

Effect of immunisation against angiotensin II with CYT006-AngQb on ambulatory blood pressure: a double-blind, randomised, placebo-controlled phase IIa study



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Summary

Background Hypertension can be controlled adequately with existing drugs such as angiotensin-converting enzyme inhibitors or angiotensin II receptor blockers. Nevertheless, treatment success is often restricted by patients not adhering to treatment. Immunisation against angiotensin II could solve this problem. We investigated the safety and efficacy of CYT006-AngQb—a vaccine based on a virus-like particle—that targets angiotensin II to reduce ambulatory blood pressure.

Methods In this multicentre, double-blind, randomised, placebo-controlled phase IIa trial, 72 patients with mild-to-moderate hypertension were randomly assigned with a computer-generated randomisation list to receive subcutaneous injections of either 100 µg CYT006-AngQb (n=24), 300 µg CYT006-AngQb (24), or placebo (24), at weeks 0, 4, and 12. 24-h ambulatory blood pressure was measured before treatment and at week 14. The primary outcomes were safety and tolerability. Analyses were done by intention to treat. This study is registered with ClinicalTrials.gov, number NCT00500786.

Findings Two patients in the 100 µg group, three in the 300 µg group, and none in the placebo group discontinued study treatment. All patients were included in safety analyses; efficacy analyses did not include the five dropouts, for whom no data were available at week 14. Five serious adverse events were reported (two in the 100 µg group, two in the 300 µg group, and one in the placebo group); none were deemed to be treatment related. Most side-effects were mild, transient reactions at the injection site. Mild, transient influenza-like symptoms were seen in three patients in the 100 µg group, seven in the 300 µg group, and none in the placebo group. In the 300 µg group, there was a reduction from baseline in mean ambulatory daytime blood pressure at week 14 by $-9.0/-4.0$ mm Hg compared with placebo ($p=0.015$ for systolic and 0.064 for diastolic). The 300 µg dose reduced the early morning blood-pressure surge compared with placebo (change at 0800 h $-25/-13$ mm Hg; $p<0.0001$ for systolic, $p=0.0035$ for diastolic).

Interpretation Immunisation with CYT006-AngQb was associated with no serious adverse events; most observed adverse events were consistent with local or systemic responses similar to those seen with other vaccines. The 300 µg dose reduced blood pressure in patients with mild-to-moderate hypertension during the daytime, especially in the early morning.

Funding Cytos Biotechnology AG.

Introduction

Pharmacological treatment of hypertension with both angiotensin-converting enzyme inhibitors and angiotensin II type I receptor blockers has proven very successful. Nevertheless, only about a third of patients with hypertension in the USA are estimated to have their blood pressure under control.¹ In addition to inadequate diagnosis and prescription, low adherence to prescribed treatment is a major reason for the high proportion of patients with uncontrolled arterial hypertension.² Key factors affecting patients' adherence to treatment include the presence of side-effects and concerns over taking long-term medication in the absence of symptoms.³

Active immunisation to induce antibodies against angiotensin could simplify treatment. An ideal regimen would be a few injections per year, the ease of which should encourage better adherence to treatment. Previous attempts to treat hypertension by immunotherapy targeted angiotensin I; however, a phase II clinical trial in patients with hypertension showed no reduction in blood pressure.⁴

We chose to target angiotensin II with a vaccine, CYT006-AngQb (referred to here as AngQb) and in 2007 reported the results of preclinical studies and a phase I clinical trial.⁵ AngQb is a conjugate vaccine, composed of angiotensin II chemically linked to recombinant virus-like particles derived from the

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	Placebo (n=24)	100 µg (n=24)	300 µg (n=24)
Sex (male/female)	22/2	20/4	23/1
Ethnic origin (white/black)	24/0	23/1	24/0
Age (years)	50.5	52.0	52.5
Height (cm)	177.8	174.1	175.3
Bodyweight (kg)	88.2	82.5	85.3
ABP day (mm Hg; systolic/diastolic)	150.9/98.2	149.2/95.5	145.9/95.3
ABP night (mm Hg; systolic/diastolic)	133.7/84.4	130.3/80.7	130.0/82.5
Supine blood pressure (mm Hg)	151.3/93.4	149.4/89.8	148.6/92.3

ABP=ambulatory blood pressure.

Table 1: Baseline characteristics

	100 µg group		300 µg group	
	Placebo (n=12)	AngQb (n=24)	Placebo (n=12)	AngQb (n=24)
ABP day (mm Hg; systolic/diastolic)	151.5/97.8	149.2/95.5	150.3/98.6	145.9/95.3
ABP night (mm Hg; systolic/diastolic)	132.4/83.2	130.3/80.7	135.0/85.6	130.0/82.5
Supine (mm Hg; systolic/diastolic)	150.6/91.6	149.4/89.8	152.0/95.2	148.6/92.3

ABP=ambulatory blood pressure.

Table 2: Baseline blood pressure measurements by dosage group

RNA-phage Qβ. Initial tests showed that the vaccine was safe, well tolerated, and induced a high titre of anti-angiotensin II-specific antibodies. Here, we report results of the phase IIa clinical trial testing of AngQb in patients with mild-to-moderate hypertension.

Methods

Patients

The study cohort consisted of men and non-reproductive (ie, surgically sterilised or post-menopausal) women, aged 18–65 years. Eligible participants had mild-to-moderate hypertension and were either newly diagnosed, previously clinically diagnosed but not treated, or clinically diagnosed patients treated with antihypertensive therapy that could be safely withdrawn for the duration of the study.

Mild-to-moderate hypertension was defined according to WHO criteria (140–179 mm Hg systolic blood pressure, 90–109 mm Hg diastolic blood pressure, or both), with blood pressure measured as the average of the last two of three consecutive readings taken after 5 min in the supine position. Exclusion criteria were severe essential hypertension, secondary hypertension, current pharmacological treatment that could affect blood pressure, renal insufficiency (serum creatinine >159 µmol/L), a history of cerebrovascular disease, type 1 diabetes or poorly controlled type 2 diabetes, a body-mass index greater than 32, total cholesterol over 6.9 mmol/L, triglycerides over 3.5 mmol/L, an

autoimmune disease or severe allergy, a history of hepatitis B or C, HIV/AIDS or other immunosuppressive disorders, a history of malignancy, drug or alcohol abuse within the previous 2 years, pregnancy or breastfeeding, present history of mental diseases, participation in any drug trial within 3 months of the start of the current trial, or previous participation in a clinical trial with a Qβ-based vaccine.

The study protocol and other relevant documents were reviewed and approved by the ethics committee of the Landesärztekammer Brandenburg, Germany, and the ethics committee of the Ärztekammer Hamburg, Germany. The Paul Ehrlich Institute (Langen, Germany), was notified in accordance with German legal requirements before the start of the trial. The study was done in accordance with International Conference on Harmonisation and Good Clinical Practice guidelines and the Declaration of Helsinki and subsequent revisions. Written consent was obtained from all participants. The study was overseen by a safety monitoring board of six people knowledgeable in the field, one of whom represented the sponsor of the study.

Procedures

The study was a multicentre, randomised, placebo-controlled, double-blind, time-lagged, parallel-group comparison of two doses of the AngQb vaccine. The treatment regimen consisted of three subcutaneous injections of either AngQb 100 µg or 300 µg, or placebo (sodium phosphate saline buffer), formulated with the adjuvant aluminum hydroxide. Injections were administered in weeks 0, 4, and 12. The third injection at week 12 was approved for each patient by the safety monitoring board provided that the anti-angiotensin II antibody titre had fallen by 25% from the peak response after the second injection. Progression from the 100 µg to the 300 µg group was also approved by the safety monitoring board after the assessment of available

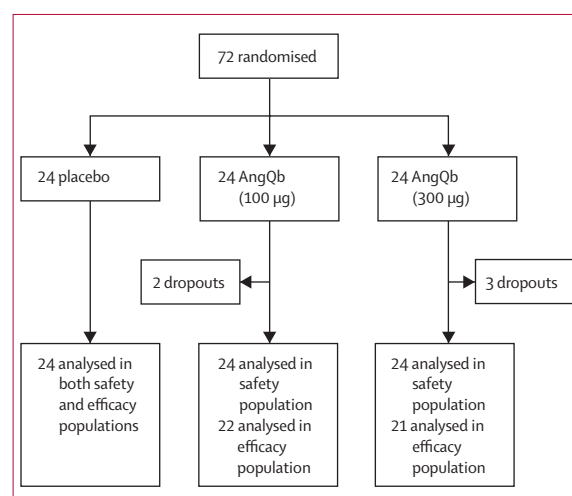


Figure 1: Trial profile

safety and tolerability data. A prerequisite for this decision was that half of the patients had received their second injection, and the antibody response was reversible 8 weeks after the second injection in these patients.

The first 36 patients enrolled were randomly assigned to receive either AngQb 100 µg or placebo. Randomisation was done by use of a randomisation list generated by a validated program (F.A.C.T.S. system) that automated the random assignment of treatment group to randomisation number. The next group of 36 patients were randomised to receive either AngQb 300 µg or placebo. The primary study outcomes were safety and tolerability. Secondary outcomes were pharmacodynamic effects (immunogenicity and effects on the renin-angiotensin-aldosterone system) and clinical efficacy. After enrolment, patients returned for visit 1 within 28 days. Patients who were taking antihypertensive drugs stopped therapy at least 2 weeks before injection of study drug. 24-h ambulatory blood pressure was measured at baseline. After each dose, patients stayed overnight in the hospital for medical observation. During the 16-week study, which included a 4-week post-treatment observation period, patients visited the clinic 11 times for examination. Additionally, patients were asked to report side-effects by telephone throughout the study. The post-treatment 24-h blood pressure monitoring was done at week 14—ie, 2 weeks after the last dose. An open-label follow-up period of 8 months followed the double-blind phase of the study. During follow-up, patients resumed anti-hypertensive treatment when required.

24-h ambulatory blood pressure monitoring was done with Spacelabs 90217 Ultralite ABP Monitors (Del Mar Reynolds GmbH, Feucht, Germany) every 15 min during the day (0600 h–2200 h) and every 30 min during the night (2200 h–0600 h). The averages of day, night, and 24-h measurements were calculated. Supine blood pressure (office blood pressure) was measured with Dinamap Pro 100 (GE Healthcare, Munich, Germany) during all visits to the clinic. Three consecutive measurements were taken after 5 min lying supine and 1 min after standing. Blood and urine samples were obtained at all visits for measurement of antibody titre and urinalysis, which included proteinuria measurements (Combur 10 Test, Roche Diagnostics GmbH, Mannheim, Germany). Physical examination, including 12-lead ECG tests, and haematology and blood chemistry analysis was done at weeks 0, 4, 12, and 16. Plasma concentrations of active renin were measured at baseline and 2 weeks after the second and third doses. Blood samples drawn after 1 h supine and a monoclonal antibody specific for active renin were used in the assay.⁶

Anti-angiotensin II antibody concentrations were measured as antibody titres with an angiotensin II-specific ELISA described previously.⁵ The half-life of

	Placebo (n=24)	100 µg (n=24)	300 µg (n=24)	p value*
Number of patients with adverse event	24 (100%)	24 (100%)	24 (100%)	..
Mild	23 (96%)	24 (100%)	24 (100%)	1.0
Moderate	5 (21%)	9 (38%)	9 (38%)	0.39
Severe	1 (4%)	1 (4%)	4 (17%)	0.35
No suspected relation with study treatment	16 (67%)	17 (71%)	19 (79%)	0.72
Suspected relation with study treatment	22 (92%)	24 (100%)	24 (100%)	0.32
Serious adverse event (no suspected relation with study treatment)	1	2	2	1.0
Serious adverse event (suspected relation with study treatment)	0	0	0	..

Data are number of patients (%). Patients can contribute to all three categories of adverse event severity (mild, moderate, or severe). *p values were calculated with Fisher's exact test for a difference between treatment groups.

Table 3: Summary of adverse events

the late phase of the antibody response after the third injection was fitted to a mono-exponential decay, using data from weeks 28 to 48. The average affinity of antibodies was measured as an apparent average affinity as described.⁵ In this ELISA assay, the concentration of the coating reagent is minimised to keep the amount of antibody binding to the solid phase to a minimum so that the equilibrium between inhibitor and antibody in solution is not disturbed. Since it would not have been practical to do this measurement for all patient sera, the optimum coating concentration in the inhibition ELISA was determined by use of pooled sera from seven high antibody responders and an apparent average dissociation constant (k_d) was measured for this pool. To confirm the value obtained, the half maximum inhibitory concentration (IC_{50}) for angiotensin II was determined for sera collected from ten patients at week 14. The sera were selected to have an antibody concentration similar to the value of the pool, and in this situation, the IC_{50} closely approximates the apparent average affinity.

The concentrations of activated complement factor C3a and of immune complexes were quantified by assays from BD Biosciences (Heidelberg, Germany) and OSTEOmedical (Bünde, Germany), respectively, 1 week after each dose. The determination of T cells, NK cells, B cells, CD4+ T cells, CD8+ T cells and the ratios of CD69+/CD4+ T cells, CD69+/CD8+ T cells, HLA-DR+/CD4+ T cells, HLA-DR+/CD8+ T cells, and CD4+/CD8+ T cells was done with flow cytometry as described previously^{7–9} at screening and weeks 0, 1, 5, and 13. Lymphocytes, monocytes, neutrophils, eosinophils, and basophils were determined at the same time points with a haematology analyser (Diamond Diagnostics, MA, USA).

Statistical analysis

Double-blind treatment allocation was maintained until database lock after the last patient had completed the

16-week blinded phase of the trial. A sample size of 24 patients on each dose and placebo was considered sufficient to assess safety and tolerability. The sample size was calculated to detect a 5 mm Hg reduction in office diastolic blood pressure, assuming an SD of 6 mm Hg, with a power of 80%. Safety and efficacy data were analysed in the intention-to-treat population—ie, individuals who received at least one injection (safety analyses) or those with an ambulatory blood pressure measurement at baseline and at week 14 (efficacy analysis). The difference in antibody response between the 100 µg and 300 µg groups was analysed by two-way

See Online for webtable

	Placebo (n=24)	100 µg (n=24)	300 µg (n=24)	p value
Injection-site erythema	20 (83.3 %)	24 (100%)	22 (91.7%)	0.15
Injection-site induration	16 (66.7%)	23 (95.8%)	19 (79.2%)	0.045
Injection-site oedema	8 (33.3%)	18 (75.0%)	21 (87.5%)	0.0003
Injection-site pain	11 (45.8%)	17 (70.8%)	17 (70.8%)	0.16
Headache	8 (33.3)	6 (25.0%)	15 (62.5%)	0.024
Fatigue	1 (4.2%)	5 (20.8%)	5 (20.8%)	0.20
Rhinitis	1 (4.2%)	3 (12.5%)	4 (16.7%)	0.51
Nasopharyngitis	2 (8.3%)	3 (12.5%)	3 (12.5%)	1.00
Injection-site pruritus	0	3 (12.5%)	1 (4.2%)	0.31
Rigors (chills)	0	2 (8.3%)	2 (8.3%)	0.54
Influenza-like illness	0	1 (4.2%)	4 (16.7%)	0.12
Toothache	0	1 (4.2%)	2 (8.3%)	0.77
Raised body temperature	0	1 (4.2%)	3 (12.5%)	0.31
Injection-site warmth	2 (8.3%)	0	2 (8.3%)	0.53
Pain in extremity	3 (12.5%)	1 (4.2%)	1 (4.2%)	0.61
Haematuria	2 (8.3%)	0	1 (4.2%)	0.77
Limb injury	0	0	2 (8.3%)	0.32
Erythema	0	2 (8.3%)	0	0.32
Hypertension aggravation	3 (12.5%)	6 (25.0%)	1 (4.2%)	0.14

Data are number of patients (%).

Table 4: Adverse events with an incidence of 5% or higher

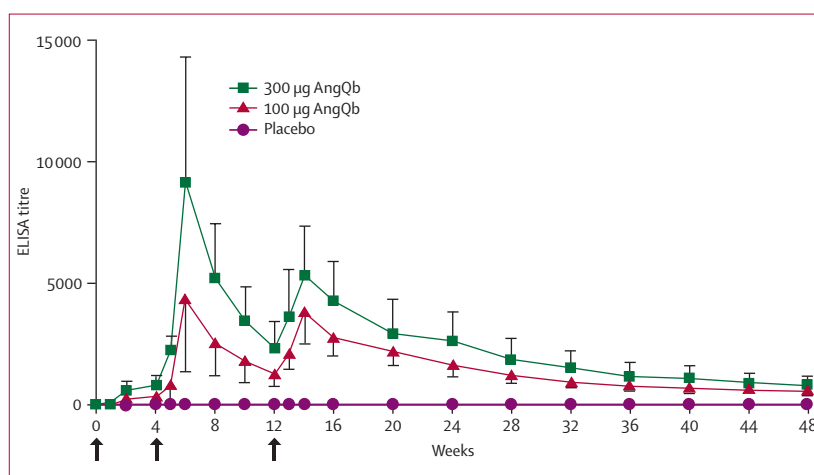


Figure 2: Profile of antibody titres in patients immunised with 100 µg or 300 µg AngQb, or placebo
Injections were administered at weeks 0, 4, and 12 (arrows). IgG antibody titres are expressed as geometric mean titres. Error bars represent SE.

repeated-measures analysis of variance (ANOVA) on the logarithm of the antibody titre.

Changes from baseline in blood pressure measurements were compared with placebo by analysis of covariance (ANCOVA) with the 24-h baseline as covariate. Changes from baseline in blood pressure between 0500 h and 0800 h were analysed in the 300 µg group in comparison with placebo by use of a repeated-measures two-way ANOVA on mean hourly ambulatory blood-pressure measurements for the corresponding time points. Changes from baseline in plasma active renin levels were assessed with Student's *t* test. For the analysis of plasma concentrations of active renin, data from patients with values more than three SDs away from the mean were not included (values from one patient in the 100 µg and one patient in the 300 µg group). Two-sided *p* values below 0.05 were considered significant. All analyses were done with SAS version 9.1.3.

This study is registered with ClinicalTrials.gov, number NCT00500786.

Role of the funding source

The sponsor participated in study design, data analysis, data interpretation, and writing of the report. The sponsor was not involved in data collection except for the measurement of antibody titres, which were measured before database lock under blinded conditions. The corresponding author had full access to all data and final responsibility to submit for publication.

Results

The phase II group reported here is part of a phase I and II study, of which the phase I part has been reported.⁵ From March 1, 2005, to May 29, 2006, 72 patients were enrolled. Baseline patient characteristics are summarised in tables 1 and 2. Five patients left the study before week 16, none of whom were in the placebo group (figure 1). From the 100 µg group, one patient withdrew for an unspecified reason. One patient had an episode of syncope 10 min after receiving his first injection and did not receive any further injection thereafter but was followed-up for safety. From the 300 µg group, one patient withdrew consent, one withdrew because of unspecified reasons, and one patient was withdrawn because of vertigo (in the absence of orthostatic hypotension) and unsatisfactory compliance with study rules.

Table 3 shows a summary of adverse events. Most side-effects were transient and mild, local injection-site reactions (table 4). There were 253 adverse events in the 100 µg group, 234 in the 300 µg group, and 155 in the placebo group (webtable). The higher frequency of adverse effects with active treatment was due to the higher occurrence of local reactions (table 4). Symptoms compatible with a systemic reaction to the vaccine—influenza-like illness, rigors, raised body temperature, or pyrexia—presented within hours of dosing in

ten (21%) participants on active treatment (three in the 100 µg group, seven in the 300 µg group), and resolved 1–2 days after injection (table 4). Heart rate and 12-lead ECG data were unchanged, and laboratory indices, including urinalysis and measurements of proteinuria showed no treatment-related or clinically important deviations from the normal range (data not shown). All adverse events reported during 8 months of follow-up (100 µg: 40 events in 16 patients; 300 µg: 35 events in 15 patients; placebo: 38 events in 17 patients) were considered not to be related to treatment.

All volunteers receiving AngQb responded with high IgG titres against angiotensin II after only one injection (n=48; figure 2). The antibody response was strongly boosted after the second injection, but was reversible with an average half-life of 3.2 weeks (95% CI 2.8–3.7; n=43), which was similar to the half-life noted in the phase I clinical trial of AngQb.⁵ Consequently, all patients, except the five who withdrew, received a third injection. The antibody titres were boosted again, and reached peak levels 2 weeks after the third injection but without surpassing the level reached after the second injection. The 300 µg dose induced a significantly higher angiotensin II-specific IgG response than did the 100 µg dose ($p=0.0098$; figure 2). The average half-life after the third injection was 17 weeks for the late phase of the decline (95% CI 15–19; n=42).

There was no difference in rate of antibody decrease between dose groups. As expected, all 24 patients receiving placebo showed no detectable antibody response against angiotensin II. The apparent average affinity of the antibodies (k_d), determined in pooled sera drawn on week 14 from seven high-antibody responders, was 1.4 nmol/L (average of four measurements, 95% CI 0.6–2.2). The IC_{50} for angiotensin II in individual sera from ten patients ranged from 1 to 5 nmol/L.

Induction of antibodies against endogenous angiotensin II could theoretically lead to immune complex deposition. Therefore, we measured the concentration of immune complexes containing C1 and C3, and the level of complement factor C3a. We did not note any changes in mean levels of C1, C3, and C3a beyond fluctuations also recorded in the placebo group (data not shown).

We monitored blood immune-cell subpopulations at baseline and 1 week after dosing to detect any signs of silent immune pathology. In particular, we monitored the population of activated T cells, measured by the CD69 and the HLA-DR markers. We found no changes from baseline in mean number of HLA-DR+ T cells and CD69+ T cells, and in the mean level of any of the measured immune cell populations and their ratios beyond one SD of the corresponding change from baseline of the placebo group (data not shown). Furthermore, we did not observe treatment-related or clinically significant increases in the number or proportion of the large subsets of immune cells tested, and in particular of activated T cells (data not shown).

	100 µg		300 µg	
	Placebo (n=12)	AngQb (n=22)	Placebo (n=12)	AngQb (n=21)
Systolic blood pressure day	-3.4 (2.3)	-1.5 (1.7)	3.4 (2.8)	-5.5 (2.1)*
Diastolic blood pressure day	-1.6 (1.8)	0.0 (1.3)	1.1 (1.7)	-2.9 (1.2)†
Systolic blood pressure night	-2.6 (3.2)	1.1 (2.3)	-2.5 (4.0)	-1.2 (3.0)
Diastolic blood pressure night	1.7 (2.0)	1.3 (1.5)	-1.8 (2.3)	-0.8 (1.7)

Data are mean (SE). * $p=0.012$ compared with baseline. † $p=0.024$ compared with baseline.

Table 5: Change from baseline in ambulatory blood pressure for treatment groups

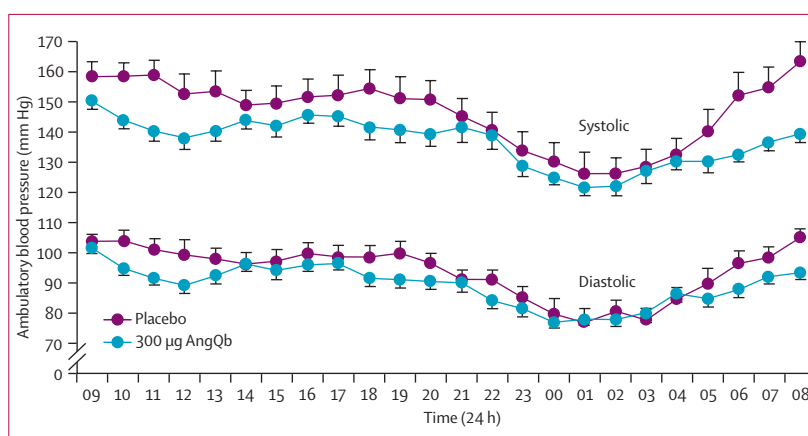


Figure 3: Profiles of mean hourly ABP measurements of the 300 µg (n=21) and corresponding placebo group (n=12) at week 14

Blood pressure surge between 0500 h and 0800 h was reduced compared with placebo after baseline correction ($p=0.012$ and $p=0.036$, respectively).

	Placebo (n=23)	100 µg (n=21)	300 µg (n=20)
Renin baseline (pg/mL)	5.3 (2.4)	3.7 (2.5)	5.1 (2.8)
Change in renin from baseline at week 6 (pg/mL)	0.3 (2.1)	0.2 (1.8)	0.7 (2.3)*
Change in renin from baseline at week 14 (pg/mL)	0.7 (2.5)	0.5 (1.4)	1.2 (2.1)†

Data are mean concentration (SE). * $n=19$. † $p=0.02$ compared with baseline.

Table 6: Plasma active renin measurements

We analysed effects on blood pressure in all patients completing the study for whom ambulatory blood pressure measurements were available at baseline and at week 14 (n=67). Since there was a difference in office diastolic blood pressure at baseline between the two treatment groups ($p=0.0041$), and given the sequential design, we analysed the effects on blood pressure within each treatment group separately. There was, however, no significant difference at baseline between active treatment and placebo within either treatment group. In the 300 µg group, the difference from placebo in the change from baseline in mean ambulatory blood pressure at week 14 was $-9.0/-4.0$ mm Hg ($p=0.015$ for systolic blood pressure, $p=0.064$ for diastolic blood pressure; table 5).

Changes from baseline in mean ambulatory blood pressure during the day in the 100 µg group and during

the night in both the 100 µg and 300 µg groups were not significant compared with placebo (data not shown). We analysed further the 24-h profile of mean hourly ambulatory blood pressure measurements in the 300 µg group. There was a significant reduction of the early-morning blood pressure surge between 0500 h and 0800 h compared with placebo in the 300 µg group after baseline correction (figure 3), with a change at 0800 h of -25 mm Hg in systolic blood pressure ($p < 0.0001$) and -13 mm Hg in diastolic blood pressure ($p = 0.0035$). Office blood pressure showed considerable variability, and no significant differences between active treatment and placebo groups were noted (data not shown). Change from supine to standing position did not lead to orthostatic hypotension (data not shown).

We expected that sequestration of angiotensin II and a consequent fall in blood pressure would induce renal renin secretion, and thus increase plasma renin activity. Measurement of this activity is hampered by the presence of anti-angiotensin II antibodies in the plasma.⁵ We thus measured the plasma concentration of active renin molecules, which is generally well correlated with plasma renin activity.⁶ There was no significant difference between placebo and either of the active treatment groups for the change in mean plasma renin. However, there was a significant increase in mean renin from baseline at week 14 in the 300 µg group (from 5.1 to 6.3 pg/mL, $p = 0.02$), which was coincident with blood-pressure reduction (table 6).

Discussion

Immunisation with AngQb was associated with no serious adverse events, and the number of injection-site reactions—common for vaccines—did not differ significantly from the placebo group. Transient symptoms indicative of a systemic response to vaccine were noted in about a fifth of patients receiving AngQb. The frequency of such events is lower than that reported for another vaccine (NicQb) which uses the same carrier but a different antigen (nicotine).¹⁰

The occurrence of influenza-like symptoms has been associated with administration of several vaccines, and is thought to originate from the activation of the innate immune system that occurs after immunisation.^{10–13} We nevertheless carefully monitored the level of immune-cell subpopulations to rule out asymptomatic immune pathology. For all immune-cell subpopulations, including activated T cells, we did not find treatment-related or clinically significant changes. The immunisation therefore does not lead to any uncontrolled immune stimulation, confirming the good clinical tolerability and safety profile. However, confirmation of safety will require further clinical assessment in phase IIb and phase III.

Our data also indicate that active immunisation with AngQb, at a dose of 300 µg, raised a sufficiently high level of antibodies to significantly decrease blood

pressure. This effect was observed during daytime when the renin-angiotensin-aldosterone system is stimulated¹⁴ by daily activity and upright posture. Moreover, the drop in blood pressure was especially pronounced in the early morning, when the renin-angiotensin-aldosterone system is most active and when most cardiovascular events occur.^{14–16} Indeed, AngQb reduced the early-morning blood pressure surge, which has been associated with increased incidence of stroke¹⁷ and intracerebral haemorrhages.¹⁸ This effect was not anticipated at the beginning of the study.

By contrast, small-molecule inhibitors of the renin-angiotensin-aldosterone system, while lowering blood pressure over 24 h, do not affect the surge in early-morning blood pressure.^{19,20} This difference could be due to the distinct pharmacokinetic profiles of the two types of intervention. Plasma concentrations of small-molecule inhibitors are characterised by a steep rise with subsequent peaks and troughs, while the level of anti-angiotensin II antibodies increases comparatively slowly over days without much fluctuation. This difference could explain the modest increase in plasma active renin that we noted. A low reactive rise in renin could indeed be advantageous, since renin and prorenin are proposed to increase cardiovascular risk factors directly through binding to the renin/prorenin receptor.²¹

We recorded a dependence of both the mean antibody titre and blood-pressure reduction on dose. The antibodies induced by the vaccine sequestered angiotensin II and thereby led to a decrease in blood pressure, an effect confirmed by the induction of plasma active renin. Renin induction was presumably caused by reduced angiotensin II type I receptor stimulation relieving feedback suppression of renin secretion by the baroreceptor reflex and by reduced renal perfusion pressure.

All patients who received AngQb had an immune response against angiotensin II after only one dose of the vaccine, confirming data obtained in the phase I trial,⁵ and is in agreement with results obtained with other vaccines using the same virus-like particle carrier.^{10,22} At week 14 after immunisation, the anti-angiotensin II antibodies were of high affinity, with a range of 1–5 nmol/L, which is compatible with the observed efficacy.

The induced antibody response was reversible, with a half-life of about 4 months after the third injection. This half-life is longer than that reported for the phase I clinical trial after one injection. Importantly, it is compatible with a treatment regimen of a few injections per year, when the vaccine could be administered during regular control visits of hypertensive patients to their doctors. Such a regimen is likely to promote adherence to treatment, but will need to be supported by clinical data. Currently, the blood-pressure reducing effect of AngQb is comparable with that of low doses of renin inhibitor.¹⁹ Optimisation of the immunisation

regimen with shorter dosing intervals and increasing dose is expected to lead to higher antibody titres and consequently to a more robust anti-hypertensive effect. This exploratory study still had a limited sample size, and the efficacy of AngQb was shown in an otherwise healthy hypertensive population. Later stage clinical trials will be needed to show efficacy and safety in a broader hypertensive population.

Contributors

PM, FW, MFB, JN, GTJ, ACT, HS, and HDV contributed to the study protocol and design. PM, MFB, ACT, PaM, HS, TP, JN, RS, and HDV contributed to data analysis and interpretation, FW and SI to clinical data collection, and PaM, JN, RS, and ACT to laboratory data collection. ACT, PM, PaM, JN, GTJ, and MFB participated in manuscript writing.

Conflict of interest statement

ACT, PaM, GTJ, PM, HS, TP, and MFB are employees of Cytos Biotechnology AG, and hold stock or stock options in Cytos Biotechnology AG. RS and JN have received funding from Cytos Biotechnology AG.

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