Original Article

Immunological evaluation of OMP-F of native Iranian *Pseudomonas aeruginosa* as a protective vaccine

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Abstract

Introduction: This study involved 300 *Pseudomonas aeruginosa* strains isolated from patients admitted in four Tehran hospitals. Using standard O-specific typing sera, they were all grouped into 16 strains out of 17 known *P. aeruginosa*. The strains were lyophilized and each was given a code according to the Collection of Standard Bacteria, Pasteur Institute of Iran (CSBPI) for further investigations.

Methodology: Among all clinical samples, CSBPI: 16-190 was the most prevalent *P. aeruginosa* serotype which showed a high agglutination titer (4+, 320) against homologous O-specific typing sera. This serotype was selected for extraction of *P. aeruginosa* major outer membrane vesicles (OMP-F). OMP-F vesicles were extracted and purified according to the Deoxycholate Ultracentrifuge Differentiation Technique. Purity and molecular weight of OMP-F were determined by SDS-PAGE and the ability of OMP-F vesicles to induce high titers of antibody in rabbit, which was shown as a sharp antibody-antigen precipitation line in the agarose gel immune-diffusion technique.

Results: Passive immunization of mice with anti-rabbit OMP-F antisera induced a high level of protection when the mice were post-challenged with 2×LD50 of live *P. aeruginosa* CSBPI: 16-190. Furthermore, active immunization of mice with 50 µg of OMP-F could protect mice against 2×LD50 of live homologous (100% protection) and 15 heterologous native Iranian *P. aeruginosa* serotypes with 50-100% level of protection.

Conclusions: These investigations indicate that purified OMP-F of CSBPI: 16-190 can be regarded as a safe protective immunogen in vaccinothrapy against all *P. aeruginosa*immunotype isolated in Iran.

Key words: *Pseudomonas aeruginosa*; OMP-F; purification; protective immunogen

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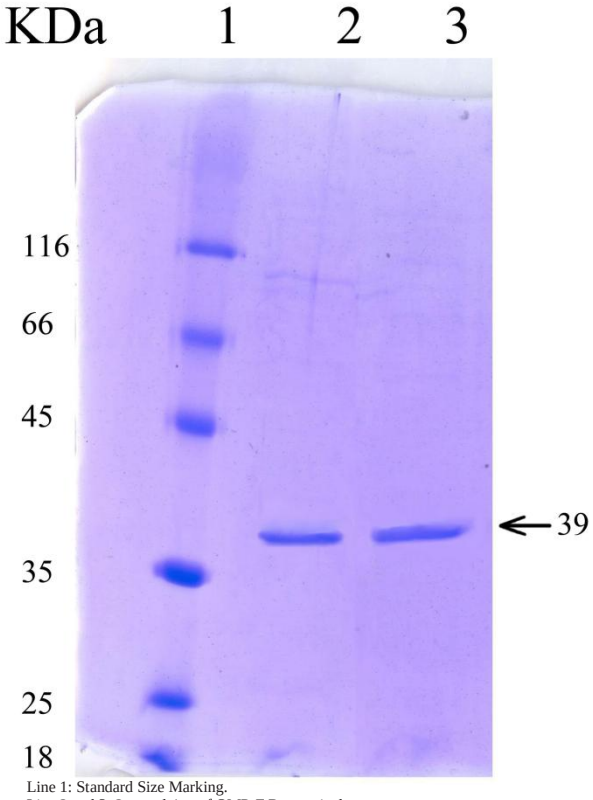
Introduction

Pseudomonas aeruginosa, a ubiquitous environmental Gram-negative microorganism, is a major cause of hospital-acquired infections [1,2]. It is one of the most causatives against of nosocomial infections worldwide and prophylaxis is a standard goal for its control and prevention [3]. Being parasitic and saprophytic in nature, *P. aeruginosa* generally is responsible for acute and chronic lung infections in artificially ventilated [4] and in cystic fibrosis patients [5], and for septicemia in immunocompromised patients, including transplant and cancer patients, as well as patients with severe burn wounds [6]. Using the International Antibody Typing Standard (IATS), *P. aeruginosa* can be classified into 17 serotypes [7], but about 16 serotypes are prevalent in Iran [8]. Serotype CSBPI: 16/190 was one of the most cross-reactive of serotypes.

Among Iranian isolates, LPS or detoxified derivatives from *P. aeruginosa* provide good protective activities in animal models, but their pathophysiological adverse effects limit their application in vaccines. Therefore, major outer membrane proteins (OMP-F) from serotype CSBPI: 16/190 with a molecular weight of 37-39 KDa can be regarded as a safe and reliable vaccine candidate. Different antigens of *P. aeruginosa*, such as the outer membrane proteins (OMPs), LPS, toxins, pilli and flagella, have been investigated as possible targets for the development of vaccines. Vaccination with outer membrane protein antigens has been shown to be efficacious against *P. aeruginosa* infection in a number of studies using killed whole cells [9], isolated outer membrane proteins [10-13] and purified outer membrane preparations [14]. The *P. aeruginosa* major constitutive porin protein, OMP-F, which has previously been shown to be antigenic [10,15] and has high homology among *Pseudomonas* strains [12,16], was also chosen as a vaccine target [17]. This protein has been shown to provide protection in a mouse

Figure 1. SDS-PAGE Electrophoresis profile of OMP-F of *P. aeruginosa* CSBPI: 16/190



Line 1: Standard Size Marking.
Line 2 and 3: 2ug and 4ug of OMP-F Respectively.

model of systemic infection [10], a mouse burn infection model, and in rodent models of acute [18] and chronic lung infection [12]. In the present study, OMP-F was obtained from Iranian serotype CSBPI: 16/190 through a mild extraction procedure as described by Classen *et al*. [19], then the immunological characteristics of the OMP-F was evaluated through passive and active immunization challenges with homologous and heterologous *P. aeruginosa* serotypes in mouse models.

Methodology

Bacterial strain

Strain CSBPI-16-190 was selected from 300 *P. aeruginosa* biochemically and serologically confirmed clinical isolates [8]. The selection was based on the cross-reactivity between the antibody raised against the heat-inactivated CSBPI-16-190 and other *P. aeruginosa* isolates belonging to serotypes 1-15. Active protection was tested using 300 *P. aeruginosa* serotypes 1-15 isolated from clinical samples [8]. This strain was further selected for extraction of OMP-F.

Culture conditions

P. aeruginosa CSBPI-16-190 was cultured into 10 liters of synthetic *P. aeruginosa* medium [20] using a Novo-paljas Bioreactor (Contact-flow B.V., Bilthoven, Netherlands) at optimum cultural conditions. At the end of logarithmic phase of growth, the cells were harvested by centrifuge at 3000 rpm for 45 minutes at 4°C.

Extraction of OMP-F

OMP-F was extracted from *P. aeruginosa* CSBPI-16-190; using the method described by Classen *et al*. [19] with mild modifications as follows:

Cells were washed three times with phosphate buffered saline (PBS, pH = 7.2) and suspended in 7.5 times its wet-weight with 0.1 M Tris-HCl buffer containing 10 mM EDTA. After two hours of shaking at room temperature, 20 times (V/V) 0.1 M Tris-Buffer containing 10 mM EDTA and 100 grams of sodium deoxycholate were added to the cell suspension. After 10 minutes of shaking, the suspension was centrifuged at 8000 rpm for one hour at 4°C. The cells were then discarded and the

Table 1. Passive immunization in mice with hyper immune serum against 2×LD50 of *P. aeruginosa*

Group	Challenge Dose	No. of Survival	Survival Rate %	P-Value
Test	**$2\times LD_{50}$**	**10/10**	**100%**	**0.001**
Control	**$2\times LD_{50}$**	**0/10**	**0%**	**0.001**

Table 2. Active immunization of OMP-F vesicle of *P. aeruginosa* CSBPI: 16/190 in mice challenged with 2×LD50 of live cell homologous and heterologous isolates

Challenge Strain	Challenge Dose	No. of Survival	Survival Rate %
CSBPI:1/101	3×10^8	5:10	50
CSBPI:2/160	3×10^8	8:10	80
CSBPI:3/172	3×10^8	5:10	50
CSBPI:4/89	6×10^8	8:10	80
CSBPI:5/60	6×10^8	10:10	100
CSBPI6/109	5×10^8	5:10	50
CSBPI:7/107	4×10^8	8:10	80
CSBPI:8/98	5×10^8	7:10	70
CSBPI:9/105	7×10^8	6:10	60
CSBPI:10/55	4×10^8	7:10	70
CSBPI:11/106	5×10^8	6:10	60
CSBPI:12/195	3×10^8	10:10	100
CSBPI:13/108	3×10^8	6:10	60
CSBPI:15/14	5×10^8	6:10	60
CSBP1:16/190	3×10^8	10:10	100
CSBPI:17/110	6×10^8	6:10	60

Figure 2. Double immunodiffusion in 1% agarose of OMP-F of *P. aeruginosa*

Well 1 and 3: 500ug Purified OMP-F
Well 2: Rabbit hyper immune antibodies against OMP-F
Well 4: 500ug OMP (Por A) of *Neisseria meningitides* type B as Negative control

supernatant was centrifuged at 42000 rpm for two hours.

The sediment was dissolved in 15 ml DW containing 3% sucrose, and then filter-sterilized and lyophilized. SDS-PAGE electrophoresis was performed in 10% gel using standard-size markers (Figure 1) and the protein content was measured using the Lowry method with BSA as a reference.

Production of hyper immune antibody

Hyper immune antibody was prepared by intramuscular injection of 500 µg of the *P. aeruginosa* OMP- F vesicle along with Fronds incomplete adjuvant into a group of three New Zealand white rabbits weighing 1.5 - 2 kg, on days 0, 7, 14, 21 and 28. Seven days after the last injection, serum was collected from each immunized rabbit and pooled. The precipitation reaction of hyper immune antiserum against the purified OMP-F was evaluated by double agarose gel immunodiffusion technique.

Passive immunization

Passive immunization was performed as described by Gilleland *et al*. [12] (Table 1). A group of five young adult mice weighing 18-20 grams received 0.1ml of hyper immune antiserum against OMP-F by I/V injection. PBS (pH = 7.2) was used as the control [12].

Active immunization

Active immunization against homologous and heterologous serotypes was performed using animal models (mice) by subcutaneous injection of 50 µg of OMP-F according to the methods of Pier *et al*. [21]. Active protection of OMP-F vesicle of CSBPI: 16-190 *P. aeruginosa* was evaluated against homologous and heterologous isolates (Table 2).

Pyrogenic test

Pyrogenic tests were performed according to the British Pharmacopeia (2007) annex 2.6.8, through

intravenous injection of purified OMP-F into the group of three rabbits weighing 2 to 2.5 kg.

Abnormal toxicity test

The purified OMP-F was tested for abnormal toxicity by intra peritoneal injection of 100 µg purified OMP-F into five mice weighing 17-22 g and 500 µg purified OMP-F into two guinea-pigs weighing 250-350g. The test was considered satisfactory if the animals survived for at least seven days without weight loss.

Results

OMP-F was extracted from CSBPI: 16/190 as a result of the high cross-reactivity between this serotype and all the 300 *P. aeruginosa* isolates. Furthermore, this serotype was the most common pathogenic isolate from Iranian hospitals [8].

The molecular weight of OMP-F was found to be 37-39 KDa by SDS-PAGE electrophoresis (Figure 1) and the high precipitation line of reaction in agarose gel immuno diffusion was observed between purified OMP-F and its hyper-immune rabbit antibodies (Figure 2).

The results of the toxicity and pyrogenicity tests showed that OMP-F was safe and did not cause any adverse effects after injection into the animals.

As shown in Table 1, passive immunization of mice with hyper immune OMP-F antiserum protects mice against 2×LD50 of the live homologous serotype. Moreover, good protective activity of the OMP-F was observed by active immunization of mice against 2×LD50 of live homologous and heterologous serotypes (Table 2). It seems that there is a close immunological cross-reactivity among OMP-F isolated from different *P. aeruginosa* isolates.

Discussion

Today, the protective activities of subunit vaccines—mainly major outer membrane proteins of many Gram-negative bacteria—are well-known [12,22,23]. There is no reliable *P. aeruginosa* vaccine produced so far, although many attempts have been made [10,14,24,25]. In 1984, Gilleland *et al*. used the porin of *P. aeruginosa* as a protective vaccine in mice [10]. Pier *et al*. induced good protective activity by immunizing mice with capsular polysaccharide of *P. aeruginosa* [21]. However, this activity was not observed in man and in 2000 Lee *et al*. [26] reported that OMPs elicit antibodies with protective efficacy against *P. aeruginosa* infection in burn patients. In 2006, Cripps *et al*. used heat-killed *P. aeruginosa* as a vaccine against the infection, but this immunogen caused serious adverse effects.

In the present investigation, out of 300 *P. aeruginosa* strains isolated from clinical samples of hospitalized patients in Iran, serotype CSBPI: 16/190 was selected to extract OMP-F because this serotype was the most pathogenic isolate prevalent in Iran and also showed O-antigenic similarities with all 300 isolates [8]. Our results also indicate that purified OMP-F induced not only good protective efficacy against homologous serotypes of *P. aeruginosa* infection, but also protected the mice against all 15 heterologous isolates, suggesting that there is good immunological cross-reactivity among purified OMP-F of different serotypes. Therefore, OMP-F extracted from serotype CSBPI: 16/190 was considered to be a reliable and safe immunogen with broad spectrum activity in regard to its application in therapeutic vaccines or prophylaxis. Thus purified OMP-F isolated from serotype CSBPI: 16/190 can be a safe and effective candidate subunit vaccine against infection caused by different *P. aeruginosa* serotypes.

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