

Argocytes Containing Enzyme Nanoparticles Reduce Toxic Concentrations of Arginine in the Blood

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A method for incorporation of arginase nanoparticles into mouse erythrocytes has been developed and the possibility of reducing blood arginine concentration in animals with experimental hyperargininemia with arginase-loaded erythrocytes (argocytes) has been studied. Argocyte infusion to animals with hyperargininemia led to a rapid decrease in blood arginine concentration within 1 h and this effect of argocytes persisted for at least 4 h. This was paralleled by an increase in plasma concentrations of urea and ornithine. Hence, plasma arginine is hydrolyzed by arginase incorporated into argocytes; argocytes are functionally active and can serve as a defense system in pathological hyperargininemia, while the method developed by us can be regarded as a new nanobiotechnology for medicine and veterinary.

Key Words: *erythrocytes; arginase encapsulation; plasma arginine; argocytes; nanobiotechnology*

Arginine is an essential amino acid, a component of all proteins and a common product of their degradation. Excessive exogenous entry or production of arginine resulting from dysfunction of enzyme systems (mainly of genetic origin) can cause hyperargininemia (HA), abnormal elevation of blood arginine level.

The main enzyme involved in arginine metabolism is arginase catalyzing L-arginine hydrolysis to ornithine and urea at the final stage of urea cycle: (L-arginine+H₂O→L-ornithine+urea) and expressed mainly in hepatocytes [7]. In humans and higher primates, arginase is present in erythrocytes, though in low amounts [3]. In the liver, arginase finishes the multi-enzyme cycle of ammonium detoxification, while in other tissues it mainly regulates the concentrations of arginine and ornithine — precursors of glutamate, glutamine, agmatine, polyamines, creatinine, proline, urea, and NO [6].

Congenital arginase deficiency can cause HA with toxic effects of arginine on the liver and brain of newborns. Hyperargininemia is a metabolic disorder with

symptoms of progressive neurological and intellectual disorders, spastic paralysis, growth delay and episodic hyperammonemia, brain atrophy [11]. Congenital HA is fatal [8].

Arginine is a substrate for not only arginase, but also for NO-synthase, catalysing NO formation in all tissues, including blood cells [4]. The product of NO reaction is the main regulator of arterial muscle tone, a vasodilatory factor. Its deficit leads to blood pressure elevation, while its excess is fraught with DNA damage [12]. Hence, arginase and NO-synthase compete for available arginine and via their products regulate the production of numerous metabolites, including essential, signal, and destructive free radical compounds.

The technology of blood arginine concentration reduction is little developed and very slightly touched upon in publications. The only therapy recommended for congenital HA is arginine-free diet. This therapy seems to be unreliable, ineffective, and works with a great delay (several days).

We failed to find reports on basic studies of blood arginine reduction. The closest analogs of studies of this kind are recent studies of the effects of erythrocytes with encapsulated glutamine synthetase nanopar-

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ticles in on blood ammonia concentration [1,5] and urease and alanine dehydrogenase nanoparticles on blood urea concentrations [2]. The problem of rapid (within several hours or minutes) modification of blood arginine concentrations is still unsolved. No agents rapidly eliminating high arginine concentrations from the blood are known.

Human and animal erythrocytes should be prepared for passive transfer of encapsulated drugs [1, 5,10]. Arginase activity in rat and mouse erythrocytes, similarly as in erythrocytes of patients with HA, is low, and therefore, these cells can serve as the model for HA studies and can be used as arginase carriers in enzyme therapy.

We evaluated the possibility of using argocytes (mouse erythrocytes with encapsulated arginase nanoparticles) for prolongation of enzyme activity *in vivo* and removal of high arginine concentration from the blood of animals with experimental HA.

MATERIALS AND METHODS

The study was carried out on mice weighing 26-30 g. Erythrocytes were isolated from pooled blood and loaded with arginase by encapsulation [1,5] based on hypotonic dialysis, “hardening”, and isotonic “sealing” [9]. The blood was collected into a tube with sodium citrate. Arginase was encapsulated in isolated thoroughly washed erythrocytes. These erythrocytes, named argocytes, were resuspended in donor animal native plasma or in 0.9% NaCl and used in experiments within 24 h.

Hyperargininemia was induced by a single intraperitoneal injection of neutral arginine solution (1 g/kg). Argocytes were suspended in authentic plasma or 0.9% NaCl (1:1) and injected to mice into the caudal vein (0.4 ml) directly after arginine injection. Erythrocytes subjected to similar manipulations as argocytes, but without arginase, served as the control. The blood

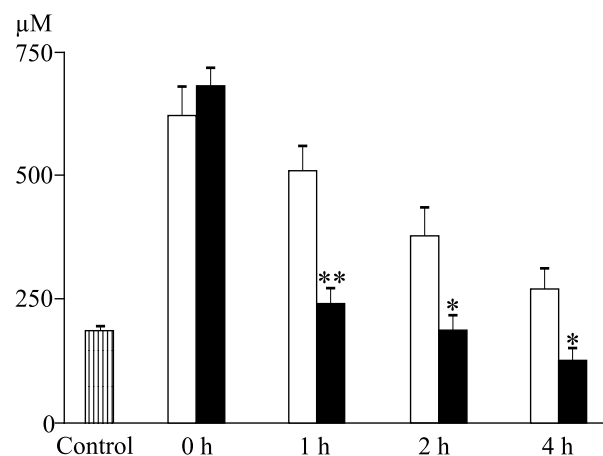


Fig. 1. Plasma arginine level during the first 4 h after arginine injection with erythrocytes (light bars) or argocytes (dark bars). * $p<0.05$, ** $p<0.01$ in comparison with erythrocytes.

was collected from the retro-orbital venous plexus directly and 1, 2, and 4 h after all injections. Arginine, ornithine, and urea were measured spectrophotometrically by the enzyme methods.

RESULTS

The developed method allowed incorporation of the enzyme with activity of 2.77 $\mu\text{mol}/\text{min}/\text{ml}$ erythrocytes (10-fold higher than its activity in erythrocytes of patients with congenital arginase deficiency).

Arginine injection did not change animal behavior and caused moderate HA. Arginine concentration in the blood increased by 3.3-3.7 times (to 0.6-0.7 mM vs. 0.188 mM normally under our conditions; $p<0.0001$) 1 h after arginine injection.

Injection of argocytes drastically accelerated metabolic removal of arginine in comparison with its natural elimination after injection of native erythrocytes (Fig. 1). Arginine concentration reached the normal level ($p=0.1002$) in comparison with the control) as

TABLE 1. Dynamics of Plasma Ornithine and Urea Concentrations after Simultaneous Injection of Arginine with Argocytes or Erythrocytes ($M\pm m$)

Plasma metabolite	Group	Control	Time after injection			
			0	1	2	4
Ornithine, μM	Arginine+erythrocytes	41 \pm 3	123 \pm 19**	192 \pm 23***	235 \pm 21***	249 \pm 19***
	Arginine+argocytes		141 \pm 14***	213 \pm 14***	265 \pm 17***	305 \pm 9****
Urea, μM	Arginine+erythrocytes	4.18 \pm 0.25	5.01 \pm 0.37	4.98 \pm 0.40	5.55 \pm 0.27**	5.84 \pm 0.18***
	Arginine+argocytes		5.29 \pm 0.32*	5.82 \pm 0.29**	6.38 \pm 0.35***	7.04 \pm 0.20****

Note. * $p<0.05$, ** $p<0.01$, *** $p<0.001$ in comparison with the control; * $p<0.05$, ** $p<0.01$ in comparison with injection of arginine+erythrocytes.

soon as 1 h after argocyte injection and persisted at this level and even lower after 2 and 4 h. The concentration of arginine after erythrocyte injection normalized only in 4 h and was 2-fold higher than that after argocyte injection.

Plasma concentrations of ornithine and urea in the same animals increased after arginine injection and this trend persisted for 4 h of observation (Table 1). This indicated arginine conversion into urea and ornithine in the arginase-catalyzed reaction. Differences by these parameters between the two groups were quantitatively negligible and were significant only after 4 h. Hence, argocytes stimulated plasma arginine elimination in animals with HA, which was paralleled by intensive formation of ornithine and urea. Argocytes can be used *in vivo* as a defense system in pathological HA. The developed method can be regarded as a new nanobiotechnology for medicine and veterinary.

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