Complex N-Acetyl-L-Cysteine Compounds with Biometals as Self-Defense Factors of Biological System

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We studied the effects of complex N-acetyl-L-cysteine compounds with transitional biometals on the inflammatory and adaptation reactions. Some compounds were superior to known antihypoxants and actoprotectors and exhibited significant antiinflammatory activity.

Key Words: *N-acetyl-L-cysteine; biometals; inflammation; adaptation*

Live organisms respond to health-threatening physical, chemical, or biological exposure by a complex universal reaction overcoming the pathological or extreme states. Two universal defense reactions are known: inflammation and adaptation. Inflammatory reaction can be easily detected at the biochemical level by the content of cytokines and other effectors (the composition and content of these effectors depend on the phase of inflammation) [3]. The development of adaptation reaction is also associated with the formation of chemical compounds not characteristic of the normal state [2]. Exogenous chemical agents regulating natural defense reactions and normalizing the homeostasis parameters can be also regarded as defense factors. These agents should reproduce the structure of true metabolites or simulate it [6].

L-Cysteine is a component of the majority of proteins and glutathione. In proteins this amino acid plays a structural and functional role. The reactivity of the sulfhydryl group in L-cysteine is of priority significance. Its reversible transformation into disulfide group allows intra- and intermolecular restructuring of proteins, which largely determines their behavior in biological systems. Sulfhydryl group participates in many enzymatic reactions. Its role is particularly important in reactions of coupled catalysis providing synchronization of events in metabolic, energetic, and information biological flows. Redox transformation of the sulfhydryl group of organic components in cells promotes the maintenance of homeostasis parameters [7].

N-Acetyl-L-cysteine (L-NAC) is a natural metabolite of L-cysteine characteristic of animal and plant organisms. For example, the content of this metabolite in rat brain approaches the content of glutathione. It seems that this compound can be regarded not only as an L-cysteine depot, but also as a low-molecular-weight compound with functions of its own, for example, defense functions. This is seen from antioxidant effect of L-NAC in biological systems [7]. Biological activity of L-NAC in some of them is determined solely by redox activity (without its pre-transformation into glutathione), as even an increase in glutathione level in a cell does not depend on the form of the stereoisomer used (L- or D-) [5].

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Transitional metals in biological systems function as active center of metal enzyme and their excess is deposited by specialized proteins. Under pathophysiological conditions transitional metals are redistributed. For example, mobilization of copper from depot and its redistribution into other organs and tissues sharply increase during inflammatory reaction [8]. The level of free ions of transitional metals (transit pool) is extremely low [1]. Ions of transitional metals are delivered to the target organ from their depot by special transporters. Some antiinflammatory drugs (indomethacin, *etc.*) can participate in copper metabolism as its transporters, which to a certain measure explains their therapeutic effect [8]. Some facts indicate that L-NAC can normally act as a chelator. For example, this agent regulates activity of AP-1 and NF-kB transcription factors as Zn^{2+} ion chelator [4], but not as active trap, as was considered previously.

Complex compounds of L-NAC with biometals can be regarded as metal and ligand metabolites. For this reason the probability of protective effect is higher for a complex compound than for metal or ligand alone.

The aim of our study was to confirm experimentally that complex L-NAC compounds with transitional biometals can be regarded as agents with defense function in disease or under extreme conditions.

MATERIALS AND METHODS

Complex L-NAC compounds with biometals were synthesized at Institute of Experimental Diagnosis and Therapy of Tumors within the framework of research aimed at theoretical and experimental validation of the concept of physiologically compatible antioxidants [3,6]. Some biological tests were carried out at Department of Fundamental Medical Knowledge, Acad. I. G. Petrovskii State University, Bryansk (Head: Professor N. N. Samoilov).

The capacity of complex L-NAC compounds to function as adaptogens under extreme conditions was studied on outbred male mice (20-23 g). Each experiment was carried out on an experimental group with an appropriate control (8-12 animals per group). The doses varied from 10 to 50 mg/kg. Water solutions or stabilized suspensions were injected intraperitoneally 1 h before the experiment. Controls were injected with the same volumes of saline via the same route at the same terms. Physical working capacity of mice was evaluated by the duration of treadmill running (6-track treadmill) at the transporter band movement velocity of 29-32 m/min or by the duration of swimming (water temperature 28°C) with a load (7% body weight) fixed to the base of the tail. Acute hypobaric hypoxia was induced in a Vita electrovacuum device by "elevating" the animals to altitude of 10,000 m above sea level at a mean rate of 50 m/sec at 20-22°C. Acute normobaric hypoxia with hypercapnia was induced by placing the animals into a 0.25-liter vessel with ground-glass stopper. Antihypoxic effect of the substances was evaluated by the duration of animal stay under experimental conditions until complete respiratory arrest.

The capacity of complex L-NAC compounds to participate in the regulation of inflammatory reaction was evaluated on a model of carrageenan edema of the limb in outbred rats (200-250 g); 0.1 ml 1% carrageenan (Serva) was injected subplantarly into the right paw. The test substance was administered into the stomach 1 h before carrageenan. The paw volume was measured with a plesthysmometer (Ugo Basil) 4 h after carrageenan injection. The therapeutic effect of the test preparation was evaluated by inhibition of the inflammatory reaction in comparison with the intact paw of the same animal and with the reaction of control (untreated) rats. The increment in paw volume, resultant from the development of inflammatory reaction, was estimated by the formula:

$$\frac{(V_r - V_l)}{V_l} \times 100,$$

where V_r is the volume of the right paw and V_l volume of the left paw. Inhibition of the inflammatory reaction (in percent) was estimated by the formula:

$$100-(V_{exp}-100/V_c),$$

where V_{exp} and V_c are the increment in paw volume during the experiment and in the control, respectively.

The results were statistically processed using Student's *t* test and Statgraph software.

RESULTS

Eleven L-NAC derivatives with the ligand modified by Zn^{2+}, Co^{2+}, Co^{3+}, Fe^{2+}, or Ti^{4+} ions were used in the studies by three biotests.

The results of biological testing for the capacity of complex L-NAC compounds with transitional biometals to function as protective agents under extreme conditions and in disease are presented in Tables 1 and 2.

TABLE 1. Effects of L-NAC Metal Derivative on the Duration of Running and Swimming in Mice under Standard Conditions and on Animal Survival in Hypoxia

Preparation	Running		Swimming		Hypoxia type			
					acute hypobaric hypoxia		acute normobaric hypoxia with hyperecapnia	
	dose, mg/kg	duration, % of control	dose, mg/kg	duration, % of control	dose, mg/kg	duration of stay in enclosure, % of control	dose, mg/kg	duration of stay in enclosure, % of control
Metal derivatives								
bis(N-acetyl-L-cysteinato)cobalt(II)	25	149±3*						
bis(N-acetyl-L-cysteinato)cobalt(III)	10	164±3*	5	164±4*				
bis(2-methyl-5-ethyl-3-hydroxypyridinium) bis(N-acetyl-L-cysteinato)esculetinato-titanium(IV)	25	63±10*						
bis(N-acetyl-L-cysteinato)iron(II)	10	151±7*	10	175±3*	10	126±6*	25	57±2*
bis(N-acetyl-L-cysteinato)aquazink(II)	25	64±8			50	286±12*	50	417±18*
bis(N-acetyl-L-cysteinato)aquazink(II)sulfate					50	244±14*	50	385±16*
bis(N-acetyl-L-cysteinato)esculetinato-titanium(IV)	25	109±16					25	96±9
Reference drugs								
bemithyl	50	132±6*	50	124±2*				
bromantane	50	125±4*	50	128±3*				
amtizol					25	161	25	96
acisole	10	94±15			10	220	10	100
mexidol	50	110±14			100	90±18	100	124±5*
sodium hydroxybutyrate	50	76±10*			100	105±21	100	100±9
nooglutil	50	95±17			25	172±7*	50	118±4*
emoxipin	50	81±12			100	125±6	100	131±4*
ethomersol	50	113±14			100	151±9*	100	125±6*

Note. *$p<0.05$ compared to the control.

TABLE 2. Effects of L-NAC Metal Derivatives on Carrageenan Edema of Rat Paw

Group	Volume of right paw, cm^3	Volume of left paw, cm^3	Difference, cm^3	Increment, %	Inhibition, %
Control, carrageenan	2.12±0.10	0.86±0.10	1.26±0.20	146.5	
Carrageenan+N-acetyl-L-cysteinatozink(II)	1.60±0.15	1.07±0.07*	0.53±0.18	49.5	66.2
Carrageenan+N-acetyl-L-cysteinatocobalt(II)	1.53±0.03	1.07±0.08*	0.46±0.10	42.9	70.6
Carrageenan+N-acetyl-L-cysteinatocobalt(III)	1.50±0.04	1.03±0.05*	0.46±0.08	44.6	69.5
Carrageenan+bis(N-acetyl-L-cysteinato)zinc(II)	1.37±0.07	0.96±0.04*	0.40±0.05	41.7	71.5
Carrageenan+bis(N-acetyl-L-cysteinato)cobalt(II)	1.55±0.02	1.03±0.08*	0.51±0.07	49.5	66.2
Carrageenan+bis(N-acetyl-L-cysteinato)cobalt(III)	1.57±0.20	0.89±0.10*	0.68±0.20	76.4	47.8
Carrageenan+bis(2-ethyl-6-methyl-3-hydroxypyridinium) bis(N-acetyl-L-cysteinato)esculetinatotitanium(IV)	1.49±0.03	1.10±0.01*	0.39±0.03	35.4	75.8
Carrageenan+bis(N-acetyl-L-cysteinato)esculetinatotitanium(IV)	1.72±0.03	0.97±0.01*	0.75±0.03	77.3	47.2
Indomethacin	1.28±0.06	0.92±0.02	0.36±0.07	39.1	73.3

Note. *$p<0.002$ compared to the control.

Well-known chemical compounds used in medicine as adaptogens were selected as reference drugs. Three of the tested L-NAC derivatives (bis (N-acetyl-L-cysteinato)cobalt(II), bis(N-acetyl-L-cysteinato)cobalt(III), and bis(N-acetyl-L-cysteinato) iron(II)) were not inferior to the known actoprotectors bromantane and bemithyl (Table 1). The best results were observed in the swimming test. Of the new antihypoxants, L-NAC derivatives with zinc (bis(N-acetyl-L-cysteinato)-aquazink(II) and bis(N-acetyl-L-cysteinato)aquazink(II)sulfate) were significantly more active than all seven reference drugs, while bis(N-acetyl-L-cysteinato)iron(II) exhibited low activity under conditions of acute hypobaric hypoxia, but was no less active than the reference drugs in acute hypoxia with hypercapnia.

All test compounds, new L-NAC derivatives, exhibited antiinflammatory activity, only two of eight compounds being 1.5 times less active than the reference drug indomethacin, while the rest showed similar activity (Table 2).

Hence, testing of complex compounds of zinc, cobalt, and titanium confirmed our hypothesis that chemical modification of natural antioxidant L-NAC by transitional biometals allows classifying it as a protective agent. This can be regarded as scientific validation for the existence of minor metabolites with protective effects, represented by complex compounds of natural antioxidants with transitional biometals and suggests the use of these metabolites as principally new safe drugs.

Eight of eleven (73%) tested compounds exhibited biological activity. Our results experimentally confirmed the concept of physiologically compatible antioxidants [6]. The postulated metabolites with protective function exhibited multiple activity (only 3 compounds were active in one test, while the rest five were active in two or three tests) and the modulatory pattern of biological activity, this suggesting the regulatory effect of the studied compounds on the multicomponent defense systems of animals.

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