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MEMBRANE STABILIZING EFFECT OF CURCUMIN ON CHRONIC CADMIUM INTOXICATION

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A new mechanism of Curcumin membrane stabilizing effect on white rats tissue cells' membranes (brain, liver, kidneys, lungs, spleen, small intestine and heart) with chronic cadmium intoxication was defined. The white rats were divided into 3 groups (4 rats in each): 1) rats received water by drinking – control (C); 2) rats received $CdSO_4$ with drinking water (0,3 mg/kg/day) during 28 days – (Cd); 3) rats received $CdSO_4$ and Curcumin (200 mg/kg/day) at the same time during 28 days (Cd+Cur).

Compared to the control (C) group the specific content of total fractions of new membrane components (total fractions of superoxide-producing and thermostable associate isoforms between NADPH containing lipoprotein (NLP) and NADPH oxidase (Nox)) – NLP-Nox significantly increased in the second group, which caused a corresponding destabilization of biomembranes.

In the Cd+Cur group, under the influence of Curcumin, depending on the organ, there is a tendency to approach the specific contents of total fractions of NLP-Nox isoforms of above mentioned tissues to control in varying degrees. The membranostabilizing effect of Curcumine was revealed.

Cadmium intoxication – biomembranes – NLP-Nox associate isoforms – Curcumin

Որոշվել է Կուրկումինի թաղանթակայունացնող ազդեցության նոր մեխանիզմը սպիտակ առնետների հյուսվածքային թաղանթների վրա (ուղեղ, լյարդ, երիկամներ, թոքեր, փայծաղ, բարակ աղիք և սիրտ) կադմիումով քրոնիկական թունավորման ժամանակ: Սպիտակ առնետները բաժանվել են 3 խմբի՝ 1) առնետները ստացել են խմելու ջուր՝ ստուգիչ խումբ (U), 2) առնետները ստացել են խմելու ջրով $CdSO_4$ (0,3 մգ/կգ/օր)՝ 28 օրվա ընթացքում (Cd), 3) առնետները ստացել են $CdSO_4$ և Կուրկումին (200 մգ/կգ/օր) միաժամանակ՝ 28 օրվա ընթացքում (Cd+Cur):

Ստուգիչ (U) խմբի հետ համեմատած՝ նոր թաղանթային բաղադրամասերի տոտալ ֆրակցիաների տեսակարար պարունակությունը (սուպերօքսիդ արտադրող և ջերմակայուն ասոցիատների իզոձևերի տոտալ ֆրակցիաները NADPH պարունակող լիպոպրոտեինի (ՆԼՊ) և NADPH օքսիդազի (Nox) միջև)՝ ՆԼՊ-Nox-ը նկատելիորեն բարձրացել էր երկրորդ խմբում, որն առաջացրել էր թաղանթների համապատասխան անկայունացում:

Cd+Cur խմբում, կախված օրգանից, Կուրկումինի ազդեցության տակ վերը նշված հյուսվածքների ՆԼՊ-Nox իզոձևերի տոտալ ֆրակցիաների տեսակարար պարունակություններից, առկա է տարբեր աստիճանի՝ ստուգիչին մոտենալու միտում: Բացահայտվել է Կուրկումինի թաղանթակայունացնող ազդեցությունը:

Կադմիումային ինտոքսիկացիա – կենսաթաղանթներ – ՆԼՊ-Nox ասոցիատի իզոձևեր – Կուրկումին

Определен новый механизм мембраностабилизирующего действия Куркумина на мембраны клеток тканей белых крыс (мозг, печень, почки, легкие, селезенка, тонкий кишечник и сердце) при хронической кадмиевой интоксикации. Белых крыс разделили на 3 группы (по 4 крысы в каждой): 1) крысы, получившие питьевую воду – контрольная группа (К); 2) крысы получившие $CdSO_4$ с питьевой водой (0,3 мг/кг/сут) в течение 28 дней – (Cd); 3) крысы, получившие $CdSO_4$ и куркумин (200 мг/кг/сут) в одно и то же время в течение 28 дней (Cd+Cur).

По сравнению с контрольной группой (К) удельное содержание суммарных фракций новых мембранных компонентов (суммарные фракции супероксид продуцирующих и термостабильных изоформ ассоциатов между NADPH содержащим липопротеином (НЛП), и NADPH-оксидазой (Nox)) – НЛП-Nox, значительно увеличилось во второй группе, что вызвало соответствующую дестабилизацию биомембран.

В группе Cd+Cur, в зависимости от органа, под влиянием Куркумина наблюдается тенденция к приближению удельных содержаний суммарных фракций изоформ НЛП-Nox вышеуказанных тканей к контролю различной степени. Выявлено мембраностабилизирующее действие Куркумина.

Кадмиевая интоксикация – биомембраны – изоформы ассоциатов НЛП-Nox – Куркумин

Cadmium causes oxidative damage of tissue cells – inflammation. Cadmium (Cd) is a toxic metal, targeting the lung, liver, kidney and testes following acute intoxication, and causing nephrotoxicity, immunotoxicity, osteotoxicity after prolonged exposures. Reactive oxygen species (ROS) are often implicated in Cd toxicology. It was known, that evidence for the generation of free radicals in intact animals following acute Cd overload and discussed the association of ROS in chronic Cd toxicity and carcinogenesis. The protective effects of vitamin C, zinc, and *N*-acetylcysteine, individually or in combination with Cd, to monitor their amelioration capability against Cd-induced oxidative damage in Wistar rats [4, 6, 10, 12].

Cd accumulates in plants and animals with a long half-life of about 25-30 years and its low rate of excretion from the body cause its storage in soft tissues (liver and kidneys) with a diversity of toxic effects such as nephrotoxicity, hepatotoxicity, endocrine and reproductive toxicities. Cd exposure may be related to various types of cancer, including breast, lung, prostate, pancreas, and kidney cancers. At the cellular level, cadmium affects cell proliferation, differentiation, apoptosis and other cellular activities. Mitochondria damage is highly plausible given that these organelles play a crucial role in the formation of ROS and are known to be among the key intracellular targets for cadmium. Cd-dependent interference in DNA repair mechanisms as well as the generation of reactive oxygen species are important causes of its cellular toxicity. At the cellular level, cadmium affects cell proliferation, differentiation, apoptosis and other cellular activities [3,5,9].

Observed increases in the level of reactive oxygen species in tissues during cadmium intoxication give grounds to use Curcumin as a natural antioxidant with immunomodulator activity [1, 7, 8].

On the other hand, with the recent discovery of new protein components from biomembranes of animal and plant origin [11] – isoforms of superoxide-producing thermostable associates between NLP and Nox – NLP-Nox, as new protein components of biomembranes, which are responsible for their stability, it becomes possible to determine the specific content of the total fraction of isoforms of thermostable associates, as new factors of biomembrane stability during cadmium intoxication, with the detection of a membrane stabilizing effect of Curcumin during chronic cadmium intoxication in rats. This is the aim of the work.

Materials and methods. The white rats were divided into 3 groups (4 rats in each group): 1) water drinking control (C) group; 2) the group received with drink water $CdSO_4$ (0,3 мg/kg/day) during 28 days (Cd) group; and 3) rats, receiving $CdSO_4$ and Curcumin (200 mg/kg/day) body weight at the same time during 28 days (Cd+Cur).

Isolation and purification of the total fractions of the isoforms of O_2^--producing associate NLP-Nox from membranes of the rats' organs' tissue cells.

The isoforms of O_2^--producing associates NLP-Nox from rats' brain, liver, lung, small intestine, heart, liver, kidneys, spleen were isolated and purified by the universal method developed by the authors [11], after freezing and defrosting of these organs' aqueous homogenates, they were incubated at pH 9,5 and 37°C, for 1,5 hours. After centrifugation at 5000×g, for 10 min, pH of supernatant was adjusted to 4,8. Precipitate of the fraction of NLP-Nox was soluble in water at pH 9,5, and after centrifugation the supernatant subjected to ion-exchange chromatography on cellulose DE-52 at pH 9,5. The Nox-NLP associates do not absorbed on this column and was eluted free. After concentration of these total fractions of NLP-Nox, its gel-filtration on the separately column of Sephadex G-200 at pH 9,5 were carried out. The total fractions of the isoforms of NLP-Nox were eluted with symmetrically elution diagram, and after deionization of isoforms of NLP-Nox associate were subjected and incubated in boiling water during 10 min. After centrifugation the supernatant undergo to vacuum lyophilization. After weighting the isoforms of associate NLP-Nox stored in anaerobic condition at -10° C.

NLP-Nox associates' electrophoresis was carried out on 10% PAAG (Polyacrylamide Gel) for proteins of acidic and basic character.

Determination of NADPH in the composition of isoforms of received total fractions of NLP-Nox from C, Cd and Cd+Cur groups.

The presence of NADPH in the composition of total fraction of the isoforms of NLP-Nox was determined by spectrofluorimetric method, by determination of the fluorescence intensity (F) in comparative units at 430 nm with excitation at 370 nm [13]. The specific content of NLP-Nox was determined by its weighting, after deionization and vacuum lyophilization and was conveyed by mg in 1g tissue (mg/g).

During the investigation, the DE-52 cellulose ("Whatman", England), Sephadex G-200 ("Pharmacia", Sweden), adrenaline ("Sigma", USA), the spectrofotometer "Cary 60" (USA), spectrofluorimeter "Perkin-Elmer" (USA), centrifuge K-70D and K-24 "Janetzki" (Germany) were used.

The statistical treatment of the received results was carried out by the variation statistical method of Student-Fisher, by determining the criteria of reliability "p", m±M.

Results and Discussion. The isoforms of total fraction of the presented above O_2^-- producing associates NLP-Nox from rats' tissues (brain, liver, lung, small intestine, heart, kidneys, spleen) of C, Cd and Cd+Cur groups, did not undergo to PAAG electrophoresis and remained on the entry of the gel tubes in an aggregated state. Indirectly, the purity of these associates is evidenced by the fact, that during electrophoresis of the opalescent solutions of these associates on 10% PAAG tubes strips of accompanying water-soluble proteins for acidic and basic nature were not detected. On the other hand, the symmetry of the elution diagrams of total fractions of the isoforms of NLP-Nox associates after gel-filtration through Sephadex G-200 and unchanged optical spectral index (A_{280}/A_{400}) also shows the purity of these associates. The total fractions of the isoforms of presented above associates practically do not lose their nativity and O_2^--producing activity after heating in boiling water during 10 min, to denature of possible traces of antioxidant and other proteins. On the optical absorption spectra of the total fractions of the isoforms of NLP-Nox from presented above tissue formations in C, Cd and Cd+Cur groups in oxidized and reduced state, at pH 9,5 the characteristic absorption spectra of associated Nox in composition of NLP-Nox in visible regions are observed. In the UV region in spectra of isoforms of presented associates the optical absorptions at 265, 270 and 280 nm are observed.

The presented above isoforms of NLP-Nox associates are biological systems, ferments for which the substrate is not a free Nox, but connected with the NLP, in the composition of associate NLP-Nox. NLP and Nox together form a thermostable and continuousily O_2^- producing biosystem, for which the electrone bridge is a Fe(III) in heme group of the Nox, in the composition of the total fractions of the associates of NLP-Nox. This biosystem reduces only O_2, forming O_2^-. The specific contents of NLP-Nox from presented above tissues are shown in the tab. 1.

After heating of these associates in boiling water during 10-12 min, the effect of denaturation and decrease of superoxide production is not detected. The higher termostability of the O_2^--producing NLP-Nox associates from small intestine of C and Cd rats can be connected with the pulsate rise in temperature up to 280-300^{0}C, during nanosecond, for transmission of redox metabolic processes [2].

As presented in tab. 1, in comparison to C group data, under chronic influence of $CdSO_4$ the specific amount of isolated total fraction of NLP-Nox from tissue membranes increases. It is possible, that lipid peroxidation processes in presented membranes were increased.

Table. The specific contents of the isoforms of NLP-Nox (mg) in 1g tissues (mg/g) in C, Cd and Cd+Cur groups, n=6

Tissue	C	Cd	Cd+Cur
Brain	83,3±5,3	95,8±8,9 p<0,001	87,8±6,4 p<0,05
Heart	48,2±3,4	78,5±5,9 p<0,02	61,6±5,4 p<0,001
Lung	10,47±0,2	34,7±2,8 p<0,005	21,0±1,3 p<0,003
Liver	12,4±1,1	22,6±1,8 p<0,001	15,7±1,2 p<0,01
Kidneys	40,5 ± 17,4	85,6±5,5 p<0,005	54,2±5,1 p<0,001
Spleen	40,5±3,3	54,7±4,7 p<0,005	48,6±4,4 p<0,003
Small intestine	2,4 ±1,8	41,8 ±5,6 p<0,002	31,7± 3,9 p<0,005

As a result the releasing of the isoforms of NLP-Nox increased correspondingly. Thus, cadmium ions act as a stimulant for the releasing of the NLP-Nox from presented above biomembranes. In fact, the chronic administrated Cd(II) acts as a destabilizer of biomembranes. On the other hand, Curcumin in this concentration presented membranostibilizing effect, and it shows: 1) by decreasing the releasing process of the isoforms of NLP-Nox from presented biomembranes (lipid peroxidation of these membranes decreases); 2) by decreasing the lipid peroxidation of corresponding lipids of these membranes, which due to the stabilization of the structure of the NLP-Nox on the surface of biomembranes.

Thus, a new mechanism of toxic effect of chronically administrated cadmium ions has been revealed. Cadmium ions initiate oxidative stress in biomembranes by stimulating releasing of new superoxide producing components – the isoforms of associates NLP-Nox from these biomembranes. Curcumin indicates membrane stabilizing effect, decreases the releasing of NLP-Nox isoforms from these membranes into soluble phases.

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