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Antihypoxic and Antioxidant Effects of Exogenous Succinic Acid and Aminothiol Succinate-Containing Antihypoxants

I. V. Zarubina, M. V. Lukk, and P. D. Shabanov

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Pronounced antihypoxic and antioxidant effects of preventive injection of succinic acid, aminothiol antihypoxants gutimine and amtizol, and succinate-containing aminothiol antihypoxants gutimine succinate and amtizol succinate to Wistar rats with acute hypoxic hypoxia have been demonstrated. Exogenous succinic acid was inferior to aminothiol compounds by antihypoxic effect, but superior to them by its effect on the level of LPO products. Succinate in the aminothiol molecule modulated the intensity of their antihypoxic and antioxidant effects. It did not modulate the antihypoxic activity of amtizol, but reduced the antihypoxic effect of gutimine, presumably because of the physicochemical characteristics of aminothiols. Comparison of the intensities of antihypoxic and antioxidant effects of the studied drugs showed no direct relationship between these effects.

Key Words: *antihypoxants; succinic acid; succinate; lipid peroxidation; antioxidant system*

Effective aminothiol antihypoxants with energy stabilizing and antioxidant activities have been developed at Department of Pharmacology of Military Medical Academy. Clinical trials have confirmed their efficiency in diseases and critical states in which hypoxic and ischemic disorders play the leading role [1,7]. Search for new antihypoxants and their use in medical practice imply the use of new approaches to the problem. One of them is the use of succinates for antihypoxant substrates; this approach is expected to have a favorable impact for intracellular medium oxygenation, stabilize the structure and functional activity of the mitochondria, and prevent excessive lipid peroxidation and suppression of the antioxidant systems. This probability has been confirmed not once [3,5]. Succinate is a usual component of antihypoxants (succinate-containing antihypoxants). A good example of these drugs is mexidol, containing succinate in its structure (2-ethyl-6-methyl-3-hydroxypyridine succinate) [5]. This implies that addition of succinate to antihypoxants boosts their pharmacological activity.

We studied antihypoxic and antioxidant effects of exogenous succinic acid and aminothiol antihypoxants with or without succinate.

MATERIALS AND METHODS

Experiments were carried out on male Wistar rats (160-180 g; n=152) from Rappolovo Breeding Center. Acute hypoxic hypoxia was created by "elevation" of rats in a pressure chamber at a velocity of 50 m/sec to an "altitude" of 11,000 m and 30-min exposure of animals at this altitude with evaluation of survival or elevation to the "altitude" of 8000 m and exposure for 30 min with evaluation of biochemical parameters. Succinic acid, antihypoxants gutimine and

Department of Pharmacology, S. M. Kirov Military Medical Academy, St. Petersburg, Russia. ***Address for correspondence:*** I.V.Zarubina@inbox.ru. I. V. Zarubina

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amtizol (acknowledged as the reference antihypoxant by the Pharmacological Committee of the Ministry of Health of the Russian Federation) and their succinates (gutimine succinate and amtizol succinate) were used in the study. All drugs were injected intraperitoneally 30 min before "elevation" in the optimal doses: amtizol, gutimine, and succinate in a dose of 25 mg/kg, amtizol succinate and gutimine succinate in a dose of 50 mg/kg. Controls were injected with an equivalent volume of the solvent (saline). The material for biochemical studies was collected directly after "descent". Antihypoxic activity was evaluated by rat survival and ATP levels in the brain, heart, liver, kidneys, and skeletal muscles (hip). The antioxidant effects of the drugs were evaluated by tissue levels of MDA, reduced glutathione, and SOD activity [1,2].

The data were statistically processed by Student's *t* test and Fisher's test.

TABLE 1. Antihypoxic Activities of Succinate, Amtizol, Amtizol Succinate, Gutimine, and Gutimine Succinate

Group	Survival	%	Protection coefficient	P (Fisher's precise test)
Hypoxia	4 (15)	27	-	-
Amtizol	13 (15)	87	1.47	0.001
Amtizol succinate	9 (10)	90	1.50	0.003
Gutimine	8 (10)	80	1.42	0.01
Gutimine succinate	6 (10)	60	1.26	0.09
Succinic acid	5 (10)	50	1.18	0.2

Note. Total number of animals is shown in parentheses.

RESULTS

All the studied drugs improved animal resistance to critical hypoxia and prevented hypoxia-induced reduction of ATP level in the brain, heart, liver, kidneys, and skeletal muscles (Tables 1, 2). The energy-stabilizing effects of antihypoxants directly correlated with their effects on animal survival. Both parameters characterized the antihypoxic activity of the drugs, which formed the following series from most to least effective: amtizol succinate≈amtizol≈gutimine>gutimine succinate>succinic acid.

Exogenous succinic acid exhibited the least antihypoxic effects on the survival and cell energy, presumably, because of poor penetration in the studied organs' cells. Introduction of succinate in the antioxidant molecule virtually did not modify the antihypoxic activity of amtizol (amtizol succinate) and reduced significantly the antihypoxic effect of gutimine (gutimine succinate) [2]. This phenomenon could be explained by physicochemical characteristics of gutimine, a compound with the pK_a=5.9 basicity [4]. Chemical relation of gutimine to succinic acid seemed to be poorly hydrolyzed in water solutions and hence, in tissues. This resulted in a worse penetration of gutimine succinate into the cell in comparison with gutimine *per se* and led to reduction of its antihypoxic activity. Amtizol is a compound with low basicity (pK_a<2). Succinate in water solutions of amtizol is an equimolar mixture of amtizol amine and succinic acid [6]. Obviously, for this reason amtizol transport into the cell did not suffer because of slight hydrolysis of its succinate. Hence, antihypoxic effects of amtizol and amtizol succinate were virtually the same.

Studies of antioxidant activity of antihypoxants showed two components of their effects: inhibition of LPO stimulation and prevention of antioxidant system suppression (Table 3). The LPO-inhibiting capacity of the drugs decreased in the following order: succinic acid>gutimine succinate>amtizol>amtizol succinate≈gutimine. Exogenous succinic acid exhibited

TABLE 2. Effects of Acute Hypoxia and Antihypoxants on ATP Content (μmol/g tissue) in Organs of Rats (n=10; $M\pm m$)

Drug	Brain	Kidneys	Liver	Heart	Skeletal muscles
Hypoxia	2.47±0.12	2.19±0.10	1.94±0.10	2.73±0.03	2.58±0.10
Amtizol+hypoxia	2.83±0.13*	2.73±0.07*	2.22±0.04*	4.24±0.09*	3.59±0.12*
Gutimine+hypoxia	2.82±0.09*	2.86±0.04*	3.43±0.08*	4.11±0.10*	3.96±0.38*
Succinic acid+hypoxia	2.65±0.13*	2.72±0.08*	1.97±0.05*	3.57±0.08*	3.60±0.14*
Amtizol succinate+hypoxia	2.95±0.06*	2.80±0.07*	2.48±0.13*	3.75±0.09*	3.65±0.14*
Gutimine succinate+hypoxia	2.49±0.09	2.86±0.06*	2.53±0.06*	4.11±0.06*	3.78±0.12*

Note. Here and in Table 3: *p<0.05 in comparison with the parameter in hypoxia.

TABLE 3. Effects of Antihypoxants on LPO and Antioxidant Defense Parameters in Hypoxic Hypoxia (8000 m; n=10; $M\pm m$)

Parameter	Organ	Hypoxia	Amtizol	Gutimine	Amtizol succinate	Gutimine succinate	Succinic acid
MDA, μmol/g tissue	Brain	99.90±2.09	29.90±2.61*	55.99±8.30*	61.21±2.16*	41.20±6.70*	27.67±2.60*
	Kidneys	49.26±2.43	50.12±2.22	40.06±5.30	43.80±0.89*	27.10±4.20*	21.73±3.10*
	Liver	25.99±2.45	19.91±2.11*	21.94±4.81	18.21±1.35*	20.31±4.50	17.34±2.40*
	Heart	33.39±2.54	10.23±3.81*	14.31±7.00*	13.96±1.56*	7.50±2.70*	7.80±1.10*
	Skeletal muscles	33.73±3.25	12.50±2.41*	15.86±2.70*	20.50±1.81*	11.30±2.0*	4.87±1.90*
Reduced glutathione, μmol/g tissue	Brain	17.90±0.89	46.56±1.21*	43.47±1.20*	41.50±1.12*	37.30±1.60*	42.14±0.90*
	Kidneys	32.58±1.52	61.08±1.69*	59.68±4.30*	55.46±0.82*	51.47±1.60*	49.80±5.40*
	Liver	63.42±0.89	84.65±1.32*	115.05±10.0*	86.28±1.52*	71.70±2.90*	81.56±7.80*
	Heart	20.92±0.89	33.04±2.22*	42.59±2.70*	40.20±1.23*	35.70±1.40*	41.89±2.60*
	Skeletal muscles	19.25±0.44	25.87±2.53*	31.79±2.80*	28.92±0.70*	18.19±2.10	25.80±1.30*
SOD, U/mg protein	Brain	0.62±0.16	1.52±0.15*	0.16±0.03*	1.35±0.17*	0.63±0.29	0.85±0.21*
	Kidneys	0.71±0.02	1.82±0.06*	0.42±0.09*	2.61±0.05*	1.06±0.23*	1.79±0.27*
	Liver	0.82±0.12	3.13±0.17*	0.87±0.14	2.65±0.15*	1.14±0.23*	2.43±0.42*
	Heart	0.39±0.05	1.37±0.04*	0.14±0.02*	1.43±0.06*	0.56±0.16*	0.64±0.08*
	Skeletal muscles	0.46±0.02	1.66±0.06*	0.10±0.02	1.03±0.02*	0.50±0.15*	0.82±0.35*

the highest efficiency in LPO suppression. Succinate in the gutimine molecule stimulated its antioxidant activity in comparison with gutimine and did not modify them in amtizol.

The absence of pronounced organ specificity of the antihypoxant effects (prevention of LPO stimulation) is worthy of note, though the greatest shifts after injections were found in the brain and heart. The drugs similarly reduced the levels of LPO products. The effects of antihypoxants were most pronounced in organs with most severe LPO disorders and the least in organs with least stimulated LPO. All the studied drugs (except gutimine) exhibited positive effects on the antioxidant defense factors decreased in the following order: amtizol>amtizol succinate>succinic acid>gutimine succinate>gutimine. Injection of gutimine increased the level of reduced glutathione in all organs and inhibited (in addition to the hypoxic effect) SOD activity. Addition of succinate to gutimine completely abolished the inhibitory effect of gutimine on SOD. Succinate added to the amtizol molecule virtually did not change its antioxidant activity.

Comparison of the antihypoxic and antioxidant effects of the studied drugs showed no direct relationships between these activities (Table 4). A strong antihypoxic effect of gutimine did not correlate with its pronounced negative effects on antioxidant defense enzymes. On the other hand, gutimine succinate with its lower antihypoxic activity in comparison with gutimine exhibited a more pronounced antioxidant effect.

The highest antioxidant activity of exogenous succinic acid was paralleled by its lowest (of the studied drugs) antihypoxic effect. Only amtizol and amtizol succinate combined high antihypoxic and antioxidant activities under conditions of hypoxia, the antioxidant activity consisting mainly in prevention of suppression of antioxidant defense factors.

TABLE 4. Antihypoxic and Antioxidant Effects of Antihypoxants

Drug	Antioxidant activity	Antihypoxic activity	
		LPO suppression	effect on AOS
Amtizol	+++	+	+++
Gutimine	+++	±	±
Amtizol succinate	+++	±	+++
Gutimine succinate	+	++	+
Succinic acid	±	+++	++

Note. AOS: antioxidant systems. "±" slight effect, "+" moderate effect, "++" strong effect, "+++" very strong effect.

Antioxidant activity of the studied antihypoxants suggests them for correction of hypoxic disorders and disorders of free radical oxidation processes of other nature. The antioxidant effect of antihypoxants under these conditions is clearly preventive, which suggests their use as effective preventive antioxidants. This is confirmed by discovery of the signal molecule function in succinic acid [8,9], triggering a chain of adaptive changes in the cells under conditions of hypoxia, including the oxidative response.

Hence, exogenous succinic acid is inferior to aminothiol compounds gutimine and amtizol by its antihypoxic effect. Succinate is inessential for antihypoxic activity of amtizol and reduces the antihypoxic effect of gutimine, presumably because of physicochemical characteristics of aminothiols. Comparison of the intensity of antihypoxic and antioxidant activities of the studied drugs showed no direct correlation between these effects.

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