# A Water-Soluble Form of Estradiol with Estrogenic and Cardiotropic Activity

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It is demonstrated that a water-soluble form of estradiol (disodium salt of estradiol diphosphate), apart from having an estrogenic influence on the uterus, is effective against severe blood loss and has a cardiotropic activity.

**Key Words**: *water-soluble estradiol; estrogenic and cardiotropic activity*

Estrogens (female sex hormones) and their analogs have found wide application in the treatment of female and male endocrine and nonendocrine disorders [1]. Since these hormones and their analogs have low hydrophily, oil solutions are the common form of the drug used at the present time [4].

However, oil solutions cannot be injected intravenously, being suitable only for intramuscular and subcutaneous administration.

The use of oils as solvents for steroid hormones has a number of drawbacks. Unsaturated fatty acids contained in these oils are prone to peroxidation with the subsequent formation of toxic compounds with a broad spectrum of biological activity, which may generate untoward effects during clinical application. Furthermore, there is the possibility that in an environment predisposed to peroxidation steroid hormones can become involved in this process and transformed so that the spectrum of their hormonal and pharmacological activities changes. The presence of biologically active fat-soluble antioxidants in oils can also distort the clinical picture in view of the biological activity intrinsic to these compounds. The possibility of a direct chemical interaction between antioxidants and estrogens should also be considered. It is noteworthy that the distribution of hormones in the organism and their effect on internal organs are probably limited by weak diffusion in the surrounding tissues due to the "coalescence" of oil molecules. This hampers the creation of "high-impact" hormone concentrations in the blood and reduction of the dose.

Therefore, the development of a new water-soluble form of sex steroid hormones suitable for intravenous administration, assessment of their biological activity in animal models, and the search for new areas of clinical application are urgent tasks.

Our aim was to study some pharmacobiological properties of the water-soluble form of estradiol diphosphate (ED) synthesized in our laboratory.

## MATERIALS AND METHODS

Spectroscopy of the specimens containing estrogenic preparations was performed in a Specord M 42/M400 spectrophotometer using a rectangular quartz cuvette with an optical pathway length of 1 cm.

Hormonal and biological activity of ED was assessed in fertile rats and mice weighing 180-220 and 15.9±1.7 g, respectively. The estrogenic activity of ED was assessed by the gravimetric method: by measuring the uterine mass as described elsewhere [7]. ED in normal saline (pH 7.4) was injected intraperitoneally in a dose of 0.1, 1.0, 10, and 100 μg per mouse for 3 days or in a single dose of 10 μg/mouse with daily monitoring of the

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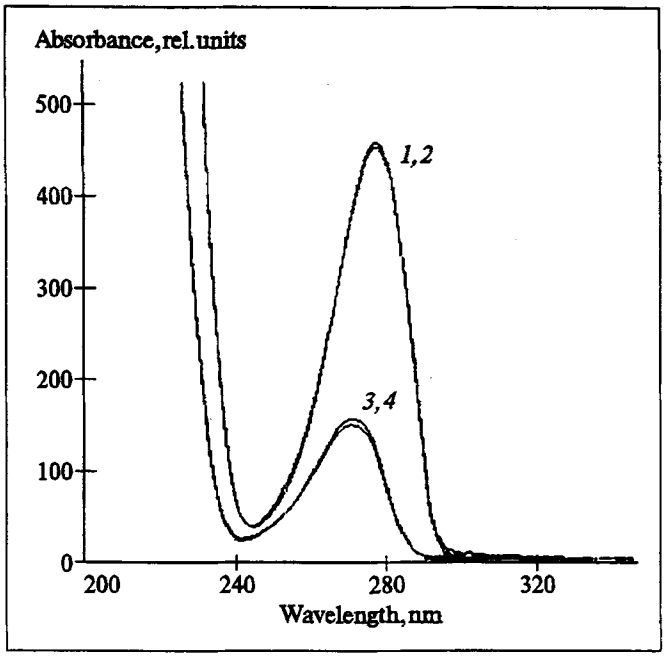


**Fig. 1.** Absorbance spectra of estradiol and ED. *1, 2*) ethanol (96%) and water:ethanol (1:1) ED solutions; *3, 4*) water and water:ethanol (1:1) ED solutions. The estradiol and ED concentration is $2.5 \times 10^{-4}$ M.

process during a 5-day period. Control animals received intraperitoneal injections of normal saline.

The effect of ED on cardiac function and hemodynamic parameters was studied in rats after a 60-min infusion in the femoral vein [3]. The following parameters were measured: left ventricular pressure, amplitudes of contraction and relaxation, systemic arterial pressure, and heart rate from the changes in the *R-R* interval on the electrocardiogram.

Lipid peroxidation was assessed by the accumulation of products reacting with 2-thiobarbituric acid in canine heart homogenate [8].

Free and total activities of β-galactosidase and β-glucosidase in rat heart homogenates were determined as described elsewhere [9], the protein concentration was measured after Lowry.

In experiments on rats, hemorrhagic shock was simulated by a single bleeding from the carotid artery in a volume equal to 2.5% of the body weight.

ED (sodium salt) was administered intravenously in a dose of 10 mg/kg 10 min prior to and after bleeding.

The survival rate was observed over 1-h and 24-h periods. Statistical analysis was performed by conventional methods. The differences were considered to be significant at $p<0.05$.

## RESULTS

We have synthesized a water-soluble form of estradiol, one of the most active sex hormones, as a disodium salt of ED.

Estradiol benzoate or dipropionate ester is a white crystalline powder practically insoluble in water and poorly soluble in ethanol and vegetable oils [1]. Estradiol esters slowly degrade in the tissues, are absorbed slowly, and elicit a prolonged effect on the organism. For this reason, they can be administered at relatively long intervals between injections.

The water-soluble form of estradiol was synthesized in a reaction with phosphorus oxychloride in a pyridine solution at 0°C, this being followed by treatment with an alkaline solution at pH 8.0. The resultant compound is a white crystalline powder readily soluble in water and insoluble in spirits.

The absorbance spectrum of ED does not differ appreciably from that of the conventional drug form. However, quantitatively the maximum ED absorbance is 3 times as high as that of the conventional form. ED is characterized by a shift toward the short-wave region (6 nm). The quantitative differences in the spectra of these preparations are not due to polarity of the medium, since they are retained in water:ethanol solutions (1:1). Both preparations are readily soluble in the water-ethanol mixture.

The water-soluble ED exhibits pronounced estrogenic activity, as evidenced by the increase in the relative mass of the uterus in response to administration of the drug to mice.

**TABLE 1.** Effect of ED (10 mg/kg) on Rat Heart Function (% of the Baseline Value)

| Parameters | Observation period, min | | | | |
|---|---|---|---|---|---|
| | 5 | 15 | 30 | 45 | 60 |
| Left ventricular pressure | 5.40±2.60 | 10.69±4.70* | 14.12±5.88* | 8.65±5.34 | 5.77±5.48 |
| Contraction amplitude | 5.28±2.65 | 10.41±4.78 | 14.31±5.97* | 8.65±5.43 | 5.81±5.57 |
| Relaxation amplitude | 5.95±2.69 | 10.70±4.69 | 13.97±5.80* | 8.62±5.51 | 6.24±5.63 |
| Systemic arterial pressure | 5.74±1.64* | 9.86±3.16* | 12.29±5.56 | 7.38±4.04 | 4.10±4.17 |
| *R−R* interval changes | 6.78±1.69* | 11.86±4.01* | 16.10±4.64* | 10.17±4.07* | 7.63±4.17 |

**Note.** Contraction amplitude is $dp/dt_{max}$; relaxation amplitude is $dp/dt_{min}$. Asterisk indicates $p<0.05$ compared with the baseline values.

For example, when ED was injected in a dose of 10 μg/mouse, the pharmacological response was characterized by the following dynamics (in terms of the relative mass of the uterus): 0.064±0.003, 0.097±0.002, 0.093±0.003, 0.100±0.006, and 0.110±0.005 on days 1, 2, 3, 4, and 5, respectively.

For assessing the estrogenic activity as a function of ED dose the preparation was administered in doses of 0.1, 1.0, 10.0, and 100.0 μg/mouse for 3 days. The relative mass of the uterus increased from 0.06±0.007 (control) to 0.21±0.03, 0.29±0.002, 0.5±0.002, and 0.54±0.03, respectively. From this it follows that the estrogenic activity of ED depends on the dose and the time after administration.

Administration of ED to intact rats in a dose of 10 mg/kg increased left ventricular contractile activity. Systemic arterial pressure also increased and the heart rate dropped. These parameters reached the maximum toward the 30th min after ED administration (Table 1).

Water-soluble forms of glucocorticoid hormones and their synthetic analogs are widely applied in emergency states [5]. The possibility of using other steroids (for example, sex hormones) has hardly been considered due to their lipophilic properties that preclude intravenous administration. A study of the pharmacological activity of ED (water-soluble form) has revealed that, in addition to its estrogenic activity, ED modulates the functional-metabolic parameters of the cardiovascular system and increases the survival rate of animals after severe blood loss.

In experiments with male rats subjected to acute hemorrhage, ED administered in a dose of 10 mg/kg before and after bleeding reduced the mortality rate: the survival rate was 90-95% 1 h and 70-75% 24 h after bleeding in the experimental group and 73 and 53%, respectively, in the control group.

The increase in survival rate after heavy bleeding and improved function of the cardiovascular system induced by ED may be due to its action on the membrane structures in the myocardium and on the processes causing their damage.

For example, one hour after a single injection of ED (10 mg/kg) the percentage of free activity of the lysosomal enzyme β-galactosidase dropped from 74.5 to 46.0% and for β-glucosidase from 89.7 to 57%, indicating that ED stabilizes lysosomal membranes in the rat heart.

Three hours after administration of ED (10 mg/kg) to dogs, the content of 2-thiobarbituric acid-reactive substances in heart homogenate tended to decrease (from 8.5±1.9 to 5.4±0.5 nmol/g tissue). Previously, we reported that ED reduces both the ability of mitochondria to accumulate $Ca^{2+}$ and the rate of $Ca^{2+}$ uptake and release by myocardial mitochondria [2].

The findings indicate that the water-soluble form of estradiol, in addition to possessing estrogenic activity, protects the organism from severe blood loss and exhibits a cardiotropic activity, which may be of practical significance.

## REFERENCES

1. H. Schambach (ed.), *Hormone Therapy* [Russian translation from German], Moscow (1988).
2. L. D. Luk'yanova, R. A. Eliseev, T. N. Makarenko, and A. I. Matyushin, *Byull. Eskp. Biol. Med.*, **118**, No. 12, 616-618 (1994).
3. A. I. Matyshin, I. V. Levandovskii, V. M. Gukasov, and D. M. Zelenov, in: *Modeling, Pathogenesis, and Therapy of Hypoxic States* [in Russian], Gorki (1989), pp. 124-127.
4. M. D. Mashkovskii, *Drugs* [in Russian], Moscow (1993).
5. G. Rikker (ed.), *Shock* [Russian translation from German], Moscow (1987).
6. V. I. Dedukhova and E. N. Mokhova, *FEBS Lett.*, **295**, 51-54 (1991).
7. G. Kranzfelder, M. Schneider, *et al.*, *J. Cancer Res. Clin. Oncol.*, **97**, 167-186 (1980).
8. A. E. Kutabchi, D. R. Challoner, and R. M. Williams, *Proc. Soc. Exp. Biol.* (New York), **127**, 647-651 (1968).
9. B. Patel and A. L. Tappel, *Biochem. Biophys Acta*, **191**, 86-94 (1969).