Randomized double-blind placebo-controlled study of an angiotensin immunotherapeutic vaccine (PMD3117) in hypertensive subjects

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ABSTRACT

Immunization against components of the renin–angiotensin system offers a potential alternative to daily medication in some patients with hypertension or heart failure. Our primary objective was to determine whether a sustained antibody titre to Ang I (angiotensin I) can be achieved in hypertensive patients. The secondary objective was to determine whether the antibodies block the renin system. Patients (n = 27) with essential hypertension responsive to an ACEi (angiotensin-converting enzyme inhibitor) or ARB (angiotensin blocker) were randomly assigned to receive three or four injections of the Ang I vaccine PMD3117 or aluminium hydroxide (Alhydrogel™) over a 6 week period. Antibody titre was measured prior to each injection and every 30 days until disappearance. Indices of renin blockade were changes in renin and aldosterone (blood and urine) and a within-patient comparison of the pre- and post-vaccination rise in 24 h ambulatory blood pressure after 2 weeks of withdrawal of ACEi or ARB. The anti-(Ang I) antibody titre rose from the second injection in both regimes and peaked on day 64. Median half-life was 85 (95% CI, 44 and 153) days (where CI is confidence interval). Vaccination did not influence blood pressure, but significantly blunted the fall in plasma renin following withdrawal of ACEi or ARB. At 42 days after the first injection, aldosterone excretion was decreased by PMD3117 to 6 (95% CI, 1 and 31)% of values in patients receiving Alhydrogel™ (P = 0.012). In patients with essential hypertension, PMD3117 generated a prolonged antibody response to Ang I. Biochemical measurements show evidence of blockade of the renin system, but higher titres will be required to achieve a decrease in blood pressure.

INTRODUCTION

Immunization against a component of the renin–angiotensin system might obviate the need for daily drug administration in patients with hypertension or other indications for chronic treatment with drugs that block the renin system. The PMD3117 vaccine consists of a 12-amino-acid analogue of Ang I (angiotensin I) in which the decapeptide is extended by acetylcysteine–glycine at the N-terminal and covalently linked to KLH (keyhole limpet haemocyanin). The vaccine is formulated as an aqueous suspension by adsorption on to the registered adjuvant aluminium hydroxide (Alhydrogel™). In laboratory animals, an earlier formulation of the

Key words: angiotensin, hypertension, immunotherapeutic, vaccine.

Abbreviations: ABPM, ambulatory BP monitoring; ACE, angiotensin-converting enzyme; ACEi, ACE inhibitor; Ang I, angiotensin I; Ang II, angiotensin II; ARB, angiotensin blocker; BP, blood pressure; CI, confidence interval; KLH, keyhole limpet haemocyanin; SBP, systolic BP.

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vaccine decreased the BP (blood pressure) response to infused Ang I [1], and the 24 h BP was lower in spontaneously hypertensive rats immunized with PMD3117 than in sham immunized rats [2].

No study of active immunization with renin or angiotensin has been undertaken in humans [3]. Our primary objective was to determine whether a sustained antibody response would be induced by PMD3117, using two different dosing regimes. Secondary objectives were to assess the safety and local tolerability of the vaccine and to determine if the antibody response included blocking antibodies by assessing changes in renin and aldosterone secretion. We also compared the rise in BP during 2 weeks of ACEi [ACE (angiotensin-converting enzyme) inhibitor] or ARB (angiotensin blocker) withdrawal prior to vaccination with a second period of withdrawal 7 weeks later.

**METHODS**

**Subjects**

Patients with essential hypertension of either gender, aged between 18 and 70, were recruited in three centres. They had BP responsive to an ACEi or ARB taken for at least 1 month before entry to the study. In order to assess responsiveness, these drugs were withdrawn at entry to the study for 2 weeks. The criterion for continuation in the study was a 24 h ABPM (ambulatory BP monitoring) averaging 140–180/85–120 mmHg, with the 24 h average SBP (systolic BP) being at least 8 mmHg higher than before discontinuation of ACEi or ARB. Patients who, at entry, were receiving a thiazide diuretic in addition to ACEi or ARB were also eligible; the diuretic was continued during and after the period of ACEi withdrawal. No other antihypertensive treatment was permitted.

**Experimental treatment and randomization**

Two groups of different vaccine-dosing schedules were compared with placebo control groups in a randomized parallel and double-blind design (Figure 1). Before randomization, patients were stratified according to whether diuretic was administered as well as ACEi or ARB. It was planned that one group of eight patients received three doses of 100 µg of peptide equivalent vaccine at 21 day intervals, whereas a second group of eight patients received the same (100 µg) dose of vaccine on four occasions at 14 day intervals. Each active group was compared with a placebo group (four subjects in each placebo group) given adjuvant only at 21 or 14 day intervals. The volume of each injection was 2 ml, given by deep subcutaneous injection.

Patients restarted their previous dose of ACEi or ARB on the day of randomization and continued using a thiazide diuretic if already receiving this at entry to the study. At day 50 after randomization, ACEi or ARB was again discontinued for a 2 week period.

**Follow-up and measurements**

Patients in group one and their placebo controls received their three injections of vaccine or placebo on days 1, 22 and 43 after randomization; patients in group two...
received their four injections on days 1, 15, 29 and 43. Clinical assessments and blood sampling were performed on these days and on days −15 (screening) and 64 (at the end of the second period of drug withdrawal). Urine collections over 24 h at each of these visits were an optional additional measurement undertaken in 11 out of 24 patients. After day 64, investigators and patients were unblinded; follow-up continued until the anti-(Ang I) antibody levels had declined (antibody titre < 1000) and patients had re-started their pre-study antihypertensive medication.

Supine BP was recorded with an Omron 705CP. Blood samples were drawn, after at least 30 min in the supine position, for measurement of the IgG antibody titres to Ang I and KLH and of renin and aldosterone concentrations. ABPM for 24 h was measured with a Spacelabs monitor model 90207 before and after the two periods of drug withdrawal, namely days −14, 0, 50 and 64. On these four days and on the days of vaccination, there was an optional 24 h urine collection for measurement of aldosterone excretion.

Biochemical analyses

Antibodies were determined by ELISA, with absorbance (A) measured in serial dilutions of sera. A positive antibody response was defined as A > 0.1 in the 1:1000 diluted serum sample. NAc-AngI–BSA, an analogue of Ang I conjugated to BSA, was used as a substrate to quantify antibodies to Ang I; the background A, if any, due to antibodies present to BSA was subtracted for each sample. For antibodies to KLH, KLH itself was used as a substrate in the ELISA and a background A was measured using snake venom as a substrate. Renin and aldosterone concentrations were measured by immunoradiometric assay and RIA respectively.

Tolerability and safety

Local tolerability assessments at the site of vaccination involved semi-quantitative measures of pain, swelling, erythema, warmth and tenderness. Between visits, subjects filled in a daily diary card recording any local reactions or other adverse events. Routine biochemistry and haematology was also monitored at each visit.

Statistical analyses and study size

The primary objective was to determine optimal vaccination schedule, based on the antibody titre, and the study was powered to detect a 2-fold difference in the anti-(Ang I) titre between the two groups, estimated by analysis of covariance with BMI (body mass index) as covariate (β = 0.9 for P < 0.05). To quantify the rate of decline of antibody titres, a half-life was calculated as (date of day 90 sample − date of day 63 sample) × log(2)/log(titre on day 63/titre on day 90). For exploration of secondary outcomes, analyses of covariance were performed comparing values at day 64 for the two vaccine schedules and controls and using values at day 1 as a covariate. The study was powered to detect a 50% decrease in the rise in SBP in the PMD3117-treated patients when the first and second periods of ACEi or ARB withdrawal were compared by repeated measures ANOVA.

The research protocol was in accord with Institutional Guidelines at the three participating centres and was approved by their Research Ethics Committees. All patients gave written informed consent.

RESULTS

Twenty-seven patients were randomized, of whom 24 completed all scheduled injections. One patient each in the active and control groups was withdrawn, because of skin reactions described below. One further patient started diuretic therapy after randomization and is included only in the antibody analyses. Patient demographics are shown in Table 1.

IgG antibody response to Ang I

The titres at each visit are shown in Figure 2. Significant antibody induction was detectable after the second injection with both regimes, with no significant difference between the two regimes. The antibody titres to Ang I and KLH were significantly correlated (r = 0.68, P = 0.002), as well as showing similar time courses within each patient. The median half-life for anti-(Ang I) antibodies for the period following day 64 was 85 (95% CI, 44 to 153; where CI is confidence interval) days. This was shorter than the median half-life for anti-KLH antibodies of 321 days, with five subjects showing an increase in anti-KLH antibody titres after day 64.

Ambulatory and clinic BP

Vaccination did not influence clinic BP readings (Figure 3) or the rise in 24 h mean ABPM observed after 2 weeks of withdrawal from ACEi (or ARB) therapy. There were similar increases in BP between days −14 and day 1 and between day 50 and day 64 and, in particular, the rise on the latter occasion was no smaller in patients assigned to PMD3117 injections (Figure 3). However, within the latter group, the 24 h mean SBP on day 64 was 7.5 (95% CI, 2.1 to 13.0) mmHg lower in the four-dose PMD3117 subjects than in three-dose PMD3117 subjects.

Renin and aldosterone

Renin measurements are shown in Figure 4. Repeated measures ANOVA over the whole vaccination period showed no significant difference between groups. Significant elevation was present, however, at day 64 in the

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Table 1  Patient demographics
Values are means (range).

<table>
<thead>
<tr>
<th>Patients receiving Alhydrogel™ vaccination</th>
<th>Patients receiving PMD3117 vaccination</th>
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<tbody>
<tr>
<td></td>
<td>Three doses</td>
</tr>
<tr>
<td>n</td>
<td>5</td>
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<tr>
<td>Age (years)</td>
<td>55 (45–60)</td>
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<td>Height (metres)</td>
<td>177 (171–185)</td>
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<tr>
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<td>86 (64–101)</td>
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<tr>
<td>Female</td>
<td>1</td>
</tr>
<tr>
<td>Race</td>
<td></td>
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<tr>
<td>Caucasian</td>
<td>5</td>
</tr>
<tr>
<td>Afro-Caribbean</td>
<td>1</td>
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<tr>
<td>Asian</td>
<td>1</td>
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<tr>
<td>Antihypertensive therapy</td>
<td></td>
</tr>
<tr>
<td>ACEi</td>
<td>4</td>
</tr>
<tr>
<td>ARB</td>
<td>1</td>
</tr>
<tr>
<td>(ACEi or ARB) + diuretic</td>
<td>2</td>
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PMD3117 group as determined by an ANOVA comparison of log renin between the two groups. The degree of attenuation correlated with the antibody titre to Ang I (Figure 5). There were no significant changes in plasma aldosterone, for which several measurements on ACEi or ARB were below assay detection. Urine aldosterone was decreased in the PMD3117 group, falling to 6 (95% CI, 1 to 31)% of control values at 6 weeks of vaccinations with PMD3117 (Figure 6).

Tolerability and safety
There were 128 (12.8 per patient) adverse events in the Alhydrogel™ group and 195 (11.5 per patient) in the PMD3117-treated patients. One of the former and five of the latter reported slight swelling and erythema around the injection site. No patient showed a rise in serum creatinine outside the normal range. One patient in each group receiving PMD3117 or Alhydrogel™ had non-sustained proteinuria as determined using a
Effect of an angiotensin immunotherapeutic vaccine in hypertensive subjects

Figure 4 Effect of vaccination on plasma renin
PMD3117 vaccination was associated with a tendency to higher values of plasma renin concentration than in control subjects, although the difference was significant \((P = 0.033)\) only at day 64, after the post-vaccination period of ACEi/ARB withdrawal. (For the sake of clarity, the renin value at day 22 is the mean of the day 22 value in patients receiving three doses of PMD3117, and the day 15 and 29 values in patients receiving four doses of PMD3117.)

Figure 5 Influence of vaccination upon rise in renin during ACEi or ARB withdrawal
The fall in plasma renin during 2 weeks of ACEi/ARB withdrawal was lower in the PMD3117 group after 6 weeks of vaccinations than on the first period of withdrawal. The degree of attenuation was correlated with the anti-(Ang I) antibody titre, remaining significant \((P < 0.05)\) when titre, age and baseline renin were entered into a multiple regression analysis with the change in log renin \((\Delta \log \text{renin})\) as the dependent variable. Renin was log-transformed to achieve normality for the regression analysis, and the antilogs calculated to permit non-transformed renin values to be shown.

dipstick, which was quantified as 0.22 and 0.47 g/24 h respectively.

DISCUSSION

The main question in the present study was whether immunization with a modified small endogenous peptide can induce an antibody titre in the patients for whom a vaccine may offer a therapeutic option and whether the titre would be of sufficient duration to suggest that subsequent booster vaccination would be required only once or twice a year. Previous volunteer studies [3a] with shorter vaccination schedules than in the present study and using lower amounts of the modified Ang I peptide had suggested a need for the higher and longer dosing schedules which we employed. These have indeed induced a titre in all patients and suggested no benefit from using more than three injections to induce immunization. Furthermore, the prolonged half-life of approx. 100 days means that 4-monthly injections would coincide with a similar proportional fall in antibody titre as seen for drug levels of the average ACEi between each daily dose.

A secondary objective was to determine whether the antibodies induced had a blocking effect on the renin–angiotensin system. On the analogy of known effects of ACEi and ARB treatment, the most likely effects of blockade are an increase in renin [due to release of the negative feedback by Ang II (angiotensin II)], a fall in aldosterone and fall in BP [4]. The present study was powered to find only large differences between the PMD3117 and control groups. Therefore the possibility of recognizing blocking actions of the antibodies was increased by incorporating a crossover element in the protocol, so that the changes in BP and biochemistry following ACEi or ARB withdrawal could be compared before and after vaccination. Crossover analyses of ABPM can detect differences of approx. 5 mmHg in as few as 20 subjects [5]. In addition, the range of antibody titres allowed us to examine the influence of titre upon the biochemistry. It is clear from these analyses that the
antibody titres induced in the present study have less effect on either BP or biochemistry than treatment with therapeutic doses of ACEi or ARB. The trend to higher renin values in the PMD3117 group may reflect increased release from the negative feedback of Ang II upon renin secretion, but achieved marginal significance only at the final time point when renin was no longer influenced by concurrent treatment with ACEi/ARB. The apparently marked effect on urine aldosterone excretion should also be treated cautiously, because of the small number of patients who undertook 24 h urine collections. However, the significant differences that were observed provide evidence of some blockade of the renin-angiotensin system that can be enhanced with increasing titres. The differences between the three- and four-dose vaccination regime were small and, therefore, further increases in titre are most likely to come from modifications to the vaccine formulation.

Previous studies in experimental animals of passive immunization against components of the renin-angiotensin system have yielded variable results, although none of the experiments employed Ang I as sole immunogen [6,7]. An argument against the immunization strategy has been that increased secretion of renin would result in saturation of an angiotensin antibody [8,9]. Active immunization is less likely than passive immunization to suffer from this problem; experience from use of ACEi and ARB shows that the increased renin and Ang I secretion in response to inhibition or blockade of Ang II are insufficient to overcome the inhibition. In the present study, the achieved antibody titre led to a small further increase in renin levels above those due to ACEi or ARB, but this did not prevent the observed decrease in urinary aldosterone. The vaccine had no effect on BP, presumably because the titre generated by the current formulation of the vaccine is too small to provide sufficient inhibition or blockade of angiotensin production or action.

Pre-clinical and Phase I experience with PMD3117 has demonstrated good tolerability and safety to date, and concern about immune-complex disease with vaccination is usually unfulfilled [10]. Could blockade of the renin-angiotensin system pose a danger at times of sudden volume depletion, such as following trauma or surgery? Probably no more than in patients receiving ACEi or ARB, since the effects of either of these would persist for most of the first 24 h that constitute the critical period before volume depletion is corrected.

The potential use of such a vaccine can be debated. Although BP changes will provide the most convenient assessment of efficacy in the short term, an angiotensin vaccine might find alternate or greater use for those conditions where ACEi or ARB are indicated to improve outcome in the absence of short-term surrogate measures of benefit. For example, these drugs are used in only half of patients with heart failure [11]. A vaccine would be unlikely to be associated with first-dose hypotension, and there would be less concern about compliance with a vaccine than with a daily drug in patients where monitoring is problematic. The long-term safety of a vaccine may also be of less concern in patients with severe heart failure than in patients with asymptomatic hypertension. However, it is possible that the longer-term potential of an angiotensin vaccine will be less as an alternative to the excellent oral medications available for blockade of the renin system and more as a model for testing the principle of active immunization against undesirable endogenous molecules for which oral blockade does not exist. It is possible that this novel therapeutic approach could be directed against many systemic or local mediators, or cell surface molecules.

In summary, we have demonstrated the possibility of generating by active immunization a long-lasting therapeutic antibody response in patients to a small endogenous peptide. The vaccination was well tolerated over the course of the study. Further changes to the vaccine formulation are in progress which will enhance immunogenicity and allow potential for sustained BP decrease to be investigated.

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