EATM regulates the NADPH-oxidases of erythrocyte membranes and serum of patients with types 1 and 2 diabetes *ex vivo*

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INTRODUCTION

Embryonal antitumor modulator of Mkrtchyan (EATM) reveals a hypoglycemic effect in streptozotocin-induced diabetes in rats. At the same time EATM regulates the level of pro- and antioxidant metalloproteins in tissues of rats with streptozotocin-induced diabetes. The decrease in the level of NAPH-oxidase (Nox) isoforms in blood serum, erythrocyte membranes (EM) and subcellular formations of animal spleen is also observed.

OBJECTIVES

• To determine the influence of EATM on the process of releasing of Nox of erythrocyte membranes (EM) and serum of patients with diabetes type 1 and 2.

MATERIALS AND METHODS

The study included 12 patients with type 1 diabetes, 12 patients with type 2 diabetes and 12 healthy volunteers. Isolation of Nox fractions of purified EM and blood serum was performed by ion-exchange chromatography. The Nox amount was determined at 530 nm. O_2^--producing activity of Nox isoforms was determined by nitrotetrazolium blue method [Simonyan G. et al., 2001]. The ferriHb-reducing activity of Nox isoforms was determined by measuring the gradual reduction of ferriHb to ferroHb at 555 nm [Loehneysen K.et al., 2010]. The statistical analysis was performed by one-way ANOVA. The study was approved by the Local Ethics Committee of the YSMU.

RESULTS

We showed that the Nox release in EM of type 2 and 1 diabetes is higher by 83.3±7.4% and 58.4±6.5%, respectively, compared with donor blood (fig.1) . The addition of EATM resulted in suppression of the process of the Nox release in EM in diabetes type 2 and 1 and healthy donors by 45,5 ± 6,2%, 31,6 ± 4,4% and 25.1 ± 3,7%, respectively (fig.2). In the same conditions the increase of releasing of the extracellular Nox (eNox) from the blood serum in diabetes type 2 and 1 is up by 109.2±8.7% and 45.4±5.5% respectively. Addition of EATM reduced activity of eNox by 47,9 ± 4,4%, 31,3 ± 2.9% and 29.3±3,2%, respectively.

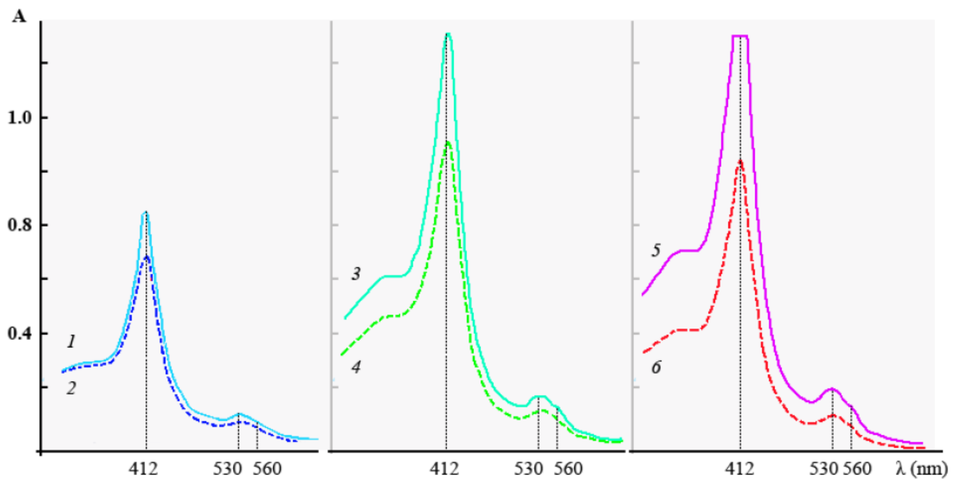

Fig.1. Optical absorption spectra of Nox released from blood of patients with type 1 and 2 diabetes and healthy donors: 1, 2 – EM of donor blood; 3,4 – EM of blood sample drawn from type 1 diabetes patients; 5,6 – EM of blood samples from type 2 diabetes patients. Maximum optical absorbance for Nox at 560, 530 and 412 nm (oxidazed) are shown to be similar to the rest of the Nox spectra.

RESULTS

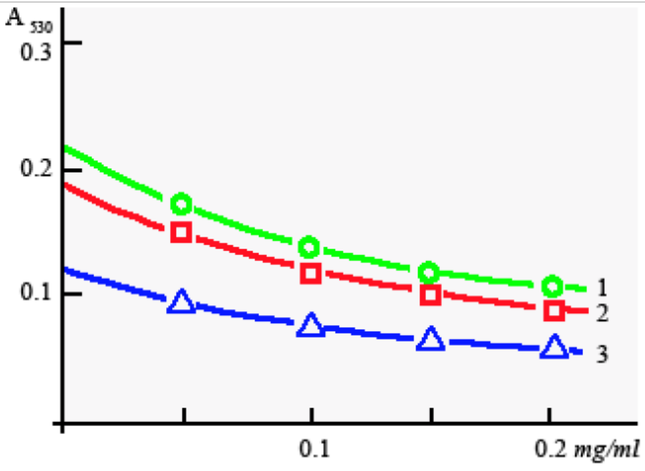

Fig.2. Changes in density of maximum optical abortion of Nox from the blood of patients with type 1 and 2 diabetes and healthy donors at 530 nm under the influence of EATM various doses. The density of EM Nox optical abortions in type 2 diabetes (1), type 1 diabetes (2) and donor blood (3) is demonstrated

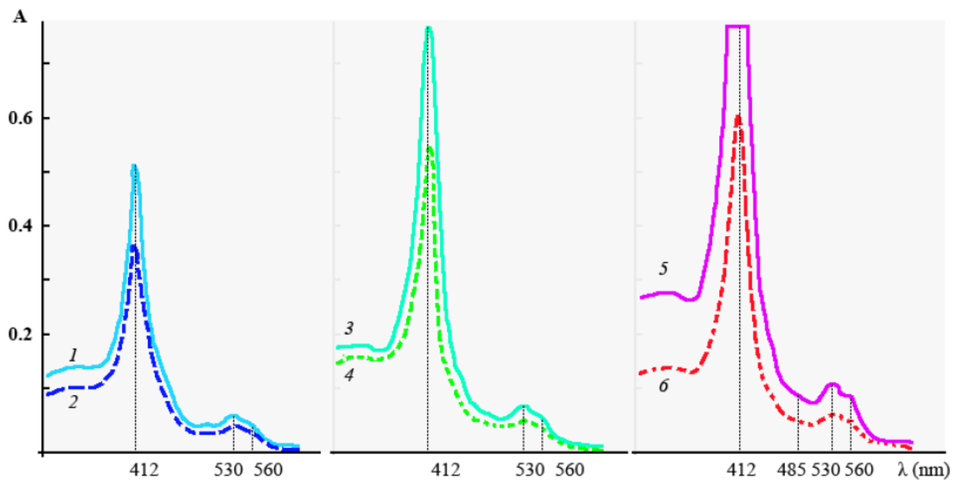

Fig.3. Optical absorption spectra of the released eNox from serum of patients with diabetes types 1 and 2, as well as healthy donors after 4-day aerobic incubation at 4ºC and pH 8 in the absence (solid lines) and presence (dashed lines) of 0.15 mg/ml EATM. Absorption spectra of eNox from serum of patients with type 2 diabetes (5, 6) and type 1 (3, 4) and donor blood (1, 2). The example of the spectrum 1 of eNox shows the characteristic maximum optical absorption at 560, 530, 485 and 412 nm (oxidized), which are similar to the rest of eNox spectra.

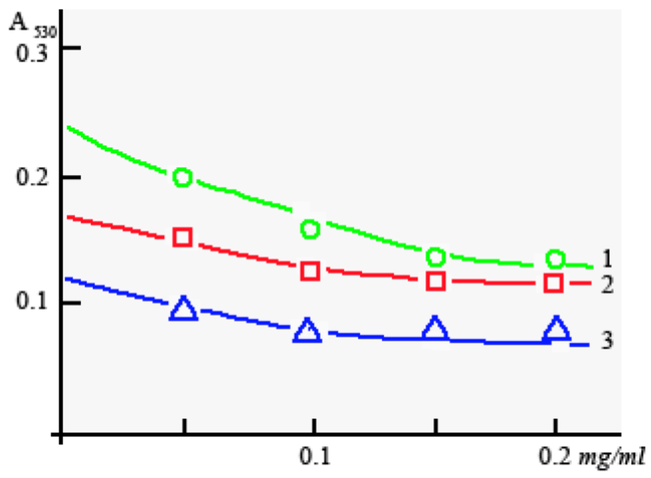

Fig.4. Changes in density of maximal optical absorption of eNox from blood serum of patients with diabetes type 1 and 2, as well as healthy donors, at 530 nm and different doses of EATM. Density of optical absorption of eNox from blood serum in diabetes type 2 (1), type 1 (2) and donor blood (3).

CONCLUSIONS

- The suppression of the releasing Nox and eNox of EM and serum exosomes in diabetes type 1 and 2 may be apparently a novel mechanism of membrane stabilizing effect of the EATM *ex vivo*.
- This gives some scientific term for EATM as an agent, which supports the stability of the EM and exosomes in the blood of diabetes type 1 and especially type 2 patients *in vivo*..

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