Investigation the Immunoadjuvant Activity for Polysorbate 80

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ABSTRACT— ***Immune compromization is the mainstay of the problems that threat health setting in the world. Assessment of surfactant (polysorbate 80) in immunological effects can focus the light on new solutions for these problems. The immunoadjuvant activity of polysorbate 80 was assessed in animal labs by testing the levels of mediators for both humoral and cell-mediated immunity against the tested bacteria which were the somatic antigens of MRSA(methicillin resistsnt Staph.aureus) and Pseudomonas aeruginosa. Polysorbate 80 exhibits a high level of immunoadjuvant activity for both humoral and cell-mediated immunity against bacterial antigens. Polysorbate 80 has a great immunoadjuvent activity against the multidrug resistant bacteria.***

Keywords — Polysorbate 80, Pseudomonas aeruginosa, immunoadjuvant

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1. INTRODUCTION

Surface-active agents (surfactant) are defined as amphiphilic or amphipathic molecules, *i.e.*, they have one part that has an affinity for non-polar media and one part that has an affinity for polar media. These molecules form oriented monolayers at interfaces and show surface activity (they lower the surface or interfacial tension of the medium in which they are dissolved) [1]. Polysorbates generally refer to ethoxylated derivatives of fatty acid esters of sorbitan (commercially referred to them as Tweens®). They are perhaps one of the most commonly used nonionic surfactants, since they are sugar-based surfactant and considered as the safest surfactant (renewable surfactant) [2]. Polysrbate 80 (ps 80) (Tween 80® or polyoxyethylene [20] sorbitan monooleate) is an oleate ester of sorbitol co-polymerized with about 20 moles of ethylene oxide for each mole of sorbitol [3].

In the immunological field, ps 80 is preferably used in the concentration of about 0.001 % to about 2.0% (w/v) most preferably, the ps 80 is present for 0.02%. The addition of ps 80 to the pharmaceutical formulations overcomes the problem of precipitating or aggregating antibody when preparing formulations with higher protein contents [4] Sometimes ps 80 cause reduction of intracellular glutathione level but with an elevation of intracellular calcium level therefore, it can be suggested that ps 80 modifies some of membranes, intracellular physiological and certain immunological parameters without affecting the cell viability [5]. This study aimed to investigate the immunoadjuvant capacity of ps 80 against clinical bacterial isolates of MRSA(methicillin resistsnt *Staph.aureus*) and *Ps.aeruginosa*.

2. MATERIALS AND METHODS

2.1 Animals

Albino Swiss male rats brought from the Embryo Research and Infertility Institute- University of Baghdad. They were caged for acclimatization for 3 months in the Animal House- College of Medicine – Babylon University. Rats weight was ranged 150-250 g. The animals were kept on normal pellet diet, standard room temperature and normal diurnal rhythm.

2.2 Bacterial isolates

Clinical isolates of MRSA(methicillin resistsnt *Staph.aureus*) and *Ps.aeruginosa* were provided from department of Medical Microbiology College of Medicine Babylon University. Both of them were clinically isolated from patients with urinary tract infection.

2.3 Study design

Animals grouped randomly into seven groups. Each group was consisted of (3) rats as shown in the table (1).

Table 1: Classification of rats groups according to treatments received and bacterial antigens

Group number	Treatment received	Bacterial antigens
1	Bacterial antigen only	*P. aerugenosa*
2	Surfactant only	*P.aerugenosa*
3	Bacterial antigen+ surfactant	*P. aeruginosa*
4	Bacterial antigen only	MRSA
5	Surfactant only	MRSA
6	Bacterial antigen + surfactant	MRSA
7	Control (neither treatment received nor belong to any bacterial class)	

2.4 *The preparation of bacterial somatic antigens*

Somatic antigens of (MRSA and *P.aeruginosa*) were prepared by according to [6] and [7].

2.5 *Schedule of immune challenge*

After grouping , the rats were given a specified amount of treatment (surfactant, bacterial antigen or both) via intra peritoneal routes. One of the groups received neither surfactant nor bacterial antigens as control group. Surfactant solutions were given to MRSA and *P.aeruginosa* groups in a concentration of 1% and 6% polysorbate 80 respectively as both ps 80 concentrations were the most effective concentrations that potentiate the *in vitro* antibacterial efficacy of some antibiotics. Surfactant solutions were prepared and administered under aseptic conditions [8][9].

2.6 Immunologic effects of polysorbate 80

A. *Phagocytic index*

Phagocytic index was performed according to the procedure that was outlined by [10].

B. Estimation of interferon- gamma(IFN- γ)

The procedure of IFN- γ estimation carried out according to the information supplied by Biosourse® Co.

C. *Determination of complement components*

The complement components C3 and C4 were measured by using *the technique of single radial immunodiffusion test (SRID)*according to the [11].

D. Determination of antibacterial antibody titer

Antibody titer was determined by using of standard agglutination tube method.This method was carried out according to [6].

2.7 Statistical analysis:

Data were presented in Mean ± standard deviation (SD). T-test, analysis of variance (ANOVA) and regression and correlation analysis at different p values usually ($p<0.05$) considered the least significance level used to analyze the results according to [12].

3. RESULTS

3.1 Neutrophile function (phagocytic activity)

The results of phagocytic activity of the groups challenged with *P. aeruginosa* somatic antigen (*P.* antigen), ps 80 or both were expressed in the table (2).The results showed that group 4 (*P.* antigen+ ps 80 combination) group exhibited a highly significant elevation in phagocytic activity ($p<0.01$) when compared with the all other groups.

Table 2: Effects of polysorbate 80 (6) % (v/v) on phagocytic activity against *Pseudomonas aeruginosa*

expressed as phagocytic Activity percentage (PA %). The data expressed as (mean ±SD)

No.	Group	Phagocytic index % (mean ± SD)	Significance (P<0.01)
1	Control	2.66 ± 0.57	A*
2	Ps 80 6% (v/v) alone	2.73 ± 1.15	A
3	*P* antigen alone	8.66 ± 1.15	B
4	*P* antigen+ ps80 combination	21.33 ± 1.15	C

*The identical letters indicate that non-significant relationship is present, while the different letters indicate significant one.

The results of phagocytic activity of the groups challenged with MRSA somatic antigen (MRSA antigen), polysorbate 80 or both were expressed in the table (3). The results showed that group 4 (MRSA antigen+ ps 80 combination) exhibited a highly significant elevation in phagocytic activity ($p<0.01$) when compared with the all other groups.

Table 3: Effects of ps 80 (1) % (v/v) on phagocytic activity against MRSA antigen

expressed as Phagocytic Activity percentage (PA %). The data expressed as (mean ± SD)

No.	Group	Phagocytic index % (mean ± SD)	Significance(p< 0.01)
1	Control	2.66 ± 0.57	A*
2	Ps 80 1% (v/v) alone	2.73 ± 1.15	A
3	MRSA Antigen alone	11.33 ± 0.57	B
4	MRSA antigen+ ps80 combination	18.33 ± 0.57	C

*The identical letters indicate that non-significant relationship is present, while the different letters indicate significant one.

3.2 Effects of polysorbate 80 on the TH1 response (IFN- γ concentration)

No significant changes in the production of serum IFN- γ level was observed when ps 80 was used in combination with antigen as immune adjuvant when compared with other groups for both isolates.

3.3 The effects of polysorbate 80 on the production of complement system

The results of C3 level of the groups challenged with *P. aeruginosa* somatic antigen (*P* antigen), ps 80 or both were expressed in the table (4). The results showed that group (4) (*P* antigen+ ps80 combination) exhibited a significant elevation in C3 level ($p<0.05$) when compared with the all other groups.

Table 4: Effects of polysorbate 80 (6) % (v/v) on C3 concentration (mg/dl)

against *P. aeruginosa* . The data expressed as (mean ± SD)

No.	Group	(Mean± SD)	Significance P<0.05
1	Control	128.40 ± 3.20	B*
2	Ps 80 6% (v/v) alone	121.70 ± 2.70	B
3	*P* Antigen alone	122.13 ± 2.85	B
4	*P aeruginosa* + ps80 combination	153.00 ± 3.00	A

*The identical letters indicate that non-significant relationship is present, while the different letters indicate significant one.

The results of C3 level of the groups challenged with MRSA somatic antigen (MRSA antigen), polysorbate 80 or both were expressed in the table (5). The results showed that group (4) (MRSA antigen+ ps80 combination) exhibited a significant elevation in C3 level ($p<0.05$) when compared with the all other groups.

Table 5: Effects of polysorbate 80 (1) % (v/v) on C3 concentration in (mg/dl)

against MRSA and the data expressed as (mean ± SD)

No.	Group	Mean ± SD	Significance (P<0.05)
1	Control	148.40±3.20	A*
2	Ps 80 1% (v/v) alone	187.20±2.80	B
3	MRSA Antigen alone	198.80±2.70	C
4	MRSA+ ps 80 combination	218.70±3.20	D

*The identical letters indicate that non-significant relationship is present, while the different letters indicate significant one

The results of C4 level of the groups challenged with *P. aeruginosa* somatic antigen (*P.* antigen), ps 80 or both expressed in the table (6). The results showed that group (4) (*P.* antigen + ps 80 combination) exhibited a highly significant elevation in C3 level ($p<0.01$) when compared with the all other groups.

Table 6: Effects of polysorbate 80 (6) % (v/v) on C4 concentration (mg/dl)

against *P. aeruginosa* . The data expressed as (mean ± SD)

No.	Group	Mean ± SD	Significance (p<0.05)
1	Control	12.20 ± 2.90	B
2	Ps 80 6% alone	16.93 ± 2.41	B
3	*P*. Antigen alone	14.80 ± 2.70	B
4	*P*. antigen + ps 80 combination	30.70 ± 2.60	A

The identical letters indicate that non-significant relationship is present, while the different letters indicate significant one.

The results of the effects of ps 80 on C4 concentration for the rat groups challenged with MRSA somatic antigen or ps 80 or both were expressed in the table (7) which shows that ps 80 when combined with antigen significantly elevate C4 level when compared with administration of antigen alone.

Table 7: Effects of polysorbate 80 (1) % (v/v) on C4 concentration (mg/dl)

against MRSA. the data expressed as (mean ± SD)

No.	Group	Mean± SD	Significance (p<0.05)
1	Control	42.20 ± 1.79	-
2	Ps 80 1% alone	42.20 ± 1.79	-
3	MRSA antigen alone	36.90 ± 1.79	A
4	MRSA antigen + ps 80 combination	44.90 ± 1.79	B

The different letters indicate significant relationship while the relation with (-) indicates non-significant one.

3.4 The effect of polysorbate 80 on TH2 response (humoral immunity)

For the rat groups challenged with *P. aeruginosa* somatic antigen or ps 80 or both were expressed in the table (8), which showed that the titer of anti- *P. aeruginosa* somatic antigen antibody observed with group (4) is significantly higher than other groups.

Table 8: Effects of polysorbate 80 (6) % (v/v) on antibody titer against *P. aeruginosa*.

NO.	Group	Mean ± SD	Significance (p<0.01)
1	Control	16 ± 0.087	A*
2	Ps 80 6% (v/v) alone	16 ± 0.087	A
3	*P.* antigen alone	64 ± 0.087	B
4	*P.* antigen + ps 80 combination	256 ± 0.087	C

*The identical letters indicate that non-significant relationship is present, while the different letters indicate significant one.

For the rat groups challenged with MRSA somatic antigen or ps 80 or both were expressed in the table (9), which showed that the anti-MRSA somatic antigen antibody titer observed with group (4) is significantly higher than all other groups

Table 9: Effects of polysorbate 80 (1) % (v/v) on antibody titer against MRSA

NO.	Group	Mean ± SD	Significance (p<0.01)
1	Control	16 ± 0.211	A*
2	Ps 80 1% alone	16 ± 0.211	A
3	MRSA antigen alone	128 ± 0.211	B
4	MRSA antigen + ps 80 combination	512 ± 0.211	C

*The identical letters indicate that non-significant relationship is present, while the different letters indicate significant one.

4. DISCUSSION

Challenging the rats with somatic bacterial antigens reveals significant ($p<0.01$) elevation of phagocytic activity when compared with control group. This result agrees with [13].

It was found that ps 80 enhanced the phagocytic activity by increasing membrane Ca^{2+} permeability lead to an increase in the intracellular Ca^{2+} concentration [12][14]. Ps 80 is an ionophore, able to sequester and transport across membrane various ions many of which are biologically important like (Ca^{+2}, Na^{+} and K^{+}) [15]

It has been found that Ca^{2+} influx into cells is primarily important in the neutrophils activating effect of an ionophore and neutrophil activation with the ionophore proceeds by the following scheme: Ca^{2+} influx → activation of Ca^{2+} receptor protein, calmodulin → activation of calmodulin-regulated enzymes → metabolic changes, activation [16].

From what has been discussed above, it may be concluded that ps 80 has a direct effect of activation of neutrophils when used as an immune adjuvant through the elevation of intracellular Ca^{2+} level [17].

In this study, it has been found that the level of IFN- γ did not significantly change with or without ps 80 when combined with bacterial antigen. These findings are in agreement with [14], who found that enhancement of IL-5 consider as TH2 product but not IFN- γ secretion in response to *in vitro* stimulation indicates that ps 80 induces a shift in the balance of T helper cells towards Th2 cells or the activation and migration of eosinophils, but does not affect Th1-dominant reactions *in vivo*.

Complement system plays a direct role against bacterial infection by direct killing via the formation of MAC (major attack complex) or opsonization to phagocytic cells [18][19].

In this study, it was found that ps 80 elevate the level of both C3 and C4 for both isolates when combined with the antigens of both bacterial isolates as immune adjuvant than when antigen administered alone. This may be explained as following: First :It was found that ps 80 induce oxidative stress through the reduction of intracellular glutathione level [20]. As well as, polysorbate 80 participate in peroxide formation in vitro when combined with proteins. It was found that oxidative stress implicates and enhances the complement activation [21]. Second: The general impact of surfactants in promotion of protein secretion is likely to involve interactions with the lipid components of cell membranes in a manner, which facilitates secretion. It should note that most of the observations related to the positive effects of surfactants on secretion of extracellular enzymes relate to eukaryotic organisms, which release enzymes from intracellular organelles through exocytosis. This observation suggests that surfactants may promote this exocytosis by interaction with cell and organelle lipid membrane components [22]. Third: expression of many complement components in the liver is

significantly elevated during acute phase response and induced by pro-inflammatory cytokines such as IL-6, IL-1, TNF-α and IFN-γ and IL-6 [23]. IL-6 stimulates hepatic protein synthesis during acute phase response and acts as endogenous pyrogen. In addition IL-6 correlated with the levels of anaphylatoxins C3a and C4a peptides that are generated during the activation of complement system [24]. One of most important mode of action of ps 80 is the stimulation of cytokine synthesis, especially of IL-5 and IL-6 [25], so this elevate acute phase proteins such as complement system.

Heat-inactivated *P. aeruginosa* is an effective means to induce anti- *P. aeruginosa* immunity enhance antigen-specific humoral immunity and may be useful for vaccines designed to induce antibodies against extracellular bacteria. Administration of heat killed *P. aeruginosa*, as a model of a simple bacterial vaccine, protects against a lethal tissue challenge with *P. aeruginosa*. This protection depends on the presence of T helper cells and is associated with a *P. aeruginosa*-specific antibody response. Antigens from MRSA cell wall as well as cell wall associated proteins have been shown to be immunogenic, that are immunogenic these antigens are able to create polyclonal immunoglobulin G (Ig G) against these antigens [26] As in the whole killed cell intraperitoneal, intranasal immunization included both specific humoral and cellular immune responses suggesting that a combination of both is required for optimal vaccine induced protection against *P. aeruginosa* infection [27] where as whole killed cell vaccines, particularly administered orally, are well immune-tolerated [28].

The mechanism of induction of antibodies upon the challenge with bacterial antigen vaccine: Upon infection or immunization, antigens are captured and processed by antigen-presenting cells, in particular dendritic cells, which migrate to local lymph nodes, where interact with naive T cells [29]. B-cells with antigen-specific receptors move to the boundary between B-cell follicles and T-cell zones of lymphoid tissue, allowing the close contact to antigen-specific T cells necessary for optimal B-cell activation [30].

Non-ionic surfactants are well-known immune adjuvants that enhance humeral immunity one of them is ps 80 which act through the followings [30]: i- Stimulate the production of both immunoglobulin M (Ig M) and immunoglobulin G (Ig G); .ii- Surfactants are able to enhance the secretion of antibodies by already activated B cells, which leads to accelerated expansion of the spectrotype especially IgG isotypes; .iii- Polysorbate 80 induces a shift in the balance of T helper cells towards Th2 cells.

According to the reported data, ps 80 can enhance TH2 but not TH1 response. In addition, ps 80 has the ability to increase the phagocytic activity and complement system therefore further study required for the use of ps 80 as immunotherapeutic agent during extracellular bacterial infection involving toxigenic bacteria.from the results of the present work one could conclude that Polysorbate 80 has a great ability to enhance the antibacterial activity and immunological activity against the multidrug resistant bacteria.

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