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INTERMITTENT THREE-MONTH TREATMENT WITH UKRAIN IN INTACT AND OVARIECTOMIZED RATS. PART III: EFFECT ON THE NATIVE ELECTRON PARAMAGNETIC RESONANCE SIGNAL INTENSITY OF THE FEMUR

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Summary: *Ukrain, an acid alkaloid derivative of* Chelidonium majus L., *was administered intraperitoneally to ovariectomized and sexually mature female control rats at doses of 7, 14 and 28 mg/kg once daily for 10 days, followed by a 10-day break. This procedure was repeated five times. At the end of the long-term treatment with Ukrain (24 h after the last dose of the drug) the left femora of the rats were harvested. Dried bones were studied by electron paramagnetic resonance (EPR) method. Significant changes in the intensity of the native signal were observed within the groups (ANOVA,* p = *0.0001); the lowest value was observed in the ovariectomized rats treated with 28 mg/kg of Ukrain (decrease of 40.8% compared to the ovariectomized group,* p = *0.005). A significant increase in the intensity of the signal was observed in the intact 7 mg/kg Ukrain-treated group (34.7% compared to controls,* p = *0.004).*

Introduction

In the electron paramagnetic resonance (EPR) method, a constant frequency of microwave power is absorbed by the specimen (*e.g.*, rat femoral bone) which is placed in the resonator cavity as a function of the increasing magnetic field. Each absorption line can be characterized by an intensity, a peak-to-peak line width and a dimensionless constant (the so-called "g" factor) related to the magnetic field strength and the microwave frequency. Native EPR signal intensity is usually expressed in arbitrary units (AU) directly related to the amount of free radicals in the investigated tissue (1).

Our study aimed to assess the possible changes in the relative native EPR signal intensity in female intact and ovariectomized rats treated with different doses of Ukrain.

Materials and methods

Animals. Sexually mature female Wistar rats (340 g ± 30 g) were used for the study. The animals were housed at room temperature (20-21 °C) on a natural day-night cycle with free access to food (1.1% Ca) and water.

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Drugs. Ukrain was received in its purest state from the Ukrainian Anti-Cancer Institute, Vienna, Austria. Water for injection was obtained from Polfa, Poland.

Experimental procedures and treatments. The intact and ovariectomized rats received i.p. injections of Ukrain (7, 14, or 28 mg/kg at a volume of 0.5 ml/100 g) once daily for 10 days, followed by 10-day break. This procedure was repeated five times. The control and sham-operated animals received the same volume of water for injection. Operative procedure was performed from the dorsal approach (2). At the end of the experiment (24 h after the last dose of the drug) the rat left femora were harvested, dried at room temperature for 4 days and weighted.

Bone EPR spectrometry. Dried rat femoral bones were studied by EPR. The EPR spectra were registered at room temperature by an X-band spectrometer with a modulation frequency of 100 kHz. The EPR spectrum, showing the first derivative single absorption line, was plotted as a function of the magnetic field. The bones showed a 1.16 mT wide free radical singlet at the g = 2.074 region of the spectrum.

Statistical analysis. After assessment for normality using the Kolmogorow-Smirnow test, statistical differences between the means within the groups were calculated with ANOVA. *Post hoc* comparisons of means were performed using Duncan's test (Statistica 5.0). $p = 0.05$ was regarded as significant.

Results

Study results are shown in Table I and Figure 1. Significant changes of signal intensity were observed within the groups (ANOVA, $p = 0.0001$). The lowest

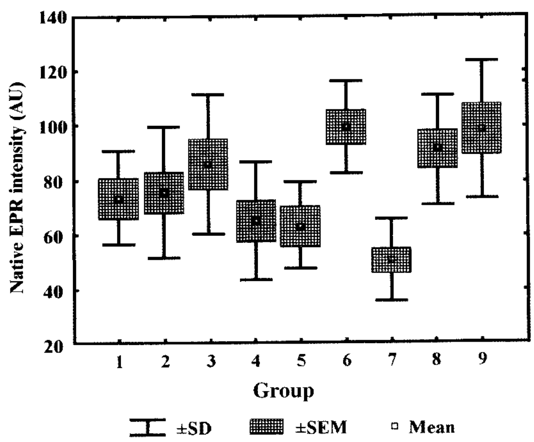

Fig. 1 Native EPR signal intensity expressed in arbitrary units (AU) in the study groups: 1) control, 2) sham-operated, 3) ovariectomized, 4) intact + 28 mg/kg Ukrain, 5) intact + 14 mg/kg Ukrain, 6) intact + 7 mg/kg Ukrain, 7) ovariectomized + 28 mg/kg Ukrain, 8) ovariectomized + 14 mg/kg Ukrain, 9) ovariectomized + 7 mg/kg Ukrain.

Table I *Native EPR signal intensity and weights of dried rat femora in the study groups*

Group	1	2	3	4	5	6	7	8	9
EPR intensity (UA±SD)	74.78 (17.23)	75.54 (24.03)	85.78 (25.63)	65.04 (21.60)	63.54 (15.71)	99.39 (16.67) $p = 0.04$ *vs.* 1 group	50.76 (15.09) p =0.01 *v.*3 group	91.12 (20.00)	98.58 (25.04)
Weight of dried femora (mg) (±SD)	875.6 (74.0)	854.4 (67.2)	883.4 (103.3)	810.8 (100.6)	853.6 (130.4)	818.2 (103.0)	802.8 (83.8)	736.1 (88.9)	850.8 (92.9)

Groups: 1) control, 2) sham-operated, 3) ovariectomized, 4) intact + 28 mg/kg Ukrain, 5)intact + 14 mg/kg Ukrain, 6) intact + 7 mg/kg Ukrain, 7) ovariectomized + 28 mg/kg Ukrain, 8-ovariectomized + 14 mg/kg Ukrain, 9) ovariectomized + 7 mg/kg Ukrain value of this parameter was observed in the ovariectomized rats treated with 28 mg/kg Ukrain (decrease of 40.8% compared to only ovariectomized rats). A significant increase in the signal intensity was observed in intact the 7 mg/kg Ukrain-treated group (34.7% *vs.* controls).

Discussion

To our knowledge no previous studies have been performed on the influence of Ukrain on the results of electron paramagnetic resonance (EPR) of rat bones. Our present investigations could therefore be regarded as a pilot study clearly showing the influence of this drug on the level of the native EPR signal, which corresponds to the amount of free radicals in the tissue studied. The highest intensity of this parameter was demonstrated in the femora of animals treated with the lowest doses of Ukrain (7 mg/kg). The lowest signal intensity was found in the group of ovariectomized rats treated with the highest dose, *i.e.*, 28 mg/kg of Ukrain. In this study, doses of 7 mg/kg of Ukrain produced an increase in native EPR signal intensity, which was significant for intact rats. An intermediate dose of 14 mg/kg showed no influence on EPR native signal in ovariectomized or in intact rats. It seems logical that lower EPR signal reflects diminished free radicals in the investigated bone tissue. At least in this respect, a distinct difference in effect could be attributed to low and high doses of Ukrain.

Ukrain exerts its pharmacological action on many cells and organs (3-5) by also influencing hormone status (6). Free radicals are shown to participate in numerous biochemical reactions as signal substances, as well as in bone metabolism (6-10). The toxic effect of irradiation could be also attributed at least partly to the augmented concentration of free radicals and it has been shown that some plant-derived compounds show radioprotective effect (11). It has recently been demonstrated that Ukrain also shows radioprotective effect (12-15). It is possible that this is achieved via a free radical system and that free radical depletion induced by high dose of Ukrain could have a beneficial effect.

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References

(1) Symons M. "Chemical and Biochemical Aspects of Electron-Spin Resonance Spectrosopy". Van Nostrand Reinhold Company, New York, 1978.

(2) Waynforth H.B., Flecknell P.A. "Experimental and Surgical Techniques in the Rat". Academic Press, Harcourt Brace & Company, Publishers, San Diego, 1992.

(3) Hohenwarter O., Strutzenberger K., Katinger H., Liepins A., Nowicky J.W. *Selective inhibition of in vitro cell growth by the antitumoral drug Ukrain.* Drugs Exptl. Clin. Res., **18** (Suppl.), 1, 1992.

(4) Nowicky J.W., Greif M., Hamler F., Hiesmayr W., Staub W. *Biological activity of Ukrain in vitro and in vivo.* Chemoterapia, **6**, (Suppl. 2), 683, 1987.

(5) Jagiello-Wójtowicz E., Kleinrok Z., Nowicky J.W., Jablonski M., Gorzelak M., Chodkowska A., Feldo M., Matuszek B. *Effect of six-month treatment with Ukrain on early osteoporosis induced by ovariectomy in rats. Part I: Preliminary studies of bone parameters.* Drugs Exptl. Clin. Res., **22** (Suppl.), 101, 1996.

(6) Jagiello-Wójtowicz E., Kleinrok Z., Nowicky J.W., Baran E. *Effect of six-month treatment with Ukrain on early osteoporosis induced by ovariectomy in rats. Part III. Preliminary studies of some hormone levels.* Drugs Exptl. Clin. Res., **22** (Suppl.), 109, 1996.

(7) Mancini L., Moradi-Bidhendi N., Brandi M.L., MacIntyre I. *Nitric oxide superoxide and peroxynitrite modulate osteoclast activity.* Biochem. Biophys. Res. Commun., **243**, 785, 1998.

(8) Avitabile M., Rasa R., Campagna N.E., *et al. Calcium release from the mineral matrix of the mandibular bone due to hydrogen peroxide exposure.* Minerva Stomatol., **45**, 401, 1996.

(9) Fraser J.H., Helfrich M.H., Wallace H.M., Ralston S.H. *Hydrogen peroxide, but not superoxide, stimulates bone resorption in mouse calvariae.* Bone, **19**, 223, 1996.

(10) Evans D.M., Ralston S.H. *Nitric oxide and bone.* J. Bone Miner. Res., **11** , 300, 1996.

(11) Shimoi K., Masuda S., Shen B., Furugori M., Kinae N. *Radioprotective effects of antioxidative plant flavonoids in mice.* Mutat. Res., 350 , **153**, 1996.

(12) Boyko V.N., Belskiy S.N. *The influence of the novel drug Ukrain on hemo- and immunopoiesis at the time of its maximum radioprotective effect.* Drugs. Exptl. Clin. Res., **24**, 335, 1998.

(13) Boyko V.N., Zholus R.B. *A comparative evaluation of the influence of the complex drug Ukrain and its components on the effects of radiation.* Drugs. Exptl. Clin. Res., **24**, 331, 1998.

(14) Boyko V.N., Zholus R.B., Legeza V.I. *A study of the influence of different types of radioprotectors on the survival of mice treated with ionizing radiation over a wide dose range.* Drugs Exptl. Clin. Res., **24**, 343, 1998.

(15) Boyko V.N., Levshina Ye.V. *A study of the influence of a novel drug Ukrain on in vivo effects of low-dose ionizing radiation.* Drugs. Exptl. Clin. Res., **24**, 339, 1998.