METHODS

Comparative Study of Vasaprostan and Alprostan in Spontaneous Platelet Aggregation Test in Mice

D. V. Gol'dshtein, E. I. Maevskii, and V. N. Pogorelova

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We propose a method for evaluation of spontaneous aggregation of mouse platelets based on routine clinical blood tests for humans. Comparative analysis of Vasaprostan and Alprostan drugs showed that this test can be used for testing of new drugs on animals.

Key Words: *Vasaprostan; Alprostan; SHK mice; spontaneous platelet aggregation*

Vasaprostan (Schwartz Pharm AG) and Alprostan (Lechiva) containing the same substance (prostaglandin E1 — alprostadil), but prepared in different forms are used in clinical practice. Vasaprostan is a dry powder-like white substance packed in glass ampoules containing 20 μg alprostadil in complex with alphadex and lactose. Each ampoule of Alprostan contains 100 μg alprostadil dissolved in 0.2 ml absolute ethanol. The difference in the dosage form can be essential for the intensity of prostaglandin E1 effect [1]. We studied the effects of these drugs on spontaneous aggregation of mouse platelets.

MATERIALS AND METHODS

In vitro experiments were carried out on blood samples from healthy male NMRJ mice kept under standard conditions: 20±2°C, free access to water, and PK-121-2 fodder (Informkorm).

Whole blood was stabilized in 3.8% sodium citrate in 9:1 ratio. Equal volumes of stabilized blood were transferred into 4 plastic (polystyrene) tubes. Normal saline (0.9% NaCl; 75 μl/ml blood) was added into tubes 1 and 2. Equal volumes of Alprostan or Vasaprostan in normal saline at alprostadil concentration of 2 μg/ml were added into tubes 3 and 4. Tube 1 was left in the holder for 4 min; tubes 2, 3, and 4 were shaken for 4 min at a frequency of 1.5 Hz.

After 4 min 2 volumes of 0.5% glutaraldehyde in 0.9 NaCl (fixative) was added into all tubes for 10 min. Fixed blood was then centrifuged for 1 min at 125*g* for precipitation of erythrocytes and platelet aggregate. The supernatant containing free platelets was collected from all tubes and diluted with saline (1:10). Samples of platelet-containing plasma were placed into a Goryaev cell where the cells were precipitated for at least 10 min in a humid atmosphere.

Ready preparations were examined under a Peraval Interphako optic microscope (Zeiss) fitted with a WAT-505ex digital camera (Watec). The image was recorded in a PC by means of a VideoIn software. Objective 63 (0.83 magnification) gave a final 1000-fold magnification of the visual field on the monitor. Platelets were counted in a cell containing 16 minor squares with an area of $^1/_{400}$ mm^2 each. Thirty cells were thus processed for each sample. The mean counts of platelets per square were compared and the effects of the test drug on spontaneous aggregation were evaluated.

RESULTS

Shaking of platelet plasma induced additional aggregation of platelets leading to a decrease in the count of free cells: the number of free platelets in the control

Institute of Theoretical and Experimental Biophysics, Russian Academy of Sciences, Pushchino. ***Address for correspondence:*** pogorelov@iteb.ru. V. N. Pogorelova

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(free status) was 59±2 *vs.* 47±2 after shaking. The counts of free platelets appreciably increased in tubes incubated with the test drugs. The effect of Alprostan consisted in prevention of shaking-induced aggregation, which normalized the level of free cells (59±2). Vasaprostan decelerated platelet aggregation more intensely, the number of free platelets in the preparation treated by Vasaprostan even surpassed the control values. Presumably, this means that Vasaprostan not only inhibits aggregation, but also causes disaggregation of formed complexes.

Hence, a method for the study of spontaneous platelet aggregation was developed in our experiment on mouse blood samples; the method is based on routine clinical blood tests [2]. The method can be used for studies of activities of potential antiaggregants on animals (preclinical studies) [3].

REFERENCES

1. E. I. Maevskii, A. N. Murashev, O. G. Aksenova, *et al.*, *Tromboz, Gemostaz, i Reologiya*, No. 4, 41-45 (2002).
2. *Fundamentals of Diagnosis of Hemostasis Disorders* [in Russian], Moscow (1999).
3. *Manual of Experimental (preclinical) Studies of New Drugs* [in Russian], Moscow (2000).