# Experiments on Alcocytes Containing Enzyme Nanoparticles for Reducing Toxic Blood Concentration of Ethanol

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Translated from *Kletochnye Tekhnologii v Biologii i Meditsine*, No. 1, pp. 58-60, January, 2012
Original article submitted April 28, 2011.

We developed a method of introduction of alcohol dehydrogenase and aldehyde dehydrogenase into mouse and human erythrocytes. The possibility of using erythrocytes loaded with the two enzymes (alcocytes) for reducing ethanol concentration in animal blood was studied. Injection of alcocytes to mice led to accelerated decrease in ethanol concentration as soon as after 5 min and this capacity of alcocytes persisted for at least 2 h. Alcocytes prepared from fresh or preserved human blood did not survive in mice. Thus autologous alcocytes is functionally active and can be used as a protective system in acute alcohol intoxication. The developed method can be regarded as a new medical biotechnology.

**Key Words:** *erythrocytes; encapsulation of alcohol dehydrogenase; encapsulation of aldehyde dehydrogenase; alcocytes; biotechnology*

Alcoholism is very important medical problem. According to WHO data, up to 10% adults in developed countries suffer from various forms of alcoholism and concomitant diseases and the annual incidence of alcohol abuse-related deaths is up to 1000 per million people.

Blood concentration of endogenous ethanol in non-drinking individuals is up to 100 mg/liter (up to 2 mM) [4], which is absolutely harmless. Ethanol concentrations up to 500 mg/liter (10 mM) are toxic and 5 g/liter (100 mM) and higher are lethal [6,7].

Toxic concentrations of ethanol are neutralized by its oxidation to acetate. The primary role in ethanol oxidation is played by alcohol dehydrogenase (ADH); activity of this enzyme is maximum in the liver and is not detected in erythrocytes.

Toxic effects are caused by not ethanol, but acetaldehyde, a product of its oxidation. Metabolic elimination of acetaldehyde by its conversion into acetate is catalyzed by aldehyde dehydrogenase (ALDH), which is also abundant in the liver and is present in trace amounts in erythrocytes [2].

Fundamental studies on reduction of ethanol concentration in the blood are sparse. We previously used specially prepared erythrocytes for reduction of ammonia concentration in the blood *in vivo* [1].

Here we selected conditions for incorporation of ADH and ALDH into mouse erythrocytes and evaluated the capacity of the prepared alcocytes to reduce ethanol concentration in the blood and survival of mouse and human alcocytes in mouse circulation.

## MATERIALS AND METHODS

Erythrocytes isolated from mixed blood of experimental mice were loaded with ADH and ALDH by the method of encapsulation based on hypotonic dialysis and isotonic sealing described previously by us [1,3]. These erythrocytes loaded with ADH and ALDH (alcocytes) were mixed 1:1 with authentic blood plasma or 0.9% NaCl and injected to mice into the lateral caudal vein in a volume of 0.4 ml per 25 g body weight.

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0007-4888/12/1531-0170 

Erythrocytes were also isolated and alcocytes were prepared from preserved or fresh human blood.

Ethanol (24% aqueous solution) was injected intraperitoneally in a dose of 2 g/kg immediately after alcocyte injection.

The animals were decapitated 5-1440 min (5 min-24 h) after alcocyte and ethanol injection, mixed blood was collected, and ethanol concentration in acid extracts was measured.

An aliquot of freshly isolated alcocytes was preincubated with FITC. The animals were decapitated 5-1440 min after injection into the lateral caudal vein, mixed blood was collected, and erythrocytes were isolated and analyzed by cytofluorometry for measuring their lifetime in circulation. The lifetime was evaluated by the decrease in relative content of injected erythrocytes and alcocytes.

In some experiments, blood (0.1 ml) obtained from the rentroorbital venous plexus (RVP) of the same mice at different time intervals was used.

## RESULTS

The developed method allowed us to introduce ADH and ALDH in human and mouse erythrocytes in amounts sufficient for elimination of exogenous ethanol *in vitro* from the incubation medium at a rate of 30-500 nmol/min×ml erythrocytes depending on medium composition.

After intravenous injection of human alcocytes to mice, the decrease in blood ethanol concentration (elevated to 30-40 mM) was not accelerated 5 min after its intraperitoneal injection. Therefore we studied survival of human erythrocytes in mouse circulation. Figure 1 shows changes in the count of human erythrocytes isolated from fresh donor blood and labeled with FITC in mouse circulation. Their number decreased from 100% (in injected erythrocyte suspension) to approximately 14% in 5 min, 3-5% in 30-120 min, and 0-0.2% in 24 h after their administration to mice. The decrease in the number of labeled erythrocytes was accompanied by increasing hematuria, which attested to erythrocyte lysis.

Thus, human erythrocytes isolated from fresh or preserved donor blood did not survive in mouse circulation over 30 min and disappear after 24 h.

Injection of mouse erythrocyte or alcocytes induced neither changes in their behavior nor hematuria, at least over 24 h. Figure 2 shows the dynamics of FITC-labeled mouse alcocyte count in mouse circulation. The relative content of mouse alcocytes in mouse circulation decreased by 4-5 times 5 min after injection, most likely due to dilution of the injected suspension with own erythrocytes *in vivo*. This proportion remained at the level of 20-25% during the next day. This suggest that mouse alcocytes survive in mouse

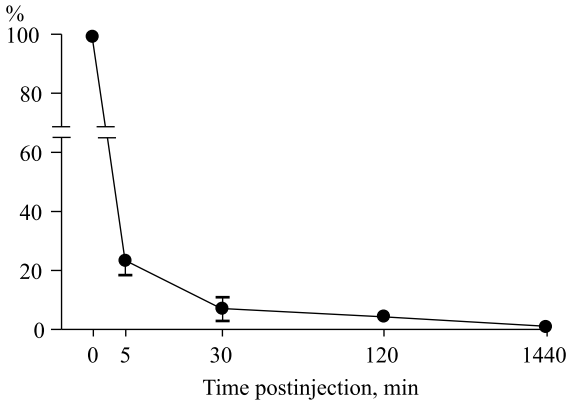


**Fig.1.** Changes in relative content of human erythrocytes isolated from fresh donor blood and labeled with FITC in mouse circulation. Mean values for 2-5 samples are presented. Here and in Figs. 2 and 3: ordinate shows percent of labeled cells.

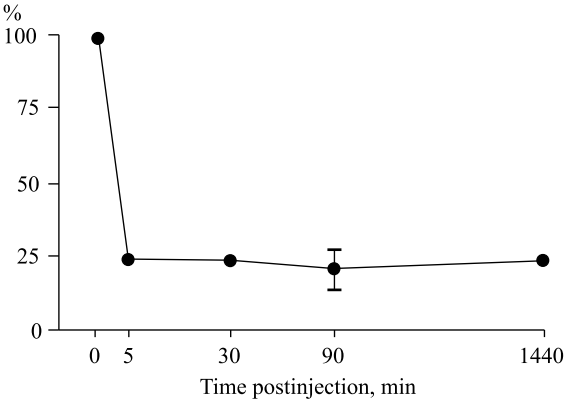


**Fig.2.** Changes in the content of FITC-labeled mouse alcocyte count in mouse circulation. Mean values for two experiments are presented.

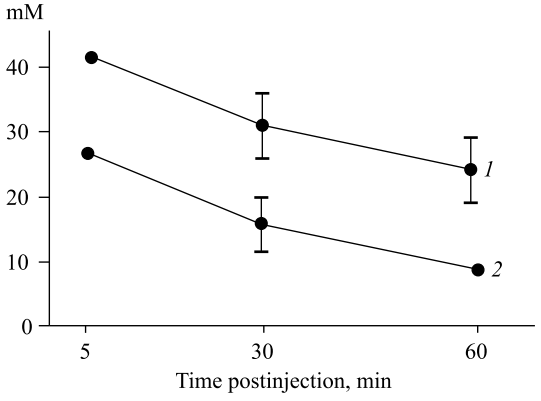


**Fig.3.** Changes in ethanol concentration in RVP blood in control (*1*) and experimental (*2*) animals 5, 30, and 60 min after injection of alcocytes (or erythrocytes in the control) and ethanol. Mean values for 3 animals are presented. $p<0.05$ in comparison with the control.

circulation over 24 h and can be used for reducing ethanol concentration in mouse blood.

Figure 3 shows the dynamics of ethanol concentration in the blood from RVP during the first 60 min

after simultaneous injection of ethanol and alcocytes (or erythrocytes in the control) to mice. The procedure of alcocyte preparation and experimental conditions were the follow: 285 U/ml ADH and 9.2 U/ml ALDH were added to 1 ml suspension of mouse erythrocytes (hematocrit 65%) and then dialyzed for 3 h. The blood was collected from RLP in each (the same) mice at different time intervals. It was found that thus prepared autologous alcocytes significantly reduced ethanol concentration in the blood from RVP at all terms of observation, *i.e.* 5, 30, and 60 min after injection. The rate of ethanol elimination in the control and experiment was 444 and 544 nmol/min×ml blood during the first 25 min and 227 and 243 nmol/min×ml blood during the next 30 min, respectively, which is by two orders of magnitude lower than ADH activity and by 2-5 times lower ALDH activity encapsulated in erythrocytes and 2-fold lower than the rate of ethanol elimination from the incubation medium *in vitro*.

The results presented in Fig. 3 agree with the data of the only published report [6]. However, additional experiments showed that the decrease in ethanol concentration *in vivo* under the effect of alcocytes depends on many factors. Thus, 1- or 4-h dialysis procedure leads to the formation of alcocytes that did not affect the rate of ethanol elimination from the blood. The same was observed immediately after addition of ADH and ALDH to the incubation medium in 2-3-fold lower concentrations than the concentrations specified in Fig. 3. The results obtained with mixed blood of different animals differ from the results obtained with the blood from RVP of the same animal. Therefore, experimental conditions specified in Fig. 3 are optimal. The efficiency of ethanol elimination by alcocytes is not optimal under these conditions.

Thus, in our experiments mouse alcocytes stimulated metabolic elimination of ethanol from mouse blood. Human alcocytes did not survive in mouse blood. The rate of ethanol elimination was not sufficiently high under all experimental conditions and did not correspond to the contribution of encapsulated enzymes. The mechanisms of the latter phenomenon will be elucidated in our future studies.

The study was supported by the Russian Foundation for Basic Research (grants 09-08-00420 and 09-02-00018).

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