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Effects of cadmium and monensin on the morphology of lung of mice, subjected to subacute cadmium intoxication

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The effects of cadmium (Cd) and monensin on the lungs of mice, subjected to subacute Cd intoxication were studied on ICR mouse model. The data demonstrated that Cd induced elevation of the lungs weight in Cd-intoxicated mice compared to the normal controls. The treatment of Cd-intoxicated mice with monensin recovered lungs weight to normal values, suggesting an ameliorative effect of the antibiotic on the lung function. Histopathological analysis of the lung tissue demonstrated that Cd induced circulatory and inflammatory alterations. Monensin administration to Cd-treated animals reduced the morphological alterations and restored histology of the lungs to normal in a great extent. These data were well correlated with the results from the atomic absorption that showed that monensin reduces the concentration of Cd in the lungs of the Cd-intoxicated animals by 30 % compared to the toxic control. Taken together the results presented in this study prove that monensin could be a promising chelating agent for the treatment of Cd-induced lung dysfunction.

Key words: lung, cadmium, monensin, ICR mice, chelating agents

Introduction

The development of modern industry led to a rapid increase of the concentrations of toxic elements in our environment [9]. Among them, cadmium (Cd) is extremely dangerous for the human health because of its long biological half-life (40 years for humans) [8, 9]. Humans could be exposed to Cd either by inhalation or ingestion. 90 % of inhaled Cd is absorbed by the lungs [4]. Studies on animal model have been proven that the accumulation of Cd in the lungs initiates inflammation via cytokine production [4]. High levels of lipid peroxidation have been observed in the lungs of Cd-intoxicated mice [6]. Cd has been demonstrated to induce obstructive lung disorder and at prolonged exposure – lung cancer [2, 5, 7].

To the best of our knowledge, there is no effective chelating therapy for humans exposed to Cd intoxication [1]. Recently we have demonstrated that the polyether

ionophorous antibiotic monensin significantly reduces the concentration of Cd in organs of mice, subjected to subacute intoxication [3]. These results motivate as to conduct extensive research on the potential application of this antibiotic as chelating agent for the treatment of Cd-intoxications. Herein we present novel data that demonstrate that monensin significantly attenuates Cd-induced morphological alterations in the lungs of mice, subjected to subacute Cd intoxication.

Material and Methods

Chemicals

The sodium salt of monensin was provided by Biovet Ltd. (Peshtera, Bulgaria). Tetraethylammonium hydroxide (Et_4NOH), nitric acid (HNO_3), and diethyl ether (Et_2O) were purchased from Merck (Darmstadt, Germany).

Preparation of monensic acid

Monensic acid A monohydrate was prepared from sodium monensin (711 mg, 1 mmol) applying the procedure previously described.

Animal model

The animal model is described in details in Ivanova et al [3]. Briefly sixty-day old adult male ICR mice were housed at the Institute of the Experimental Morphology, Pathology and Anthropology with Museum (Bulgarian Academy of Sciences, Sofia) under conventional conditions. The animals were divided into three groups with six mice in each group. The first (normal control) group received standard diet and had free access to distilled water during the experimental protocol. The second group animals (toxic control) was subjected to treatment with 20 mg/kg body weight $Cd(CH_3COO)_2 \times 2H_2O$ once daily for 2 weeks. The compound was dissolved and obtained in drinking (distilled) water. During the next 14 days of the experiment, the animals from this group were received distilled water and food *ad libitum*. The third group (monensin-treated mice) was administrated with $Cd(CH_3COO)_2 \times 2H_2O$ as described above followed by treatment with tetraethylammonium salt of monensic acid (16 mg/kg body weight in distilled water) during the 15^{th} to the 28^{th} days of the experimental protocol. On the 29^{th} day of the experimental protocol, all the animals were sacrificed under light ether anaesthesia and the samples were collected for the analysis. The lungs for atomic absorption spectrometry were stored at -20 °C prior to analysis. The animal studies were approved by the Ethics Committee of the Institute of the Experimental Morphology, Pathology and Anthropology with Muzeium, BAS.

Atomic absorption analysis

The organs were digested with concentrated HNO_3 (free of metal ions) as previously described [3]. The determination of Cd in the lungs was preformed by electrothermal (Zeeman Perkin Elmer 3030, HGA 600) analyzer. Certified Reference Materials were used to control for analytical accuracy.

Histopathological analysis

Lung tissue materials from the experimental animals were embedded in paraffin using routine histological practice. Tissue sections (thickness 5μm) were deparaffinised and stained with haematoxylin and eosin.

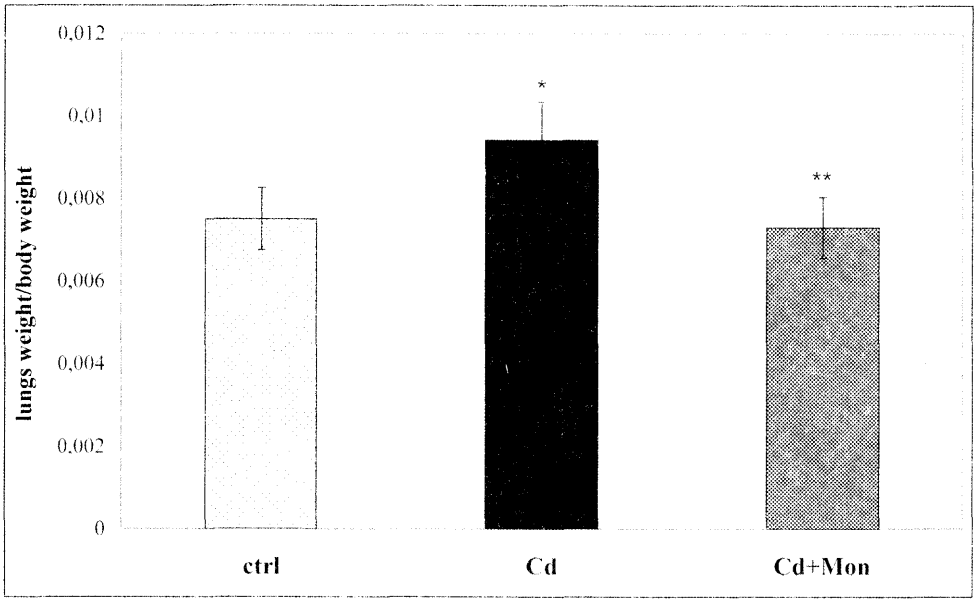

Fig. 1. Lung weight/body weight ratio of the experimental animals. Each column represents mean ± SD, $n = 6$. Asterisk (*) represents statistical differences between the Cd-treated group and normal controls ($p<0.05$); Double asterisk (**) represents significant differences between monensin-treated group and the Cd-intoxicated animals ($p<0.05$)

Statistical analysis

The obtained results are presented as mean value ± SD. Statistical significance between the experimental groups was determined using Student's *t*-test. Difference was considered significant at $p < 0.05$.

Results and Discussion

The data on the effect of cadmium and monensin on the lungs weight of mice, subjected to subacute Cd intoxications are presented on Fig. 1. As could be seen, Cd induced a significant increase (by 20 %) of the lungs weight/body weight index compared to the normal control. The treatment of Cd-intoxicated mice with monensin restored the lungs weight to normal values. These results suggest that the antibiotic most likely improved the lung function in Cd-intoxicated mice.

The morphological analysis of the lung tissue from the experimental animals demonstrated severe pathological changes of lung morphology in the subacute Cd intoxication of adult mice. Cd exerts its effect in the lung tissue by perturbing blood circulation and inducing inflammatory alterations.

The pathological changes were significantly manifested in Cd-treated animals (Fig. 3) compared to corresponding control animals (Fig. 2). Bronchioli with lesions and desquamations of the respiratory epithelium and many large focuses of inflammatory and circulatory alterations (haemorrages) were observed in the lung tissue of the Cd-treated mice (Fig. 3). After monensin administration to Cd intoxicated animals we

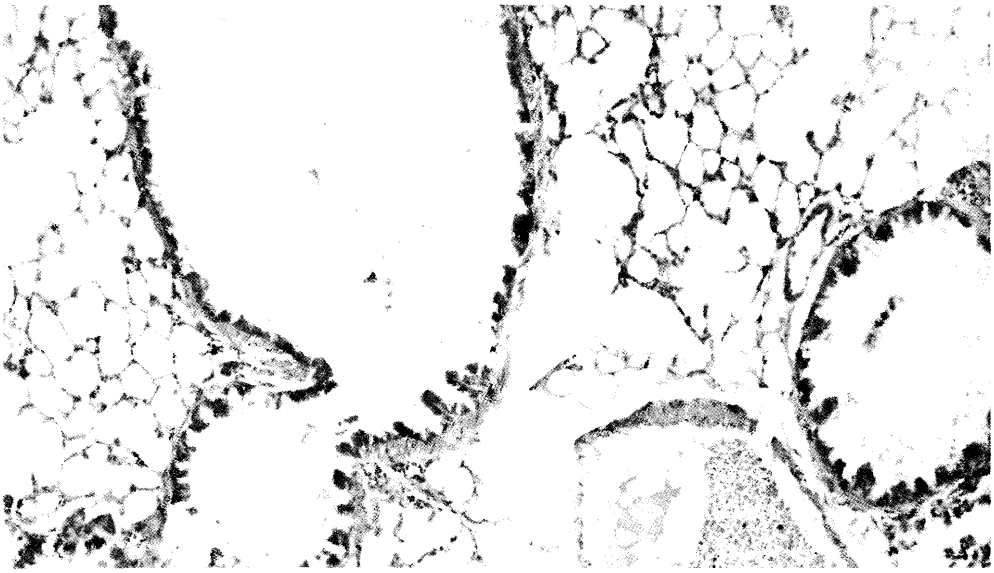

Fig. 2. Morphology of lung in a control animal (tissue section stained with haematoxylin and eosin)

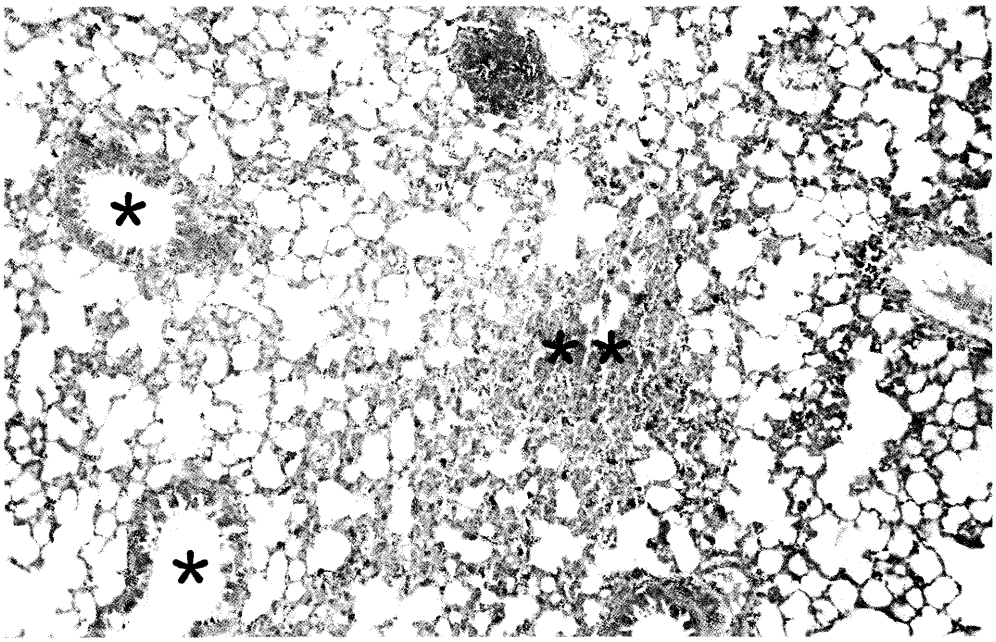

Fig. 3. Morphology of lung in a Cd-intoxicated animal. Asterisk (*) marks bronchioli with lesions and desquamations of the respiratory epithelium; Double asterisk (**) marks a large focus of inflammatory and circulatory alterations (haemorrage) (tissue section stained with haematoxylin and eosin)

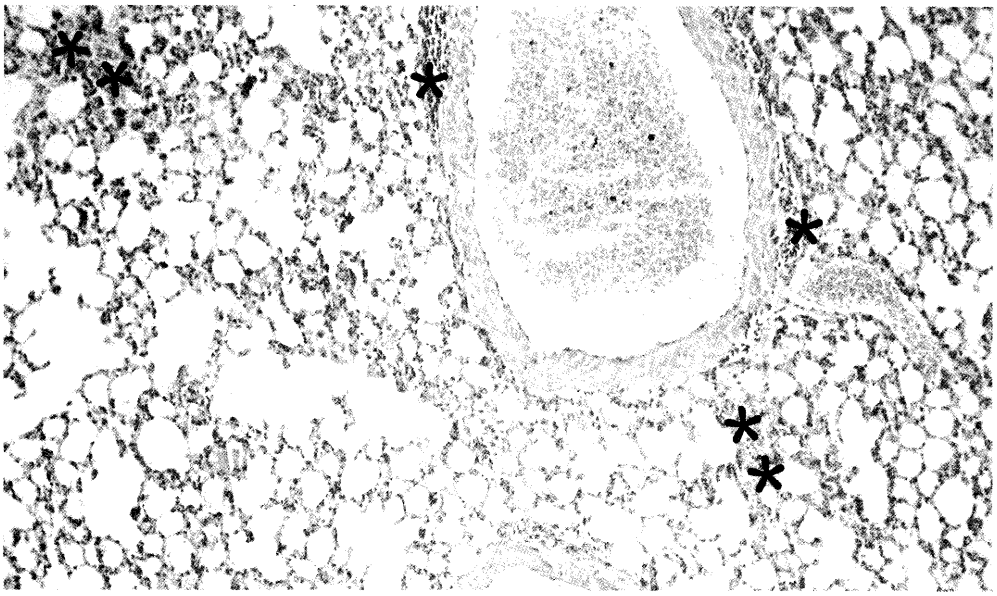

Fig. 4. Morphology of lung in monensin-treated group. Asterisk (*) marks scanty perivasal inflammatory infiltrates. Double asterisk (**) marks single small disseminated residual focuses of inflammatory and circulatory alterations (tissue section stained with haematoxylin and eosin).

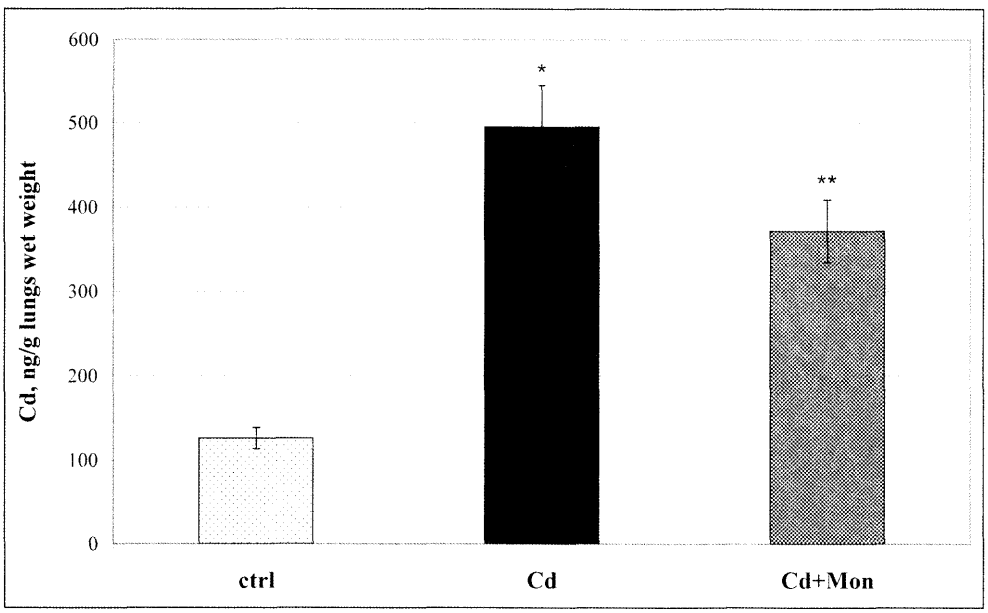

Fig. 5. Cd concentration in the lungs of the experimental animals. Each column represents mean ± SD, $n = 6$; Asterisk (*) represents statistical differences between the Cd-treated group and normal controls ($p<0.05$); Double asterisk (**) represents significant differences between monensin-treated group and the Cd-intoxicated animals ($p < 0.05$)

established reduction of the pathological alterations and tendency towards restoration of the normal morphology of the lungs to a great extent (Fig. 4).

The atomic absorption analysis of the lungs of the experimental animals revealed that monensin decreases the concentration of Cd in the lungs of the Cd-intoxicated animals by 30 % compared to the toxic control ($p < 0.05$) (Fig. 5).

In conclusion our investigation demonstrated that subacute Cd intoxication induced severe changes in lung morphology, especially in the blood circulation and in the epithelium of the bronchial tree and of the alveols. Monensin reduced lung injuries and recovered its morphology to a great extent that can suggest monensin as a candidate in chelating therapy of some heavy metal intoxications.

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