НАУЧНА ДЕЙНОСТ НА Д-Р ВИОЛЕТА ЙОРДАНОВА ДАНЧЕВА, ДМ РЕЗЮМЕТА

НАУЧНАТА ДЕЙНОСТ, СВЪРЗАНА С ДОКТОРСКАТА ДИСЕРТАЦИЯ

Автореферат

 Данчева В. Ефекти на каталитични антиоксиданти и 21-аминостероиди в експериментални модели на оксидативен стрес и възпаление [автореферат]. София: Военномедицинска академия – 2014 г.

ANNOTATION

Background: Oxidative stress has been implicated in the pathogenesis of over 50 human diseases. The pulmonary system is particularly vulnerable to reactive oxygen species (ROS)-induced injury. To protect against exposure to oxidants the lungs have a powerful antioxidant system which may delay or prevent oxidation, but also eliminate ROS. Considerable progress has been made in the last years, in developing catalytic nonselective mitochondria-targeted antioxidants such as manganese porphyrins (SOD/CAT mimetics).

Objective: The aim of the dissertation was to explore and determine the effects of catalytic antioxidants EUK-134 and Mn(III)TBAP, and 21-aminosteroid U-74389G on inflammation, lipid peroxidation and antioxidant defense system in experimental models of paraquat-induced pneumotoxicity and ionizing radiation.

Material and methods: The study was carried out on 320 male Wistar rats divided into three experimental models. In a part of the groups animals were treated with a single toxic/radiation factor (paraquat dichloride/ionizing radiation) and in the other part - combined treatment with a toxic/radiation factor and an antioxidant. Integral, cytological indicators, markers of toxic pulmonary lesions (lactate dehydrogenase (LDH), alkaline phosphatase (AIP), acid phosphatase (AcP), total protein), antioxidant defense system (superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), lipid peroxidation (malondialdehyde (MDA), lipid hydroperoxides), pulmonary fibrosis (hydroxiprolin), and immunological markers such as IL-6 and CINC-1, supported by detailed morphology were investigated in blood, BALF, lung homogenate, plasma._An immunohistochemical study of Ki-67 proliferative index was carried out in the lungs and intestines of irradiated rats. Statistical methods were used to process data.

Results: Isolated application of paraquat (80 mg/kg, group 3) significantly increased the number of leucocytes in blood on day 3, and the number of PMNs on days 1 and 3. The expression of CD11b in the same group showed a tendency to elevate on day 1.Total protein content in BALF increased abruptly on day 1 (360%). The activity of LDH was increased on day 1 (243%) and day 5 (200%), AcP activity was increased on days 1 and 3, and the activity of AIP was increased after day 3 in group 3. Paraquat significantly decreased the activities of antioxidant enzymes in lung homogenate: SOD and CAT on days 3 and 5, GPx on day 5 after treatment. The herbicide significantly increased the quantity of lipid peroxides on day 3 (164%) and L-hydroxyproline content on day 5 (175%). The combined treatment with paraquat and EUK-134 (group 4) and U-74389G (group 5) decreased significantly the number of leucocytes and PMNs in blood on days 1 and 3. The expression of CD11b in group 5 decreased on day 1, as compared with the paraquat group. Total protein content and the activities of LDH and AIP remained significantly lower as compared to group 3 on days 1 and 3, whereas AcP activity was decreased significantly on day 1. Antioxidants increased the activities of antioxidant enzymes: SOD on days 1 and 3, CAT on day 3 and 5 and GPx on day 5, whereas the quantity of lipid peroxides and L-hydroxyproline content were decreased significantly on day 5 as compared to group 3.

Isolated application of paraquat (40 mg/kg) also elevated significantly the number of leucocytes and PMNs in blood, total protein content (227.4%) and total cell count on day 1 in BALF. CD11b expression in BALF increased in paraquat group on day 1 (91.45%). The enzyme activities in BALF were significantly increased in the same group: LDH on days 1 and 5, AIP and AcP on day 15 in comparison with controls. Paraquat significantly decreased the activity of SOD on day 5, and increased the activity of GPx until the 15th day. The MDA content

was significantly elevated on days 1 and 5 in the same group. The catalytic antioxidant Mn(III)TBAP in combined group decreased significantly the number of PMNs, total protein content and total cell count on day 1. CD11b expression in BALF decreased in the same group (40.41%) as compared to paraquat group. The activities of LDH, AIP were decreased till the 5th day, and that of ACP till the 15th day. Mn(III)TBAP increased the activities of SOD on days 5 and 15, CAT on day 1, GPx on day 5. The MDA content was significantly reduced on days 1 and 5.

lonizing radiation (6 Gy total body irradiation) increased the levels of IL-6 and CINC-1 considerably on day 3 in comparison to that in controls, whereas EUK-134 decreased them on day 3 in combined group. Ionizing radiation totally supressed the proliferative activity measured by marker Ki-67 in the lungs (<1%) and intestines (<10%). Immunohistological testing in the combined group showed a decrease of the proliferative activity, as compared to the group of controls, but it was generally preserved and was greater(>50%) than the activity seen in the group treated with ionizing radiation.

Conclusion: The results obtained suggest that in the early stages after oral paraquat administration an acute pulmonary toxicity developed due to the oxidative stress and inflammation provoked by the herbicide. Protective effects of catalytic nonselective porphyrins such as EUK-134 and Mn(III)TBAP as well as 21-aminosteroid U-74389G are directed towards reduction of inflammatory response, suppression of oxidative stress and lipid peroxidation, attenuation of pulmonary fibrosis in rat experimental models treated by paraquat. Salen-manganese complex EUK-134 also attenuates inflammation and increases suppressed by ionizing radiation proliferative index in the lungs and intestines of rats.

Key words: oxidative stress, paraquat, inflammation, cytokines, EUK-134, U-74389G, Mn(III)TBAP, ionizing radiation

Публикации в чуждестранни научни списания

 Shopova V, Dancheva V, Salovsky P, Stoyanova A, Lukanov Tz. Protective effect of U-74389G on paraquat induced pneumotoxicity in rats. *Environmental Toxicology and Pharmacology.* 2007;24:167-173. (IF 1.281, инд. IF 0.256, 5 цитирания).

Abstract

Paraquat is a very toxic herbicide and a dangerous pollutant of the environment. It forms reactive oxygen species and increase the lipid peroxidation in the pulmonary cells. Our aim in this study was to estimate the protective effects of the lazaroid U-74389G possessingantilipidperoxidation activity and membrane –stabilizing effect. The experiment was carried out with 96 male wistar rats. Paraquat dichloride was administered orally at 80 mg/kg. Thelazaroid U-74389G was injected intraperitoneally twice – 2 h before receiving the paraquat with 10 mg/kg and four hours later with 5 mg/kg.

Isolated application of paraquat increased enzyme activities of lactate dehydrogenase (LDH) an acid phosphatase (AcP) and the total protein content in bronchoalveolar lavage fluid (BALF). In the same experimental group the number of polymorphonuclear cells (PMNs) in BALF is elevated significantly on days 1 and 3. The combined treatment with paraquat and U-74389G did not increase the total protein content and the number of PMNs and it elevated the enzyme activities of LDH and AcP significantly less than the alone application of paraquat.

It is concluded that the lazaroid U-74389G reduces the pneumotoxic effects of paraquat, estimated by sensitive cytologic and biochemical markers in BALF. The protective effect of U-74389G is well-expressed until day 3 after the trearment.

Key words: rats, lung, pneumotoxicity, paraquat, U -74389G

3. Shopova V, **Dancheva V**, Salovsky P, Stoyanova A. Protective effects of a superoxide dismutase/catalase mimetic compound against paraquat

pneumotoxicity in rat lung. Respirology. 2009;14:504-510 (IF 1.853, инд. IF

0.463, 8 цитирания).

Abstract

Background and objective: EUK-134 is one of the most promising of the superoxide dismutase (SOD)/catalase mimetic compounds. The antioxidant effects of EUK-134 were tested in a rat of paraquatpneumotoxicity.

Methods: Male Wistar rats (n=72) were divided into three groups: group 1, controls; group 2, paraquat alone; group 3, paraquat + EUK-134. Paraquat dichloride was administered *per os* at a dose of 80 mg/kg. EUK-134 was injected intraperitoneally at 10 mg/kg 2 h before the paraquat and again 4 h later at 5 mg/kg.

Results: On days 1, 3 and 5 after treatment with paraquat alone the LDH activity increased (P = 0.001, P = 0.00001 and P = 0.03, respectively), and the total protein content increased (P = 0.00002, P = 0.001 and P = 0.01, respectively). The levels of acid phosphatase (AcP) in BALF fluid increased on days 1 and 3 (P = 0.006 and P = 0.04). In lung homogenates paraquat alone increased SOD activity on day 1 and decreased it on days 3 and 5. Combined treatment with paraquat and EUK-134 elevated LDH activity on day 3 (significantly less than paraquat alone) and day 5, elevated the total protein content on day 5 only, and did not change AcP activity. The combination of both agents did not alter SOD activity and decreased catalase activity on day 5 significantly less than treatment with paraquat alone (P = 0.05).

Conclusions: EUK-134, a superoxide dismutase/catalase mimetic compound decreased the pneumotoxic effect of paraquat in rats.

Key words: EUK-134, lung, paraquat, pneumotoxicity, rat.

Публикации в научни списания в България

4. Данчева В, Съловски П, Шопова В. Механизми на увреждащото действие на свободните радикали върху белия дроб. *Българска медицина.* 2003;4:3-5, **2 цитирания**.

Summary: Free radicals mechanisms leading to formation of reactive oxygen species (ROS) have been implicated in the pathogenesis of over 50 human diseases. The pulmonary system is particularly vulnerable to ROS-induced injury because of its high oxygen content. ROS are frequently associated with many pulmonary diseases. They are recognized as mediators of early cell injury inflammatory response and increased secretion of cytokines. ROS also play an important role in the regulation of gene transcription. Various chronic disease processes in the lungs are characterized by progressive accumulation of collagen undergoing fibrotic degeneration. Special emphasis is laid on the major sources of ROS in occupational and environmental exposures to asbestos, crystalline, silica, coal, metals, herbicides, cigarette smoke, ionizing radiation.

Key words: free radicals, silica, asbestos, coal, metals, lungs

 Dancheva VY, Karaivanov MP, Shopova VL, Terziev LG, Stavreva GTz. Effect of Dexamethasone on some markers for cytotoxicity and proliferation in rat intestine after total body gamma irradiation. *Journal of Biomedical & Clinical Research.* 2011;4(1):23-29.

Summary

The effect of dexamethasone on some markers for cytotoxicity and proliferation in rat intestine after total body gamma irradiation was studied. Seventy-two male rats were divided into three groups: group 1

(controls); group 2 (receiving a single 6 Gy total body irradiation); group 3 (receiving dexamethasone and 6 Gy ionizing radiation). The animals in group 3 were injected *i.p.* with dexamethasone at a dose of 3 mg/kg four hours before irradiation as well as on days 2 and 3 after exposure. The levels of IL-6 and CINC-1 were determined in the plasma by the ELISA method. Immunohistochemical and histological studies were performed in rat intestine. Ionizing radiation increased the levels of IL-6 and CINC-1 considerably in comparison to that in controls on day 3. Dexamethasone significantly decreased the level of IL-6 on day 7, as compared to both controls and irradiated group. The level of CINC-1 in group 3 wa significantly lower on days 3 and 7 than that in the control group. Immunohistochemical testing with the marker for proliferative activity Ki-67 in group 2 showed a total suppression of the proliferative activity was still present. Dexamethasone produced moderate anti-inflammatory protection from radiation injury. *Key words*: cell proliferation, dexamethasone, inflammation, interleukins, ionizing radiation

НАУЧНАТА ДЕЙНОСТ, НЕСВЪРЗАНА С ДОКТОРСКАТА ДИСЕРТАЦИЯ

Публикации в чуждестранни научни списания

6. Salovsky P, Shopova V, Dancheva V, Marev R. Changes in antioxidant lung protection after single intratracheal cadmium acetate instillation in rats. *Human & Experimental Toxicology*. 1992;11:217-222 (IF=0.638, инд. IF 0.159, 22 цитирания).

Abstract

One-hundred male white rats were given a single intratracheal dose of 0.5 mg/kg cadmium acetate. There was a fall in catalase (CAT) and superoxide dismutase (SOD) in lung homogenate throughout the 30 d after treatment. Non-protein sulphhydryl (NPSH) content, glucose-6-phosphate dehydrogenase (G-6-PD) and glutathione peroxidase (GP) were all increased from days 5 to 15. There was an increase in lactate dehydrogenase (LDH) and protein in bronchoalveolar lavage fluid (BALF) and in the relative weight of the lungs which provide evidence of severe toxic lesions of the lungs. Increased lipid peroxidation mechanism in the pulmonary damage caused by cadmium.

Key words: cadmium acetate, bronchoalveolar lavage fluid, lung homogenate, superoxide dismutase, catalase

7. Salovsky P, Shopova V, Dancheva V, Marev R, Pandurska A. Enhancement of the pneumotoxic effects of cadmium acetate by ionizing radiation in the rat. *Environmental Health Perspectives*. 1993;Suppl.101(suppl.2):260-274. (IF=1.481, инд. IF 0.296, 10 цитирания).

Abstract

Experiments involving 120 male Wistar rats were performed to study the effect of treatment with cadmium acetate and external irradiation. A single 0.5 mg/kg body weight dose of cadmium acetate was administered intratracheally. Shortly thereafter, the animals received a single whole-body exposure to 4 Gy γ rays (cesium source). Findings indicated the chemical elevated enzyme activities of lactate dehydrogenase (LDH), alkaline phosphatase (AIP), and acid phosphatase (AP), as well as protein content and percentage of neutrophils in bronchoalveolar lavage fluid (BALF); the percentage of alveolar macrophages was sharply reduced. Radiation alone produced no substantial changes in the parameters investigated. Treatment with both agents combined was found to result in a synergistic rise

of LDH, AIP, and AP activities and protein content in BALF. It was concluded that the BALF biochemical markers used are reliable indicators for identifying the type of combined effect produced in the lungs by chemical agents and ionizing radiation.

Key words: cadmium acetate, bronchoalveolar lavage fluid, lactate dehydrogenase, alkaline phosphatase, acid phosphatase, alveolar macrophages

 Salovsky P, Shopova V, Dancheva V, Pandurska A, Marev R. Bronchoalveolar lavage fluid in rats treated intratracheally with lead acetate. Bulletin of Environmental Contamination and Toxicology. 1994;52:912-918 (IF

0.563, инд. IF 0.113, 5 цитирания).

Abstract

The experiment was carried out on 48 male Wistar rats divided into two groups: controls and experimental. The lead acetate dissolved in water was administered intratracheally in a dose of 100 μ g for each animal. Findings indicated significantly elevated enzyme activities of lactate dehydrogenase (LDH), alkaline phosphatase (AIP), and acid phosphatase (AP), as well as protein content and glucose in bronchoalveolar lavage fluid (BALF) on day 1 after treatment in comparison with the control group. In conclusion we could accept that the single intratreacheal administration of 100 μ g lead acetate provoked an acute but transitory increase of some sensitive biochemical and cytological indices in BALF.

Key words: lead acetate, bronchoalveolar lavage fluid, pneumotoxicity, lactate dehydrogenase, alkaline phosphatase, acid phosphatase

9. Шопова В, Съловски П, Данчева В. Влияние интратрахеального введения Америция-241 на альвеолярные макрофаги крыс. Радиационная биология Радиоэкология. 1996; т.36, вып.3:434-439.

Абстракт

В опытах на крысах показано, что америций-241 кратковременно снижает общее число клеток и процент альвеолярных макрофагов в бронхоальвеолярной лаважной жидкости (БАЛЖ), при этом увеличиваются размер макрофагов и площадь ядер, повышается активность кислой фосфатазы и лактат дихидрогеназы в БАЛЖ.

Ключевые слова: Америций-241, альвеолярные макрофаги, нуклеоплазматический индекс.

10. Шопова В, П.Ц.Съловски, П.К.Петрова, В.Й.Данчева. Влияние ацетата кадмия и йонизируещей радиации на альвеолярные макрофаги крыс. *Гигиена и санитария.* 1997;1:37-39, 2 цитирания.

Абстракт

Эксперимент проведен на 48 белых крысах-самцах породы Вистар, разделенных на 4 группы: 1-я – контроль, 2-я – подвергшаяся однократному ү-облучению в дозе 4 Gy, 3-я – воздействию раствора ацетата кадмия, вводимого однократно интратрахеально в дозе 0.5 мг/кг и 4-я группа – комбинированное действие. Результаты позволяют утверждать что использованная нами доза ацетата кадмия дает летальный и сублетальный эффект в отношении альвеолярных макрофагов (AM) в бронхоальвеолярной лаважной жидкости (БАЛЖ, при этом уменшается числа AM и понижается их фагоцитарная активность, повышается активность кислой фосфатазы и лактат дихидрогеназы (особенно в 1-й день) в БАЛЖ. Изолированное внешнее ү-облучение не изменяло числа AM в эксперименте

Ключевые слова: кадмий, альвеолярные макрофаги, бронхоальвеолярная лаважная жидкость

11. Salovsky P, Shopova V, Dancheva V. Antioxidant defence mechanisms in the lung toxicity of Tri-n-Butyl phosphate. *American Journal of Industrial Medicine*. 1998;33:11-15 (IF=1.194, инд. IF 0.398, 11 цитирания).

Abstract

Pneumotoxic effects of the tri-n-butyl phosphate (TBP) are investigated on rats using biological markers in bronchoalveolar lavage fluid (BALF) and studyng key antioxidant enzymes in lung homogenate. Each animal from the experimental group received intratracheally 5µl TBP (20% v/v in n-dodecane). Ix rats from the control and treated groups are sacrificed on post-treatment days 1, 3, 7, 14 and 28. The lactate dehydrogenase activity, the total protein content and the total cell number in BALF are increased on day 1 after the treatment. The activities of superoxide dismutase and catalase are decreased to day 7 and those of glutathione peroxidase and glutathione reductase on day 1 only. The malondialdehyde content is elevated to day 14. It is concluded that TBP causes moderate toxic injury of the lung parenchyma. The depression of the jey antioxidant enzymes and the elevated lipid peroxidation are probably important mechanisms of the lung damage.

Key words: tri-n-butyl phosphate; lung toxicity; antioxidant defense mechanism; lipid peroxidation; bronchoalveolar lavage fluid

12. Salovsky P, Shopova V, Dancheva V. Pneumotoxic Effects of radioactive dust (RD) from a nuclear power plant in Kozloduy, Bulgaria. American Journal of Industrial Medicine. 2000;38:639-643. (IF= 1.277, инд. IF 0.426, 3 цитирания).

Abstract

Background Airborne radioactive particles isolated from the reactor halls of nuclear power plant are a potential risk to the respiratory system of the working staff. Such radioactive dust (RD) from the Nuclear Power Plant, Kozloduy, Bulgaria was investigated.

Method The RD was administered intratracheally to 60 male Wistar rats as an aqueous suspension and the biological effects are estimated, using sensitive biological markers in bronchoalveolar lavage fluid (BALF) and important parameters of the pulmonary antioxidant defense.

Results The results obtained show that RD increases the total cell number, the activities of the lactate dehydrogenase and alkaline phosphatase as well as the protein content in BALF. The activities of superoxide dismutase and catalase in lung homogenate are decreased while activity of the glutathione peroxidase and the content of non-protein sulfhydrilsis elevated.

Conclusions It is concluded that the RD causes transitory toxic effect on lung tissue and changes the correlation between the elements of the pulmonary antioxidative defense.

Key words: radioactive dust; pneumotoxic effects; bronchoalveolar lavage fluid; antioxidant defense system; radioactivity

13. Salovsky P, Shopova V, Dancheva V, Yordanov Y, Marinov E. Early pneumotoxic effects after oral administration of 1,2-Dichlororthane, *Journal of Occupational and Environmental Medicine*. 2002;44(5): 475-480. (IF=1.811, инд. IF 0.362, 10 цитирания).

Abstract

Early biochemical and histological changes in rat lungs were investigated after oral administration of 136 mg/kg 1,2-dichloroethane in oleum solution. The experiment was performed using 80 male Wistar rats.

Bronchoalveolar lavage fluid and lung homogenate were examined on post-treatment days 1, 5, 15 and 30. In bronchoalveolar lavage fluid, the activities of lactate dehydrogenase, alkaline phosphatase, and acid phosphatase were elevated on day 1. The activities of superoxide dismutase, catalase, and glutathione peroxidase, and the content of malondialdehyde in lung homogenate, were also increased on day 1. The histological investigation indicated congestion, edema, and lung intestinal inflammatory changes. It was concluded that oral administration of 1,2-dichloroethane causes mild-to-moderate transitory toxic injury of the lung. Lipid peroxidation and the levels of key antioxidant enzymes are increased in the earliest post-treatment period.

Key words: 1,2-dichloroethane, bronchoalveolar lavage fluid, antioxidant defense system, lipid peroxidation, pneumotoxicity

14. Shopova V, Terziev L, Dancheva V, Stavreva G, Atanasova M, Stoyanova A, Lukanov Tz. Effects of MnTnHex-2-PyP on markers of inflammation and lipid peroxidation in asthma mice model. *European Medical Health and Pharmaceutical Journal*. 2011;2:24-30.

Abstract

Background and objective: Investigation of the effects of MnTnHex-2-PyP on some markers of inflammation and lipid peroxidation in an asthma mice model.

Methods: The experiment was carried out on 24 female mice C57BI/6, divided into four groups: group 1, controls; group 2,injected with ovalbumin (OVA); group 3, treated with MnTnHex-2-PyP and group 4, treated with OVA and MnTnHex-2-PyP. The animals from groups 1 and 3 were injected *i.p.* on days 0 and 14 with a 100 µl phosphate-buffered saline (PBS), and those from groups 2 and 4 were injected with a 100 µl ovalbumin solution, containing 20 µg OVA. On days 24, 25 and 26 the mice from groups 1 and 2 were inhaled with PBS for 30 min, and those from groups 2 and 4 were given a 1% ovalbumin solution. One hour before inhalation, and 12 hours later the animals from groups 1 and 2 were injected *i.p.* with 100 µl PBS, and those from groups 3 and 4 received a 100 µl MnTnHex-2-Pyp solution in PBS containing 0.05mg/kg.

Results: Ovalbumin alone (group 2) increased the total cell number, total protein content, the levels of IL-4, IL-5 and 8-isoprostane in bronchoalveolar lavage. Elevations were observed in IgE level in serum, and the malone dialdehyde (MDA) content in the lung homogenate. These markers were decreased significantly in group 4 as compared to the OVA group.

Conclusions: MnTnHex-2-Pyp reduces the inflammation and lipid peroxidation in Ovalbumin-induced mice asthma model.

Key words: asthma, BALF, inflammation, ILipid peroxidation, lung homogenate, MnTnHex-2-PyP

15. Terziev LG, Shopova VL, **Dancheva VY**, Stavreva GT, Atanasova MA, Stoyanova AM. Effects of L-2-oxothiazolidine-4-carboxylic acid on the lung antioxidant defense system in an asthma mouse. *Turkish Journal of Medical Sciences.* 2012;42(5):901-905 (IF= 0,450, инд. IF 0.075, 1 цитиране).

Abstract

Aim: We aimed to study the eff ect of a glutathione precursor, L-2-oxothiazolidine-4-carboxylic acid (OTCA), on the lung antioxidant defense system in an animal asthma model.

Materials and methods: The study was carried out on 24 female C57BL/6 mice. The mice were divided into 4 treatment groups: group 1 – control group; group 2 – injected with ovalbumin (OVA) and

given an OVA inhalant; group 3 –treated with OTCA and phosphate-buff ered saline inhalant; and group 4 – injected with OVA and OTCA and given an OVA inhalant. Under sodium pentobarbital anesthesia the animals were killed by exsanguination 48 h aft er the last inhalation to obtain a lung homogenate. Th e activities of superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GP) and the content of nonprotein sulfh ydryl (NPSH) groups in lung homogenate were investigated.

Results: OVA decreased the activities of SOD (P = 0.007), CAT (P = 0.004), and GP (P = 0.05) and the NPSH content (P = 0.0008) in the lung homogenate compared with the control animals. Treatment with OVA and OTCA (group 4) resulted in a signifi cant increase in the activities of CAT (P = 0.01) and GP (P = 0.05) and the NPSH content (P = 0.002) compared to the OVA group (group 2).

Conclusion: OTCA (160 mg/kg) restored the activities of basic enzymes in the lung antioxidant defense system in an OVA-induced asthma mouse model 48 h aft er the last nebulization.

Key words: asthma, L-2-oxothiazolidine-4-carboxylic acid, lung antioxidant defense system

16. Dancheva V, Terziev L, Shopova V, Stavreva G. Effects of MnTnHex-2-PyP on Lung Antioxidant Defence System in Asthma Mice Model. *Iranian Journal* of Allergy, Asthma and Immunology. 2012;11(4):329-335 (IF=0.778, инд. IF 0.194, 1 цитиране).

Abstract

We aimed to study the MnTnHex-2-PyP effect on some markers of lung antioxidant defence system in mice asthma model.

The study was carried out on 28 C57B1/6 mice divided into four treatment groups: group 1 – controls; group 2 – injected and inhaled with ovalbumin; group 3 – treated with MnTnHex-2-PyP and inhaled with phosphate buffered saline; group 4 – injected with ovalbumin and MnTnHex-2-PyP but also inhaled with ovalbumin. On days 24, 25 and 26, mice from groups 1 and 2 were inhaled with PBS for 30 min, and those from groups 2 and 4 were given a 1% ovalbumin solution. One hour before inhalation, and 12 hours later the animals from groups 1 and 2 were injected *i.p.* with 100 µl PBS, and those from groups 3 and 4 received a 100 µl MnTnHex-2-PyP solution in PBS, containing 0,05mg/kg. The animals were killed by exsanguination 48 hours after the last inhalation for obtaining a lung homogenate. The activities of superoxide dismutase, catalase, glutathione peroxidase and the non-protein sulphhydryl group content in the lung homogenate were investigated. Ovalbumin decreased the activities of superoxide dismutase (p=0.01), catalase (p=0.002), glutathione peroxidase and non-protein sulphhydryl groups content (p<0.001) in comparison to controls. In group 4 (ovalbumin and MnTnHex-2-PyP) the activities of superoxide dismutase (p=0.044), catalase (p=0.045), glutathione peroxidase (p=0.002), and the non-protein sulphhydryl groups content (p<0.001) in comparison to controls. In group 4 (ovalbumin and MnTnHex-2-PyP) the activities of superoxide dismutase (p=0.044), catalase (p=0.045), glutathione peroxidase (p=0.002), and the non-protein sulphhydryl groups content (p<0.001) were significantly increased compared to ovalbumin (group 2).

MnTnHex-2-PyP restored the activities of basic enzymes in the lung antioxidant defence system in ovalbumin-induced asthma mice model, 48 hours after the last nebulization. *Key words*: antioxidants; asthma; MnTnHex-2-PyP

- 17. Terziev L, Dancheva V, Shopova V, Stavreva G. Antioxidant effect of MnTE-2-PyP on lung in Asthma Mice Model. *The Scientific World Journal*, vol.2012 (2012); Article ID 379360, 6 pages, doi:10.1100/2012379360, (IF= 1.730, инд.
 - IF 0.432, 3 цитирания).

Abstract

Aim. To investigate the effects of MnTE-2-PyP on some markers of antioxidant defence system in asthma mice model.

Material and Methods. The animals were divided into four groups: group 1, controls; group 2, injected with ovalbumin, group 3, treated with MnTE-2-PyP, and group 4, treated with ovalbumin and MnTE-2-PyP. The activities of superoxide dismutase, catalase, glutathione peroxidase and nonprotein sulfhydryl groups content (NPSH) were determined in lung homogenate.

Results. The activities of superoxide dismutase and catalase in group 2 decreased significantly as compared to control group. The decrease of the same enzymes in group 4 was lower and significant as compared to group 2. Changes in the glutathione peroxidase activity showed a similar dynamics. The NPSH groups content decreased in group 2. In group 4 this decrease was relatively lower as compared to group 2.

Conclusions. The application of MnTE-2-PyP mitigated the effects of oxidative stress in asthma mice model.

Key words: asthma; antioxidant enzymes; catalase; glutathione peroxidase; MnTE-2PyP; non protein sulfhydryl groups; superoxide dismutase.

18. Terziev L, Shopova V, Dancheva V, Stavreva G, Atanasova M, Stoyanova A, Lukanov Tz, Dimitrova A. Influence of MnTE-2-PyP on Inflammation and lipid peroxidation in mouse asthma model. *Open Journal of Respiratory Diseases*. 2012;2:37-42.

Abstract

Our aim was to investigate the effects of MnTE-2-PyP on some markers of inflammation and lipid peroxidation in mouse asthma model. 24 female mice were divided into four groups; group 1, controls; group 2, injected with ovalbu- min (OVA); group 3, treated with MnTE-2-PyP; and group 4, treated with ovalbumin and MnTE-2-PyP. The mice from groups 2 and 4 were injected with 10 µg OVA and 1 mg Imject Alum® in 100 µL phosphate buffered saline (PBS) on days 0 and 14. The animals from groups 1 and 3 were injected with 100 µL PBS + Imject Alum® (1:1). The animals from groups 2 and 4 were subjected to a 30 min aerosol challenge of 1% ovalbumin on days 24, 25 and 26 and those from groups 1 and 3 were subjected to aerosol challenge of PBS at the same time and duration. One hour before inhala- tion, and 12 hours later the animals from groups 3 and 4 were injected with 100 µL MnTE-2-PyP solution in PBS con- taining 5 mg/kg. The total cell number, total protein content and 8-isoprostane, IL-4 and IL-5 levels in the bronchial- veolar lavage fluid increased in group 2 as compared to the control group. Malone dialdehyde content in the lung ho- mogenate and IgE levels in the serum also increased in this group. The total cell number, total protein content, and lev- els of 8-isoprostane, IL-4, IL-5 and IgE decreased significantly in group 4 as compared to the OVA group. The parame- ters set out above in group 3 did not differ significantly from those of the control group. MnTE-2-PyP administered intraperitoneally, 48 hours after the last nebulization, reduced the inflammation and lipid peroxidation in mouse asthma model.

Key words: asthma; inflammation; interleukins; 8-Isoprostane; lipid peroxidation; MnTE-2-PyP

Публикации в научни списания в България

19.Шопова В, Съловски П, **Данчева В**. Ранни биохимични промени в белите дробове на плъхове, третирани интратрахеално с радиоактивен материал от АЕЦ-Козлодуй. Българска медицина. 1995, том III, бр.5-6:26-

29.

Резюме: Експериментът е проведен върху 60 бели мъжки плъха, порода вистар, разделени в две групи – контролна и опитна. Опитните животни са третирани интратрахеално с радиоактивен прах BG1D, изолиран от AEЦ-Козлодуй. Чрез метална сонда на всяко животно е въвеждан 0,0029gBG1D, разтворен в 0,15 ml физиологичен разтвор. Животните са убивани на 1, 3, 7 и 30 ден. Установено е, че BG1D повишава краткотрайно общия брой клетки в бронхоалвеоларна лаважна течност (БАЛТ) за сметка на полиморфонуклеарните клетки, повишава активността на лактат дехидрогеназата и алкалната фосфатаза, съдържанието на белтък и глюкоза. Предвид ниската радиоактивност на пробата, изразеният пневмотоксичен ефект се свързва главно с химическата токсичност на BG1D.

Ключови думи: радиоактивен прах BG1D, бронхоалвеоларна лаважна течност, пневмотоксичен ефект

20.Съловски П, Шопова В, **Данчева В**. Пневмотоксични ефекти на три-нбутилфосфат (ТБФ), приложен интрахепатално на бели плъхове. *Съвременна медицина*. 1998, год. XIX, кн.1:9-10.

Абстракт

Експериментът е проведен върху 120 бели мъжки плъха, порода "Вистар", разделени в една контролна и една опитна група. Три-н-бутилфосфат (ТБФ) е въведен интрахепатално под формата на разтвор в н-додекан и в обем 5 мкл на животно.. Увеличен е съществено броят на клетките в бронхоалвеоларна лаважна течност (БАЛТ) на 1-я ден, главно за сметка на полиморфонуклеарните левкоцити. В същия пункт е повишена активността на лактат дехидрогеназа и съдържанието на общ белтък. Активността на алкалната фосфатаза е повишена до 3-я ден, а тази на киселата фосфатаза показва трайна тенденция на повишение след 1-я ден до края на наблюдавания период. Получените експериментални данни свидетелстват за ясно изразен, но краткотраен токсичен отговор на белодробния паренхим към ТБФ.

Ключови думи: три-н-бутилфосфат, бронхоалвеоларна лаважна течност, пневмотоксичност

21. Шопова В, Съловски П, Данчева В. Радиационен пневмонит и фиброза.

Рентгенология Радиология, 2001, т. XL, 4: 272-275.

Summary: The likelihood of toxic pulmonary lesions development as the result of radiation therapy for pulmonary carcinoma and breast cancer is discussed. Two possible forms of radiation induced changes are described, namely: classical radiation pneumonitis (RP) terminating with lung fibrosis circumscribed in the radiation zone, and sporadic RP giving rise to bilateral lymphatic alveolitis and manifestations outside the irradiation field. Attention is called to the fact that chemotherapy augments the risk of toxic lung damage occurrence. Number of markers for early RP diagnosis, including lactate dehydrogenase activity, KL-6, procollagen III, transforming growth factor β , C-reactive protein and partial oxygen pressure are listed.

Therapeutic possibilities in coping with RP and pulmonary fibrosis are assayed. Apart from the standard therapeutic approach using corticosteroids and azatioprin, ideas are set forth concerning the application of some antioxidants, angiotensin converting enzyme inhibitors and γ -interferon. It is pointed out that radiation pneumonitis and pulmonary fibrosis treatment has an essential practical bearing on life expectancy and quality of life in a great number of cancer patients.

Key words: radiation pneumonitis, lung fibrosis, antioxidants, angiotensin converting enzyme inhibitors.

22. Данчева В, Шопова В, Ставрева Г, Стоянова А. Влияние на U-74389G

върху пневмотоксичния ефект на блеомицин в белодробен хомогенат на плъхове. *Trakia Journal of Sciences*. 2008;6(2):69-71.

Abstract

Our goal is to study the effect of U-74389G on some markers for antioxidant defense system in rat lung homogenate (LH) after bleomycin treatment. The study was carried out on 120 male rats divided into four groups: group 1 – controls; group 2 – with U-74389G; group 3 – with bleomycin; group IV – with bleomycin and U-74389G. Bleomycin was administered intratracheally in a dose of 2.5 U/kg; U-74389G – twice i.p., two hours before bleomycin and four hours later at a dose of 5 mg/kg. In LH were investigated the activities of superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GP) and malondialdehyde content (MDA). Bleomycin decreased SOD and GP on day 1and increased GP, MDA and CAT on days 5 and 15 in comparison with controls. U-74389G attenuated the toxic effects of bleomycin at investigated points.

Key words: bleomycin, U-74389G, pneumotoxicity, antioxidant

23. Stavreva G, Shopova V, Dancheva V, Stoyanova A, Terziev L. Effect of 21-aminosteroid U-74389G on markers for pneumotoxicity in rat bronchoalveolar lavage fluid after intratrachel amiodarone administration. *Journal of Biomedical & Clinical Research*. 2008;1(1):30-40.

Summary

The protective effect of 21-aminosteroid U-74389 G on amiodarone-induced pneumotoxicity in rats was studied. Previous *in vitro* and *in vivo* studies have proven the remarkable antioxidative and membrane stabilizating potency of U-74389 G. The study was carried out on 72 male Wistar rats, divided into four groups: (1) – control; (2) – treated intratracheally (i.t.) with amiodarone (AM); (3) – treated with AM and U-74389G; (4) – treated with U-74389G. AM was installed i.t. on days 0 and 2 (6.25 mg/kg with a 3.125 mg/ml water solution). U-74389G was injected on day 0 and 2 at a daily dose 15 mg/kg. The activity of lactate dehydrogenase (LDH), acid phosphatase (AcPh), alkaline phosphatase (AlPh), total protein content and cytological assays of bronchoalveolar lavage fluid (BALF) were performed on days 3, 7 and 28. AM treatment resulted in significantly increased lung weight coefficient, protein content, total cell count, polymorphonuclear cells, alveolar macrophages, and activity of LDH, AcPh and AlPh. The treatment with AD and U-74389G attenuated the markers of pulmonary inflammation and damage of the alveolar-capilary barrier (lung weight coefficient, protein content, total cell count, polymorphonuclear cells, alveolar lavage to AM group. The results obtained from our study showed that U-74389G reduced early AM-induced lung inflammatory injury.

Key words: 21-aminosteroid U-74389 G, amiodarone-induced pneumotoxicity, bronchoalveolar lavage fluid

24. Terziev L, Tzvetkova V, Shopova V, Dancheva V, Stavreva G, Atanasova M, Lukanov Tz, Stoyanova A. Efffect of L-2-Oxothiazolidine-4-carboxylic acid on markers for inflammation and lipid peroxidation in bronchoalveolar lavage fluid (BALF) in mice model of asthma. *Journal of Biomedical & Clinical Research*. 2010;3(1):40- 44, 1 цитиране.

Summary

Asthma is a serious medical and social problem, characterized by an inflammatory response and production of a large amount of reactive oxygen species. Our goal was to study the effect of a

glutathione precursor on some markers of inflammation and lilpid peroxidation in an animal model of asthma. The study was carried out on 28 $C_{57}B1$ mice, divided into four groups: group 1 - controls; group 2 – injected and inhaled with ovalbumin (OVA); group 3 - treated with L-2-oxothiazolidine-4-carboxylic acid (OTCA) and inhaled with phosphate buffered saline; group 4 – injected with ovalbumin and OTCA, as well as inhaled with OVA. Under sodium pentobarbital anaesthesia the animals were sacrificed on hour 48 after the last inhalation to obtain bronchoalveolar lavage fluid (BALF). The total cell number and cell counting, total protein content, the levels of II-4, II-5 and 8-isoprostane were investigated in BALF. OVA increased the total cell number and the levels of II-4, II-5 and 8-isoprostane. OTCA significantly decreased the total cell number, the total protein content, as well as the levels of II-4, II-5 and 8-isoprostane in comparison with ovalbumine. OTCA attenuates inflammation and lipid peroxidation in asthma provoked by ovalbumin in a mouse model.

Key words: asthma, bronchoalveolar lavage fluid, inflammation, lipid peroxidation, L-2-oxo thiazolidine-4-carboxylic acid

25. Valcheva-Kuzmanova S, Stavreva G, Dancheva V, Terziev L, Atanasova M, Stoyanova A, Shopova V. Effect of *Aronia Melanocarpa* fruit juice on indices of inflammation and fibrosis in a rat model of amiodarone-induced pneumotoxicity. *Scripta Scientifica Medica*. 2012;44 (2):37-40.

Abstract

The effect of Aronia melanocarpa fruit juice (AMFJ) on indices of inflammation and fibrosis was studied in a model of amiodarone (AD)-induced pneumotoxicity in rats. AD was instilled intratracheally on days 0 and 2 (6,25 mg/kg as a 3,125 mg/mL water solution). AMFJ (10 mL/kg) was given orally to rats either from day 1 to day 10, or from day 11 to day 27. Thus, the animal groups were: control, AD, AD+AMFJ (day 1-10), and AD+AMFJ (day 11-27). The rats were sacrificed on day 28. The levels of IL-6 and IL-10 were measured in rat serum as markers of inflammation, and hydroxyproline (HP) level was determined in lung tissue as a marker of fibrosis. AD caused a tendency to elevate IL-6 and decrease IL-10. AMFJ counteracted these effects of AD. In rats from group AD+AMFJ (day 1-10), IL-6 level was significantly lower (p<0,05) than that of AD group, lower (p<0,05) even than the control value. AD significantly increased (p<0,05) HP content in lung homogenate. AMFJ antagonized that effect, and in AMFJ-treated rats HP levels did not differ significantly from the control value. Any AMFJ effects were more prominent in rats that were treated with the juice during the first 10 days after AD instillation. In conclusion, AMFJ reduced the signs of inflammation and could have a protective effect against AD-induced pulmonary fibrosis, especially if administered in the early phase after AD instillation.

Key words: Aronia melanocarpa fruit juice, amiodarone, lung, inflammation, fibrosis, rats

26. Valcheva-Kuzmanova S, Stavreva G, **Dancheva V**, Terziev L, Shopova V, Stoyanova A. Effect of *Aronia melanocarpa* fruit juice on the activity of antioxidant enzymes in a rat model of amiodarone-induced pneumotoxicity. *Journal of Biomedical & Clinical Research*. 2012;5(2):97-103, **2 цитирания**.

Summary

The effect of *Aronia melanocarpa* fruit juice (AMFJ) on the activity of antioxidant enzymes in a model of amiodarone (AD)-induced pneumotoxicity in rats was studied. AD was instilled intratracheally on days 0 and 2 (6.25 mg/kg as a 3.125 mg/mL water solution). AMFJ (5 mL/kg and 10 mL/kg) was given orally from day 1 to days 2, 4 and 9. The activities of catalase (CAT), glutathione peroxidase (GPx) and superoxide dismutase (SOD) in lung tissue were measured on days 3, 5 and 10, respectively. AD

decreased significantly CAT activity on days 3, 5 and 10. It caused a decrease of GPx activity which was significant on day 3. It decreased SOD activity but not significantly. AMFJ antagonized the effects of AD to such an extent that the enzyme activities at all time points did not differ significantly from the control values. The effect of AMFJ is probably due to its polyphenolic ingredients which serve as powerful radical scavengers. AMFJ probably decreased of the oxidative damage of cells by AD-induced overproduction of reactive oxygen species thus preserving the capacity of cells to produce antioxidant enzymes which, in turn, could further reduce oxidative stress.

Key words: Aronia melanocarpa fruit juice, amiodarone, pneumotoxicity, antioxidant enzymes

Публикации (разписани в пълен текст с книгопис и резюме) в рецензирани научни сборници на научни звена или в сборници от проведени научни форуми

27. Salovsky P, Shopova V, Dancheva V, Galabova V. Changes in rat lungs to combined acute selinon treatment and external ionizing radiation. *Scientific works of the Higher Med. Institute, Pleven.* 1990:13-16.

Резюме: Опыты проводились на 200 белых крысах мужского пола породы Wistar, которые были подвергнуты действию (изолированно и в сочетании) селинона и внешнего облучениья 4 Gy йонизирующей радиацией. Селинон был применен однократно перорально в дозе одна ЛД₅₀. Из за летального эффекта гербицида исследования проводились до 15-ого дня. Были прослежены интегральные показатели, а в лаважной жидкости (БАЛЖ) и легочном гомогенате – активность ферментов лактатдегидрогеназы (ЛДГ), щелочной фосфатазы (ЩФ), кислой фосфатазы (КФ) – биохимических маркеров поражения определенных клеточных популяций в легких, и некоторые показатели липидной пероксидации.Быстрое снижение (в 1-ый день) ЛДХ, ЩФ, КФ в БАЛЖ под воздействиев селинона имеет подчеркнутую информативную стоимость. Гербицид подавляет активность как супероксиддисмутазы (СОД), так и небелковых тиоловых групп (НБТГ), что представляет собой один из механизмов поражения им легких. В некоторых пунктах внешнее облучение потенцирует эффект селинона.

Key words: selinon, ionizing radiation, broncoalveolar lavage fluid, lipid peroxidation, antioxidant defense system

28.Salovsky P, **Dancheva V**, Shopova V, Marev R, Pandurska A. Experimental model of a combined lung lesion by selinon and external whole-body ionizing irradiation. *Scientific works of the Higher Med. Institute, Pleven.* 1991:13-16.

Резюме: Изучены некоторые аспекты биологического эффекта в легких при изолированном и комбинированном применении селинона и внешнего облучения йонизирующей радацией. Опыт проведен на белых крысах породы Wistar. Облучение осуществлено сразу после прекращения травления. Подопытные животные были забиты на 1, 5, 15, 30 и 60-ый день после облучения. Результаты исследования бронхоальвеолярной лаважной жидкости указывают на повышение активности энзиматических маркеров, как и увеличение содержания белка в группе животных, травленных селиноном. В легочном гомогенате установлена сниження активность супероксиддисмутазы и повышение активности каталазы. Сделан вывод, что хроническое оральное введение малых доз селинона вызывает токсическое повреждение легких. Налицо косвенные данны об увеличении липидной пероксидации под воздействием селинона и радиации. Внешнее облучение в дозе 4 Gy не потенцирует этот биологический эффект. *Кеу words:* selinon, ionizing radiation, broncoalveolar lavage fluid, superoxide dismutase, catalase

29. Shopova V, Petrova P, Salovsky P, **Dancheva V**. The effect of separate and combined application of whole-body ionizing irradiation and cadmium acetate on rat alveolar macrophages. *Scientific works of the Higher Med. Institute, Pleven.* 1991:17-19, **1** цитиране.

Резюме: Доказано, что ацетат кадмия, примененный интратрахеально на крысах в дозе 0,5 mg/kg изолированно и в сочетании с внешним облучением ионизирующей радацией в дозе 4 Gy, снижает процент альвеолярных макрофагов и вызываеш дегенеративные изменения в них. Считаем, что при подобных условиях легочное повреждение связано с повреждением альвеолярных макрофагов и угнетением их функции, вероятно, обусловленными повышенной липидной пероксидацией.

Key words: cadmium acetate, ionizing radiation, broncoalveolar lavage fluid, alveolar macrophages, phagocytic activity

30.Данчева В, Съловски П, Шопова В. Модификация на биологичните ефекти на Динитроортокрезол /Селинон/ и йонизираща радиация при комбинираното им приложение у бели плъхове. *Научни доклади, Юбилейна научна сесия "Човекът и йонизиращите лъчения"- НЦРРЗ*, *София*. 2003:59-61.

Резюме

Експериментът е проведен върху 200 бели мъжки плъха, порода "Вистар", разделени в 4 групи: първа – контрола; втора – облъчена с 4 Gy йонизираща радиация; трета – третирана със селинон, въвежден ежедневно, орално в продължение на 4 месеца в доза 1/₂₀ ЛД₅₀ = 2.22 мг/кг и четвърта – третирана комбинирано със селинон и йонизираща радиация. Резултатите от изследването на бронхоалвеоларна лаважна течност (БАЛТ) свидетелстват за умерено изразени промени под влияние на двата фактора. Селинон увеличава активността на ензимите ЛДХ, КФ, АФ и съдържание на общ белтък в БАЛТ. Комбинираното третиране със селинон и йонизираща радиация показва изразен антагонистичен ефект, най-вероятно в резултат на тъканната хипоксия, предизвикана от селинона и предхождаща облъчването на опитните животни. *Ключови думи*: селинон, йонизираща радиация, бронхоалвеоларна лаважна течност

31. Данчева В, Шопова В, Ставрева Г, Стоянова А. Ефект на U-74389G върху маркери за цитотоксичност в бронхоалвеоларна лаважна течност на плъхове, третирани с блеомицин. *Сборник доклади от Международна научна конференция на СУБ, Стара Загора. Хуманна медицина.* 2007; VII:208-13.

Abstract

Objective: Our goal was to study the effect of U-74389G on some markers for cytotoxicity in rat bronchoaleveolar lavage fluid (BALF) after bleomycin treatment.

Methods: The study was carried out on 27 male Wistar rats, divided into three treatment groups: group 1 – controls; group 2 – treated with bleomycin; group 3 – treated with bleomycin and U-74389G. Bleomycin was administered intratracheally in a dose of 2.5 U/kg.

U-74389G, dissolved in CS-4, was injected twice i.p., two hours before receiving bleomycin and four hours later at a dose of 5 and 5 mg/kg body weight respectively.

In BALF were investigated the activities of lactate dehydrogenase (LDH), alkaline phosphatase (AP) and acid phosphatase (AcP).

Results: The isolated application of bleomycin increased significantly the activity of AP and AcP on day 1 in comparison with controls. In the rats treated with a combination with U-74389G the activity of LDH, AP and AcP were decreased on day 1 in comparison with bleomycin group.

Conclusions: The presence of antioxidant U-74389G before and very soon after application of bleomycin shows marked but transient protective effect on some markers for cytotoxicity in BALF. *Key words:* bleomycin, U-74389G, pneumotoxicity, BALF

32. Шопова В, Ставрева Г, **Данчева В**, Стоянова А. Ефект на алфатокоферол върху маркери за пневмотоксичност в бронхоалвеоларна лаважна течност на плъхове, третирани с амиодарон. Международна научна конференция на СУБ – Стара Загора, 2008. От: www.sustz.com/Proceeding08/.../Shopova_V.pdf

Abstract

Amiodarone (AM) has been associated with the development of pulmonary toxicity. Our goal was to study the effect of DL alpha-tocopherol on markers for pneumotoxicity in rat broncho-alveolar lavage fluid (BALF) after AM treatment. The study was carried out on 72 male rats divided into four groups: group 1 – controls; group 2 – with AM; group 3 – with AM and DL alpha-tocopherol; group IV – DL alpha-tocopherol. AM was administered intratracheally at a dose 6.25 mg/kg in a 3.125 mg/mL solution on day 0 and 2; U-74389G – at a dose of 15 mg/kg on day 0 and 2. Activities of lactate dehydrogenase, acid phosphatase, alkaline phosphatase; total cell count, total protein in BALF were investigated. *Key words*: amiodarone, pneumotoxicity, broncho-alveolar lavage fluid

33. Ставрева Г, Шопова В, Стоянова А, **Данчева В**, Йорданов Й. Сравнение на два експериментални модела на амиодарон-индуцирана белодробна фиброза при плъхове. Международна научна конференция на СУБ – Стара Загора, 2008. От: www.sustz.com/Proceeding08/.../Stavreva G.pdf

Abstract

Amiodarone (AM), an antiarrhythmic agent, has been associated with the development of pulmonary fibrosis. Several in vivo animal models have been used to study amiodarone-induced pulmonary fibrosis (AIPF). Intratracheal administration of AM to rodents has been used as a model for the AIPF. We compared two experimental models of AIPF. Wistar rats were given a single intratracheal insufflation of AM at a dose 6.25 mg/kg in a 7.8 mg/mL solution and AM at a dose 6.25 mg/kg in a 3.125 mg/mL solution. Hydroxyproline and collagen content in lung homogenate were measured; histological examination was performed.

Key words: amiodarone-induced pulmonary fibrosis, hydroxyproline, collagen.

Резюмета от международни научни форуми, публикувани в научни списания или сборници с резюмета на научни прояви

34. Salovsky P, Marev R, Shopova V, **Dancheva V**. Cadmium chloride - induced changes of the antioxidative protective systems in rat lungs. ERS Meeting,

Austria Center, August 29th-September 3rd, 1992. *The European Respiratory Journal*. 1992;5(15):250S (IF=1.062, инд. IF 0.212, 1 цитиране).

Abstract

Study is carried out on 100 male Wistar strain rats (weight 180-200 g). Cadmium chloride is applied *per* os in daily dose of 8.5 mg/kg = $1/20 \text{ LD}_{50}$ for 8 days. Studied in induces are followed-up on days 1, 5 and 15 after the end of poisoning. It is established that cadmium chloride increases the superoxide dismutase (SOD) in comparison with controls with 213% on day 1, and decreases it on days 5, 15 and 30. Catalase activity is decreased up to day 15 and glutathione peroxidase activity – up to day 5. Glutathione reductase activity is reduced only on day 1, but the non-protein thyol groups amount is reduced all during the observed period and it is most expressed on day 1 (47%). Obtained experimental data suggest an expressed tendency towards inhibition of the studied links of antioxidant defence until day 5 (excepting SOD on day 1). This indicates that the oral application of cadmium chloride induces an oxidant stress and increases the lipid peroxidation in lungs. Data show increased activities of lactate dehydrogenase, alkaline phosphatase and acid phosphatase in bronchoalveolar lavage fluid during the earliest time – points after the treatment confirm our conclusion.

Key words: cadmium chloride, antioxidant defence system, oxidative stress, lipid peroxidation

35. Salovsky P, Shopova V, **Dancheva V**, Marev R, Pandurska A. Lung antioxidant enzymes in cadmium treated rats. 11th Summer Meeting of the British Association for Lung Research, Cardiff, 06.07.1992. *Respiratory medicine*. 1992;86(6):537 (IF=1.062, инд. IF 0.212).

Abstract

The aim of the present study was to follow up the behaviour of some lung antioxidant enzymes in cadmium chloride-treated rats. The experiment was carried out on 60 male Wistar rats. Cadmium chloride was instilled intraperitoneally in a single dose of a total of 0.5 mg/kg. It was established that superoxide dismutase (SOD) activity in lung homogenate is decreased on days 15 and 30 after poisoning, and catalase (CAT) activity is decreased on days 5, 15 and 30. Glutathione peroxidase (GP) and glutathione reductase (GR) activities are increased on days 1 and 5 and are then moderately decreased during the later time points. Our experimental data show that an intraperitoneal instillation of a total of 0.5 mg/kg cadmium chloride suppresses the activity of key antioxidant enzymes in the lung and decreases the antioxidant capacity of this organ on day 5 after treatment.

Key words: cadmium chloride, antioxidant defence system, antioxidant capacity, oxidative stress

36. Salovsky P, **Dancheva V**, Shopova V. Changes in pulmonary antioxidant defence mechanisms during separate and combined treatment with paraquat and ionizing radiation. Прв Македонски пулмо-алерголошки конгрес со мегународно учество, 6-10.09.1993, Охрид, Македония, Сборник резюмета:392.

Abstract

White male Wistar rats are treated with $1/100 \text{ LD}_{50}$ (0.46 mg/kg) paraquat, orally, five days weekly for a period of 4 months. Immediately after termination of the poisoning the animals were exposed to a single whole-body irradiation with 4 Gy ionizing radiation on a linear accelerator Neptun 10 P with photon energy 9 MeV and radiation power 2 Gy/min. Lung homogenate was prepared in the ratio 1:10 with ice-cold 0.25 M sucrose solution containing 50 mM Tris-HCl. It was ascertained that the paraquat and the

ionizing radiation decrease the activity of the enzymes superoxide dismutase, catalase, glucose-6phosphate dehydrogenase and the content of the non-protein thiol groups in lung homogenate in the earliest stages. These results suggest a decreased pulmonary antioxidant capacity and for increased lipid peroxidation in this organ. The combined treatment with both factors has a marked synergic effect concerning the observed biochemical indices.

Key words: paraquat, ionizing radiation, antioxidant defence system, lung homogenate

37. Shopova V, Petrova P, Salovsky P, **Dancheva V**. The effect of separate and combined application of whole-body irradiation and cadmium acetate on rat alveolar macrophages. Прв Македонски пулмо-алерголошки конгрес со мегународно учество, 6-10.09.1993, Охрид, Македония, Сборник резюмета:393.

Abstract

Male white Wistar rats were treated with a single dose of 0.5 mg/kg body wt cadmium acetate intratracheally and with 4 Gy external gamma irradiation separately and in combination. Animals were sacrificed by cervical dislocation on post-treatment days 1 and 15. The following parameters were investigated in the bronchoalveolar lavage fluid (BALF): total cell number and differential cell count, morphological changes in alveolar macrophages (AM), AM phagocytic activity by modified nitroblue-tetrazolium test. Evaluation was done in conventional units by the method of Kaplaw. It was proved that cadmium acetate applicated separately and in combination with 4 Gy external irradiation sharply decreased AM percentage in BALF and provoked multiform degenerative changes. Both parameters AM phagocytic activity and capacity were decreased in the same groups. It could be suggested that one of the possible ways for lung injury after the treatment with cadmium acetate is associated with AM injury.

Key words: cadmium acetate, bronchoalveolar lavage fluid, alveolar macrophages, phagocytic activity

Salovsky P, Shopova V, Dancheva V, Radionova Z. Changes on bronchoalveolar lavage fluid in rats treated intratracheally with Americium-241.
5th International Conference Bronchoalveolar Lavage, 29th June-1st July, 1995, Dublin, Ireland. Abstracts:S08.

Abstract

Introduction: Americium-241 (²⁴¹Am) is a high toxic radionuclide. It is a dangerous contaminant in the nuclear industry. The aim of the present study is to follow time-dependant changes in alveolar lavage fluid (BALF) after intratracheal administration of ²⁴¹Am in rats.

Methods: The experiment was carried out on 48 male Wistar rats.²⁴¹Am was intratracheally instilled with a metal probe in dosage of 2000 Bq per animal. The bronchoalveolar lavage was made *in situ* by triple lavage via the trachea. The cells were eliminated by centrifugation x 300g for 10 min and the supernatant was used for biochemical analysis.

Results: Lactate dehydrogenase (LDH) activity was increased on day 30 and that one of gammaglutamyl transferase (γ GT) on days 3, 7, 15 and 30. Alkaline phosphatase activity was increased on day 3 and decreased on day 7. The protein content was elevated on days 15 and 30.

Conclusion:

1. The intratracheal administration of 2000 Bq ²⁴¹Am per rat provokes significant changes in BALF.

- 2. The yGT is more sensitive biochemical marker than LDH.
- 3. The bronchoalveolar lavage is a valuable method for the study of the biological effect of ionizing radiation.

Key words: Americium-241, bronchoalveolar lavage fluid, lactate dehydrogenase, alkaline phosphatase, gamma-glutamyl transferase

39. Salovsky P, Shopova V, **Dancheva V**, Radionova Z. Antioxidant defence system after intratracheal treatment with 241Am in rat lung. *VII International Congress of Toxicology, July 2-6, 1995, Seattle, Washington, USA. 1995. The International Toxicologist.* 7(1):88-P-21 (IF 3.032, инд. IF 0.758).

Abstract

In the animal experiment described here, we have examined the antioxidant system in the lungs in the early periods of time after intratracheal application of 2000 Bq ²⁴¹Am per animal in water solution. The animals used were white male Wistar rats killed on days 3, 7, 15 and 30. It was observed that ²⁴¹Am increased SOD activity in lung homogenate on day 15, while the same time-point CAT activity was decreased compensatively. GP and G-6-PD activities were considerably increased on days 3 and 15. The content of NPSH groups was reduced after day 15 and corresponded to the maximum of the accumulated tissue dose in the lungs. The intratracheal application of 2000 Bq ²⁴¹Am/rat caused considerable changes in key-steps in the antioxidant protection in the lungs.

Key words: Americium-241, lung homogenate, antioxidant defence sysrem, pneumotoxicity

40. Salovsky P, Shopova V, Dancheva V, Radionova Z. Combined effects of cadmium and lead on some biochemical markers in rat bronchoalveolar lavage fluid. 34th European Congress of Toxicology (Eurotox), Prague, Czech Republic, August 27-30, 1995. *Toxicology Letters*. Suppl 1/78:73 (IF 1.242, инд. IF 0.310, 4 цитирания).

Abstract

Rats were treated intratracheally alone with single doses of 10 µg cadmium chloride and 100 µg lead acetate respectively, and in combination of both metals. Bronchalveolar lavage was performed on days 1, 5 and 15 after treatment. The activities of lactate dehydrogenase, alkaline phosphatase and acid phosphatase as well as protein and glucose content in bronchoalveolar lavage fluid were increased in all treated groups mainly on day 1. These changes were more expressed in the combination of the two agents. It was concluded that the simultaneous treatment with cadmium and lead shows a clear tendency to additive toxic damage of rat lungs in the early periods of time. *Key words*: cadmium, lead, lavage, biochemical markers

41. Stavreva G, Shopova V, Atanasova M, Dancheva V, Georgieva M, Dimitrova A, Stoyanova A, Tisheva S. Changes of elastin turnover in Wistar-Kioto rats with amiodarone-induced pneumotoxicity. ESC Congress, 30 August - 3 September, 2008, Munich, Germany. *European Heart Journal*. 2008;29(Suppl 1):634 (IF 8.917, инд. IF 1.114).

Abstract

Objectives: Amiodarone (AD) is an efficacious, first-line antidysrhythmic agent with a propensity to cause pulmonary toxicity, including potentially fatal fibrosis. Elastin is insoluble cross linked polymer of

tropoelastin (monomer units) which forms fibers endowing tissues with elasticity. It is one of the main structural proteins of the aorta, lung and skin in humans and vertebrates. In the present study,

the potential effect of intratracheally instilled AD (alone and combined with U-74389G or a-tocopherol) on elastin turnover was examined via assessment of anti- α -elastin (AEAb) and anti-tropoelastin antibodies (ATEAb) in male Wistar-Kioto rats (WKY).

Methods: The study was carried out on 48 male WKY weighing 220-250 g. The animals were divided into four treatment groups: (1) – controls; (2) – treated intratracheally with AD; (3) – treated with AD and 21-aminosteroid U-74389G; (4) – treated with AD and a-tocopherol. AD was installed intratracheally on days 0 and 2 (6.25 mg/kg with a 3.125 mg/ml water solution). U-74389G (dissolved in CS-4) was injected i.p. at a dose 15 mg/kg and a-tocopherol (as an emulsion) was injected i.p. at a dose 50 mg/kg on day 0, 1 and 2. Serum samples were tested for the levels of antibodies directed against α -elastin and tropoelastin on day 3th and 28th using enzyme-linked immunosorbent assays (ELISA).

Results: AEAbs in the sera of WKY from the group, treated with AD and atocopherol were significantly increased (p=0.001) on day 3th compared to the levels measured in the other groups. ATEAbs in the sera taken on day 3th were elevated in the group, treated with AD plus U-74389G and decreased in groups, treated with AD and AD plus a-tocopherol. ATEAb to AEABb ratio was significantly decreased in the group, treated with AD and a-tocopherol (p=0.02) and elevated in the group, treated with AD plus U-74389G on day 3th. No significant differences in all groups on 28th day compared to control group.

Conclusions: These results suggested an altered elastin metabolism. There was a tendency to diminished elastin synthesis due to AD, applied alone. AD combined with a-tocopherol showed a negative effect on elastin turnover (increased elastin degradation and reduced elastin production) on day 3th. Elevated ATEAb to AEABb ratio in the group, treated with AD plus U-74389G showed a stimulation of elastin synthesis.

Key words: amiodarone, fibrosis, U-74389G, anti-α-elastin, α-tocopherol

42. Stavreva G, **Dancheva V**, Shopova V, Stoyanova A, Yordanov Y, Ivanov Y, Popova T. Effect of U-74389G and alpha-tocopherol on amiodarone-induced pulmonary fibrosis in rats. *European Respiratory Society, Annual Congress, October4-8,2008,Berlin,Germany.*

www.ersnet.org/learning_resources_player/abstract...08/.../419.pdf

Abstract

Pneumotoxicity is an adverse effect of great concern in patients on amiodarone (AD) pharmacotherapy. This is primarily due to the potential for development of pneumonitis and pulmonary fibrosis, a condition for which there is no effective treatment and the prognosis is poor. We tested the potential effect of 21-aminosteroid U-74389G and a-tocopherol against AD-induced pulmonary toxicity in the rat model.

Methods: The study was carried out on 64 male Wistar rats weighing 220-250 g. The animals were divided into four treatment groups: (1) – controls; (2) – treated intratracheally with AD; (3) – treated with AD and 21-aminosteroid U-74389G; (4) – treated with AD and a-tocopherol. AD was instilled intratracheally on days 0 and 2 (6.25 mg/kg with a 3.125 mg/ml water solution). U-74389G was injected at a dose 15 mg/kg and a-tocopherol was injected at a dose 50 mg/kg on day 0, 1 and 2, intraperitoneally. Pulmonary fibrosis was assessed biochemically by measuring hydroxyproline (HP) content in lung homogenate (LH) and histopathologically on day 7 and 28 after AD administration.

Results: AD altered HP levels on day 7 and did result in significant (50%) increase on day 28 after treatment in comparison with controls. The content of HP in AD + U-74389G ($0,68\pm0,08$ mcg/ml LH) in AD + a-tocopherol ($0,83\pm0,31$ mcg/ml LH) groups were decreased compared to AD alone ($1,07\pm0,17$ mcg/ml LH) on day 28. Intratracheal AD resulted in increased histopahological damage on day 28, as indicated by thickening of interstitial spaces, and these damages were attenuated by both combinations. **Conclusions:** The antioxidants U-74389G and a-tocopherol can substantially protect animals from amiodarone-induced pulmonary fibrosis.

Key words: amiodarone, U-74389G, pulmonary fibrosis, a-tocopherol, lung homogenate

43. Stavreva G, **Dancheva V**, Stoyanova A, Terziev L, Popova T, Ivanov Y. Effect of Mn(III tetrakis (4-benzoic acid) porphyrin on inflammation and fibrosis in amiodarone-induced pneumotoxicity in rats. European Respiratory Society, Annual Congress, September 12-16, 2009, Vienna, Austria. *European Respiratory Journal.* 2009:34(Suppl 53):558s (IF 5.527, инд. IF 0.921).

http://www.ersnetsecure.org/prblic/prg_congres.abstract?ww_i_presentation=41101

Abstract

New catalytic antioxidants metalloporphyrin have been proved to have protective role in inflammatory conditions, inhibiting inflammatory gene expression in response to reduced generation of reactive oxygen species such as superoxide, peroxide, peroxynitrite and lipid peroxyl radicals. We examined the effect of manganese(III)*tetrakis*(4-benzoicacid)porphyrin (MnTBAP) on amiodarone (AD)-induced pulmonary toxicity in the rat model.

Methods: The study was carried out on 48 male Wistar rats, divided into four groups: (1)–controls; (2)– treated intratracheally (*i.t.*) with AD; (3)–treated with AD and MnTBAP; (4)–treated with MnTBAP. AD was administrated *i.t.* on days 0 and 2 (6.25 mg/kg). MnTBAP was injected intraperitoneally at a dose 10 mg/kg on day 0, 1 and 2. Cytologic and biochemical (activity of lactate dehydrogenase (LDH), acid phosphatase (AcPh), alkaline phosphatase (AlPh) assays of bronchoalvealar lavage fluid (BALF) was performed on day 3. Pulmonary fibrosis was assessed by measuring hydroxyproline (HP) content in lung homogenate (LH) on day 28 after AD administration.

Results: AD treatment resulted in significantly increased protein content; total cell count; polymorphonuclear cells; activity of LDH, AcPh and AlPh; and content of HP. The treatmen twith AD and MnTBAP decreased the markers of pulmonary inflammation and citotoxicity in BALF compared to AD group. The content of HP in AD+MnTBAP (2.25±0.16 mcg/ml LH) group was decreased compared to AD alone (3.34±0.15mcg/ml LH) on day 28 (p<0.05). MnTBAP reduced early AD-induced lung inflammatory injury and can protect animals from AD-induced pulmonary fibrosis.

Key words: amiodarone, MnTBAP, bronchoalvealar lavage fluid, lung homogenate, inflammation, fibrosis

44. Stavreva G, Dancheva V, Popova Tz, Terziev L, Stoyanova A, Yordanov Y, Shopova V, Ivanov Y. Effect of prednisolone and imatinib mesylate on inflammation and fibrosis in amiodarone-iduced pulmonary toxicity in rats. European Respiratory Society, Annual Congress, September 18-22, 2010, Barcelona, Spain. *European Respiratory Journal. 2010:36(Suppl 54):223s* (IF 5.922, инд. IF 0.740).

Abstract

The experimental model of amiodarone (AM)-induced lung toxicity is one of the relevant models to study idiopathic pulmonary fibrosis (IPF). AM administered intratracheally, induces inflammatory response and activation of fibroblasts, and subsequent fibrosis. We tested the antiinflammatory and antifibrotic effect of prednisolone (PR) and imatinib mesylate (IM) against AM-induced pulmonary fibrosis in a rat model. The study was carried out on 72 male Wistar rats weighing 220-250 g. The animals were divided into six treatment groups: control; treated intratracheally with AM; treated with AM and PR or IM from day 1 to day 10; treated with AM and PR or IM from day 10 to day 28. AM was instilled on days 0 and 2 (6.25 mg/kg with a 3.125 mg/ml water solution). PR (10 mg/kg) and IM (50 mg/kg) were given orally.

Pulmonary fibrosis was assessed biochemically by measuring hydroxyproline (HP) and collagen (C) content in lung homogenate (LH) and histopathologically on 28 after AM administration.

AM resulted in significantly increased HP and C content in LH on day 28 in comparison with controls. The content of HP in animals, treated with AM+IM, given after day 10 (4.76±0.36 mcg/ml LH) and AM+PR, given after day 1(4.76±0.36 mcg/ml LH) was decreased compared to AM alone (4.79±0.18 mcg/ml LH) on day 28 (p<0.05). Intratracheal AM led to moderate interstitial and perivasal fibrosis, thickening of interstitial spaces and cellular infiltration; these damages were attenuated by above-mentioned dosing regimes.

The results obtained from our study showed that IM, given from day 10, attenuated fibrosis, whereas corticosteroid PR was effective during the inflammatory phase of the model.

Key words: amiodarone, prednisolone, imatinib mesylate, pulmonary fibrosis, hydroxyproline, collagen