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ANTIOXIDANT ACTION OF ACETYLCISTEINE EXPERIMENTALLY PROVED IN VITRO

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Abstract: *Peroxydases are to be found in biological liquids and have the propriety of catalyzing the discomposure of oxygenated water, formed at these levels. The first part of the present paper presents an analysis of the latest data in the specialty literature regarding the exposure of the lung to oxidative stress, as the effect of free radicals upon the induction of pulmonary pathology is already known. The second part presents a study based on kinetic analysis. The study proves that the acetylcisteine is an activator upon the 3,3' -diaminobenzidine-H_2O_2-peroxydase system. The imunoenzymatic technique called ELISA enables some qualitative and quantitative determinations, based on the action of the enzyme upon a specific substratum that changes its structure and the possibility of absorbing the radiation in ultraviolet (UV) spectrum or visible spectrum (VIS). The authors have characterized the kinetic of enzymatic reactions; they have investigated the dependence of extinction according to time and the dependence of velocity according to concentration. The team has also determined the percentage of enzymatic activity, so as to highlight the extent to which the acetylcisteine has an activating character upon the given system. Following the experiments, the team of researchers has shown the activating influence of acetylcisteine upon the 3,3' -diaminobenzidine-H_2O_2-peroxydase system.*

Key words: *acetylcisteine, stress oxidative, enzymatic activity.*

1. Introduction

The lung is exposed to endogenous (recruitment of inflammatory cells) and exogenous sources of oxidative stress (air pollution, cigarette smoke, ozone). Oxidative stress is a significant part of the pathogenesis of obstructive lung diseases such as parenchymal lung diseases, and asthma [5], [11].

The inflammatory cells existing in the airways have a great capacity of production of ROS (reactive oxygen species). The neutrophils , the monocytes and the activated macrophages can produce hyperoxide radicals and other reactive oxygen species by means of the NADPH-dependent complexes [1], [3], [10]. Neutrophils and macrophages, are generally considered to be the most frequent source of endogenous , reactive oxygen species (ROS) [6-8]. Proinflammatory substances as cytokines, and lipid mediators may be activating neutrophils to generate ROS.

Other sources of ROS include mitochondrial electron transport chain, cytochrome P450, and xanthine oxidase [2], [12], [15].

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Lung tissue is protected against these oxidants by a variety of antioxidant mechanisms for example the superoxide dismutases (SODs) -which convert superoxide radicals to hydrogen peroxide, catalase (CAT), and glutathione peroxidase (GPXs) [4], [9].

When the balance between oxidants and antioxidants shifts in favor of the former, oxidative stress occurs [13,14], [16].

In this paper, the influence of acetylcisteine on the 3 ,3'-diarninobenzidine- H_2O_2-peroxidase system was experimentally studied using the photometric method [17,18].

2. Principle of the Method

The first part of the present paper presents an analysis of the latest data in the specialty literature regarding the exposure of the lung to oxidative stress, as the effect of free radicals upon the induction of pulmonary pathology has been already known. The second part presents a study based on kinetic analysis. The study proves that the acetylcisteine is an activator upon the 3,3'-diaminobenzidine-H_2O_2-peroxydase system.

The imunoenzymatic technique called ELISA enables some qualitative and quantitative determinations, based on the action of the enzyme upon a specific substratum that changes its structure and the possibility of absorbing the radiation in ultraviolet (UV) spectrum or visible spectrum (VIS).

Known amounts of acetylcisteine were introduced into the 3,3'-diaminobenzidine-H_2O_2 peroxidase system. The following parameters were determined: kinetic parameters, the dependence of the optical density (OD) versus time and the dependence of the velocity of reaction versus concentration. Five determinations were made for each concentration [19, 20].

The data obtained for each concentration value was processed and shown in graphics using EXCEL.

3. Substances and Apparatus

Well-known firms produce the utilized substances:

- Peroxidase (KC. 1.11.1.7) – Meck (Dannstadt, Germany);
- H2Ch3% - Fannacom (B_v, Romania);
- Phosphate buffer saline (PBS) – Ortho Diagnostic System Inc. (New Jersey, USA);
- 3,3'-diaminobenzidine - Fluka (Buchs, Switzerland);
- Acetylcysteine 200 - ACC, capsules - Producer: Hexal Pharma.

The pH was kept at a constant value (7.4) using the phosphate buffer saline (PBS), providing the best conditions for activating the peroxidase. Experiments were made at room temperature and the photometric analyses were made at a wavelenght of 546 nm.

4. The Work Mode

In the 3,3'-diaminobenzidine-H2O2-peroxidase system the concentration of the enzyme, $[E] = 8\times10^{-8}$ M, and the concentration of the substratum, [S] = 0.946 mM, were kept at a constant value and the concentration of acetylcisteine was variable.

First we determined the dependence of the optical density versus time at variable concentration of drug. A brown quinoid product was obtained from the enzymatic reaction, which was analyzed in the visible spectrum [21, 22].

The volumes and solutions used in the experiment are given in Table 1.

Table 1

The volumes of the reaction agents used in the kinetic study with Acetylcysteine.

Trial	Distilled H 2O (µl) volume	Acetylcysteine (µl) volume	Acetylcysteine(µg/ml)
A	900	0	0
B	800	100	50
C	700	200	100
D	600	300	150
E	500	400	200
F	400	500	250
G	300	600	300
H	200	700	350
I	100	800	400

The percents of the enzymatic activity based on compound concentration within the system were calculated and graphically represented. Then, graphics were generated, analyzing the dependence of optical density versus time and the dependence of velocity of reaction versus concentration (program used: EXCEL

5. Results and Discussions

The authors have characterized the kinetic of enzymatic reactions; they have investigated the dependence of extinction according to time and the dependence of velocity according to concentration. The team has also determined the percentage of enzymatic activity, so as to highlight the extent to which the acetylcisteine has an activating character upon the given system.

The data obtained was processed and graphics were generated to represent the dependence of the optical density versus time for each concentration value.

A linear increase can be observed in Figures 1-3. The equation and the correlation coefficient (R2) were given for each line.

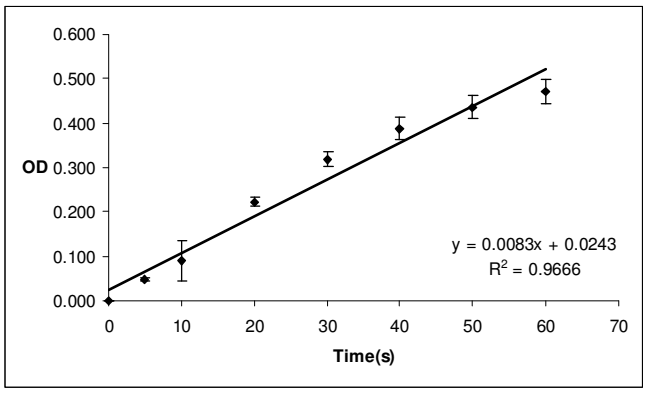

Diagram 1. *Optical density dependence contingent on time for Trial A* [acetylcysteine 0 µg/ml]

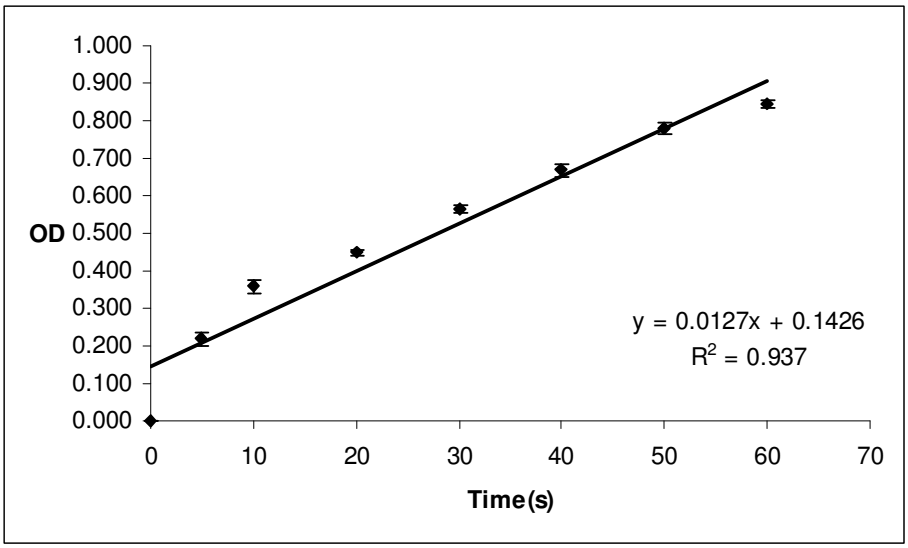

Diagram 2. *Optical density dependence contingent on time for Trial D*
[acetylcysteine 150 µg/ml]

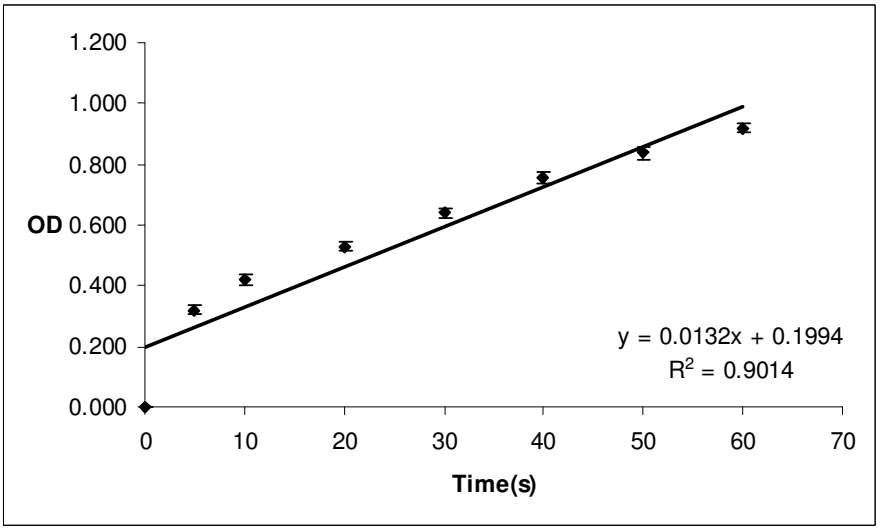

Diagram 3. *Optical density dependence contingent on time for Trial E.*
[acetylcysteine 200 µg/ml]

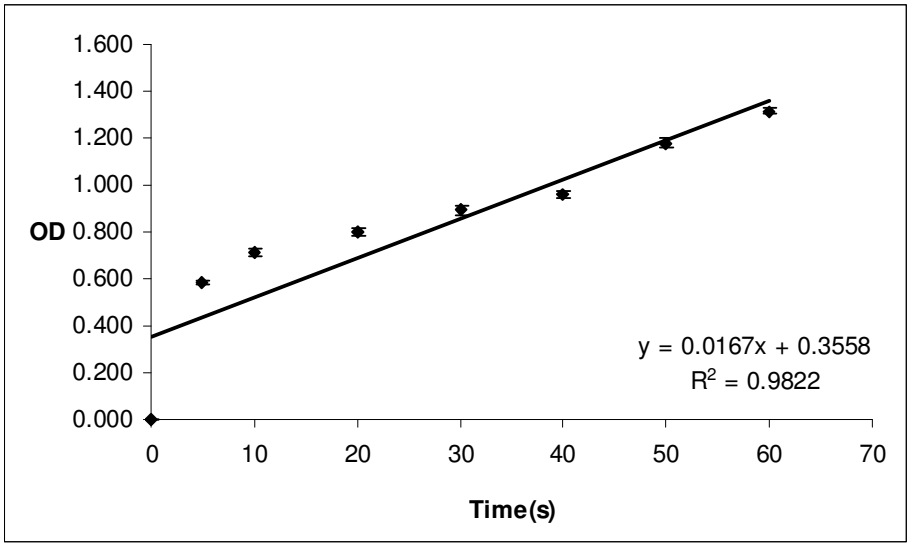

Diagram 4. *Optical density dependence contingent on time for Trial I* [acetylcysteine 400 µg/ml

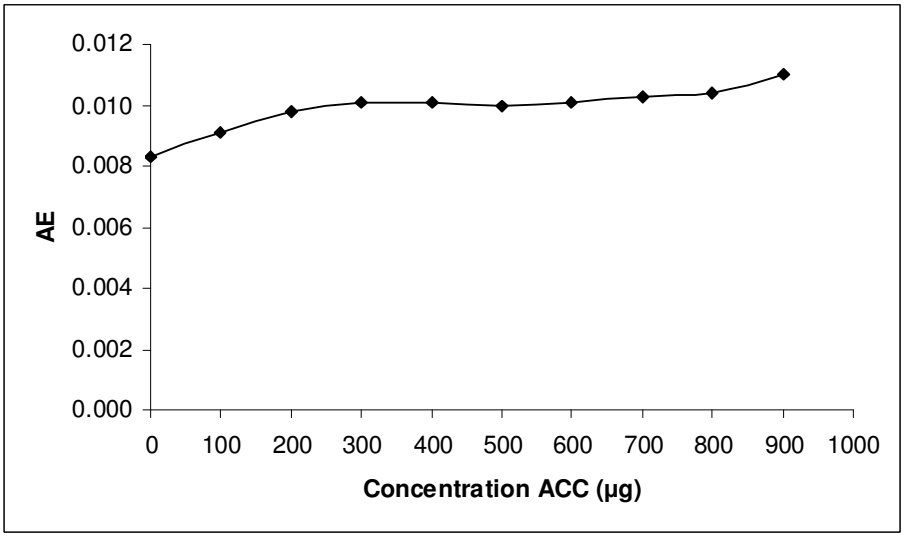

Diagram 5. *Dependence of the reaction rate on the concentration of acetylcysteine*

Diagram 4 represent the dependence of the velocity of reaction (AE) versus the concentration of acetylcysteine. As a result, it was observed an increase in velocity of reaction versus the concentration of acetylcysteine Moreover, between the concentration values of 0-350 µg/ml of acetylcysteine the increase is linear.

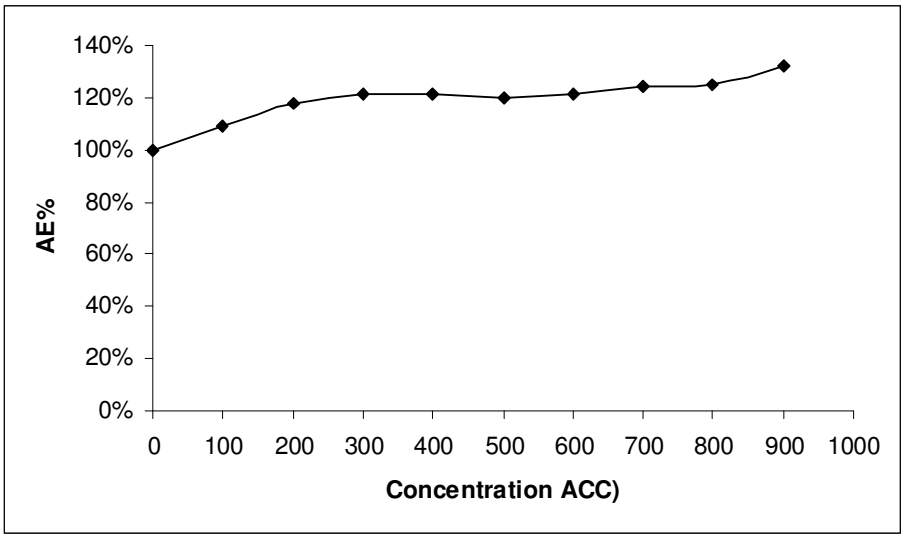

Diagram 6. *Dependence of the change in the percentage of the enzymatic activity on the concentration in the system with acetylcysteine*

It was observed that the percentage of the enzyme activity (% EA) increases with the increase of concentration, reaching values beyond 100%.

6. Conclusions

Following the experiments, the team of researchers has shown the activating influence of acetylcisteine upon the 3,3'-diaminobenzidine-H_2O_2-peroxydase system.

1. The study made demonstrates the activating action of acetylcysteine over the 3.3'– diaminobenzidine – peroxide – peroxidase enzyme system.
2. Experimental data were validated statistically.
3. Observance of the enzymatic kinetics described by Michaelis - Menten was noticed.
4. An increase of the reaction rates and of the enzymatic activities in direct proportion with the increase of the concentrations of acetylcysteine was noticed.
5. In the case of the Acetylcysteine in the 0-350µg/ml concentration interval, this increase is linear. Above this value, the diagram takes the form of a level.
6. The percentage of enzymatic activity increases over 100%, which demonstrates the activating action of the drug over the peroxidase, reaching 200%.
7. In-vitro experimental demonstration of the action of Acetylcysteine over the peroxidase leads to the hypothesis of the existence of some antioxidant effect of this drug.

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