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# Adjuvancy and reactogenicity of *N*-acetylglucosaminyl-*N*-acetylmuramyl-dipeptide (GMDP) orally administered just prior to trivalent influenza subunit vaccine. A double-blind placebo-controlled study in nursing home residents

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*One hundred and fifty-three nursing home residents received 0, 5, 25 or 50 mg* N-*acetylglucosaminyl*-N-*acetylmuramyl-dipeptide (GMDP) orally, and trivalent influenza subunit vaccine intramuscularly. One day after intervention, there was a strong increase of total leucocytes, monocytes and neutrophils in the groups receiving 25 or 50 mg GMDP. A GMDP dose dependent increase in systemic, but not in local, vaccine side-effects was observed. No significant differences in post-vaccination haemagglutination inhibiting serum antibody titres were observed between the four groups, indicating that oral administration of GMDP together with influenza vaccination, does not lead to a higher vaccine efficacy. Copyright © 1996 Elsevier Science Ltd.*

**Keywords:** GMDP; influenza vaccine; adjuvant

*N* - acetylglucosaminyl - *N* - acetylmuramyl - dipeptide (GMDP), an analogue of muramyl-dipeptide, has adjuvant properties and low toxicity in animal models and human beings[1]. These characteristics suggested its use as a vaccine-adjuvant in the elderly, a prominent target group for influenza immunization, as the elderly generally show a less favourable antibody response to influenza vaccine than young adults[2].

## SUBJECTS, MATERIALS AND METHODS

### Intake procedure

In the Fall of 1994, the 275 residents of a Dutch nursing home were invited to participate in the study. Exclusion criteria were: allergy to chicken protein; terminal stage disease; kidney diseases or failure; and medication that may influence the immune system. Candidates, or in case of dementia their relatives, signed an informed consent prior to the study. Approval for the study was obtained from the Ethics Committees of the Erasmus University Rotterdam and the nursing home.

### Vaccine and adjuvant

Commercial trivalent subunit influenza vaccine (Influvac®, Solvay-Duphar BV, Weesp, The Netherlands) contained 15 μg haemagglutinin of the influenza viruses A/Shangdong/9/93 (H3N2), A/Taiwan/1/86 (H1N1) and B/Panama/45/90, respectively, according to the recommendations of the World Health Organization for the winter season 1994/1995[3]. GMDP manufactured and formulated into tablets under good manufacturing practice conditions, were supplied by Peptech (UK) Ltd, Cirencester, UK.

### Administration of vaccine and GMDP, and recording of the side-effects

One hundred and fifty-three participants received 0, 5, 25 or 50 mg GMDP orally, according to a

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**Table 1** Demographic parameters

| GMDP (mg) | Number of subjects entering the study | Number of deaths | Subjects completing the study | | | |
|---|---|---|---|---|---|---|
| | | | Number | Age (years)[a] | Gender[b] | PV[c] |
| 0 | 43 | 1 | 42 | 78.4 (75.0–81.7) | 10 (23.8) | 32 (76.2) |
| 5 | 44 | 1 | 43 | 83.0 (80.0–86.0) | 9 (20.9) | 32 (74.4) |
| 25 | 42 | 2 | 40 | 81.3 (78.7–83.9) | 13 (32.5) | 30 (75.0) |
| 50 | 24 | 1 | 23 | 77.3 (77.3–81.6) | 6 (26.1) | 23 (100.0) |
| All | 153 | 5 | 148 | 80.3 (78.7–81.9) | 38 (25.7) | 117 (79.1) |

[a]Arithmetic mean (95% CIs); [b]number (%) of male subjects; [c]number (%) of subjects vaccinated within 5 years prior to study

**Table 2** Frequency of side-effects

| Symptom | Total (%) ($n$=148) | Doses GMDP (%) | | | | Logistic regression ($P$-value)[a] | | |
|---|---|---|---|---|---|---|---|---|
| | | 0 mg ($n$=42) | 5 mg ($n$=43) | 25 mg ($n$=40) | 50 mg ($n$=23) | GMDP | Gender | Disease |
| Any local reaction | 55 (37.2) | 17 (40.5) | 19 (44.2) | 13 (32.5) | 6 (26.1) | N.S.[b] | 0.0030 | N.S. |
| Any systemic reaction | 44 (29.7) | 7 (16.7) | 7 (16.3) | 20 (50.0) | 10 (43.5) | 0.0003 | N.S. | 0.0001 |
| Moderate and severe inconvenience | 30 (20.3) | 3 (7.1) | 6 (14.0) | 16 (40.0) | 5 (21.7) | 0.0038 | N.S. | 0.0001 |

[a]Logistic regression with gender (male, 1; female, 0), dose GMDP (0 mg, 1; 5 mg, 2; 25 mg, 3; 50 mg, 4) and underlying disease group (psychogeriatric, 1; somatic, 2) as independent variables. $P$-value of regression coefficient. [b]N.S., not significant (0.05)

randomization scheme, and under double-blind conditions. The vaccine was injected intramuscularly in the deltoid muscle directly after GMDP/placebo intake. During the first 3 days after intervention, subjects were seen by a trained study collaborator to record reactions, using a standardized questionnaire. The questionnaire addressed nine local symptoms (redness, swelling, itching, warmth, pain on contact, continuous pain, restriction of arm movement, induration and blue spots), six systemic symptoms (fever of 37.7°C or more, increased sweating, headache, malaise, insomnia and shivering), and the degree of inconvenience these symptoms caused (none, slight, moderate or severe).

### Serology

Prior to intervention, and 1 day and 4 weeks thereafter, venous blood was taken to determine conventional haematological and biochemical parameters and serum antibody titres (haemagglutination inhibition test, according to standard methods[4] with turkey erythrocytes). Sera with undetectable HI-titres (<10) were assigned a titre of five. Serological endpoint variables were the geometric mean of post-vaccination HI-titres, and the proportion of subjects with post-vaccination titres of 40 or more. Results were analysed statistically as described previously[2], including regression models to control confounding factors. A $P$-value of 0.05 or less was considered to indicate statistical significance.

## RESULTS

### Modification of study design

During the first 10 days of the intake period, 15 out of 91 participants showed side-effects which were clinically classified as "moderate" or "severe", including nausea (sometimes with vomiting) within the first 3 days after intervention, high or prolonged fever (≥39°C, several days), and polyarthralgia. It was decided by the clinical manager outside the study site to unblind the code for these subjects. Seven of 15 cases of moderate or severe side-effects were associated with a dose of 50 mg GMDP. It was decided and communicated to the investigator to withdraw the dose of 50 mg GMDP from the ongoing trial. The study was continued with the remaining doses of GMDP of 0, 5 and 25 mg, still under double-blind conditions.

### Mortality during the study

In the course of the intake period (1 month) 11 of 120 nursing home residents who did not take part of the study (9.2%), and five of the 153 study participants (3.3%) died. This incidence of mortality was within the normal range encountered in the home. Four of the study participants who died did so with causes unrelated to GMDP (cardiac infarction, urosepsis, sudden death). In one case, a causal relationship with GMDP could neither be excluded nor confirmed. This subject (female, 75 years of age, history of stroke, aneurysm of the aorta, cataract, dementia) was in a weak, but not terminal condition, when receiving 50 mg GMDP. She developed high fever (up to 39.2°C) 1 day thereafter which lasted for 5 days, and a deterioration of her overall condition until her death on the seventh day after intervention. Autopsy was rejected by her relatives.

One hundred and forty-eight subjects completed the study (*Table 1*), of which 38 were males and 110 females, with an age-range of 41–97 years (mean 80.3). Most of the subjects (96.6%) were 60 years or older.

### Side-effects

The most frequently reported local side-effects were redness and warmth at the site of vaccination (23.6% and 22.3%, respectively), the most frequently reported systemic side-effects were malaise and fever (27.0% and 11.5%, respectively). Any local and any systemic symptom were reported by 37.2% and 29.7% of the participants, respectively (*Table 2*). Females reported

**Table 3** Seroresponse against three influenza vaccine components

| GMDP(mg) | *N* | A-H3N2 | | A-H1N1 | | B | |
|---|---|---|---|---|---|---|---|
| | | Pre | Post | Pre | Post | Pre | Post |
| *(A) Geometric mean titre (95% CIs)* | | | | | | | |
| 0 | 42 | 12 (9–17) | 40 (27–59) | 34 (19–61) | 94 (57–153) | 57 (34–95) | 171 (116–252) |
| 5 | 43 | 14 (9–21) | 28 (17–45) | 15 (10–23) | 43 (25–71) | 54 (33–90) | 171 (110–261) |
| 25 | 40 | 14 (9–21) | 52 (32–87) | 24 (15–40) | 59 (36–97) | 41 (25–67) | 180 (112–289) |
| 50 | 23 | 16 (9–28) | 55 (32–94) | 30 (13–57) | 64 (33–126) | 52 (28–97) | 136 (68–274) |
| All | 148 | 14 (11–17) | 41 (32–51) | 24 (19–31) | 62 (48–81) | 51 (39–65) | 167 (133–210) |
| *(B) Number of subjects (%) with titre ≥40* | | | | | | | |
| 0 | 42 | 8 (19.0) | 24 (57.1) | 21 (50.0) | 34 (81.0) | 25 (59.5) | 37 (88.1) |
| 5 | 43 | 12 (27.9) | 21 (48.8) | 14 (32.6) | 25 (58.1) | 26 (60.5) | 38 (88.4) |
| 25 | 40 | 10 (25.0) | 27 (67.5) | 18 (45.0) | 26 (65.0) | 24 (60.0) | 36 (90.0) |
| 50 | 23 | 5 (21.7) | 15 (65.2) | 12 (52.2) | 16 (69.6) | 17 (73.9) | 19 (82.6) |
| All | 148 | 35 (23.6) | 87 (58.8) | 65 (43.9) | 101 (68.2) | 92 (62.2) | 130 (87.8) |

significantly more local side-effects than males (44.5% vs 15.8%). Psychogeriatric patients (including those with dementia) reported significantly less systemic side-effects than somatic patients (15.5% vs 48.4%). Systemic symptoms, and moderate or severe inconvenience, but not local symptoms, showed a clear dependency on the dose of GMDP. Frequencies of side-effects in the 50 mg dose arm may have been under-reported as the study population, until the withdrawal of the 50 mg dose, consisted of less females and more psychogeriatric subjects than thereafter (distribution bias).

Three subjects (50, 25 and 25 mg GMDP) complained of joint pains lasting several days and completely resolving thereafter. Clinically, this symptom appeared as a transitory poly-articular arthritis as is seen in serum-sickness. Within the first 3 days after intervention, some other symptoms occurred whose relationship with intervention was regarded unclear or unlikely. Three subjects (all 0 mg GMDP) developed dizziness, one subject (5 mg GMDP) had abdominal pain, one subject (0 mg GMDP) showed walking difficulties, one subject (25 mg GMDP) had diarrhoea (another patient on the same ward, not treated with GMDP, had diarrhoea as well), one subject (25 mg GMDP) suffered a stroke and another subject (25 mg GMDP) a myocardial infarction.

### Biochemistry and haematology

GMDP had no meaningful effect on potassium, sodium, creatinine, alkaline phosphatase, $\gamma$-GT and total protein. ASAT, total bilirubin and ALAT were slightly elevated in the 25, and 50 mg GMDP arms one day after intervention, but back to baseline values after 4 weeks.

Of the haematological variables, the red blood cell parameters (erythrocytes, haematocrit and haemoglobin) did not show meaningful changes after intervention. Data on thrombocytes were inconclusive. White blood cell counts markedly increased after intake of GMDP: before intervention, the mean leucocyte count in the whole study population was $6.74 \times 10^9\ l^{-1}$ (95% CI: 6.42–7.05). One day after intervention, the leucocyte means for the four study arms (0, 5, 25 and 50 mg GMDP) were 6.41, 7.97, 10.55 and $11.73 \times 10^9\ l^{-1}$, respectively. The 25 and 50 mg GMDP groups showed significantly higher counts of monocytes, and neutrophils, and significantly lower counts of basophils and eosinophils, compared to those with placebo or 5 mg GMDP. Data on lymphocytes were inconclusive. After 4 weeks all white blood cell counts had returned to baseline-levels.

### HI-antibody prior to and after vaccination

*Table 3* shows the pre- and post-vaccination status of the serological variables. Prior to vaccination, and 4 weeks after vaccination, the variables were similar through the four dose groups for any influenza strain ($P0.05$). An effect of GMDP on the serological variables could not be detected. Post-vaccination endpoint variables were subjected to linear regression, and logistic regression, respectively, as described previously[2], with pre-vaccination titres, age, gender, influenza vaccinations within the previous 5 years, influenza-like illness during the last winter season, underlying disease category, baseline number of lymphocytes and number of neutrophils before and 1 day after vaccination, as independent variables. Pre-vaccination titres and status of previous vaccinations contributed significantly to post-vaccination variables. Corrected post-vaccination titres were calculated by eliminating the influence of pre-vaccination titres and status of previous vaccinations. These were also similar through the four dose groups (not shown).

## DISCUSSION

Taken together, our data showed that GMDP is bio-available after oral administration as it induces a mild, clinically irrelevant hepatic activity, and a marked, dose-dependent increase of circulating total leucocytes, monocytes and neutrophils. This leucocyte-stimulating effect of GMDP has been described previously[1].

Furthermore, oral administration of GMDP, in combination with inactivated influenza vaccine, causes an increase in the frequency of vaccine-associated systemic side-effects (like fever, headache, malaise), but not of local side-effects (like redness or pain of the injection site). Moreover, three cases of a mild transitory poly-articular arthritis occurred in the 25 mg and 50 mg dose arms (4.8%). This dose-dependent increase of side-effects was not expected as trials in human beings with GMDP alone had not revealed any side-effects attributable to GMDP (unpublished data).

Finally, sero-response to influenza vaccine in the present study population of aged nursing home residents was, at least for the influenza A strains, sub-optimal and deserved augmentation: Percentages of post-vaccination titres $\geq 40$ were only 58.8% and 68.2% for A-H3N2 and A-H1N1, respectively.

A single oral dose of GMDP did not result in an improved sero-response on influenza vaccination in elderly nursing home residents.

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