REVIEW

Immunization against angiotensins for the treatment of hypertension

Patrik Maurer, Martin F. Bachmann*

Cytos Biotechnology AG, Wegistr. 25, CH-8952 Schlieren, Switzerland

Received 3 April 2009; accepted with revision 4 June 2009

KEYWORDS
Hypertension; Vaccine; Immunotherapy; Angiotensin

Abstract Current vaccination approaches against hypertension target angiotensin I and angiotensin II, key components of the renin–angiotensin system. The effectiveness and long-term safety of blockade of the renin–angiotensin system with antihypertensive small-molecule drugs is well documented. Phase I/II testing of the angiotensin I vaccine PMD3117 demonstrated safety and immunogenicity in humans. While angiotensin I-specific antibodies were induced, blood pressure was not lowered, presumably due to insufficient antibody levels. A second vaccine, which targets angiotensin II, has been clinically tested. Administration of CYT006-AngQb to subjects with mild to moderate hypertension was safe and well tolerated. After three administrations of 300 μg of the vaccine, ambulatory blood pressure was significantly reduced compared to placebo. The vaccine was particularly effective early in the morning as systolic and diastolic blood pressure were lowered by −25 mm Hg and −13 mm Hg, respectively. Further studies are required to show long-term safety and to assess how robust and long-lived the blood pressure reduction is. It will also be important to ascertain whether the strong reduction of blood pressure in the early morning, when most cardiovascular events occur, might result in long-term benefits over current therapies.

© 2009 Elsevier Inc. All rights reserved.

Contents

Hypertension ............................................................ 0
Blood pressure and the renin–angiotensin system ....................................... 0
Therapeutic vaccination .......................................................... 0
Vaccination against renin ......................................................... 0
Early studies with vaccines targeting angiotensins .................................. 0
Clinical development of an angiotensin I vaccine ...................................... 0
Clinical development of an angiotensin II vaccine ...................................... 0
Safety of anti-angiotensin vaccines ..................................................... 0
Conclusions ................................................................................... 0

* Corresponding author.
E-mail address: martin.bachmann@cytos.com (M.F. Bachmann).

1521-6616/S - see front matter © 2009 Elsevier Inc. All rights reserved.

doi:10.1016/j.clim.2009.06.003

Please cite this article as: P. Maurer, M.F. Bachmann, Immunization against angiotensins for the treatment of hypertension, Clin. Immunol. (2009), doi:10.1016/j.clim.2009.06.003
Hypertension

High blood pressure is the most common treatable risk factor for cardiovascular diseases. Stroke and ischemic heart disease result in 7.6 million premature deaths worldwide [1]. Approximately half of these events can be attributed to high blood pressure. Prevalence of hypertension varies between 5% in rural India and 70% in Poland. Interestingly, even within western countries there are substantial differences in disease prevalence. In the US and Canada 28% of the adult population are hypertensive (defined as a blood pressure of 140/90 or higher or current use of antihypertensive medication) while 44% of Europeans living in Finland, Italy, England, Sweden, Spain or Germany are affected [2]. The reasons for this difference are not clear. Multiple factors such as nutrient intake, obesity, physical activity and genetic susceptibility contribute to high blood pressure. The high incidence of disease results in a substantial burden to the health care system. A contributing factor to disease outcome and health care cost is the fact only a small proportion of hypertensives are treated (~30% in Europe) and of those treated only a minority (30%) achieves blood pressure control. Accordingly, adequate control of hypertension control is alarmingly low; about 9% in Europe and 30% in the US [3]. One reason for poor control of hypertension is lack of compliance to intake of medications [4]. Patients often do not take their prescribed medication; it has been estimated that 50–80% are non-compliant. A majority of patients have reservations about long-term drug therapy and compliance is further worsened when patients experience medication side effects such as dry cough, peripheral oedema and sexual dysfunction. In contrast, hypertension is for the most part asymptomatic.

Blood pressure and the renin–angiotensin system

Blood pressure is principally influenced by three factors: 1) cardiac output regulated by sympathetic nerve system activation, 2) sodium and fluid retention in the kidney and 3) vascular resistance. The latter is tightly regulated by the renin–angiotensin system (RAS), a hormone system that regulates blood pressure by constricting blood vessels. The active component of the system is angiotensin II, an eight amino acid peptide that binds the AT1 receptors (AT1R) on vascular smooth muscle cells. Receptor engagement by angiotensin II results in aldosterone secretion in adrenal cortex which leads to sodium resorption in the kidney. Accordingly, blood pressure is increased when angiotensin II levels are high. Angiotensin II is derived from the precursor protein angiotensinogen via the action of two proteases. Angiotensinogen is constitutively produced by the liver and cleaved in the serum by renin to form angiotensin I, a ten amino acid peptide. This precursor peptide is further cleaved by angiotensin-converting enzyme (ACE) to form angiotensin II (Fig. 1).

Hypertension has long been successfully treated by small-molecule drugs that target various components of the renin–angiotensin system: renin inhibitors block the production of angiotensin I, ACE inhibitors interfere with the production of angiotensin II and angiotensin-receptor blockers (ARBs) inhibit the signalling of angiotensin II via the AT1 receptor. Additionally, diuretics, beta-blockers and calcium-channel blockers are also used for the long-term reduction of blood pressure.

Therapeutic vaccination

Despite the availability of numerous effective drugs for treating hypertension, reduction of the global burden of disease has not been achieved. National health agencies are in agreement that new approaches and further measures are needed. Therapeutic active vaccination targeting components of the RAS is a potential option. It offers the possibility for reduction of blood pressure with two to three immunizations per year. Such an approach could circumvent the need for daily medication and substantially reduce the issue of patient compliance. In the following, we review different active vaccination approaches for immunotherapy against hypertension.

Vaccination against renin

To date, vaccines tested for their ability to treat hypertension have solely targeted components of the renin–angiotensin
system. Interestingly, vaccination provided the first direct evidence for the participation of the renin–angiotensin system in hypertension. In 1941 Johnson and Wakerlin reported that repeated subcutaneous or intramuscular injection of hog renin into hypertensive dogs was followed by the development of anti-renin antibodies which were capable of lowering the blood pressure [5].

In 1951 hog renin was also tested in man [6]. Renin, purified from hog kidneys, was injected twice a week into eight subjects who received between 7 and 66 doses of 70–140 mg of hog renin. No effects on blood pressure were observed despite the presence of anti-hog renin antibodies. This result was explained by the fact that anti-hog renin antibodies were not cross-reactive with human renin; they neutralized hog renin in-vitro, but not human renin. In retrospect, it was perhaps fortunate that the antibodies did not cross-react with human renin. Indeed, in the eighties active immunization against renin was re-investigated by Michel et al. [7]. With the help of monoclonal antibodies they purified human renin and immunized marmosets in the presence of complete Freund’s adjuvant, a strong adjuvant not approved for human use. The marmosets developed high titers of anti-renin antibodies causing a significant reduction in blood pressure. However, one to four months after immunization the animals became sick and died within a month. An autopsy revealed strong glomerulonephritis, characterized by the presence of immunoglobulin in the kidney and infiltrating macrophages. In addition, granulomatous formations in lung and kidney were observed but these were attributed to the use of Freund’s adjuvant. Similar findings in rats led the authors conclude that the risk of autoimmune disease would prevent the development of a renin vaccine for the treatment of humans [8]. This conclusion is supported by the notion that renin is present in the kidney in substantial amount in a membrane-bound form. It is reasonable to expect that polyepitopic antibodies induced by vaccination with specificity for a highly abundant membrane-associated protein could cause kidney disease.

Early studies with vaccines targeting angiotensins

Vaccination was also used to investigate the role of the angiotensin peptides in hypertension. Christlieb et al. tested a derivative of angiotensin II containing D1N and I5V mutations as an immunogen [9]. Previously, this angiotensin II derivative had been used in the clinics under the name Hypertensin (Ciba) for mechanistic studies and for treatment of drug overdoses [10,11]. To overcome the inherent poor immunogenicity of small peptides, Hypertensin was coupled to the carrier protein rat serum albumin. The conjugate vaccine was formulated with Complete Freund’s adjuvant and repeatedly injected into rats. When a renal clip was applied to the animals, seven of the 11 animals with high antibody levels were shown to have strongly reduced blood pressure [9]. Furthermore, following intravenous injection of renin or angiotensin, immunized rats were protected against an increase in blood pressure. In contrast the vaccine failed to block hypertension in spontaneously hypertensive rats. In this alternative model, blood pressure was not decreased following vaccination with anti-Hypertensin. Curiously, the anti-Hypertensin antibodies were able to block an increase in blood pressure when Hypertensin peptide was administered [12]. This apparently conflicting observation was confirmed by others and at the time was interpreted to mean that angiotensins did not have a direct role in renal hypertension [12–15]. This view was eventually rectified when ACE inhibitors and ARBs became available. With the advent of these small-molecule drugs for the treatment of hypertension, vaccination fell by the way side. Nevertheless, in the late eighties Reade et al. [16] re-investigated angiotensin I vaccines. Five different vaccines were tested, two of which induced antibodies against angiotensin I. In one vaccine glutaraldehyde was used for coupling angiotensin I to limulus hemocyanin, in the other carbodiimide was used for coupling. No reduction of blood pressure was observed despite the presence of antibodies capable of neutralizing an intravenous angiotensin I challenges [16].

Clinical development of an angiotensin I vaccine

In 2003, Protherics plc (UK) reported results from their preclinical and clinical evaluation of two angiotensin I vaccines [17]. Angiotensin I peptides were coupled either to tetanus toxoid or to keyhole limpet hemocyanin as carrier proteins. In rats, both vaccines attenuated the normal blood pressure increase following intravenous administration of angiotensin I. In normotensive human volunteers, one injection of either vaccine did not induce an angiotensin I-specific IgG response; only after two injections anti-angiotensin I antibodies were detected. In order to measure any effect these antibodies may have had on blood pressure, volunteers were intravenously administered angiotensin I. However no significantly significant differences in blood pressure relative to placebo-treated control subjects were observed. Only in one subject with the highest antibody response was a larger amount of exogenous angiotensin I required to increase blood pressure than at baseline. These results indicated that in order to achieve significant blood pressure reduction, higher antibody levels were needed.

In a subsequent double-blind, placebo-controlled study, the angiotensin I vaccine PMD3117 was tested in hypertensive subjects [18]. PMD3117 comprises a 10 amino acid angiotensin I peptide with an additional acetylCysGly at its N-terminus. The thiol of the added cysteine is used for covalent linkage to keyhole limpet hemocyanin (KLH). The vaccine was formulated with the human compatible adjuvant Alum. One group of subjects received three subcutaneous doses of vaccine corresponding to 100 µg of peptide antigen or placebo at 21 day intervals. A second group received the same dose on four occasions at 14 day intervals. Vaccination was generally well tolerated. Swelling and erythema was noted around the injection site for 5 of 17 patients treated with PMD3117 compared to 1 of 10 patients on placebo. Angiotensin I-specific antibodies were detected in sera, with no significant difference in titre between the two regimens. However, vaccination did not influence clinical blood pressure readings. A second read-out was 24 h-ambulatory blood pressure measurements (ABPM). Before start of vaccination, ACE inhibitors and angiotensin receptor blockers were temporarily withdrawn for a period of two
weeks and the change in ABPM measured. Patients were returned to antihypertensive medication and vaccinated. The change in ABPM was again measured in a two-week withdrawal period initiated one week after the last immunization. Similar rises in 24 h ABPM upon withdrawal of antihypertensives were observed before and after treatment. Thus it was concluded that the antibody levels generated with PMD3117 were too small to provide sufficient blockage of angiotensin I [18].

Consequently, in order to increase the antibody response Protherics plc, which was acquired by BTG plc in December 2008, initiated a further study in which they tested the angiotensin I vaccine in combination with a novel adjuvant, CoVaccine HT™. In the double-blind, placebo-controlled study it was planned to enroll 124 subjects and to inject the vaccine three times at 21 day intervals. Primary endpoint was the change from baseline to week 8 in mean daytime diastolic blood pressure measured by ABPM. Data are expected to be available in June 2009 (clinicaltrials.gov accession number NCT00702221). Dosing was suspended in April 2009 as a precaution following several injection site reactions and flu-like symptoms. Data review from all patients did not reveal differences between treatment and control groups. It was concluded that the adverse events were most likely related to the adjuvant rather than the vaccine. Changes in blood pressure were not reported. BTG plc announced that a new phase Ila study will be initiated which will explore safety and efficacy using different doses of adjuvant and vaccine.

Clinical development of an angiotensin II vaccine

Cytos Biotechnology AG (Switzerland) has developed an angiotensin II-specific vaccine termed CYT006-AngQb. CYT006-AngQb comprises an angiotensin II peptide, with an N-terminal CysGlyGly extension, that is covalently coupled to virus-like particles (VLP) derived from the coat protein of the bacteriophage Qb. The use of recombinant VLP conjugate vaccines is an example of rationale vaccine design that maximizes immune response by incorporating key immunological features of viruses [19]. Highly repetitive and ordered structures such as viral surfaces are potent inducers of B-cell responses [20]. Such a structure can be imposed on any antigen by directed coupling to surface lysines of VLPs using heterobifunctional cross-linkers [21]. Additionally, coupling to carrier protein was achieved either by the use of carbodiimide which couples the peptide via the C-terminal carboxyl group [12] or by glutaraldehyde which couples the peptide via the N-terminal amino group [14]. The C- or N-termini of angiotensin II of these vaccines were in close proximity to the surface of the carrier proteins which most likely limited their accessibility to angiotensin II-specific B-cell receptors. Both mutations and the way of coupling are likely to influence the ability of the antibodies to bind the peptides. The design of CYT006-AngQb obviates these problems and enhances IgG responses.

A phase I study in healthy normotensive volunteers showed that CYT006-AngQb vaccination was safe and induced angiotensin II-specific antibodies in all subjects [27]. Subsequently, a double-blind placebo-controlled phase Ila study in 72 patients with mild to moderate hypertension was initiated [28]. Subjects were randomized into three groups, one receiving placebo, the second 100 μg CYT006-AngQb and the third 300 μg CYT006-AngQb. Three injections were given at weeks 0, 4 and 12. Vaccination was safe and no treatment-related serious adverse events were observed. Most side effects were mild local injection site reactions. Mild and transient influenza-like symptoms were reported in 21% of patients after vaccination. As seen in healthy volunteers, a single vaccination with CYT006-AngQb was sufficient to induce angiotensin II-specific antibodies in all subjects. The second injection significantly increased antibody titers and peak levels were reached at week 6. Thereafter antibody levels declined but were boosted again by the third injection. Antibodies induced by this regimen were of high affinity for angiotensin II (Kd of 1.4 nM). Fourteen weeks after the first immunization, ambulatory blood pressure was measured. In the 300 μg dose group a significant decrease from baseline was observed for mean day systolic and diastolic blood pressures (−8.9 mm Hg and −4.0 mm Hg, respectively, compared to placebo). The group treated with 100 μg CYT006-AngQb showed a smaller decrease that did not reach statistical significance versus placebo. In agreement with the proposed mechanism of action, total angiotensin II and renin levels were increased in sera as angiotensin II was sequestered by the antibodies. When 24 h profiles of ABPM were analysed, a pronounced effect of vaccination was observed between 05:00 a.m. and 08:00 a.m (Fig. 2). Typically blood pressure reaches a minimum around 01:00 and 02:00 a.m. and thereafter increases until early in the morning, a phenomenon termed early-morning blood pressure surge. In the 300 μg CYT006-AngQb dose group systolic blood pressure at 08:00 a.m. was lowered by −25 mm Hg and diastolic blood pressure by −13 mm Hg compared to placebo [28]. A high early-morning surge in blood pressure is prognostic for increased risk of stroke and intracerebral haemorrhage [29]. Moreover, myocardial infarction and sudden cardiac death rates are increased during early morning [30]. Suppression of early-morning blood pressure surge by an active vaccination approach may thus represent an effective means for controlling the morning blood pressure surge. Larger studies will be needed to determine if this translates into clinical improvements.
Cytos Biotechnology AG has initiated two further phase II studies in hypertensive subjects. In the first study 5 injections of 300 μg CYT006-AngQb were given at weeks 0, 2, 4, 6 and 10. A reduction of ambulatory blood pressure was observed, however it was less pronounced than in the previous study. The reason for the reduced efficacy is currently under investigation. First results indicate that the quality of the antibody response may have been adversely affected by the new vaccination regimen used.

Safety of anti-angiotensin vaccines

Cytos’ hypertension vaccine targeting angiotensin II has so far been tested in 106 volunteers. No serious adverse events related to vaccination have been observed [27,28]. The mild local side effects and the transient flu-like symptoms are acute responses due to the VLP component of the vaccine. Similar reactions were also observed after injection of VLP vaccines conjugated with nicotine [23,24] and house dust-mite peptide [25].

The potential for vaccination to induce immune complexes and immune-complex diseases such as vasculitis or glomerulonephritis previously reported in marmosets immunized with renin [7] has been raised as a possible safety concern [31]. As discussed above, renin is a 340-amino acid protein present in high concentrations in the kidney. It its highly likely that in the experiments reported by Michel et al. vaccination against renin induced a polyclonal anti-renin IgG response and these antibodies formed immune complexes with renin and subsequent renal inflammation. In contrast to renin, angiotensin II is an 8 amino acid peptide, a length which virtually precludes the simultaneous binding of two antibodies to one peptide and thus the formation of immune complexes. This is confirmed from a crystal structure of angiotensin II with a Fab fragment of a monoclonal antibody. Angiotensin II adopts a folded conformation and is bound deeply inside a cleft formed by the CDRs [32]. In this structure only 2 amino acids of angiotensin II are exposed; such a surface would not allow binding of a second antibody. Accordingly, it is highly unlikely that large immune complexes are formed. Indeed preclinical and clinical studies have shown no evidence of immune-complex disease for any of the vaccines targeting angiotensin I or angiotensin II described above [12,13,15,16,27]. From our own experience, even chronic toxicity studies performed with CYT006-AngQb in rats repeatedly immunized over 26 weeks showed no histopathological findings indicative of immune-complex disease. Moreover, no clinically relevant changes in urinalysis and proteinuria were reported for hypertensive patients treated with CYT006-AngQb [28]. In addition, measurements of complement factors C1 and C3 and of circulating C3a did not indicate immune-complex formation after vaccination.

The induction of an aseptic meningoencephalitis in 6% of Alzheimer patients vaccinated against amyloid β (Aβ)1–42 [33,34] raised safety concerns for all vaccines targeting self-proteins [35]. The adverse events in the Alzheimer’s disease trial were assumed to be caused by the induction of an inflammatory CD4+ T cell response against β-amyloid deposits in the brain and the vasculature [36]. Induction of inflammatory CD4+ T cells appears to have been fostered by a change in the formulation of the vaccine which included the strong adjuvant QS21 and an aggregated antigen of 42 amino acids [37,38]. Induction of T cell responses appear to have been circumvented by careful design of a second generation vaccine [39–41]. Indeed vaccines targeting Aβ have again received regulatory approval and are currently being clinically tested (clinicaltrials.gov accession numbers: CAD106 (Novartis) NCT00733863, NCT00795418; ACC-001 (Wyeth) NCT00479557, NCT00752232, NCT00498602; V950 (Merck) NCT00464334). The safety-design elements are also incorporated into CYT006-AngQb. A key requirement for antigen-specific CD4+ T cell activation is high-affinity peptide binding to MHC class II molecules. High-affinity binding requires a 9 amino acid core sequence. Amino acids 1 and 9 are critical anchoring residues. In addition, flanking amino acids also contribute to binding affinity. Ultimately, 12–14 amino acids are usually in contact with MHC class II mole-

Figure 2  Blood pressure reduction after vaccination with CYT006-AngQb Subjects were vaccinated with CYT006-AngQb (triangles) or placebo (circles) and 24 h-ambulatory blood pressures were recorded at week 14. Upper curves show systolic and lower curves show diastolic blood pressure (mean ± SEM). Subjects treated with CYT006-AngQb have significantly reduced blood pressures during day and in particular during the morning surge between 05:00 and 08:00 am.

Please cite this article as: P. Maurer, M.F. Bachmann, Immunization against angiotensins for the treatment of hypertension, Clin. Immunol. (2009), doi:10.1016/j.clim.2009.06.003
molecules. Thus for CYT006-AngQb with its 8 amino acid angiotensin peptide, it is highly unlikely that high-affinity binding to MHC class II molecules is achieved. Consequently, activation of CD4+ T cells should not occur. Indeed, monitoring of subpopulations of immune cells after immunization with CYT006-AngQb showed no indication of activated T cells or other subsets of cells in humans [28].

A number of prophylactic vaccines induce life-long protection against viral infections. Hence, it is often assumed that therapeutic vaccines will also lead to life-long neutralization of the respective target-antigen; this is not the case. Immune responses induced by vaccination against angiotensin peptides, have been shown to steadily decline after reaching peak levels and are thus readily reversible [27,28]. Following immunization, anti-angiotensin I and angiotensin II antibodies decline with half-lives of 12 and 17 weeks, respectively [18,28]. It is noteworthy that the antibody response against angiotensin is not boosted by endogenous angiotensin. Due to its length and soluble nature angiotensin II is not able to cross-link BCR on memory B cells. This, together with the lack of T cell epitopes within endogenous angiotensin II precludes any self-perpetuating immune response.

It has been argued that after severe blood loss, anti-angiotensin antibody half-lives in the range several months could cause problems [35]. This is because the RAS contributes to maintenance of blood pressure during volume depletion. However, in vaccinated subjects, free angiotensin II is in equilibrium with antibody-bound angiotensin II and complete neutralization of angiotensin II does not occur. Hence the RAS is expected to be still able to respond to episodes of acute volume depletion. Furthermore, rescue measures taken in the event of severe trauma are not expected to be affected by the antibodies raised by CYT006-AngQb. Inhibition of the RAS by ARBs, ACE inhibitors or renin inhibitors is very efficient and not more easily overcome than inhibition achieved with antibodies. Since rescue therapy following severe blood loss needs to be applied within hours, the difference in half-life between small molecules and antibodies becomes irrelevant. It should be noted that the recently registered renin inhibitor Aliskiren has a half-life of 40 h. Finally, the RAS is not the only system supporting blood pressure and blood volume in case of haemorrhage/dehydration. The sympathetic nervous system and vasopressin systems also provide support.

Conclusions

Blocking the renin-angiotensin axis using small molecular drugs is a very well validated treatment of hypertension. Evidence is now emerging that targeting angiotensin I or II by vaccination may also be a promising option to lower blood pressure. Indeed, a recent study has demonstrated that vaccination may result in blood pressure reduction comparable to those achieved by classical therapies. Nevertheless, despite this notable success, the efficacy of blood pressure lowering vaccines has remained a challenge. The cardinal reason for the encountered difficulties appears to be not only the quantity but also the quality of the induced antibodies. The currently developed vaccines therefore have been carefully designed to induce strong and highly specific antibody responses. Choice of adjuvant as well as vaccination regimens likely will also be highly critical.

In contrast to the challenge of efficacy, the vaccine approach has proven safe so far. Neither preclinical nor clinical studies with vaccines against angiotensins have shown any findings indicative of safety problems. Nevertheless, it should be borne in mind that the number of subjects treated with angiotensin vaccines is still small. As for any other drug, safety of hypertension vaccines must be carefully monitored throughout development.

The question has been raised whether it is ethical to develop new drugs for indications where effective drugs are currently available [42]. The health burden caused by poorly controlled hypertension is a clear indication that additional therapeutic options are needed. Promising observations from early clinical studies with CYT006-AngQb, such as a reduction of the early-morning surge in blood pressure, could provide an advantage over current therapies. Moreover the smooth onset of action and the absence of peak-trough levels in drug levels may also be beneficial. Again, this must be established in large efficacy studies. If shown to be safe and effective, vaccination is expected to increase compliance, an important factor in blood pressure control rates. It is argued that poor compliance should be addressed by greater investments in behavioural and social research [42]. We fully agree that such efforts are needed. However, the lack of any pronounced success of such efforts during last twenty years strongly suggests alternative approaches are needed. Vaccination against angiotensin is such an approach that warrants further studies.

Disclaimer

P. Maurer, and M.F. Bachmann are employees of Cytos Biotechnology AG and hold shares and/or options.

Acknowledgments

We thank Gary Jennings, Alain Tissot and Philippe Müller for helpful discussions.

References

Immunization against angiotensins for the treatment of hypertension


Please cite this article as: P. Maurer, M.F. Bachmann, Immunization against angiotensins for the treatment of hypertension, Clin. Immunol. (2009), doi:10.1016/j.clim.2009.06.003
