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Influence of Urethane on Rheological Properties of the Erythrocytes in Rats

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Author's contribution

The sole author designed, analyzed and interpreted and prepared the manuscript.

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ABSTRACT

Therapeutic strategies using various preparations and their combinations with different mechanisms of action are gaining increasing attention. But the experiments are carried out in accordance with the work ethic on animals using anesthetics, which change the internal environment. We examined effect of urethane on rheological properties of erythrocytes in rats. Our results demonstrated negative effect resulting in worsens deformability and aggregability of the erythrocytes. Therefore studies on the effect of drugs to hematological parameters and blood rheology which were performed on urethane anesthetized animals, should be considered invalid and incorrect.

Keywords: Erythrocyte; deformability; aggregability; urethane.

1. INTRODUCTION

The modern experiments on animals and humans are undertaken according to the conditions set forth in the Code of Ethics of the Word Medical Association (Declaration of Helsinki), in accordance with the guidelines laid down by the NIH in the US regarding the care and use of animals for experimental procedures, with approved by the ethics committee of the Institute of Transfusion Medicine, Academy of Military Medical Sciences, etc.

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The experiments on the development of injective drugs with a positive impact on the rheological properties of the blood are held with taking into account Helsinki Agreements on animals anesthesia with urethane. Nevertheless it was demonstrated long ago, that anesthesia inevitably affects the internal environment of an organism. Urethane, being a drug with immunotoxic and mutagenic properties, leads to changes in many hematological parameters. Thus, the intraperitoneal injections of urethane lead to increases in blood pressure, hematocrit, as well as number of the erythrocytes, their rigidity and platelet aggregation [1-6]. Suchwise, the study of initial rheological blood parameters under anesthesia is very doubtful. Unluckily, there is no information about the direct studies of the effect of narcotics on the deformation and aggregation properties of erythrocytes in the available literature.

2. MATERIALS AND METHODS

To clarify the question of the correctness of using of anesthetics in the study of the rheological properties of blood, we have attempted to examine the effect of urethane on deformation and aggregation properties of erythrocytes. The work was carried out on laboratory Wistar rats of both sexes (18 males and 18 females) at the age of 3 months of age. Thus we do not take into account gender differences. All animals were adapted to handling for 3 weeks, they accustomed to arms before each feeding. The rats were anesthetized with the intraperitoneal injections of 25% ethylurethane (2,8M) in dose of 1 g/kg, diluted on distilled water. Blood sampling was taken from the tail vein into heparinized clinitubes D957G. We counted the number of erythrocytes (**RBC**), hematocrit (**EPV**) and our proposed index haemoconcentration **K=(1-EPV)/RBC**, physically representing the volume of plasma on 1 erythrocyte in peripheral blood. The deformability of erythrocytes was studied by method of osmotic gradient ektacytometry [7]. Using osmotic deformability profiles (osmoscans), the method allows to assess deformability index ($\mathbf{I_e}$), degree of cell, or hemoglobin hydration (**O'**), osmolality, at which the minimum $\mathbf{I_e}$ observed ($\mathbf{O_{min}}$), which is an accurate measure of shape of the oxygen carriers, deformability in the point of isotropic erythrocyte swelling ($\mathbf{I_{min}}$), which is the measure of membrane permeability for water [8].

The reversible tendency to form aggregates was determined by piezo-dynamic method from analyzing the light transmission data through the whole heparinized blood samples at hematocrit 40% in a glass micro-chamber of 25 mcm height and of 16 mm diameter [9]. The erythrocyte disaggregation shear stress was achieved by voltage increase on piezo-crystal connected with surface of the micro-chamber. As the shear rate increases, the cells break down to monodisperse system. The time of full disaggregation ($\mathbf{T_{full}}$) was the measure of the strength of erythrocyte aggregates. The rate of spontaneous aggregation ($1/\tau$) was determined from a half-period (τ) reduction of the photometric signal through completely disaggregated blood after power-down. Aggregation index ($\mathbf{I_a}$) was calculated by dividing the full disaggregation time on a half-period time ($\mathbf{T_{full}/\tau}$). The mean cellular fragility (**MCF**) was determined by Dacie. The plasma osmolality was measured using micro-osmometer (Model 3300). The experiments were done at constant temperature of 25°C. All results were expressed as the means±SD. Student's paired t-test was used to compare the data. Statistical significance was declared when $p<0.05$.

3. RESULTS

As we can see from Table 1, when calculating changes at all 36 rats, the anesthesia induced increase of RBC, EPV and caused haemoconcentration in accordance with the decrease of the index K. Individual corpuscular volume of the erythrocyte was not changed and fragility was authentically increased.

But if the data to present in the individual examination, as is evident from Fig. 1, it appears that RBC was increased approximately in 72% of the animals and only in 28% was decreased. The situation is similar with respect to **EPV** (Fig. 2)**, K** (Fig. 3) and **MCF** (Fig. 9).

Table 2 shows the results of measuring erythrocyte deformability parameters. The deformation properties of red blood cells after anesthesia were deteriorated. The decrease of the water permeability of membranes ($\mathbf{I_{min}}$) and increase the intrinsic viscosity (**O'**) led to a deterioration of erythrocyte deformability ($\mathbf{I_e}$). Erythrocyte shape remains unchanged ($\mathbf{O_{min}}$).

Returning to our method of data analysis, it can be noted that some of the animals have reacted to the injection of urethane with deterioration of deformation parameters, and the other part have responded with some improvement in them (Figs. 4-7).

Table 3 shows the results of measuring aggregation parameters of the erythrocytes. As can we see, after the urethane injection neither rate of spontaneous aggregation ($1/\tau$) nor strength of erythrocyte aggregates (T_{full}) did not changed, but the integrated index of aggregation (I_a) was significantly increased. Back to the reactions of some rats (Fig. 8): in answer to injection of urethane into animals of the control group, aggregability of their erythrocytes was increased in 67% of cases and was decreased in 33% of cases.

Measurement of plasma osmolality showed that this parameter remained constant after anesthesia like in control 292±2 mosmol/kg H2O.

4. DISCUSSION

Our study has shown that parenteral administration of physiological doses of urethane worsens hematological and rheological properties of red blood cells in rats. However, 30% of these animals have somewhat improved characteristics compared to control values. It is known that the state of tension in adrenocortical system is accompanied by disturbances of rheological properties of red blood cells. Before the experiment, we tried to adapt animals to handling, but apparently, some of them were still stressed during the taking blood samples from the tail. So, the blood parameters after injection of urethane were slightly improved due to inadequate controls. Either way, anesthesia inevitably affects the internal environment. But, many experimenters, following the Helsinki Agreements, pay no attention to such undesirable effect. Unfortunately, this fact was safely forgotten.

We tried to remind for the researchers, who evaluate the rheological parameters in urethane anesthetized animals, about the influence of the analgesia on the deformability and aggregability of the erythrocytes in rats. As results of our experiments, we can say that the use of urethane in physiological concentrations significantly makes worse deformation and aggregation properties of red blood cells in rats. Moreover, the reactions to anesthesia can depend on the functional state of animal and be quite unpredictable. Due to this fact the question raises of the legality anesthetic using in studying the drug influence on the rheological properties of mammal's blood. According to our experiments urethane should not be used as an anesthetic for animals intended for blood obtaining.

Table 1. Erythrocyte indices

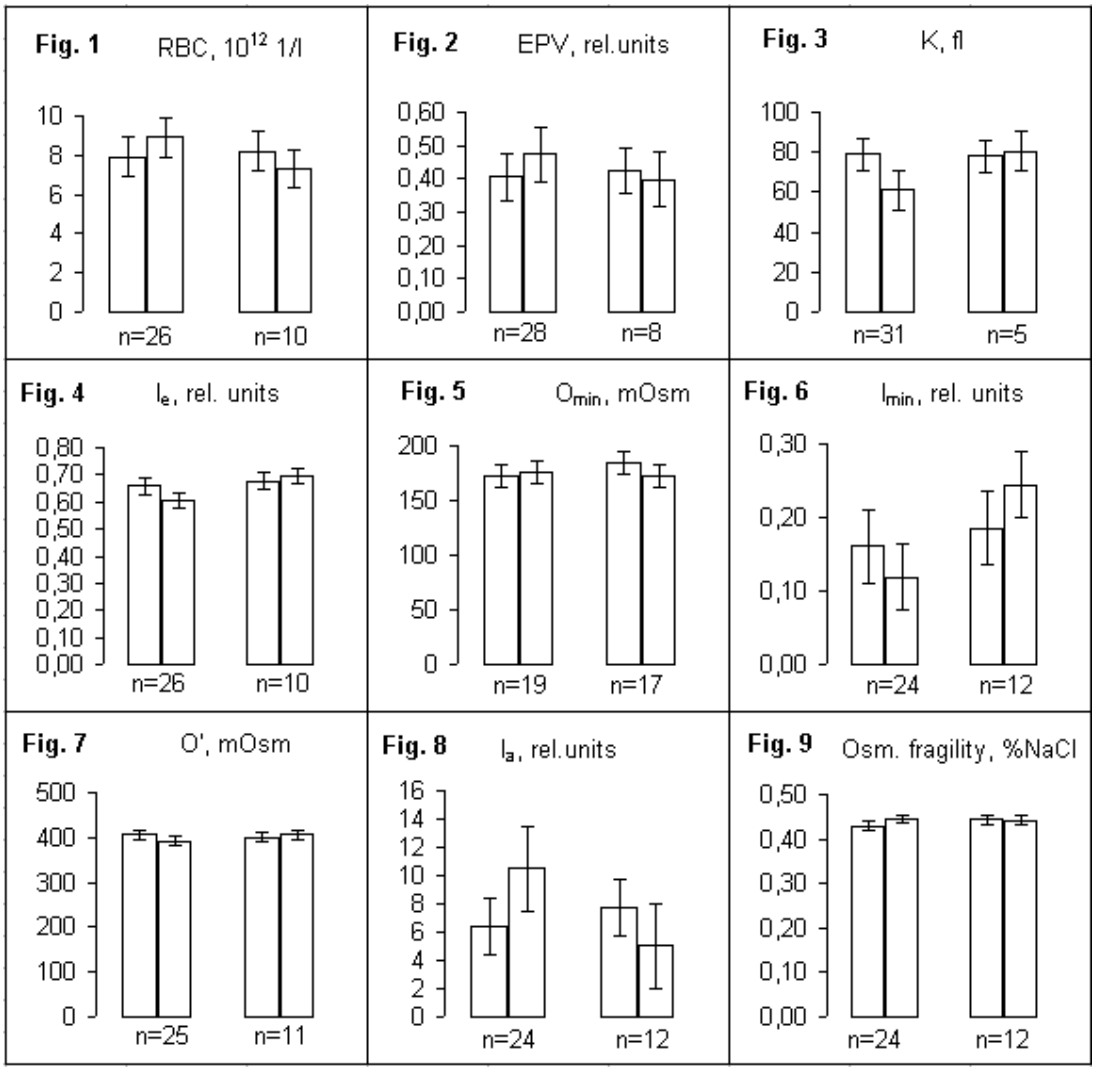
Animals	RBC,10^{12} 1/l	EPV, relative units	MCV, 10^{-15} l	K,	MCF, % NaCl
Control, n=36	7.9±0.8	0.41±0.03	52±6	78±15	0.436±0.013
Urethane, n=36	8.5±1.2	0.46±0.04	54±5	64±10	0.444±0.010
Student's test	P=0.014	P<0.001	P>0.05	P=0.001	P=0.044

Table 2. Indicators of erythrocyte deformability

Animals	I_e,	I_{min},	O_{min},	O',
	Relative units		Mosmol/kg H_2O	
Control, n=36	0.663±0.067	0.166±0.056	178±15	405±12
Urethane, n=36	0.633±0.081	0.144±0.069	174±8	396±17
Student's test	P=0.001	P=0.035	P>0.05	P=0.034

Table 3. Indicators of erythrocyte aggregation

Animals	T_{full}, C	$1/\tau$, 10^{-3} C^{-1}	I_a, rel. units
Control, n=36	50.0±6.2	30.8±15.6	6.8±2.6
Urethane, n=36	53.1±7.5	43.4±22.4	8.8±4.4
Student's test	P>0.05	P>0.05	P=0.028



Figs. 1-9. Histograms of studied parameters of the erythrocytes before and after anesthesia

5. CONCLUSION

Studies on the effect of drugs to hematological parameters and blood rheology which were performed on urethane anesthetized animals, should be considered invalid and incorrect.

COMPETING INTERESTS

Author has declared that no competing interests exist.

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