The Sunflower Oil (SFO) as mucosal Immuno adjuvant In Rabbit *O. caniculus*

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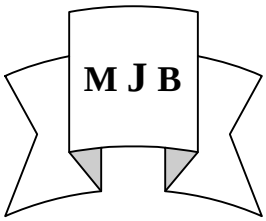

Abstract

The wheat gluten was separated by alkalinzation methods method, wheat gluten peptide antigen (WGPA) was found as a proleine rich gluten. WGPA solutions were used as a test imunogen for immunopriming of rabbits (*O. caniculus*). Six groups of rabbits, each of two were assigned as; Intramuscular Subcutaneous IM-SC saline control group (I), Oral saline control group (II), IM-SC/ WGPA group (III), Oral/ WGPA group (IV), IM-SC-/ WGPA-SFO group (V) and Oral/ WGPA-SFO group (VI). WGPA specific immunesera were separated decomplementized, saved in aliquots at -18 C^o till use. The immunoadjuvant effect was scored by comparing titers in the group V and III as well as VI and IV. The WGPA specific polyclonal antibody titers in the sera of the groups V were as 40960 and III 5120. Likewise, in the groups VI and IV were 40960 and 10240 respectively. The mucosal specific anti WPA antibody titer in group III. Was similarly, it was 3776 in the group VI as it was reaching 1920 in the group IV.

Thus, SFO could be used as a candidate enhancer of mucosal immune responses to the mucosally applied vaccines, for animal walfare at first, then after sophistication for human walfare.

الخلاصة

استخدمت طريقة القلونة لفصل كلوتين الحنطة، وتبين بأن مستضد ببتيدكلوتين الحنطة غني بالبرولين.

استخدم محلول مستضد ببتيدوكلوتين الحنطة كممنع اختياري في التحفيز المناعي للارانب. حيث وسمت ست مجاميع كل من ارنبين. مجموعة سيطرة العضلة-تحت الجلد (I) ومجموعة سيطرة الفموي (II) ومجموعة كلوتين الحنطة-عضلة/تحت الجلد(III) ومجموعة كلوتين حنطة.فموي (IV) ومجموعة كلوتين الحنطة-زيت زهرة الشمس عضلة/تحت الجلد (V) ومجموعة كلوتين حنطة-زيت زهرة الشمس.فموي (VI). امكن التعرف على حصول التحول المناعي لمقارنة المجموعة (III) مع (I) والمجموعة (IV) مع (II). وحصول

الفعل المناعي المساعد بمقارنة المجموعة (VI) مع المجموعة (IV) والمجموعة (V) مع (III). حيث كان عيار الضد المتخصص بكلوتين الحنطة في المصل في المجموعة السادسة ٤٠٩٦٠ بالمقارنة مع الرابعة ١٠٢٤٠ وفي المجموعة الخامسة ٤٩٦٠ بالمقارنة مع الثالثة ٥١٢٠. اما بالنسبة للضد المخاطي فكان عبارة في المجموعة الخامسة ٣٧٧٦ والثالثة ١٠٣٢ والمجموعة السادسة ٣٧٧٦ والرابعة ١٩٢٠. لهذا سيكون زيت زهرة الشمس مساعد مناعي مرشح لدعم الاستجابة المناعية المخاطية للقاحات المستخدمة على الاسطح المخاطية والفائدة صحة الحيوان وبعد التوثيق لفائدة صحة البشر.

Introduction

The search for immunoadjuvants are the subject of past, present and future [1,2]. The immunoadjuvants are natural, semisynthetic and synthetic materials. They are of several classes as; inorganic gel, water-in-oil emulsions lipophilic compounds, microbes and/or microbial sub fractions [1,2,3,4,5]. These adjuvants when mixed with or injected simultaneously with the antigen using different routes can led to an enhancement the humoral and/or cellular immune responses [6,7] through the increase of the antigen surface area, antigen targeting and/or modulation of cytokine network [5]. These informations were on the systemic effects of the adjuvants. Recently, however, some workers tend to investigate the validity of mucosal immuno adjuvants [8-12]. The objective of the present work was to report on the mucosal adjuvanicity of sunflower oil (SFO).

Materials and Methods

1- Test immunogen;

The wheat gluten peptide antigen (WGPA) was separated by alkaline extraction from wheat seed mel [12,13]. The protein solutions obtained from the alkaline extraction were neutralized and adjusted to 350 mg/ml for immunization of rabbits and coating canned sheep erythrocyte [15].

2- Test Adjuvant;

Sun flower oil (SFO) imported make considered as the test adjuvant.

3- Test Animals;

Rabbits (*O. caniculus*) bought from local market and cheaked for ecto, endo and haemoparasite as well as ant WGPA natural antibodies. From the parasite and antibody free 12 rabbits were elected and adapted for housing conditions. Then kept ad libitum during experimentation period.

4- Immunization protocols;

The 12 rabbits (O.caniculus) from local breed were grouped into six groups each of two. These groups were as follows:

I- Saline IM-SC dosing control.
II- Saline Oral dosing control.
III- IM-SC WGPA dosing.
IV- Oral WGPA dosing.
V- IM-SC/ SFO WGPA dosing.
VI- Oral/ SFO WGPA dosing.

5- Serology;

Blood samples were collected from the test and control animals by cardiac puncture. Sera were separated, decomplementized and preserved at – 18 Co till use [15]. The mucosal immunoglobulin were separated from mucosae of the gut in accordance with Shnawa and Thewaini [17]. Mucosal immunoglobulins were treated with 2ME as in [16].

Results

I- Immunogen Nature;

It was found that WGPA consist of prolein and glutamine. The ratio of prolein to glutamen was 6:1, as the analysis of thin layer chromatography showed.

II- Total serum proteins concentrations;

The mean total serum protein (MTSP) concentrations for the saline control groups I & II were ranged from 60-77 mg/ml. while, the MTSP for IM-SC (III) and oral group IV were 78.92 and 140.94 mg/ml respectively. While the MTSP for IM-SC-SFO and Oral-SFO were 160.68 and 171.9 mg/ml respectively. (Table 2).

III- Gut Mucosal Immunoglobulin concentrations;

The mean gut mucosal immunoglobulin concentrations were 9.41, 9:15, 20.66 27.71, 26.79 and 27.74 mg/ml for the groups I, II, III, IV, V, & VI accordingly. WGPA stimulate an immune response that increased the gut mucosal immunoglobulin concentration III & IV and such increase was enhanced by systemic or mucosal incorporation with SFO (Table 2).

IV- Gluten s Specific Antibodies;

IV- A- Serum:

WGPA specific serum antibody titers were 20 for saline controls. Whereas it was 5120 for IM-SC and 10240 for oral priming group. The incorporation of WGPA with SFO through IM-SC or oral rotes gave anti WGPA antibody titers were 40960, and 40960 respectively. Systemic

immunoadjuvanicity was within the order of eight folds (V & III), while the mucosal adjuvanicity was within the order of four folds(VI & IV). Systemic SFO act as indirect mucosal adjuvant and mucosal SFO act as an indirect systemic immunoadjuvant (Table 3).

IV-B Mucosal:

WGPA specific mucosal antibody titers were 2 for saline controls (I & III). While, it was 1032 for IM-SC primed (III) and 1920 for orally dosaged animals (IV). The mucosal anti WGPA antibody titers, however, were 3776 for WGPA-SFO through IM-SC Priming and through oral dosaged animals. SFO incorporation in IM-SC rout led to three fold increase in mucosal antibodies than WGPA IM-SC alone. Likewise, WGPA-SFO oral dosing has led to increase around two folds, this was based on the mean mucosal antibody titer values. If the gut represented by duodenum in WGPA-SFO through IM-SC routes, the mucosal immunological adjuvanicity was 8 folds than in WGPA-IMSC alone.

For appendix if it represents mucosal adjuvanicity, it was of eight folds order in the group V than in group III and two folds in group VI than in group IV.

The mean titers ratios for the systemic response to that mucosal responses were 10/1, 4.5/1, 5.55/1, 10.7/1 and 10.7/1 for the groups I, II, III, IV, and VI respectively. Thus WGPA immunization has led to drop the titer ratios III & IV to I & II, the immunoadjuvant effect include a rise up of these ratios to 10.7/1 both for the groups V & VI.

2-mercaptoethanol reduced the serum antibody titers with in 1-2 folds. For mucosal antibodies, there was in some occasions around one fold reduction in the titers. (Table 3).

Discussion

The WGPA was a peptide of six to one for proleine glutamine ratio. Such peptide composition was reported by other worker [14].

The specific immune priming of rabbits with WGPA induced an increase in the MSTP and MMG concentrations [18-23]. This may be due to synthesis and secretion of immune proteins from the activated B lymphocytes[23]. WGPA may function as direct B cells mitogen or T lymphocyte independent immunogen (2B), leading to WGPA specific polyclonal antibodies.

SFO had been proved to be a parantral immunoadjuvant [14]. The parantral SFO effected an immunoadjuvant influences on mucosal surfaces (group V) and mucosal application induced mucosal adjuvanicity (group VI) and systemic adjuvanicity as compared to group IV [22]. Thus, SFO play a role as parantral and mucosal immunoadjuvant. [21,22], whatever the nature of the priming route. SFO as, a mucosal immunoadjuvant may be acting as a candidate enhancer of mucosal immune responses to mucosally applied vaccines [18-22].

The mechanisms of action of immunoadjuvants can be distinguished at two levels, first on the antigen level in which they modify the metabolism of the antigen. While the second on cells which either affect the activation of macrophage, B cell and T cells as well as activation and inhibition of membrane enzymes [22].

Other worker, however, proposed other mechanisms such as depot hypothesis, avoidance of tolerance, cellular accumulation and selective effect on the immunocomptent cells [2]. The same author in 1989 however, have proposed other set of mechanisms as; depot hypothesis, effect on cell migratin, antigen targeting, complement activation and enhancement of the function of cells of the immune system [3]. At 1998, another set of the proposed mechanisms as; increase of antigen surface area, antigen targeting and/or cytokine modulation [5]. Recently, there was new opinion for the mechanisms of the new immunoadjuvant functions as; prolongation of retion of the immunogen, increasing the effective size of the immunogen so promoting phagocytosis and presentation by macrophages; stimulation of the influx of macrophages and promoting local cytokine production [23].

Therefore, the immunoadjuvant effect of SFO can be explained on the baises of the following possible mechanisms;

Oily adjuvants can act by changing the molecular structure of the immunogen or by affecting the access of the antigen to different hydrolic enzymes thus reducing the antigen catabolism or by affecting the interaction between the antigen and the target cell [22]. Oily adjuvant may be acting in the way of depot forming releasing slowly the

antigen to cells of the immune system [2, 3, 23].

The mucosal immune response is important for protection against many infection. Cholea toxin and *E coli* heat labile entertoxin (LT) are most potent adjuvants described for the induction of mucosal immunity. Unfortunately, neither can be used in human due to the associated toxicity. SFO, however, can be characteristic of an ideal mucosal immunoadjuvants [18-21], since it is effective, safe and practicl for use and accomplish its desired goal without toxicity SFO as hydrophobic substance forming emulsions tend to augment antibody production and cell mediated immunity [24].

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Table 1 Immunization Protocols.

Groups	Name of Immune Priming	Number of Rabbits	Does	Route	Injection Manue
I	Saline control	2	Physiologic saline	IM-SC *	Weakly, for four weeks followed by a weak leave and bleed.
II	Saline control	2	Physiologic saline	Oral	Weakly, for four weeks followed by a weak leave and bleed.
III	Test Immunogen	2	350 mg/ml WGPA	IM-SC *	Weakly, for four weeks followed by a weak leave and bleed.
IV	Test Immunogen	2	350 mg/ml WGPA	Oral	Weakly, for four weeks followed by a weak leave and bleed.
V	Systemic Adjuvanicity	2	350 mg/ml WGPA+0.5 ml SFO	IM-SC *	Weakly, for four weeks followed by a weak leave and bleed.
VI	Mucosal Adjuvanicity	2	350 mg/ml WGPA+0.5 ml SFO	Oral	Weakly, for four weeks followed by a weak leave and bleed.

* Nodular and Para nodular areas.

Table 2 Protein Determination of the test rabbits.

Animal Group		Mean of total serum protein concentrations mg/ml	Mean of mucosal immunoglobual concentration mg/ml
Saline C	I	76.77	9.41
Saline C	II	60.01	9.15
IM-S	III	78.92	20.66
Oral	IV	140.94	27.71

IM-SC V SFO	160.58	26.79
Oral VI SFO	171.9	27.74

Table 3 Gluten Specific Immune Conversion induced by WGPA (III & IV), WGPA + SFO (V&VI) in rabbits.

	Saline	Saline	WGPA Specific Response		Systemic Adjuvanicity	Mucosal Adjuvanicity
	I IM-SC Saline	II Oral Saline	III IM-SC WGPA	IV Oral WGPA	V IM-SC WGPA+SFO	VI Oral WGPA+SFO
Serum	20	20	B 5120	10240	40960	40960
			A 1470	5120	30728	20450
Duodenum	2	2	B 512	2048	4096	4096
			A 256	2048	2048	2048
Jejunum	2	2	B 1536	1536	3072	2048
			A 512	536	2048	2048
Ileum	2	2	B 1536	2048	3072	4098
			A 256	1536	3072	2048
Appendix	2	2	B 512	2048	4096	4096
			A 256	2048	2048	4096
ST/MT	2	2 10/1	1032 4.5/1	1920 5.55/1	3776 10.7/1	3776 0.7/1