Available online on www.ijtpr.com
International Journal of Toxicological and Pharmacological Research 2015; 7(6); 264-268

ISSN: 0975-5160

Research Article

Immunostimulatory Activity of Intracellular Lectin Extract from Actinomycete *Micromonospora aurantiaca*

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Available Online:4th October, 2015

ABSTRACT

Objective: Many immunomodulators have also been discovered among the primary metabolites of microorganisms, such as cell wall components. In the present study, the immunomodulatory effect of intracellular lectin extract from *Micromonospora aurantica* GF44c strain was evaluated *in vivo*. Methods: The immunomodulatory potential of intracellular lectin on the phagocytic activity was measured by the carbon clearance rate test, at different doses (30, 50, and100 mg/kg) respectively. Results: *Micromonospora aurantica* lectin extract increased significatively the phagocytic activity in when compared with the control and thus the clearance rate of carbon was faster after the administration of the actinomycete extract $P<0.05$. Conclusion: From the above findings, it is concluded that intracellular lectin extract possesses potential for augmenting activity of reticuloendothelial system more at high dose (500 mg/kg).

keywords: Immunostimulation, Actinomycete, *Micromonospora aurantica*, lectin, Phagocytic activity , Carbon clearance rate.

INTRODUCTION

Immune system is composed of many interdependent cell types that collectively protect the body from bacterial, parasitic, fungal and viral cells as well as growth of tumor cells[1,2]. Immunomodulation denotes any change in the immune response and may involve induction, expression, amplification, or inhibition of any part or phase response. Stimulation of immune response is required in certain patients, whereas suppression of the immune response is needed in other conditions[3]. Immunomodulators are a diverse array of recombinant, synthetic and natural preparations, often cytokines. Some of these substances, such as granulocyte colony-stimulating factor (G-CSF), interferons, imiquimod and cellular membrane fractions from bacteria are already licensed for use in patients. Immunomodulatory regimens offer an attractive approach as they often have fewer side effects than existing drugs, including less potential for creating resistance in microbial diseases. Novel immunomodulating agents are used for the treatment of various conditions, such as infections, organ transplantation, cancer, rheumatoid arthritis, etc.[4,5]. Hence, screening for better agents and evaluating their immunomodulatory potential is becoming a field of major interests all over the world.

Actinomycetes are one of the largest and most diverse groups of bacteria. They can be found in practically every environmental niche on the planet. One reason for their success is their ability to produce a vast range of secondary metabolites with biological activities, the different active constituents of actinomycetes such as antibiotics, lectins, peptides, have been reported to modulate the immune system in different experimental models[6-9]**.**

In this context, the GF44c actinomycete strain has been isolated from clinical specimens, and was identified as belonging to the rare genus of *Micromonospora*[10].

Micromonospora species are best known for synthesizing antibiotics, especially aminoglycoside, enediyne, and oligosaccharide antibiotics. Thus, their impact on medicine is considerable, and indeed *Streptomyces* and *Micromonospora* species produce many of the best-known antibiotics[11]. Therefore the metabolisme profile indicates *Micromonospora* species may be a good source of immunomodulatory agents.

The present investigation was under taken to preliminarily evaluate immunomodulatory potential of the intracellular lectin extract where it was obtained from *Micromonospora aurantiaca* GF44c, using phagocytic responses by carbon clearance test *in vivo* experimental model.

MATERIALS AND METHODS

Actinomycete strain and fermentation

Clinical isolate of *Micromonospora aurantiaca* GF44c (GenBank accession: JQ972874). was obtained from Dr. Zerizer H, Laboratory of microbiological engineering and applications, University of Constantine 1, Algeria[10]. The actinomycete were grown in two liters of ISP_2 medium [yeast extract (0.4%), malt extract (1%), and glucose

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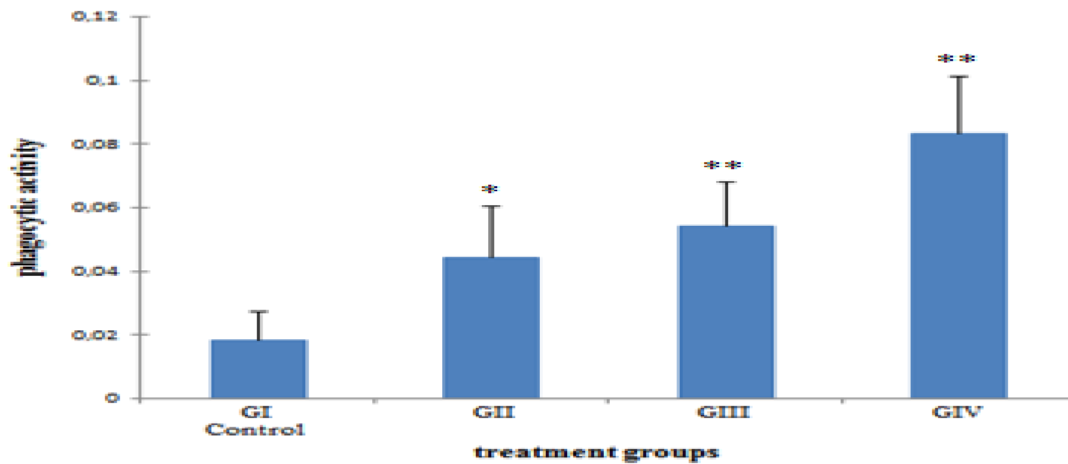
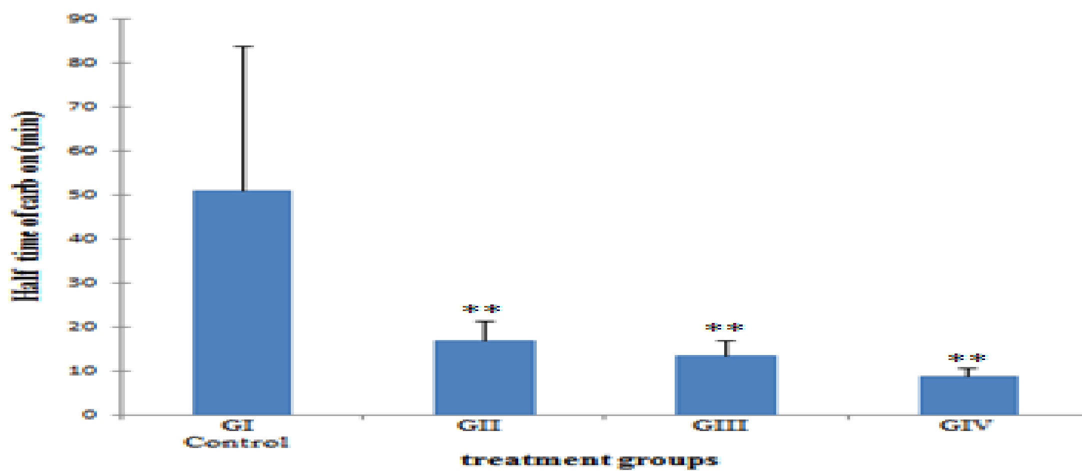
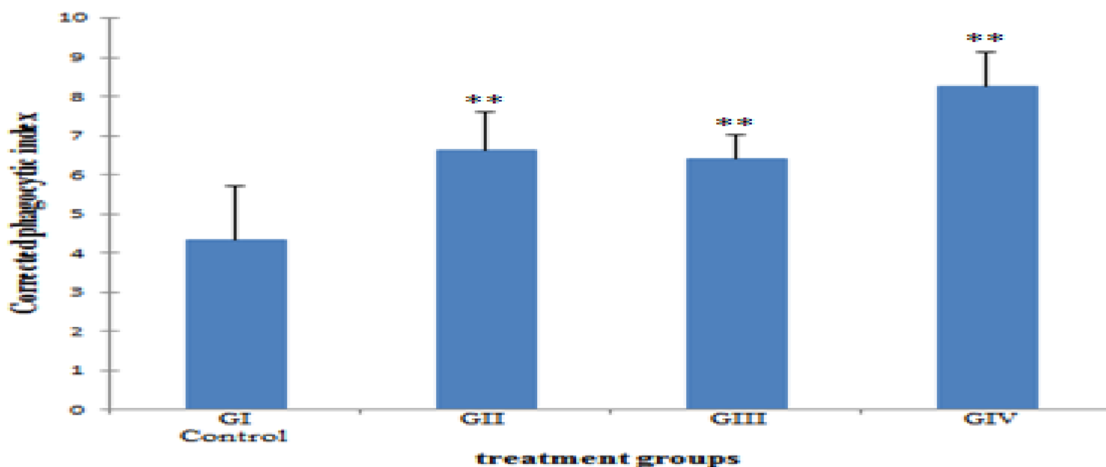


(All values are mean±SEM, n=7,*P<0.05, **P<0.01 when compared to control group)
Figure. 1: Effect of intracellular lectin from *GF44c* strain on phagocytic activity.



(All values are mean±SEM, n=7, *P<0.05, **P<0.01 when compared to control group)
Figure. 2: Effect of intracellular lectin extract from *GF44c* strain on removal speed of crabon from blood.



(All values are mean±SEM, n=7, *P<0.05, **P<0.01 when compared to control group)
Figure. 3: Effect of crude lectin from *GF44c* strain on corrected phagocytic index.

(0.4%), pH 7.5], for 6 days on a rotary shaker (200 rpm), at 28°C.

Lectin extraction and purification

The cells were harvested, washed 3 times in 0.01 M phosphate buffered saline (PBS), pH 7.2, and suspended to obtain a 20% suspension. The cells after treatment with lysozyme (0.2mg/mL, 10min at 4°C) were disintegrated by ultrasonic vibrations at a power of 50 to 60 watts intermittently for 3 min. This suspension was centrifuged at 25.000 x g for 30 min at 4 °C and the supernatant thus obtained was filtered. The isolation and purification of the lectin from the crude extract was obtained by a two-step procedure. This involved an initial ion-exchange chromatography of the crude extract on DEAE-cellulose,

carried out according to the method of Knight (1967)[12] using a linear 0.15-1.5 M NaCl gradient. In the second step, the fractions with the haemagglutinating activity from the first step were subjected to gel filtration on a Sephadex G-200 column.

Animals

Mus Musculus male mice (25-35 g) were obtained from central pharmacy of Algeria. The Animals were housed under standard conditions of temperature (25±1°C) and up to 12 h of light daily, fed with standard pellet diet and had free access to water. All the experiments were performed in accordance with the institutional animal ethics committee.

Phagocytic index

Mice were divided into four groups, of seven animals each. The working solution of lectin extract for experimental groups (GII, GIII, and GIV) was prepared by dissolving it in saline to yield a dose of 30, 50, and100 mg/Kg body weight respectively. Control group (GI) received 0.5mL of normal saline, while each experimental group received by i.p injection 0.5mL of the extract with different concentrations. After 48h, the mice receive an intravenous injection of Indian carbon ink at a dose of (0.1mL/10g). The blood samples were taken from the retro orbital vein by using glass capillaries, at 5 and 15 min. Blood sample (30µL) were mixed with 4mL of 0.1% sodium carbonate solution for the lysis of erythrocytes and the absorbance measured at 675 nm using a spectrophotometer[13]. Then the liver and spleen of individual mice were culled and weighed immediately. The phagocytic activity is expressed by the phagocytic index K which measures all the RES function in the contact with the circulating blood, and by the corrected phagocytic index α which expresses this activity by unit of weight of active organs: liver and spleen. The clearance rate is expressed as the half-life period of the carbon in the blood (t1/2, min). These are calculated by means of the following equations[14,15]:

$$K = \frac{(lnOD1 - lnOD2)}{(t2 - t1)} \quad ,$$

$$t_{½} = 0.693 / K \quad ,$$

$$\propto = \sqrt[3]{K}\,\frac{Body\ weight\ of\ animal}{Liver + Spleen\ wt}$$

Where OD_1 and OD_2 are the optical densities at times t_1 and t_2 respectively.

Statistical analysis

Statistics were applied by using XLSTAT 2013 for Windows. The statistical significance was assessed using one-way analysis of variance (ANOVA) followed by Dunnett's comparison test. The values are expressed as mean±SEM and P<0.05 was considered significant.

RESULTS

The faster removal of carbon particles has been correlated with the enhanced phagocytic activity. The phagocytic activity of the reticuloendothelium system (RES) was measured by the removal of carbon particles from the blood circulation. The present data showed that administration of intracellular lectin extract at concentrations of 50 and 100mg/kg, increase significantly the clearance rate of carbon by the cells of the RES (phagocytic index K) when compared with the control group P<0.0001 (Figure 1). This indicates that intracellular lectin extract enhanced the phagocytic activity by stimulating the reticuloendothelial system.

Figure 2 shows a significant decrease in half-time of carbon in blood dose-dependent in treated groups where we compare them with control (P<0.005). This result confirms that the administration of the extract stimulates reticuloendothelial system to eliminate the carbon particles, and the rate of elimination is reciprocal to the dose administered to mice.

The last part of this study showed that the corrected alpha was increased highly and significantly in treated groups when compared to the control group (p<0.005). This increase is more pronounced among the group GIV that received a dose of 100mg/Kg, and showing the involvement of organs such as the liver and spleen in the removal of carbon particles from the blood, after stimulation by intracellular lectin of *Micromonospora aurantiaca*.

DISCUSSION

Virtually every cell except the mature erythrocyte ingests particulate materials from its surrounding by receptor-mediated pinocytosis. Phagocytic ability is an important element of cellular immunity and it differs from pinocytosis by bigger size of ingested particles and stronger dependence on the inhibitory effects of cytochalasins and low temperature[16].

Phagocytosis provides the first line of defence of the host against infectious microorganisms. The primary role of phagocytosis is the removal of microorganisms, foreign particles and also the elimination of dead cells. Phagocytic defects are associated with various pathological conditions in human[17].

In the body of higher species, there are two mostly recognized 'professional phagocytosis: polymorphonuclear (PMN) leukocytes (neutrophils and eosinophils) and mononuclear phagocytes (monocytes and macrophages). Macrophages have a major role in immunomodulation. The primary target of most immunomodulators is believed to be macrophages which play a major role by engulfing pathogens (or) foreign particles and initiating innate immune response which in turn orchestrate the adaptive response. The PMN cells emerge from the marrow as mature cells, which circulate in the blood for about 10 hours before migrating to the tissues where they perform their effector functions for 1 or 2 days. In contrast, mononuclear phagocytes emerge from the marrow as immature cells monocytes, circulate in the blood and then enter tissues[16].

In the experimental animals, carbon clearance test can determine the influence of immunomodulators on change in macrophage phagocytic activity through reticuloendothelial system. This system consist of the spleen, thymus and other lymphoid tissues, together with

cells lining the sinuses of the spleen, bone marrow, and lymph nodes and capillary endothelium of the liver (Kupffers cells), and of the adrenal and pituitary glands[18,19].
The absorbance of lysed blood samples at 675 nm was proportional to the amount of residual carbon suspension in the blood. The phagocytic index was determined by carbon clearance method. When the carbon suspension was injected intravenously, the rate of clearance of carbon from blood by macrophage was governed by an exponential equation[14]. This seems to be the general way in which inert particulate matter was cleared from the blood. Increase of carbon clearance is an indicator of enhanced *in vivo* phagocytic activity and also competency of granulopoetic system in removal of foreign particles[20].
In this study, treated groups, exhibited significantly high phagocytic index. This indicates stimulation of the reticuloendothelial system. Improving the non-specific immunity and increase the phagocytic activity of mononuclear macrophages, resulting in opsonization of corbon particles with antibody and complement C3b, leading to more rapid clearance of particulate matter from the blood[21-23]. The results of this study agrees with those of Boulahrouf *et al*.[24] who reported that the administration of intracellular crude extract of *Nocardiopsis dassonvillei* in the mouse are increased the phagocytic index at different concentrations.
It is well known that the RES can be stimulated by live or attenuated or killed bacteria and by bacterial endotoxins (lipopolysaccharides) and lectins. The increased nonspecific immunity is considered in part as the result of an activation of the ingestive and bactericidal capacity of the RES macrophages[25,26]. A first a phase that reaches a maximum 3-5 days after treatment or infection. Phagocytic activity then progressively decreases, reaching a normal level around the day 7-9. The prolonged enhancement of phagocytosis was paralleled by a considerable increase in the weight of the liver and spleen form many bacteria. From some strains of microorganisms we have extracted a partially purified fraction mainly composed of lipids and polysaccharides (Muramyl dipeptide) which are responsible for RES stimulation, other substances of microbial origin, such as zymosan or dextran, also stimulate the phagocytic activity of reticulum endothelial cells in experimental animals[27].

CONCLUSION

Immunomodulation is the regulation of immune responses by stimulating them to prevent infectious diseases or by suppressing them in the undesired conditions. The present study showed us that *Micromonospora aurantiaca* lectin stimulates the reticuloendothelial system of mice, this stimulation results in an increase in phagocytic index. This intracellular lecin also requires further detailed investigations for their exact mechanism of immunomodulation.

CONFLICT OF INTEREST

The authors have declared that there is no conflict of interest.

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