

Application of electron spin resonance for evaluation of the level of free radicals in the myometrium in full-term pregnancy with normal labour and uterine inertia

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In order to identify and quantify free radicals in the tissues of patients with normal physiological and pathological states of births, we developed a method to evaluate the amount of free radicals in myometrium of subplacental area and from body of uterus, using electron spin resonance spectroscopy. Analysis of the concentration of free radicals in the myometrium in full-term pregnancy with normal labour and during uterine inertia was studied. The activities of Ca²⁺-ATPase, cytochrome *c* oxidase and succinate dehydrogenase in samples of these tissues were tested too. Low free radical concentrations in these tissues were associated with disturbances in contractile activity of myometrium along with reduction of Ca²⁺-ATPase, cytochrome *c* oxidase and succinate dehydrogenase activity. There proved to be an association between the level of free radicals in the tissues and alteration in the physiological processes.

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1. Introduction

Free radicals are important intermediates that play a key role in both normal biological functions as well as in the fundamental mechanisms involved in the pathogenesis of many human diseases. Oxygen is a common reactive species in free-radical processes. Another common gaseous chemical, which is a free radical, is nitric oxide. These are the key chemical components in vascular physiology and inflammatory responses (Proctor and Reynolds 1984). Free radicals are believed to have a role in many physiologic processes, ranging from intermediates in enzyme reaction to effectors. Electron spin resonance (ESR) or electron paramagnetic resonance (EPR) can be used to provide insight into the free radical state of cells, biochemical reactions and measure free radical levels in human tissues (Zyrianov and Sumovskaya 2001). Here

we describe application ESR for evaluation of the level of free radicals from different areas of myometrium with the pathological change that was accompanied by low myometrial contractile activity.

2. Materials and methods

Samples of myometrial and placental tissues were collected during cesarean section. Myometrium was dissected from subplacental area and from the body of the uterus. Samples were immediately placed in test tubes with liquid nitrogen and ground. The tissues were dried in vacuum and weighed. Concentration of the free radicals in these samples was analysed with EPR-2 or EPR-V (USSR) radiospectrometers at microwave frequency 9.330 GHz, microwave power 5 mW (77 K) and modulation amplitude 0.5 mT. The sample size was limited to

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Abbreviations used: Ca²⁺-ATPase, Adenosine triphosphatase; ESR, electron spin resonance.

a few mm. The stable free radicals, **a**, **a**-difenilpikril-hydrozil, served as the standard. EPR spectra were recorded at 77 K. Typical ESR signals of myometrial tissues at $g = 2.0036$ are due to the free radicals (figure 1). Cryosections of 5μ were cut at -20°C . The activities of Ca^{2+} -ATPase, cytochrome *c* oxidase and succinate dehydrogenase in samples of myometrium were indicated by histochemical analysis (Lojda *et al* 1979) using micro-

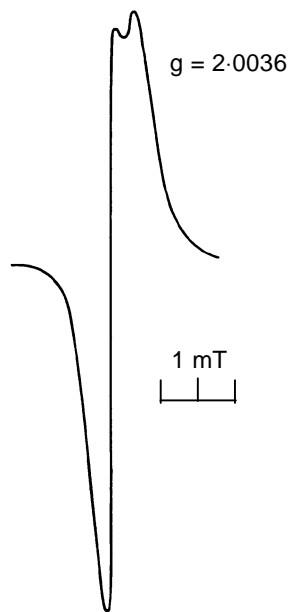


Figure 1. Typical ESR spectra of myometrial tissues. The ESR signals with the component at $g = 2.0036$ are due to free radicals. Record was made at 77 K.

spectro-photometric method (Avtandilov 1984). The disturbances in the myometrial contractile activity were measured by device (fetal/maternal monitor, Series 50-XM HR, Germany). Statistical analysis was accomplished by performing *t*-student method.

3. Results and discussion

Free radicals in the myometrial tissues were identified by ESR. A typical spectrum of an ESR signal obtained from these tissues is represented in figure 1. During the full-term pregnancy with normal physiological labour, the number of paramagnetic centers/g of dry tissue was higher in the myometrium of subplacental area [$4.82 \times 10^{15} - 0.23$ relative units (rel.un.)] than in any other areas. Concentration of the free radicals was considerably lower in the uterus body ($1.20 \times 10^{15} - 0.06$ rel.un.). A pathological state of uterine inertia is accompanied by a decrease in free radicals. The number of paramagnetic centers in tissues samples from both the subplacental area ($1.58 \times 10^{15} - 0.08$ rel.un.) and the uterine ($0.60 \times 10^{15} - 0.03$ rel.un.) decrease sharply in pathological conditions. Comparative low free radical concentrations in the subplacental area and uterus body were associated with disturbances in the contractile activity of myometrium. This symptom was also associated with low levels of free radicals in the myometrium along with the reduction of Ca^{2+} -ATPase, cytochrome *c* oxidase and succinate dehydrogenase activity (table 1) in the wall of myometrial vasculars.

The experiments showed the presence of free radical processes in myometrium. The ESR method made it possible to quantitate changes in free radical concentration in myometrial tissues in normal and pathological states. A

Table 1. Concentration of free radicals and activity of enzymes in myometrial tissues in full-term pregnancy with normal labour and uterine inertia.

Marker	Group of tissues	Normal labour	Uterine inertia
Free radicals*	1	$(4.82 \pm 1.20) \times 10^{15}$	$(1.58 \pm 0.20) \times 10^{15}$
	2	$(1.20 \pm 0.28) \times 10^{15}$	$(0.60 \pm 0.08) \times 10^{15}$
Ca^{2+} -ATPase**	1	0.47 ± 0.01	0.21 ± 0.05
	2	0.26 ± 0.03	0.12 ± 0.02
Cytochrome <i>c</i> oxidase**	1	0.36 ± 0.02	0.14 ± 0.03
	2	0.20 ± 0.03	0.06 ± 0.02
Succinate dehydrogenase**	1	0.28 ± 0.03	0.09 ± 0.02
	2	0.15 ± 0.03	0.04 ± 0.01

*Content of free radicals-paramagnetic centers/g of dry tissue. **Activity of enzymes – relative units of optical density per histochemical samples. Group of tissues: 1-myometrium of subplacental area; 2-uterus body. 16 women were in each group. Data shown are expressed as mean \pm SE of 16 individual observations from different women ($P < 0.01$ vs corresponding different states: normal labour, uterine inertia; $P < 0.01$ vs corresponding different group of tissues).

relationship between contractile activity and the concentration of free radicals has been revealed. Therefore, evaluation of free radicals in the tissues can be used as a marker for alterations in physiological processes.

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