Mechanisms of Antioxidant Effect of Natural Sesquiterpene Lactone and Alkaloid Derivatives

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We performed screening of potential antioxidants among natural lactone and alkaloid derivatives and characterized their antioxidant effects. The substances exhibiting antioxidant and metal-chelating potential, in particular, tryptamine derivatives, are promising neuroprotector agents.

Key Words: *lipid peroxidation; antioxidant; alkaloids; lactones*

Herbs and plants are used in medicine for ages, but now most attention is focused on isolation and identification of active substances, in particular, sesquiterpene lactone and alkaloids from the raw material. Alkaloids are widely used in clinical practice [6].

Targeted modification of the known natural compounds for improving or modulating the spectrum of their biological activity is a promising approach. It is known that oxidative stress is an important mechanism in the pathogenesis of various human diseases, an inevitable component of aging, and hence, plays an important role in the development of age-related neurodegenerative diseases. In light of this, the search for bioactive substances with antioxidant activity is in progress.

Here we performed screening of potential antioxidants among modified natural compounds, sesquiterpene lactones and alkaloids, and studied the mechanisms of their antioxidant activity.

MATERIALS AND METHODS

Experiments were carried out on outbred albino male rats weighing 300-400 g. The animals had free access to food and water. The animals were narcotized with carbon dioxide and decapitated using a guillotine. The brain was removed and homogenized in 120 mM KCl/20 mM HEPES on cold. For isolation of the subcellular fraction, the brain homogenate was centrifuged at 4000 rpm and supernatant was used in further experiments on the same day. The protein content in brain homogenate was measured using a biuret method [2]. LPO intensity in brain homogenate was assayed using a modified TBA test [1]. Fe^{2+}-chelating activity was evaluated using a modified method described elsewhere [5]. To exclude the possibility of binding the test substances with ferrozine, absorption spectra of ferrozine in the absence and presence of the test substance were recorded (300-800 nm) [4].

Antiradical activity was measured using a standard model stable radical diphenylpicrylhydrazine. Its reduction by compounds having labile hydrogen atom (thiols, phenols, amines, amides, *etc.*) leads to a decrease in absorption peak at 517 nm [3].

The data were processed statistically using Excel 5 software.

RESULTS

Alkaloid securinine and sesquiterpene lactones (isoalantolactone and epoxyisoalantolactone) containing an activated double bond easily binding N-nucleophiles were chosen for the experiments. Natural alkaloids tryptamine, ephedrine, anabasine, salsoline, *etc.* were used as nucleophiles. A series of new derivatives was synthesized on the basis of these two classes of compounds (Table 1).

First, we evaluated the effects of the test compounds on LPO induced by Fe^{3+} and tert-butyl hydro-

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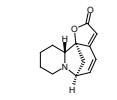
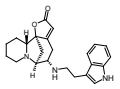
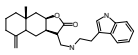
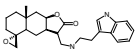
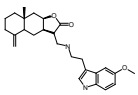
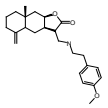
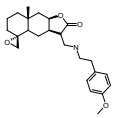
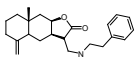
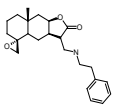
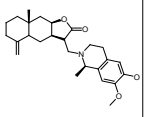
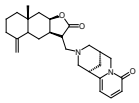
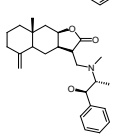
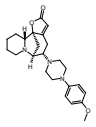
TABLE 1. Relationship between Chemical Structure and Activity of the Test Natural Compounds

Code	Formula	% of LPO inhibition, 0.1 mM substance, inductor Fe^{3+} (IC_{50}, μM)	% of LPO inhibition, 0.1 mM substance, inductor t-BHP (IC_{50}, μM)	Fe^{2+}-chelating activity (%), 0.1 mM substance (EC_{50}, μM)	Antiradical activity (%), 0.1 mM substance
L12		No activity	<10%	No activity	<10%
L01		Prooxidant activity	No activity	No activity	No activity
L04		Prooxidant activity	No activity	No activity	No activity
L12-5272a		85.6±4.5% (IC_{50}=65.9±24.1)	19.2±4.8%	49.3±3.7%	12.00±0.53%
L01-5272		65.7±16.3% (IC_{50}=31.2±11.1)	No activity	85.5±4.3% (EC_{50}=30.6±3.2)	No activity
L04-5272		65.50±3.42% (IC_{50}=31.9±10.1)	No activity	59.8±4.3% (EC_{50}=83.02±4.30)	No activity
L01-5488		65.4±54.0% (IC_{50}=13.9±1.1)	No activity	81.5±2.6% (EC_{50}=40.2±2.9)	No activity
L01-165		17.07±5.00%	No activity	38.4±3.1% (EC_{50}>100 μM)	No activity
L04-165		23.80±4.74%	13.35±2.22%	59.9±1.9% (EC_{50}=74.6±4.8)	No activity
L01-290		47.50±1.75%	10.00±0.48%	No activity	<10%
L04-290		35.14±4.93%	13.35±1.86%	No activity	No activity
L01-181		33.10±27.37%	No activity	53.7±3.9% (EC_{50}=40.2±2.9)	<10%
L04-181		11.17±2.01%	No activity	35.6±2.8%	No activity
L01-288		34.9±8.9%	12.39±4.16%	No activity	68.9±2.1%
L01-292		No activity	No activity	No activity	No activity
L01-3077		Prooxidant activity	Prooxidant activity	62.3±15.0% (EC_{50}=45.0±14.6)	No activity
L12-154		Prooxidant activity	Prooxidant activity	No activity	No activity

peroxide (t-BHP) in rat brain homogenate. We found that the initial alkaloid securinine had no activity in this test, while lactones enhanced LPO, thus exhibiting prooxidant properties. The results of testing of the modified compounds (% of LPO inhibition) are presented in Table 1.

To elucidate the mechanism of action of the test compounds we studied their metal-chelating properties and antiradical activity (Table 1). A correlation was found between Fe^{2+}-binding activity and the presence of a free nitrogen atom in the modified derivatives (cyclization of this atom determines the absence of chelating activity). The compounds exhibiting metal-chelating properties effectively reduced Fe^{3+}-induced LPO and blocked the process at its initial stage (Fenton reaction). The only exclusion was L01-3077 compound containing non-cyclized nitrogen and effectively binding Fe^{2+}, but exhibiting prooxidant properties in LPO model.

Moderate (≤20%) antiradical activity of the test compounds well correlated with the efficiency of inhibition of t-BHP-induced LPO.

Antioxidant properties of compounds devoid of metal-chelating and antiradical activity (L01-290, L04-290, Lo1-288) can be determined by other mechanisms (*e.g.* modulation of lipoxygenase family enzymes, activation of endogenous antioxidant systems, *etc.*).

Thus, appearance of LPO-inhibiting properties in chemically modified derivatives can be explained by the presence of metal-chelating activity and to a lesser extent radical-binding activity. The substances exhibiting antioxidant and metal-chelating potential are promising neuroprotector agents.

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