BIOPHYSICS AND BIOCHEMISTRY

Inhibition of Nitric Oxide Production in Tissues of γ-Irradiated Mice by Phenythoin

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Phenytoin diminished production of nitric oxide stimulated by lethal γ-irradiation in mouse liver, intestinal mucosa, lungs, heart, and brain 6 and 24 h postirradiation. This inhibition directly depended on the degree of stimulation of nitric oxide production by γ-irradiation in studied tissues.

Key Words: *nitric oxide; NO synthase; phenytoin, γ-irradiation; mice*

γ-Irradiation considerably stimulates production of nitric oxide (NO) in tissues in the early postirradiation period [2]. This effect can be attributed to activation of NO synthase (NOS). Calcium currents through cell membranes are a universal regulator of NOS activity [7]. Phenytoin has recently gained much attention as a common regulator of calcium currents through the membrane possessing a wide spectrum of pharmacological activities, including antiradiation effect [3]. However, little is known on the effect of phenytoin on NO production in mammalian tissues and NO generation induced by ionizing radiation.

The aim of the present study was to investigate the effect of phenytoin on NO production induced by lethal γ-irradiation in mice, since the existence of Ca^{2+}-dependent inducible NOS was previously demonstrated [6].

MATERIALS AND METHODS

Experiments were carried out on 40 albino randombred male mice (initial genotype was Swiss strain) aged 5 months and weighing 27-30 g. The animals were kept in a vivarium and recieved standard chow and water *ab libitum*. The mice were divided into 7 groups (5-6 animals per group). Group 1 mice were intact controls. Groups 2 and 3 were sacrificed on days 6 and 24 after whole-body γ-irradiation in a dose of 8 Gy; groups 4 and 5 mice received phenytoin 6.5 and 24.5 h before sacrifice; groups 6 and 7 animals received phenytoin 0.5 h before γ-irradiation (8 Gy) and were sacrificed 6 and 24 h postirradiation, respectively. Whole-body γ-irradiation in a lethal dose of 8 Gy was performed on a Gammasell apparatus at 80 cGy/min dose power. Phenytoin (5,5-diphenylhydantoin) was injected intraperitoneally in 0.2 ml physiological saline in a dose of 3 mg/kg body weight. Tissue samples were promptly frozen (no latter than 90 sec after sacrifice) in liquid nitrogen at 77°K in columns (10 mm long and 4 mm in diameter). In samples from the brain, liver, lungs, heart, and intestinal mucosa, the content of NO radicals was measured by electron paramagnetic resonance (EPR) using the method proposed by A. F. Vanin *et al.* [2,10]. To this end, the animals were injected with 500 mg/kg sodium diethyldithiocarbamate (DETC) and iron citrate (37.5 mg/kg $FeSO_4 \times 7H_2O$+187.5 mg/kg sodium citrate) 30 min before sac-

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rifice. These agents form NO radical traps in animal tissues, which are concentrated in cell lipids, primarily in cell membranes. Binding with NO radicals yielded paramagnetic mononitrosyl iron complexes with DETC (MNIC-DETC) characterized by a typical EPR signal with g_1=2.041 and g_2=2.025 and a superfine triplet structure at g_1. In tissues of intact and γ-irradiated animals, EPR signals from various paramagnetic compounds are present within the g-factor range of 1.91-2.2: free radicals (g=2.0), molybdenum complexes (g=1.97), reduced iron-sulfur proteins (g=1.94), magnesium complexes characterized by 6 lines of the superfine structure with 3 low-field components are located before the signal from free radicals. However, the greatest contribution to superposition of MNIC—DETC spectra is made by DETC—Cu^{2+} complex [1]. To improve reliability of our data we distracted the spectrum of pure synthetic DETC—Cu^{2+} complex from the integral spectrum of the test sample. The content of NO radicals in tissues was assessed by the amplitude (A_2) of the 1st (low-field) component of the superfine structure of the EPR spectrum of MNIC—DETC complex (Fig. 1). Measurements were standardized by recording spectrum of a standard (Mn or MgO) at the same channel simultaneously with the spectrum of test sample. The relative content of NO in samples was calculated by the formula: $A_2/(A_1 \times M)$, where A_1 is the amplitude of the second line of EPR spectrum of the standard and M is sample weight, g. X-band EPR spectra were recorded at 77°K on an ESP-300E radiospectrometer (Bruker) at 5 mW MV power and 0.32 mT amplitude modulation. The data were processed statistically using parametric methods and regression analysis.

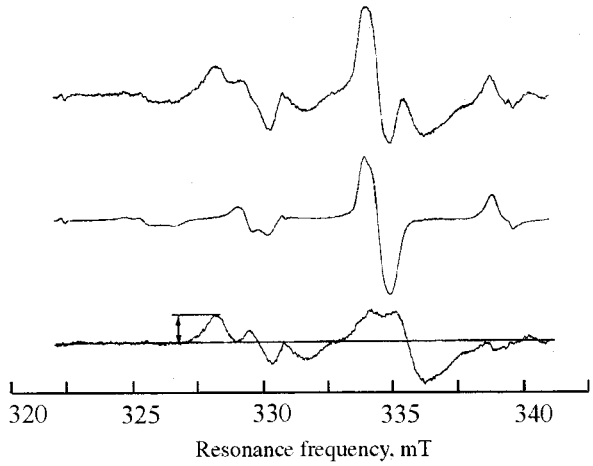

Fig. 1. Spectra of paramagnetic mononitrosyl iron complexes with diethyldithiocarbamate in the liver of mice irradiated in a dose of 8 Gy (*1*) and diethyldithiocarbamate—Cu^{2+} complex (*2*). *3*) distraction of spectrum *2* from spectrum *1*. Arrows: A_1 is the amplitude of the second line of the standard spectrum (Mn or MgO); A_2 is the amplitude of the triplet component used for quantification of NO content.

RESULTS

In group 2 mice the content of NO 6 h after γ-irradiation increased in all studied tissues (Table 1). The maximum increase was observed in the intestinal mucosa and liver, and the minimum changes were noted in the brain. In group 3 mice (24 h postirradiation), NO concentration considerably decreased in all tissues, but still surpassed the control level approximately 2-fold.

In groups 6 and 7 mice (phenytoin treatment), the release of NO was considerably decreased and this decrease positively correlated with stimulation of NO release by γ-irradiation in these tissue (Fig. 2). In non-irradiated animals (groups 4 and 5) phenytoin slightly decreased the content of NO in studied tissues. In group 5 phenytoin enhanced NO signal in the lung 24.5 h postinjection.

These findings provide new insight in the mechanisms of various effects of phenytoin, which is considered as an agent maintaining calcium homeostasis in various cells, especially in its emergency distur-

TABLE 1. Relative Content of NO in Various Tissues and Organs of Intact and γ-Irradiated (8 Gy) Mice (*M*±*m*)

Group	Tissue				
	intestine	liver	heart	lungs	brain
1	19.7±5.7	26.7±1.7	6.7±1.5	11.3±3.5	12.0±0.6
2	214±81*	192±76*	34.8±8.6*	57±13*	19.4±3.5*
3	38±8*	52±11*	17.0±3.9*	23.0±2.0*	20.0±1.0*
4	26.4±3.9	18.8±1.9*	8.5±1.2	13.6±1.5	9.2±0.7*
5	14.4±2.1	21.0±2.0	6.6±1.8	20.0±2.2*	9.8±1.5
6	61.6±15.6*	75±14.5*	20.2±2.9*	30.0±5.5*	13.8±1.7
7	15.8±3.2	27.0±2.7	15.2±3.2*	21.6±5.1	14.3±1.4

Note. *p<0.05 compared with the control (group 1).

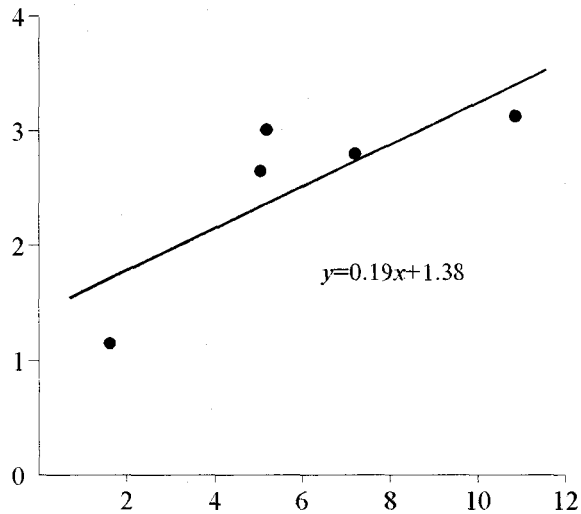

Fig. 2. Correlation between stimulation of NO production in various tissues 6 h postirradiation (abscissa: ratio of NO production in irradiated animals to respective control) and inhibitory effect of phenytoin at the same time postirradiation (ordinate: ratio of NO production in γ-irradiated animals to that in γ-irradiated animals treated with phenytoin).

bances [5,8]. If this is also true for Ca^{2+} currents induced by γ-irradiation and enhancing NO production in tissues, our findings are consistent with the modern concept of function of inducible NOS in various tissues. This suggests that disturbances in calcium metabolism in the early postirradiation period can be a trigger mechanism of NO overproduction. The effect of phenytoin probably involves more complex mechanisms, since in the nervous system this drug acts as a sodium channel blocker [9], while on the other hand, minor inhibition of calcium currents was observed only at high phenytoin concentrations far surpassing therapeutic doses. Phenytoin did not inhibited NOS in the brain [4], which allowed us to attribute the observed effects to rapid elimination of NO from tissues, rather than to inhibition of its production. Of particular interest was the hypothesis that phenytoin limits induction of NOS via inhibition of macrophage activation (which is important for reduction of the oxidative burst in combined radiation injury) and cytokine production, which through the nuclear factor NFκB inhibit expression of the corresponding gene. These data are scanty and further experiments will provide more precise interpretation of the observed inhibitory effect of phenytoin on the production of NO radicals induced by γ-irradiation in tissues; although the hypothesis of inhibition of calcium current seems to be most reliable.

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