dismutase, catalase, and total antioxidant activity being unchanged. It can be assumed that the reduced contribution of hypochlorite into free-radical processes is partially responsible for impaired antimicrobial barrier between mother and fetus in preterm labor.

Key Words: *free-radical oxidation; chemiluminescence; antioxidant activity; placenta; preterm labor*

Placental insufficiency is largely responsible for premature labor and fetal abnormalities. An important role in the pathogenesis of premature labor is played by free-radical oxidation (FRO) in the placenta, impairing the structure and permeability of cell membranes [1,5]. These destructive processes result from the imbalance between the intensity of FRO and efficiency of the antioxidant defense system and can be caused by a number of factors, in particular, endocrine insufficiency and fetal hypoxia due to impaired oxygen supply.

Mechanisms of generation of reactive oxygen species and activity of the antioxidant defense system of different placental zones in women with normal and abnormal (premature labor) pregnancy remain poorly understood.

In light of this, chemiluminescence technique in combination with other methodical approaches allows one to evaluate the role of free radicals in FRO and activity of antioxidant defense system in placental tissue in women with normal and abnormal (preterm labor) pregnancy.

MATERIALS AND METHODS

Placentae were obtained from women with normal pregnancy and term labor and from women with

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preterm (gestation week 32-36) labor. Placental fragments from the central and peripheral zones were thoroughly washed with cold physiological saline and homogenized in ice-cold 60 mM potassium phosphate saline, pH 7.4, at a 1:10 ratio (w/v). The homogenate was centrifuged for 15 min at 10,000 rpm and 4°C. The intensity of chemiluminescence (CL) was measured in an Emilite-1105 chemiluminometer at 37°C for 2 min in a reaction mixture containing 710 μl buffer (60 mM KP_2PO_4 and 105 mM KCl, pH 7.4), 50 μM luminol (10^{-5} M) and 40 μl supernatant. Chemiluminescence was induced by 20 mM hydrogen peroxide (200 μl). Intensity of luminescence was expressed in arbitrary units. Antioxidant activity was assessed by CL-reaction of riboflavin with hydrogen peroxide in the presence of Fe^{2+} [15]. The content of nonprotein sulfhydryl groups was determined spectrophotometrically using the Ellman's reagent [14]. The data were processed statistically using standard statistical software.

RESULTS

It has been generally accepted that luminol-dependent CL (LCL), a process reflecting metabolic CL, is associated with the formation of superoxide and hydroxyl and/or hypochlorite radicals [2,13]. We have previously studied the intensity of LCL in the placentae from women with normal pregnancy and term labor and from women with preterm labor; however, the role of different reactive oxygen species in this process remains unknown. To evaluate the role of radicals responsible for LCL in the placenta in term and preterm labor, we used specific scavengers of reactive oxygen species: superoxide dismutase (SOD), sodium azide, and methionine. As seen from Table 1, SOD inhibited CL in both the central and peripheral placental zones. Since SOD catalyzes the reaction of disproportionation of superoxide radical, LCL induced by this radical and the intensity of FRO in preterm labor remain unchanged. The involvement of hypochlorite, a product formed in the reaction between chloride and hydrogen peroxide catalyzed by myeloperoxidase, as evidenced by luminescence quenching by sodium azide that inhibits this enzyme in leukocytes [10] and peritoneal macrophages [9]. Taking into account the ability of sodium azide to suppress oxidative processes in tissue extracts, we studied the effect of methionine, a highly specific myeloperoxidase inhibitor, on CL intensity in tissue extracts [3]. In women with normal pregnancy sodium azide and methionine inhibited CL of tissue extracts to an equal degree.

Both in the central and peripheral zones of the placenta the inhibiting effect of sodium azide on LCL was significantly weaker in specimens obtained during preterm labor than in correspondent placental fragments obtained from women with normal pregnancy and labor. Similar changes were induced by methionine, which attests to a reduced participation of hypochlorite in FRO.

Neutrophils are the first-line defense of the organism against infectious agents. Myeloperoxidase, an enzyme catalyzing oxidation of chloride ions to highly toxic hypochlorite anion radical, is an important element of neutrophilic oxygen-dependent antimicrobial system. Sorption on bacterial cells is an essential condition of its antibiotic activity [7]. Myeloperoxidase participates in cooperative effects of other antimicrobial factors, in particular, mononuclear phagocytes, thus improving their antimicrobial potential [4]. It should be noted that monocyte-derived tissue macrophages possess no intrinsic myeloperoxidase, but uptake it from the extracellular space by endocytosis, which markedly potentiates their bactericidal activity [12]. It has been found that abnormal pregnancy (premature labor) is associated with changed functional activity of azurophil granules in placental cells [8]. Myeloperoxidase activity of

TABLE 1. Effect of Quenching Agents on Luminol-Dependent Chemiluminescence (LCL) in Placental Tissues ($M \pm m$)

Group of patients, zone of placenta	LCL quenching, %		
	SOD, 250 μg/ml	sodium azide, 0.025 mM	methionine, 0.4 μM
Norm			
Central zone	49.4±4.6 (5)	59.5±8.2 (6)	50.2±3.4 (5)
Peripheral zone	69.6±8.1 (5)	58.4±7.2 (6)	54.0±8.0 (5)
Pathology			
Central zone	52.5±4.5 (6)	15.6±1.9 (4)**	31.6±4.9 (6)*
Peripheral zone	53.7±6.3 (6)	28.0±1.8 (5)**	33.6±3.2 (6)*

Note. *$p<0.05$, **$p<0.001$ compared with the corresponding values in the norm.

TABLE 2. Content of Nonprotein SH-Groups in Placental Tissues (*M*±*m*)

Group	SH-groups, $\times 10^{-4}$ mmol/g tissue	
	central zone	peripheral zone
Norm	7.2±0.3 (16)	6.2±0.3 (16)
Pathology	5.3±0.4 (11)*	5.1±0.3 (10)*

Note. *$p<0.01$ compared with the norm.

these granules in normal and abnormal pregnancy is a subject of our further investigation.

Free-radical-induced damage to tissues and cells is normally prevented by a complex multicomponent antioxidant system. The efficiency of antioxidant defense system is characterized by the total antioxidant activity. We observed no significant differences in antioxidant activity between placental extracts obtained during term and preterm labor; SOD and catalase activities in placentae obtained during preterm labors also did not differ from the control.

By contrast, the content of nonprotein SH-groups, which are considered to be free-radical scavengers, was significantly decreased both in peripheral and central zones of preterm placentae in comparison with control samples (Table 2).

Interestingly, the reduced content of SH-groups is consistent with suppression of SOD-independent FRO (demonstrated by the CL-analysis), which is responsible for the hypochlorite generation catalyzed by myeloperoxidase. Our findings agree with the concept that SH-groups are readily oxidized by hypochlorite [11]. Depletion of tissue SH-group pools in the organism exposed to adverse factors is a common phenomenon and can be used as an index of nonspecific organism's resistance [6]. This was also confirmed by our experiments.

Thus, our findings suggest that FRO in the placenta proceeds via both the SOD-dependent and SOD-independent pathways; the latter is suppressed in premature labor, judging from reduced participation of hypochlorite in oxidative processes and a decreased content of nonprotein SH-groups. This can impair the antibacterial barrier between the fetus and mother and play an important role in the pathogenesis of preterm labor.

REFERENCES

1. V. A. Burlev, N. M. Mamedolieva, I. F. Sitnikov, *et al.*, *Akush. Ginekol.*, No. 7, 22-26 (1991).
2. Yu. A. Vladimirov and M. P. Sherstnev, *Advances in Science and Technology. Ser. Biophysics* [in Russian], Moscow (1989).
3. N. Yu. Govorova, B. P. Sharonov, and S. N. Lyzlova, *Biochemistry*, **53**, 2025-2032 (1988).
4. S. N. Lyzlova, *Vopr. Med. Khimii*, No. 5, 43-48 (1987).
5. V. M. Prokopenko, A. V. Arutyunyan, T. U. Kuz'minykh, *et al.*, *Ibid.*, **41**, No. 3, 53-56 (1995).
6. V. V. Sokolovskii, *Ibid.*, No. 6, 2-11 (1988).
7. O. Yu. Yankovskii and T. E. Dovnar, *Dokl. Akad. Nauk SSSR*, **242**, No. 6, 1445-1446 (1978).
8. L. Corash and E. Gross, *Pediatr. Res.*, **8**, 774-779 (1974).
9. I. Ginbary and R. Boriski, *Cellular Chemiluminescence*, CRC Press (1987).
10. M. B. Hallett and A. A. Cambell, *Biochem. J.*, **216**, 459-464 (1983).
11. Hu-Miao-Lin, S. Louie, C. E. Cross, *et al.*, *J. Lab. Clin. Med.*, **121**, 257-262 (1993).
12. S. Klebanoff, *Inflammation: Basic Principles and Clinical Correlates*, New York (1992).
13. P. S. Rao and J. M. Luber, *Biochem. Byophys. Res. Commun.*, **52**, 39-44 (1983).
14. J. Sedlak and R. H. Lindsaoj, *Anal. Biochem.*, **25**, 192-205 (1968).
15. B. L. Strehler and C. S. Soup, *Arch. Biochem. Byophys.*, **47**, 8-15 (1953).