The Study of Immunological And Cytogenetic Effects of Polyvinyl Alcohol

Dr.Azhar M. Halim * & Dr. Falak O. Abas

Received on: 1/6/2009
Accepted on:6/5/2010

Abstract

This study has been designed to investigate the toxicity effects of polyvinyl alcohol at different concentrations on peripheral blood lymphocytes by measuring each of blastogenic index, mitotic index and chromosomal aberration in addition to the polymorph nuclear leukocytes activity through determine both of the phagocytosis percent and phagocytosis index .The results show that PVA have no toxic effects at the concentrations 0.1,1,10,100,250 µg/ml because the cytogenetic parameters undergo not significantly raised (at $P < 0.05$) and phagocytosis percent and phagocytosis index have been increased significantly (at $P \leq 0.05$). The immune response has been found to be significantly too ($P \leq 0.05$) in the presence of PVA with Candida albicans antigen as adjuvant via increasing the foot pad swelling in the immunized mice with 50 µg/ml of C.albicans antigen.

Keywords: polyvinyl alcohol, genotoxicity, phagocytoses.

دراسة التأثيرات المناعية والوراثية الخلوية لمادة البولي فينيل الكحول

الخلاصة

صممت الدراسة الحالية للتحري عن التأثيرات السمية لمادة البولي فينيل الكحول بتراكيز مختلفة على الخلايا اللمفاوية للدم المحيطي باعتماد كل من معامل التحول الارومي BI ومعامل الانقسامMI والزيغ الكروموسومي CA اضافة الى قياس فعالية الخلايا العدلة متعددة اشكال النوى PMN عن طريق تحديد النسبة المئوية للالتهام PP ومعامل الالتهام PI . وجد ان مادة PVA لاتمتلك سمية في التراكيز المستخدمة 0.1,1,10,100,250 مايكروغرام /مل) لان كل من مؤشرات الوراثة الخلوية الثلاثة BI,MI,CA ارتفعت ارتفاعا غير ذا دلالة معنوية عند مستوى $P < 0.05$ في حين كانت الزيادة في معامل البلعمة والنسبة المئوية للبلعمة معنوية عند مستوى احتمالية $P \leq 0.05$, و الاستجابة المناعية ارتفعت بدلالة الزيادة المعنوية في كل من التفاعل المناعي العاجل والاجل بوجود مادة البولي فينيل الكحول كمساعد مناعي مع المستضد المستخلص من خميرة الكانديدا حيث زاد معدل انتفاخ القدم الخلفية لفئران ممنعة بعد حقنها بمزيج من المساعد المناعي ومستضد خميرة الكانديدا 50 مايكروغرام / مل زيادة معنوية عند مستوى احتمالية. $P \leq 0.05$.

***Environmental Research Center, University of Technology/ Baghdad**

https://doi.org/10.30684/etj.28.14.11
2412-0758/University of Technology-Iraq, Baghdad, Iraq
This is an open access article under the CC BY 4.0 license http://creativecommons.org/licenses/by/4.0

Introduction

Adjuvants have been used for more than 70 years to enhance the immune response of the host animal to an antigen [1]. Adjuvants are substances injected along with an antigen that are intended to enhance humeral and cell mediated immune response to the antigen [2, 3, and 4]. Adjuvants generally permit the use of smaller antigen dose and may modulate the immune response to the antigen, more than 100 Adjuvant preparation have been described [5].

The mechanisms by which adjuvants promote the immune response are depot effect, antigen presentation effect, an antigen distribution effect and CTL induction effect [6] experimentally we can provide a controlled release formulation comprising biodegradable polymer microspheres where in a vaccine suspended in a polymer matrix.

Poly vinyl alcohol powder is white in color, have different viscosity character Amorphous density at 25°C: 1.26 g/cm3, crystalline density at 25°C: 1.35 g/cm^3, molecular weight of repeat unit: 44.00 g/mol. PVA water soluble in the aqueous solution are colloidal and compatible with lower alcohol the PVA is insoluble in petroleum solvents, use in the plastic industry, surface coating ,film resistance to gasoline ,artificial sponges , printing inks, pharmaceutical ,and cosmetics products [7].

The diameter of PVA fibers above respiable limits and most of them are not inhalable, have a lower density as mineral fibers ranging (10-16) µm in diameter and they don't fibrillate [8].

Experiment

Materials and methods:

1- candida albicans antigen preparation :

Fresh growth of *C. albicans* was suspended with 100 ml of extract solution (Na H2PO4 0.37, Na 2HPO4 1.42, NaCl 2.5, phenol 4) gm / L following to [9] and using the method [10] for determination the total protein.

2- biodegradable polymer micro spheres preparation :

Two highly water soluble polymer micro spheres were used (PVA and starch) in ratio 20:80 % the aqueous solution of (PVA and starch) added to the *C. albicans* antigen the aqueous phase mixed with an emulsifying medium (glycerol) the mixture (antigen and adjuvant) homogenized by placed together in beaker and the two were emulsified, through use of a syringe only by pulling the material back forth rapidly the homogenized micro droplet suspension slowly to absolute methanol with stirring the micro droplet thus causing micro spheres to precipitate ,slowly evaporating the solvent leaving behind micro spheres [11].

3-chromosomal analysis:

Five concentrations of PVA (0.1, 1, 10,100 and 250) µg/ ml were used to determine the genotoxicity effect of PVA on blood lymphocytes according to [12].

4-phagocytosis assay:

five ml of venous blood were obtained by heparenized syringe and then divided into five tubes 1ml/ each, 5 concentrations of PVA with phosphate buffer saline (0.1, 1, 10,100,250µg/ ml) were added to single tube . All tubes have been

PDF created with pdfFactory Pro trial version www.pdffactory.com

incubated for one hour with 100 µl of *Candida* suspension (1×10^{3} cell/ml) at 37º C. The mixture centrifuged (3000RPM) for five minutes .One drop of the mixture (blood,PVA,and *Candida* suspension) was speared on slide ,air dried and fixed with absolute methanol and stained with Giemsa stain, ,washed with PBS and the percentage of phagocytosis was calculated using the following equation .

$$\text{Phagocytosis percentage\%} = \frac{\text{NO. Of phagocytes}}{\text{Total NO. Of phagocytes and non phagocytes}} \times 100$$

5-DTH and Arthur reaction:

Nine albino mice were immunized with biodegradable microspheres (adjuvant and *C. albicans* antigens) for three weeks. 50 µl of *C. albicans* antigens was injected in the right foot bad while the left foot pad was injected with 50 µl of PBS. The swilling of foot pad was detected by using Vernea [13].

6- Toxicity assay:

To detect the effect of PVA on poly morph nuclear leukocytes 100 µl of (0.1, 1, 10,100,250µg/ml) of PVA were mixed with 100µl of whole blood incubated for one hour at 37º C.100 µl of mixture and 100 µl of trypan blue dye (0.2gm of stain with 100 ml normal slain) at 37º C for 3 minutes and the viability percentage was calculated by the following equation:

$$\text{Viability percentage \%} = \frac{\text{NO. Of none stained cell}}{\text{Total NO. Of stained and non stained}} \times 100$$

Results and Discussion

1-chromosomal analysis:

The table (1) showed that the peripheral blood lymphocytes which exposed to graduated concentrations of PVA solutions revealed slightly increasing (in significant at $P \leq 0.05$ level) in cytogenetic parameters (BI, MI, and CA). According to WHO report about mechanisms of fibers carcinogenesis in 2008 referred to that PVA have no toxicity on genetic material *in vivo* or /and *in vitro*. As will as the studies of [14] on bacteria *Escherichia coli* and *Salmonella typhimurium* observed that PVA compounds were not genotoxic in a range of *in vivo* and *in vitro* studies.

PVA fibres, as manufactured, are above the respirable limit, and most of them are not inhalable. The only study on lung cancer risk in workers exposed to PVA fibres did not show positive results, PVA itself is not genotoxic [14].

2. Phagocytic assay:

PVA solutions have been increased the phagocytosis capacity to engulf and killed the Candida cells in vitro by poly morph nuclear leukocytes (PMN) via increasing both the phagocytosis percentage and phagocytosis index according to the increasing in concentration. Both parameters undergo slightly increasing (not significant at $P < 0.05$ level) in the first, second and third concentrations but the phagocytosis capacity was increased in the other concentrations (table 2).

Phagocytosis is non specific mechanism of defense against several types of microorganisms [15]. The increasing this parameter referred to increase in the immune response

PDF created with pdfFactory Pro trial version www.pdffactory.com

against any foreign particle, the presence of PVA gave the PMN external promoter to kill and engulf the Candida cells [4].

The activated PMN undergo several changes in the morphology (increase in the size), motility, and have high performance to adherence to the glass [16]. Also the activated PMN reduce the Nitro blue tetrazolium dye by product the super oxide [17].

3. Arthur and delayed type hypersensitivity assay:

The immediate and delayed type hypersensitivity has been raised in the immunized mice with Candida antigens conjugated with 10 and 100 µg/ml of PVA (significantly at $P \leq 0.05$ level). Because the presence of PVA gave the antigen complexity and high molecular weight, this lead to slow releasing of an antigen. Foot pad swelling of immunized mice increase according to the concentration of the PVA as compare with the negative control (PBS only) and the positive control (C.albicans antigen only) table (3).

4. Toxicity assay:

To determine the hagocytosis assay on the PMN leukocytes the viability must be ranging between 96-100 % [15]. The PVA have low toxicity on the PMN table (4) because the PVA is non toxic as it self in the human, animals, and microorganisms [13].

References

[1] Still, H.F. (2005): Adjuvants and antibody production: dispelling the myths associated with Freund'scomplete and other adjuvants. ILAR journal. 46(3): 280-293.

[2] Bryant, N.J. (1986): Laboratory immunology and serology.2nd ed. Sannders. Canada.

[3] Roitt, I, M.(1988): Essential immunology .6th ed. Blackwell Scientific Publication ,London.

[4] Kuby, J. (1994): Immunology .2nd Ed .Freeman. New York.

[5] Vogel, F.R.and Powell, M.F. (1995): a compendium of vaccine adjuvants and excipient.NewYork: Plenum press .141-227.

[6] Cox, JC.and Coulter, A.R. (1997): adjuvants classification and review of the modes of action .Vaccine VOL15:248-256.

[7] Windholz, M. (2000): Encyclopedia of chemical and drugs. The Merck index 9th .Published by Merck & CO. INC. Rahway, USA.

[8] Harrison, D.C. (1999): drugs, vaccines, hormones in polylactide coated microsepheres, HTML document.

[9] AL-Jwary, M.M. (1990): The study of some hypersensitivity agents, MSc thesis Baghdad University.

[10] Bradford, M. (1976): A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein dye binding. Anal.Biochem.72:248-254.

[11] Youmans, A; Youmans, G. (1976): preparation and effect of different adjuvants on the immunogenic activity of Mycobacterial ribosomal fraction .Journal of Bacteriology Vol.94 (4) 836-843.

[12] Verma, R. & Babu, A. (1989): Human Chromosomes: Manual of Basic techniques .Pregramon Press, New York.

[13] Triolo, A.J.; Osterholm, J.L. & Kratky, M.T. (1989): Enhancement of the Arthus reaction and suppression of delayed type hypersensitivity (DTH) by pluronic F.68 adergent frequently used prepare perflurucarbon emulsions

PDF created with pdfFactory Pro trial version www.pdffactory.com

.Int.J.Immuno pharmac.Vol.11(3):241-248.

[14] WHO (2008): Report of the World Health Organization workshop on Mechanisms of fiber carcinogenesis and assessment of chrysotile Asbestos substitutes Rome, 27–31 October 2008, P 39-41.

[15]Hudson, L. & Hay, and F. (1979): Practical immunology .2nd ed. Blackwell Scientific publication .Oxford, London.

[16]Adams, D, O; Edelson, P, J & Koren, H. (1981): Methods for studying mononuclear phagocytosis Academic Press New York.

[17] Csato, M.; Dobozy, A. &Simon, J. (1985): Study of phagocytic function with quantitative (NBT) reduction test in diabetes mellitus, Arch Dermatol.Res.Vol.268:283-288.

PDF created with pdfFactory Pro trial version www.pdffactory.com

Table (1) chromosomal analysis of peripheral blood lymphocytes exposed to Graduated concentrations of PVA

Concentration of PVA µg /ml	Blastogenic index	Mitotic index	Chromosomal aberration
0.0	**43.09±0.019**	**0.37±0.03**	**0.157±0.016**
0.1	**43.17±0.015**	**±0.046 0.37**	**±0.015 0.155**
1	**43.28±0.017**	**±0.057 0.378**	**±0.018 0.163**
10	**44.56±0.02**	**±0.018 0.39**	**±0.017 0.168**
100	**44.76±0.012**	**±0.11 0.39**	**±0.015 0.166**
250	**45.15±0.011**	**±0.12 0.41**	**±0.018 0.167**

*significant at P≤0.05 level
√the results represent M±SD for three replicates

Table (2) Phagocytic index and phagocytic percent of polymorph nuclear leukocytes exposed to graduated concentrations of PVA

Concentration of PVA µg /ml	Phgocytic index	Phgocytic Percentage %
0.0	0.15a±9.25	58.65 ± 0.83 a
0.1	9.60 ±0.48a	0.89a±58.68
1	0.58a±9.86	0.18a±58.89
10	0.11b±10.1	0.115b±59.33
100	0.35b±10.15	0.11b±59.77
250	±10.84 0.41b	0.91b±60.18

√Different letter s refer to significant deference at P≤0.05 level
√the results represent M±SD for three replicates

PDF created with pdfFactory Pro trial version www.pdffactory.com

Table (3) Arthus and DTH assay in mice treated with different concentrations of PVA and C. albicans antigen

Type of treatment	NO. of samples	Arthus reaction Foot pad swilling in millimeter	DTH assay Foot pad swilling in millimeter
Phosphate buffer saline only	3	0.158±0.01a	0.12±0.01a
50 µg /ml of C. albicans antigen	3	0.589±0.01b	0.43±0.02b
50 µg /ml of C. albicans antigen + PVA (10) µg /ml	3	0.89±0.15c	0.81±0.04c
(50) µg /ml of C. albicans antigen + PVA (100) µg /ml	3	1.21±0.11d	0.91±0.03d

√Different letter s refer to significant deference at P≤0.05 level
√the results represent M±SD for three replicates

PDF created with pdfFactory Pro trial version www.pdffactory.com

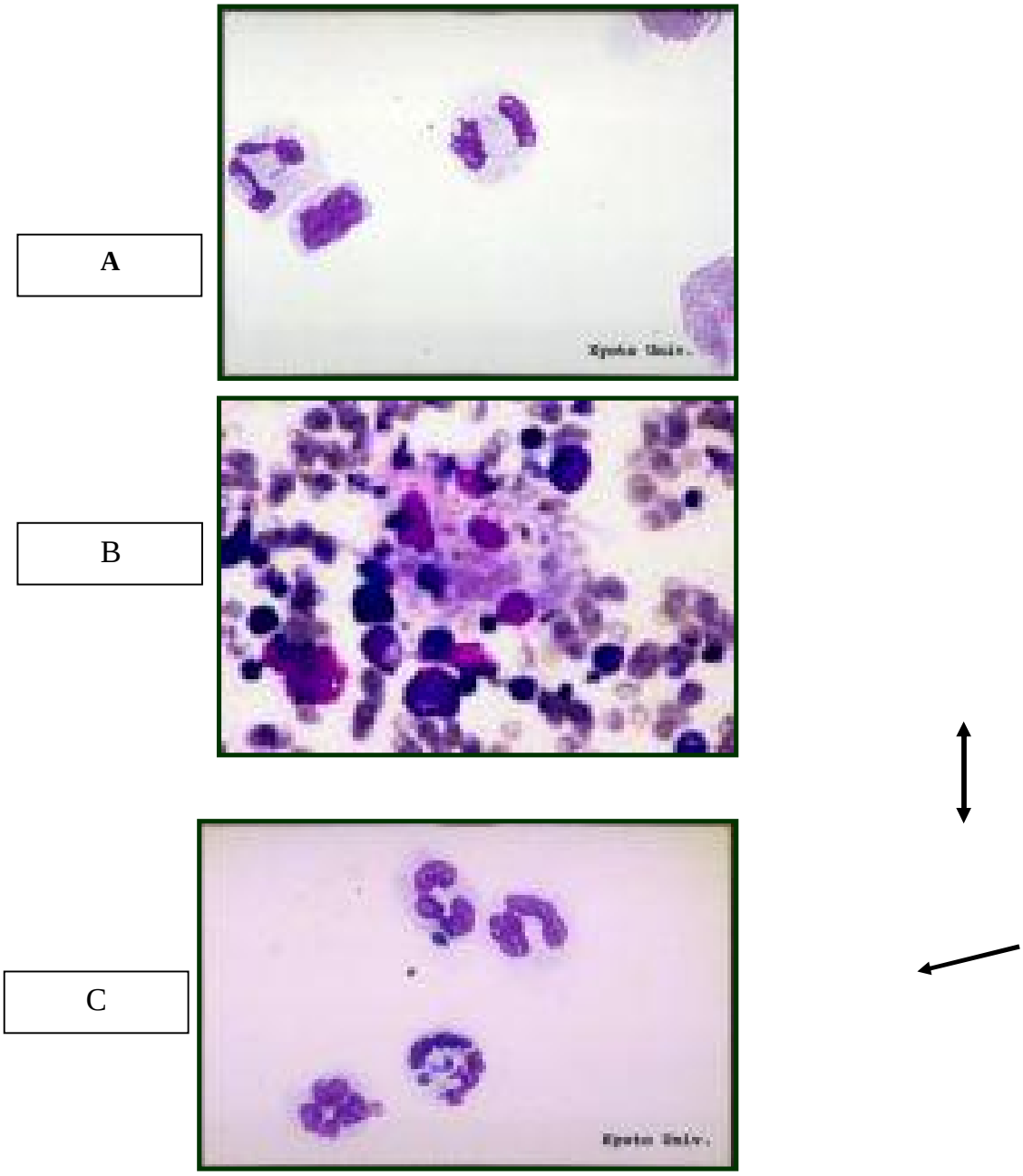

Figure (1) A- UN stimulated PMN
B- Stimulated PMN to engulf and kill the Candida cells
C- Phagocytic cells with debris of Candida

PDF created with pdfFactory Pro trial version www.pdffactory.com