The Study of Immunological And Cytogenetic Effects of Polyvinyl Alcohol

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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 ≤ 0.05). The immune response has been found to be significantly too (P ≤ 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.
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 humoral 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/cm³, 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
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 spread 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 pad 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 stain) 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 ≤ 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 *in vitro*. As well 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.
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≤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

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

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

*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

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

\*Different letter s refer to significant deference at P≤0.05 level

\*The results represent M±SD for three replicates
Table (3) Arthus and DTH assay in mice treated with different concentrations of PVA and C. albicans antigen

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

Different letters refer to significant deference at P≤0.05 level

The results represent M±SD for three replicates
Figure (1) A- UN stimulated PMN
B- Stimulated PMN to engulf and kill the Candida cells
C- Phagocytic cells with debris of Candida