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Investigation of the adjuvant effect of polyethylene glycol (PEG) 400 in BALB/c mice

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Abstract

In the formulation of peptide- and protein-based drugs, it is important that the pharmaceutical excipients used do not potentiate possible immunogenic properties of the drug substance. Polyethylene glycols (PEGs) are widely used excipients e.g. in parenteralia and in formulations for nasal application. The immunomodulating properties of PEG 400 were investigated in this study using hen egg ovalbumin (OA) as the model immunogen. OA was dissolved in saline, 10% PEG 400 in saline or undiluted PEG 400 and injected subcutaneously into the neck region of BALB/cJ mice. The levels of OA-specific IgE, IgG1 and IgG2a antibodies were measured. The 10% solution of PEG 400 did not have any immunomodulating properties, whereas the undiluted product gave rise to immunosuppression when compared with the saline control. Neither 10% nor the 100% PEG 400 preparation possessed adjuvant activity under the conditions of the study.

Keywords: PEG; Adjuvant effect; Safety; IgE antibodies; IgG antibodies; BALB/c mice

1. Introduction

Peptides with as few as seven amino acids may be immunogenic, i.e. they may elicit an antibody response, but most immunogenic peptides contain at least 11 amino acids (Kuby, 1994). The larger and more complex a protein, and the more distant its relationship to the host self proteins, the more immunogenic it is (Janeway et al., 1999). Some drugs are peptides and may as such be potential immunogens, especially in the presence of an adjuvant. Antibodies raised against a drug substance may bind to it, leading to neutralization and rapid elimination of the drug. Additionally, the presence of an adjuvant may increase the risk of development of allergy against the drug. Some pharmaceutical excipients may possess adjuvant activity. Thus, members of the non-ionic block co-polymer family, which are used in pharmaceutical products (Marti-Mestres and Nielloud, 2000), have been shown to possess adjuvant activity (Hunter et al., 1991). Low molecular weight polyethylene glycols (PEGs) are good cosolvents and are widely used as pharmaceutical excipients

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in parenteralia, for example, at concentrations of up to 30% v/v (Kibbe, 2000) and in formulations for nasal administration (Bechgaard et al., 1999).

The purpose of this study was to investigate the adjuvant activity of PEG 400. Subcutaneous (s.c.) injection of hen egg ovalbumin (OA) into the neck region of BALB/c mice was used as the screening test model, as it is able to detect the adjuvant effect of chemicals (Clausen et al., 2000). The levels of IgE, IgG1 and IgG2a antibodies in the serum of the mice were measured by ELISA. The antibody isotypes IgE and IgG1 are related to Type I allergy such as asthma and rhinitis, whereas, IgG2a is not.

2. Materials and methods

2.1. Animals

Inbred BALB/cJ female mice, 6–7-weeks-old, were from Bomholtgård Breeding and Research Centre, Denmark. The mice were housed in polypropylene cages with pine tree sawdust bedding, and they were allowed to acclimatize 2 weeks before immunization. The photoperiod was from 06:00 to 18:00 h, and the temperature and relative humidity in the animal room was 21 ± 2 °C and 50 ± 5%, respectively. Ovalbumin-free food (Altromin no. 1324, Christian Petersen, Denmark) and tap water were available ad libitum. Treatment of the animals followed procedures approved by The Animal Experiment Inspectorate, Denmark.

2.2. Sensitization procedure

The animals were sensitized s.c. in the neck region with a standard low-dose regime with the model allergen OA (grade V from Sigma). All animals were given 1 μg OA in 100 μl solvent. The composition of the solvent (vehicle) was either sterile 0.9% saline, 10% PEG 400 in 0.9% saline or 100% PEG 400 (Ph. Eur. from Merck). Before use, the PEG 400 was heat sterilized at 140 °C for 3 h. Mice in the positive control groups were injected with 1 μg OA together with 100 μl of a solution containing the known adjuvant aluminium hydroxide ($Al(OH)_3$) Alhydrogel® 2%, which was a kind gift from Superfos Biosector, Denmark. The $Al(OH)_3$ concentrations were 2.7 and 0.27 mg/ml, respectively. The 2% $Al(OH)_3$ was diluted with sterile water. After the primary immunization, the animals were given one or two booster injections s.c. in the neck region with 0.1 μg OA in 100 μl 0.9% saline. The first booster injection was given 10 days after primary immunization, the next 15 days later. Blood was collected 4 days after the respective booster injections, i.e. on days 14 and 19. Before the collection of blood by heart puncture, the mice were anaesthetized with Hypnorm® (fentanyl citrate 0.315 mg/ml and fluanisone 10 mg/ml from Janssen Pharma) and Dormicum® (midazolam 5 mg/ml from Roche) each diluted 1:1 in sterile water and then mixed. A volume of 125 μl per mouse was injected s.c. The blood samples were centrifuged for 10 min at 2500 *g* and the supernatant (serum) was stored at −80 °C until analyzed.

2.3. Determination of OA-specific antibodies in serum

The serum was analyzed by Enzyme-Linked Immunosorbent Assay (ELISA) in order to determine the levels of OA-specific IgE, IgG1 and IgG2a antibodies. The antibodies were determined as described by Larsen et al. (2001).

The 10 and 100% concentrations of the PEG 400 and the OA control group that had received the same number of boosters were compared by means of the Kruskal–Wallis test to elucidate exposure-dependent immunomodulating effects of PEG 400. If significant effects were detected, they were further elucidated by pair-wise comparison by means of the Mann–Whitney's *U*-test. Adjuvant effect was defined as a significant increase in antibody levels in a test group as compared to the saline control group, whereas significantly lower antibody levels were defined as indicating an immunosuppressive effect. Minitab Statistical Software, Release 10.51 Xtra (Minitab Inc.), was used for the Kruskal–Wallis and the Mann–Whitney's *U*-test. *P*-values less than 0.05 were considered statistically significant.

Table 1
Antibody production in the control and exposure groups

Antibody isotype	Number of boosters	Exposure concentrations of PEG 400			Kruskal–Wallis	$Al(OH)_3$ concentrations	
		0%	10%	100%	(P-value)	0.27 mg/ml	2.7 mg/ml
IgE	1	109 ± 26 ($n = 10$)	165 ± 32 ($n = 52$)	40 ± 12** [a] ($n = 52$)	<0.001	301 ± 42*[b] ($n = 70$)	254 ± 29 ($n = 89$)
	2	2098 ± 793 ($n = 10$)	1207 ± 207 ($n = 50$)	269 ± 52** [a] ($n = 52$)	<0.001	802 ± 187 ($n = 70$)	531 ± 90 ($n = 89$)
IgG1	1	10 ± 3	10 ± 2	14 ± 3	0.89	50 ± 8*** [b]	173 ± 86*** [b]
	2	150 ± 52	195 ± 24	155 ± 47** [a] [c]	<0.001	1255 ± 452** [b]	706 ± 144*** [b]
IgG2a	1	<6[d]	<6[d]	7 ± 1	–		
	2	<6[d]	<6[d]	8 ± 1	–		

Values are mean $\pm$ S.E.M (arbitrary units). Difference between the antibody production in the two exposure groups and the saline control group was evaluated by use of Kruskal–Wallis test. If the Kruskal–Wallis test showed a difference in the antibody level, further comparison was performed by use of Mann–Whitney's U-test. Statistically significant differences between PEG groups and saline control groups are indicated by *($P<0.05$), **($P\leq0.01$) or ***($P\leq0.001$).

[a] Significant reduced antibody response compared to saline control.

[b] Significant increased antibody response compared to saline control.

[c] See text for explanation.

[d] The detection limit of the ELISA assay is six arbitrary units.

3. Results

The 10% solution of PEG 400 did not give rise to any alterations in the levels of IgE or IgG1 antibodies. Thus, after both one and two booster injections, the antibody levels did not differ significantly from the saline control; i.e. neither adjuvant nor immunosuppressive effect was present (Table 1).

The concentration 100% PEG 400 reduced the IgE antibody level after both one and two boosters compared to the saline control, i.e. it induces immunosuppression. Furthermore, 100% PEG reduced the IgG1 level after two boosters, but not after one. The significant decrease in IgG1 antibody level in the 100% PEG 400 group as compared with the saline control after two boosters is not obvious from the mean values in Table 1. It should be mentioned that the Mann–Whitney's *U*-test compares the median values, which are 85 and 27 arbitrary units, respectively.

The 100% PEG 400 induced only a marginal increase in the level of IgG2a antibodies as compared with the saline control after both one and two boosters (Table 1).

Both the 0.27 and the 2.7 mg/ml concentrations of $Al(OH)_3$ possess adjuvant activity. The adjuvant effect was most pronounced on the IgG1 level (Table 1).

4. Discussion

No adjuvant or immuno-suppressive effect was seen from the 10% solution of PEG 400. The concentration 100% PEG induced suppression of IgE and IgG1 antibody formation. This may be due to cytotoxic effects at the site of injection, which may lead to reduced function of the antigen processing and presenting mechanisms.

The slight increase in the IgG2a antibodies from below six to about eight arbitrary units was not considered biologically relevant. For comparison, BALB/cJ mice treated with 10 μg OA and 250 μg of the adjuvant dimethyldioctadecyl ammonium bromide and boosted twice with 1 μg OA, produced an IgG2a level of 30,000 arbitrary units (data not shown).

OA, which is used in the present study, is a potent and commonly used model immunogen to study immunogen-specific T and B cell mediated immune function (Renz et al., 1993). Furthermore, the route of administration used, s.c. injection, is more likely than intra-venous administration, for example, to elicit an antibody response (Janeway et al., 1999). Even under these conditions, neither 10 nor 100% PEG 400 induced biologically relevant increases in levels of any of the three measured antibody isotypes. In conclusion, this study suggests that PEG 400 does not possess adjuvant activity.

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