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**Для листування:**

м. Львів, вул. Пекарська, 69, 79010  
Е-пошта: dr.kowalska.marta@gmail.com

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**ORCID ID**

Marta Kowalska:  
<https://orcid.org/0000-0002-6232-3951>

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## Functional status of prooxidant and antioxidant systems in the lungs under experimental allergic alveolitis and stress

Marta Kowalska

*Danylo Halytsky Lviv National Medical University, Lviv, Ukraine*

### ABSTRACT

We have analysed the results of the study of alterations in indices of pro-oxidant (conjugated diene and malondialdehyde) and antioxidant (superoxide dismutase, ceruloplasmin, catalase) systems in guinea pigs' lungs in experimental allergic alveolitis in the dynamics of EAA development under the conditions of immobilization stress. The study included 62 female guinea

pigs weighing 180-220 g, divided into 4 groups: 1 – intact guinea pigs (n=20); 2 – guinea pigs (n=14) with EAA under conditions of IS (1<sup>st</sup> day); 3 – guinea pigs (n=14) with EAA under conditions of IS (2<sup>nd</sup> day); 4 – guinea pigs (n=14) with EAA under conditions of IS (34<sup>th</sup> day). The results of the experimental study showed that a significant increase in conjugated diene level in animal lungs was observed at all stages of EAA development under conditions of immobilization stress as compared to a control group, indicating the activation of this marker. The same changes occurred with MDA content, indicating excessive accumulation of this lipid peroxidation product in lung tissue. Intensive synthesis of free radical compounds caused activation of some components of the enzymatic system of antioxidant defence. In particular, a moderate decrease in superoxide dismutase activity in lung tissue is observed in response to an increased level of LOPs at early stages of EAA and immobilization stress development as compared to these indices in intact animals. The same situation is observed with catalase and ceruloplasmin activity in the lungs of guinea pigs with modelled AA and IS.

**Keywords:** experimental allergic alveolitis, immobilization stress, peroxide lipid oxidation, antioxidant system

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## Функціональний стан прооксидантної та антиоксидантної систем у легенях за умов експериментального альвеоліту і стресу

Марта Ковальська

Львівський національний медичний університет  
імені Данила Галицького, Львів, Україна

### РЕЗЮМЕ

Проаналізувавши результати дослідження змін у показниках прооксидантної системи (дія нових кон'югат і малоновогодіальдегіду) та антиоксидантних (супероксиддисмутази, церулоплазміну та каталази) в легенях морських свинок при експериментальному алергічному альвеоліті в динаміці розвитку та в умовах іммобілізаційного стресу.

Дослідження проводили на 62 самках морських свинок вагою 180-220г. Поділено на чотири групи: *перша група* – інтактні морські свинки (n=20); *друга група* – морські свинки (n=14), з експериментальним алергічним альвеолітом в умовах іммобілізаційного стресу (1-й день); *третья група* – морські свинки (n=14), з експериментальним алергічним альвеолітом в умовах іммобілізаційного стресу (2-й день); *четверта група* – морські свинки (n=14), з експериментальним алергічним альвеолітом в умовах іммобілізаційного стресу (34-й день).

Результати експериментального дослідження виявили, що значне підвищення рівня дієнових кон'югат у легенях тварин спостерігалось на всіх стадіях розвитку експериментального алергічного альвеоліту в умовах іммобілізаційного стресу порівнянно з контрольною групою, що свідчить про активацію цього маркера. Такі ж зміни відбувались з вмістом малоновогодіальдегіду, що свідчить про надмірне накопичення цього продукту перекисного окислення ліпідів у легеневої тканині. Інтенсивний синтез вільно радикальних сполук спричинив активацію деяких компонентів ферментативної системи антиоксидантного захисту. Зокрема, спостерігається помірне зниження активності супероксиддисмутази в легеневої тканині у відповідь на підвищення рівня антиоксидантної системи на ранніх стадіях експериментального алергічного альвеоліту та розвиток іммобілізаційного стресу порівнянно з показниками інтактних тварин. Така ж ситуація спостерігається з активністю каталази та церулоплазміну в легенях морських свинок з моделюванням експериментального алергічного альвеоліту та іммобілізаційного стресу.

**Ключові слова:** експериментальний алергічний альвеоліт, іммобілізаційний стрес, перекисне окиснення ліпідів, антиоксидантна система

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**For correspondence:**  
69, Pekarska St., Lviv, 79010  
E-пошта: dr.kowalska.marta@gmail.com

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### ORCID ID

Marta Kovalska:  
<https://orcid.org/0000-0002-6232-3951>

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**Introduction.** Among urgent problems of health care worldwide, a significant place is occupied by exogenous allergic alveolitis (EAA), which attracts attention of internists and allergists and is associated with a high incidence of this pathology in the structure of allergic diseases and an increase in severe complicated forms of this disease [1-4]. Currently, EAA is regarded as the pathology, associated with the risk of disability, manifested by chronic respiratory insufficiency, pulmonary heart or pneumosclerosis [5].

Besides, at present there are hardly any individuals, especially in ecologically unfriendly industrial regions of Ukraine, who do not experience any signs of allergic diseases. Thus, taking into account such facts, etiopathogenic peculiarities of this nosological form of the disease should be more thoroughly studied by both clinical pulmonologists and scientists.

The problem of combined pathology is also very topical in contemporary medicine, the basic features of which being difficulties with diagnosis, especially at the initial stage of the disease, comorbid clinical course and, as a result, complicated treatment of such patients. In particular, combination of stress with other diseases is very common [3, 6-8]. In recent decades, many investigations have shown association between the nervous system and the lungs [7]. Functioning mechanisms of both systems are characterized by common pathogenic peculiarities acting in synergism, since the lungs play one of the leading roles in adaptation reactions of the body to stressful influences of various origins [5]. This is caused by the fact that the lungs are a target organ for «adaptation» hormones, because they are responsible for a large number of clearing (clearance) processes [9-15]. Accordingly, they are an optimal organ for the study of various effects, occurring due to stress and allergic processes by hypersensitivity types. Thus, the aim of the research was to study lipid peroxidation processes and the condition of antioxidant protection in guinea pigs' lungs in different periods of experimental allergic alveolitis (EAA) formation under the conditions of IS.

## MATERIALS AND METHODS

All experiments on laboratory animals were conducted following the principles of bioethics according to the regulations of *European Convention for the protection of vertebrate animals* used for experimental and other scientific purposes (Strasbourg, 1986), European Union Directive 2010/63/EU, Law of Ukraine № 3447-IV «On protection of animals from cruel treatment», general ethic principles of experiments on animals, approved by the first national congress of Ukraine on bioethics (2001).

The experiment was conducted on 62 female guinea pigs weighing 0.18-0.20 kg. The animals were divided into 4 groups:

- 1 – intact guinea pigs (n=20);
- 2 – guinea pigs (n=14) with EAA under the conditions of IS (1<sup>st</sup> day from the start of injecting antigen);
- 3 – guinea pigs (n=14) with EAA under the conditions of IS (2<sup>nd</sup> day from the start of injecting antigen);
- 4 – guinea pigs (n=14) with EAA under the conditions of IS (34<sup>th</sup> day from the start of injecting antigen).

Experimental allergic alveolitis (EAA) was induced by the method of O.O. Orehov and Y.A. Kyrylov [16]. Prior, the animals had been immunized with Freund's *complete* adjuvant (0.2 ml intramuscularly into a hind leg). In 2 weeks, 0.2 ml of 1% BCG solution was introduced intravenously every 10<sup>th</sup> day. Experimental model of immobilization stress was induced by the following principle – the animals were immobilized on the back on an operation table, with atraumatic fixing of the extremities. Duration of immobilization lasted two hours. Immobilization stress was imitated by P.D. Horizontov method [17]. Later, the animals were decapitated; the level of LOPs and activity of antioxidant system enzymes were detected in lung homogenate on the 1<sup>st</sup>, 2<sup>nd</sup>, 34<sup>th</sup> days after EAA and stress activity. The content of conjugated dienes was determined by the method of V.B. Havrylov and M.I. Myshkorudina [18], malondialdehyde (MDA) – by E.N. Korobeinikov method [19], superoxide dismutase activity – by R. Fried method [20], catalase activity – by R. Holmes [21], and ceruloplasmin – by V.H. Kolb and V.S. Kamyshnikov method [22].

All digital results were statistically processed using arithmetical mean (M), margin of error of arithmetical mean (m), and Student's criterion «t». The calculations were performed using means of statistical and graphic analysis of electron tables Microsoft Excel (Microsoft office programs). Statistically reliable were the results with  $P \leq 0.05$ .

## RESULTS AND DISCUSSION

The results of our experimental investigations showed that a significant increase in conjugated dienes in the lungs was observed on the 1<sup>st</sup>,

2<sup>nd</sup> and 34<sup>th</sup> day of EAA under the conditions of immobilization stress by 250.74 % ( $P < 0.05$ ), 151.74 % ( $P < 0.05$ ) and 217.68 % ( $P < 0.05$ ), respectively, in comparison with the control, indicating an intensive formation of free radical compounds (table 1).

The changes similar to conjugated dienes occurred with MDA. Thus, a gradual elevation of MDA level in the lungs was recorded on the 1<sup>st</sup>, 3<sup>rd</sup> and 34<sup>th</sup> days of EAA under the conditions of stress development by 118.22 % ( $P < 0.05$ ), 89.57 % ( $P < 0.05$ ) and 119.57

Table 1

### Content of conjugated dienes in guinea pigs' lungs in EAA under the conditions of IS ( $M \pm m$ )

| Form of experiment             | Duration of the experiment | Number of animals | Conjugated dienes in nmol/ml (g) |
|--------------------------------|----------------------------|-------------------|----------------------------------|
| Intact guinea pigs             | Control                    | 20                | 12.1±0.6                         |
| EAA under the conditions of IS | 1 <sup>st</sup> day        | 14                | 42.44±0.3<br>$P < 0.05$          |
|                                | 2 <sup>nd</sup> day        | 14                | 30.46±0.3<br>$P < 0.05$          |
|                                | 34 <sup>th</sup> day       | 14                | 38.44±0.3<br>$P < 0.05$          |

Note. P – statistical significance of indices difference in comparison with the results in control group.

Table 2

### Content of MDA in guinea pigs' lungs in EAA under the conditions of IS ( $M \pm m$ )

| Form of experiment                 | Duration of the experiment | Number of animals | MDA in nmol/ml (g)      |
|------------------------------------|----------------------------|-------------------|-------------------------|
| Intact guinea pigs                 | Control                    | 20                | 20.8±0.8                |
| EAA under the conditions of stress | 1 <sup>st</sup> day        | 14                | 45.39±0.4<br>$P < 0.05$ |
|                                    | 2 <sup>nd</sup> day        | 14                | 39.43±0.3<br>$P < 0.05$ |
|                                    | 34 <sup>th</sup> day       | 14                | 45.67±0.3<br>$P < 0.05$ |

Note. P – statistical significance of indices difference in comparison with the results in control group.

Table 3

### Content of superoxide dismutase in guinea pigs' lungs in EAA under the conditions of IS ( $M \pm m$ )

| Form of experiment                 | Duration of the experiment | Number of animals | Superoxide dismutase in CU/ml (g) |
|------------------------------------|----------------------------|-------------------|-----------------------------------|
| Intact guinea pigs                 | Control                    | 20                | 128.1±3.2                         |
| EAA under the conditions of stress | 1 <sup>st</sup> day        | 14                | 90.29±0.4<br>$P < 0.05$           |
|                                    | 2 <sup>nd</sup> day        | 14                | 87.33±0.4<br>$P < 0.05$           |
|                                    | 34 <sup>th</sup> day       | 14                | 70.43±0.5<br>$P < 0.05$           |

Table 4

**Catalase activity in guinea pigs' lungs in EAA under the conditions of IS (M±m)**

| Form of experiment                 | Duration of the experiment | Number of animals | Catalase activity in IU/ml (g) |
|------------------------------------|----------------------------|-------------------|--------------------------------|
| Intact guinea pigs                 | Control                    | 20                | 47.7±2.5                       |
| EAA under the conditions of stress | 1 <sup>st</sup> day        | 14                | 39.44±0.4<br>P<0.05            |
|                                    | 2 <sup>nd</sup> day        | 14                | 21.5±0.4<br>P<0.05             |
|                                    | 34 <sup>th</sup> day       | 14                | 18.35±0.3<br>P<0.05            |

Note. P – statistical significance of indices difference in comparison with the results in control group.

% (P<0.05), respectively, in comparison with intact animals, indicating excessive accumulation of lipid peroxidation products (table 2).

Intensive formation of free radical compounds caused compensatory activation of some components of antioxidant defense system. Thus, as a response to increased level of LOPs at early stages of EAA development and immobilization stress (1<sup>st</sup> day), a moderate reduction of superoxide dismutase activity in the lungs by 29.52 % (P<0.05) was observed. Further, the activity of the enzyme gradually decreased by 31.83 % (P<0.05) and 45.19 % (P<0.05), respectively, in comparison with the control indices (table 3).

Condition of pro-oxidant and antioxidant systems in the animals' lungs in EAA under the conditions of IS (in % of control)

Similar situation occurred with catalase activity in the lungs. Analysis of enzymatic activity of this marker in animals' lungs showed that a decreased in catalase activity by 17.32 % (P<0.05) was noticed in the dynamics of EAA development and stress (on the 1<sup>st</sup> day). Fur-

ther (on the 2<sup>nd</sup> and 34<sup>th</sup> days), a gradual decrease in its activity was observed by 54.93 % (P<0.05) and 61.57 % (P<0.05), respectively, in comparison with the data of intact animals (table 4).

Intensive accumulation of LOPs caused depression of ceruloplasmin activity in the lungs. It decreased by 17.97 % (P<0.05) on the 1<sup>st</sup> day of EAA development and immobilization stress in comparison with the group of intact animals. Further, a reliable reduction of ceruloplasmin activity was observed on the 2<sup>nd</sup> and 34<sup>th</sup> days of the experiment by 37.32 % (P<0.05) and 48.54 % (P<0.05), respectively, in comparison with the data of intact animals (table 5).

The obtained results indicate that a gradual accumulation of LOPs occurs in EAA under the conditions of immobilization stress, reaching its peak on the 34<sup>th</sup> day of the experiment. In its turn, at the initial stages of EAA development and stress, it caused a compensatory reaction, characterized by the activity of all investigated enzymes with their further exhaustion on the 2<sup>nd</sup> and especially 34<sup>th</sup> day of the experiment.

Table 5

**Ceruloplasmin activity in guinea pigs' lungs in EAA under the conditions of IS(M±m)**

| Form of experiment                 | Duration of the experiment | Number of animals | ceruloplasmin in mg/l |
|------------------------------------|----------------------------|-------------------|-----------------------|
| Intact guinea pigs                 | Control                    | 20                | 26.1±0.7              |
| EAA under the conditions of stress | 1 <sup>st</sup> day        | 14                | 21.41±1.1<br>P<0.05   |
|                                    | 2 <sup>nd</sup> day        | 14                | 16.36±0.5<br>P<0.05   |
|                                    | 34 <sup>th</sup> day       | 14                | 13.43±0.3<br>P<0.05   |

Note. P – statistical significance of indices difference in comparison with the results in control group.

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