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Post-irradiation effect of Broncho-Vaxom, OM-85 BV, and its relationship to anti-oxidant activities

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This study was conducted to test the efficacy of Broncho-Vaxom (OM-85 BV) in rats after exposure to radiation-induced oxidative stress. Daily administration of Broncho-Vaxom (2.5 mg/kg/day) to rats for a period of 28 days produced a progressive significant increase in the activities of superoxide dismutase (SOD) and catalase in lungs and erythrocytes. No changes were recorded in reduced glutathione (GSH) content in lungs, while an increase was recorded in erythrocytes. Significant increase was also observed in serum γ-globulin content. Intraperitoneal administration of Broncho-Vaxom to rats for 11 days before γ-irradiation and daily during the period of irradiation, delivered as 1 Gy every other day to reach 9 Gy, significantly reduced radiation-induced lipid peroxidation (LPO) measured as thiobarbituric acid-reactive substances (TBARS) in the lungs and erythrocytes. Treatment with Broncho-Vaxom modified the radiation-induced decrease of serum γ-globulins contents. It is postulated that Broncho-Vaxom, by enhancing the antioxidant system and increasing serum γ-globulin content, could play an important role in modifying radiation-induced oxidative stress.

1. Introduction

Broncho-Vaxom (OM-85 BV), a bacterial lysate, is a biotherapeutic product obtained by controlled alkaline hydrolysis of both gram-positive and gram-negative bacteria including *Staphylococcus aureus*, *Streptococcus pyogenes*, *Streptococcus pneumoniae*, *Klebsiella ozaenae*, *Klebsiella pneumoniae*, *Neisseria catarrhalis* and *Haemophilus influenzae* [1]. The compound is an immuno stimulant used for the prevention and treatment of respiratory tract infections [2].

According to Fedorocho and Mackova [3] the compound is a macrophage activator. It increases the number of T helper and natural killer cells, induces the expression of interleukin 6, interleukin 8 [4], and interleukin-12 dependent γ-interferon [5]. Furthermore, Quezada et al. [6] reported that treatment with Broncho-Vaxom increases the concentrations of IgG and IgA.

Broncho-Vaxom is said to have almost no side effects [7]. It does not induce the synthesis of heat shock/stress proteins (HSPs) [8].

Exposure to ionizing radiation results in oxidative stress characterized by an excessive generation of free radicals.

The aim of this work was to examine the effect of Broncho-Vaxom on the antioxidant defense system of lung tissues and erythrocytes of rats, as indicated by the activity of superoxide dismutase (SOD) and catalase, and the content of reduced glutathione (GSH), in parallel to changes in the process of lipid peroxidation. Changes of serum γ-globulins were followed as indicator of the condition of the immune system. The relationship of these changes to the modifying effect of Broncho-Vaxom on radiation-induced oxidative stress was also studied.

2. Investigations, results and discussion

The results illustrated in Table 1 demonstrate that the daily administration of Broncho-Vaxom (OM-85 BV) to rats (2.5 mg/kg) for 28 days significantly increases the activities of superoxide dismutase (SOD) and catalase in the lungs and erythrocytes. This increase was observed on day 22 in the lungs and reached a peak on day 28. In the erythrocytes, the increase was recorded on day 11 and reached a peak on day 28. No significant changes were recorded in the content of reduced glutathione (GSH) in

Table 1: Influence of Broncho-Vaxom (OM-85 BV) on antioxidant activities

Lungs

Days	SOD (U/g protein)		Catalase (U/g protein)		Glutathione (mg/g fresh tissue)	
	Control	Treated with OM-85 BV	Control	Treated with OM-85 BV	Control	Treated with OM-85
11	11.3 ± 0.48	11.7 ± 0.46	3.49 ± 0.16	3.33 ± 0.18	2.98 ± 0.11	3.12 ± 0.16
22	11.7 ± 0.47	14.0 ± 0.73^{ab}	3.61 ± 0.15	4.71 ± 0.21^{ab}	2.69 ± 0.10	3.12 ± 0.17
28	12.0 ± 0.49	17.3 ± 0.88^{abc}	3.51 ± 0.17	5.27 ± 0.18^{abc}	2.91 ± 0.12	2.98 ± 0.15

Erythrocytes

Days	SOD activities (U/g Hb)		Catalase activities (U/g Hb)		c) Glutathione (mg/100 ml RBCs)	
	Control	Treated with OM-85 BV	Control	Treated with OM-85 BV	Control	Treated with OM-85 BV
11	552 ± 35	690 ± 40^{a}	14.08 ± 1.6	21.90 ± 1.5^{a}	67.2 ± 3.35	81.2 ± 4.05
22	570 ± 31	772 ± 41^{a}	13.95 ± 1.7	21.80 ± 1.7^{a}	70.1 ± 3.61	98.6 ± 5.91^{a}
28	585 ± 29	1211 ± 51^{abc}	14.28 ± 1.6	41.80 ± 2.8^{abc}	69.2 ± 3.81	105.0 ± 8.11^{ab}

Each value represents the mean of 6 values ± S.E.
a: significant difference from control
b: significant difference from rats receiving OM-85 BV for 11 days
c: significant difference from rats receiving OM-85 BV for 22 days

Table 2: Influence of Broncho-Vaxom (OM-85 BV) on serum γ-globulins content (g/100ml)

Days	Control	Treated with OM-85 BV
11	0.93 ± 0.09	1.23 ± 0.08^{a}
22	0.98 ± 0.08	1.37 ± 0.04^{a}
28	0.94 ± 0.11	1.31 ± 0.07^{a}

Each value represents the mean of 6 values ±S.E.
a: significant difference from control.

the lungs. However, in erythrocytes an increase was recorded on day 22 and reached a peak on day 28. The increase of superoxide dismutase (SOD), catalase and glutathione might be due to an increase in their expression in response to the superoxide production ($O_2^{\cdot -}$) resulting from Broncho-Vaxom treatment. Superoxide dismutase (SOD) catalyzes the reduction of $O_2^{\cdot -}$ to hydrogen peroxide, which is degraded by catalase into water and O_2 [9]. Another protective mechanism against hydrogen peroxide is the glutathione peroxidase system, the activity of which depends on the presence of adequate amounts of reduced glutathione (GSH) [10].

Administration of Broncho-Vaxom resulted in a significant increase in serum γ-globulin content on days 11, 22, and 28 after treatment (Table 2). Administration of Broncho-Vaxom produced a significant increase in serum IgG and IgA levels [6]; [11] which might explain the increase in serum γ-globulins recorded.

Exposure to ionizing radiation enhances lipid peroxidation in cellular membranes [12]. The presence of an adequate amount of GSH [13]; SOD and catalase minimizes lipid peroxidation [14]. Our results revealed that exposure to ionizing radiation enhances the process of lipid peroxidation in lung tissues and erythrocytes. This is demonstrated by a significant increase in the levels of thiobarbituric acid-reactive substances (TBARS). Treatment with Broncho-Vaxom before γ-radiation exposure and during the period of irradiation significantly decreased the content of TBARS in the lungs (Table 3) and erythrocytes (Table 4). The administration of Broncho-Vaxom stimulates the antioxidant mechanisms in the biological system by increasing the levels of SOD, catalase and GSH. Oxidative stress in the biological system due to irradiation is counteracted by these antioxidants. Through this mechanism the reducing effect of Broncho-Vaxom on oxidative stress is established. However, this mechanism is dependent on the radiation dose, on the amount of Broncho-Vaxom administered and on the time at which it is administered.

The data in Table 5 show that the irradiation scheme significantly decreased serum γ-globulin as a result from radiation damage of the immunological mechanisms. Administration of Broncho-Vaxom counteracts this by enhancing γ-globulins.

According to the results of this study, it is concluded that the modifying role of Broncho-Vaxom is mediated through a dual mechanism by stimulation of the antioxidant activities, and of the immune system.

3. Experimental

Male Swiss albino rats, (100–120 g), obtained from the Egyptian Organization for Biological Products and Vaccines were used. Animals were maintained under standard conditions of ventilation, temperature and humidity. Food as standard pellets, containing all the nutritive elements, and liberal water intake were available.

Whole body γ-irradiation was performed with a Canadian Gamma cell-40 (^{137}Cs) at a dose rate of 0.7 Gy/min. Rats were exposed to fractionated dose delivered as 1 Gy every other day to reach a total dose of 9 Gy. This dose schedule chosen is nearly equivalent to an acute single dose of approximately 7 Gy, which would cause severe injury in mammals and probable lethality. Since the aim of the paper was to investigate biochemical parameters occurring at the molecular level, it was important to use a dose protocol (9 Gy protracted) without causing death.

Broncho-Vaxom (OM-85 BV) was obtained from Memphis Company for Pharmaceuticals and Chemical Industry, Egypt. The compound was diluted in saline and intraperitoneally administered to rats in a concentration of 2.5 mg/kg/day.

Animals were divided into four groups of six animals each: The control group was not exposed to radiation and not treated with Broncho-Vaxom. The treated group received Broncho-Vaxom (2.5 mg/kg/day) for 28 successive days. The irradiated group was exposed to protracted doses of 9 Gy delivered as 1 Gy every other day. The treated and irradiated group received Broncho-Vaxom (2.5 mg/kg/day) for 11 days, then the rats were exposed to the protracted doses of 9 Gy, and Broncho-Vaxom treatment

Table 3: Lung antioxidant activities and thiobarbituric acid reactive substances (TBARS) in different animal groups

Animal groups	Control	Irradiated	Treated with OM-85 BV	OM-85 BV + Radiation
SOD (U/g protein)	12.01 ± 0.49	19.36 ± 0.99^{a}	17.29 ± 0.88^{a}	17.38 ± 0.76^{a}
Catalase (U/g protein)	3.51 ± 0.17	6.56 ± 0.23^{a}	5.27 ± 0.18^{ab}	5.11 ± 0.19^{ab}
Glutathione (mg/g fresh tissue)	2.91 ± 0.12	3.11 ± 0.13	2.98 ± 0.15	3.17 ± 0.17
TBARS (nmol/g fresh tissue)	493 ± 25.6	789 ± 38.7^{a}	456 ± 29.9^{b}	501 ± 31.2^{b}

Table 4: Erythrocytes antioxidant activities and thiobarbituric acid reactive substances (TBARS) in different animal groups

Animal groups	Control	Irradiated	Treated with OM-85 BV	OM-85 BV + Radiation
SOD (U/g Hb)	585 ± 29.3	998 ± 43.3^{a}	1211 ± 51.2^{ab}	920 ± 41.5^{ab}
Catalase (U/g Hb)	14.28 ± 1.6	28.34 ± 2.1^{a}	41.80 ± 2.8^{ab}	27.80 ± 1.9^{ac}
Glutathione (mg/100ml RBC's)	69.2 ± 3.81	121.0 ± 9.11^{a}	105.0 ± 8.11^{a}	97.6 ± 6.33^{ab}
TBARS (nmol/ml serum)	14.6 ± 0.51	24.1 ± 0.86^{a}	13.6 ± 0.59^{b}	14.8 ± 0.89^{b}

Table 5: Serum γ-globulins in different animal groups

Animal groups	Control	Irradiated	Treated with OM-85 BV	OM-85 BV + Radiation
γ-Globulins (g/100ml)	0.94 ± 0.11	0.66 ± 0.08^{a}	1.31 ± 0.07^{ab}	1.05 ± 0.15^{bc}

Each value represents the mean of 6 values ± S.E.
a: significant difference from control
b: significant difference from irradiated
c: significant difference from treated with OM-85 BV

was continued daily during the course of irradiation for a total period of 28 days.

One day after the last dose of irradiation, six animals of each group were sacrificed. Samples of lung tissues and erythrocytes were obtained. The activity of SOD was determined according to Niskikimi et al. [15]. Catalase activity was assayed following Bergmeyer et al. [16]. The content of GSH was determined according to Beutler et al. [17]. The concentration of TBARS as indicator of lipid peroxidation was determined according to Yoshioka et al. [18]. For the determination of γ-globulins, serum protein electrophoretic pattern using non-denaturing polyacrylamide gel electrophoresis (PAGE) was performed [19]. Total protein content was determined according to Folin Lowry methods of protein assay.

The statistical analysis of data was done by application of t-test and one-way analysis of variance ANOVA at the 95% confidence level [20].

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