

Expert Opinion

1. Background
2. Target of vaccine
3. Renin vaccine
4. AngI vaccine
5. AngII vaccine
6. AT-1 receptor vaccine
7. Safety issues
8. Conclusions
9. Expert opinion

Vaccines in the management of hypertension

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Importance of the field: In the USA only 35% of patients with hypertension achieve adequate blood pressure control. Non-compliance is one of the main barriers to treatment. Vaccine against hypertension is an innovative treatment, injected every 4 – 6 months, to combat non-compliance.

Areas covered in this review: Pathogenesis of hypertension and progress towards developing a hypertension vaccine, including the virus-like-particle-based approach, new adjuvant molecules and the potential toxicity of hypertension vaccine.

What the reader will gain: The pathogenesis of hypertension is multifactorial. The most common cause is disruption of the Renin–angiotensin–aldosterone system (RAAS), and the first vaccine study was carried out against renin. While the vaccine reduced blood pressure in animal models, it also caused autoimmune disease. In the last decade, vaccines against angiotensin I, angiotensin II, and angiotensin II-type 1 receptors have demonstrated acceptable safety profiles in animal and human studies.

Take home message: Reduction in blood pressure can be achieved by inducing immunity against targets in the RAAS. The target antigen and selection of adjuvant are crucial factors determining effectiveness and safety of the vaccine. CYT006-AngQb (angiotensin II vaccine) reduced blood pressure in humans but the results were not reproducible with more frequent dosing. Vaccines for hypertension are still in the early phase. We hope for an effective vaccine for hypertension in the years to come.

Keywords: angiotensin, angiotensin I, angiotensin II, angiotensin II receptor, hypertension, immune drug, renin, vaccine

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1. Background

Hypertension has been a recorded health problem since the time of the early Egyptian empires. Physicians have studied the pathogenesis and treatment of elevated blood pressure for centuries, but hypertension still remains a major public health challenge. Estimated worldwide prevalence of hypertension suggests that as many as 1 billion individuals are affected, and approximately 7.1 million deaths per year are attributed to it [1]. It is estimated that there are 65 million individuals with hypertension in the USA alone, and many patients are unaware of their disease [2]. According to one report, the most common reason for office visits of non-pregnant adults in the USA in 2006 was to obtain prescriptions for hypertension treatment [3].

Unfortunately, there are no early clinical indicators for hypertension. Often the first signs and symptoms are produced by end-stage organ damage caused by long-term hypertension. For example, at the time of diagnosis, many patients already present with severe damage to the cardiovascular system (left ventricular hypertrophy, left atrial enlargement, aortic root dilatation, atrial and ventricular arrhythmias, systolic and diastolic heart failure and ischemic heart disease) and CNS (hemorrhagic and atheroembolic stroke or encephalopathy), renal failure and/or hypertensive retinopathy. Untreated hypertension is estimated to curtail

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Article highlights.

- While only 35% of patients are able to achieve adequate control of their blood pressure levels through traditional therapeutic interventions, this proportion can be improved by the use of a long-acting (4 – 6 months) medication.
- The current renin vaccine is limited by the fact that it can induce autoimmune deposits.
- Vaccines against angiotensin I, angiotensin II and angiotensin II type 1 receptor have shown promising results and good short-term safety profiles.
- Two main obstacles towards development of novel hypertension vaccinations are:
 - (1) A need to prove superiority to the current oral RAAS blockade agents.
 - (2) A need to demonstrate the long-term safety profile.

This box summarizes key points contained in the article.

life expectancy by five years, in comparison with patients who received the appropriate anti-hypertensive medications [4].

The 2007 United States Preventive Services Task Force (USPSTF) guidelines on screening for high blood pressure recommended screening every two years for persons with systolic and diastolic pressures below 120 mmHg and 80 mmHg, respectively, and yearly for persons with a systolic pressure of 120 – 139 mmHg or a diastolic pressure of 80 – 89 mmHg [5].

Hypertension can be classified according to its primary or secondary causes. The majority of patients (~ 90%) suffer from primary hypertension, which many experts now believe is a multi-factorial disease. These factors include increased salt intake, reduced nephron mass, abnormal renin–angiotensin system, increased sympathetic tone and endothelial dysfunction (Figure 1). In contrast, secondary hypertension is associated with a single definable physiological dysfunction (primary kidney disease, renovascular disease, endocrine abnormality, sleep apnea, or coarctation of the aorta) and, thus, definitive treatment options may exist [6,7].

Despite remarkable advances in new medications, the prevalence of hypertension continues to rise. From 1994 to 2004, increased public awareness of hypertension led to an increase in earlier diagnosis and treatment success. Unfortunately, approximately 30% of adults are believed to be unaware of their hypertension status, while 60% of individuals with hypertension are receiving the appropriate treatments and only 35% of patients have their blood pressure adequately controlled to less than 140/90 mmHg (from National Health and Nutrition Examination Survey (NHANES) data 1999 to 2004). The principal reasons associated with these dismal numbers are inadequate access and low compliance with treatments. One investigation reported that 11% of their patient cohort was non-compliant in adhering to an anti-hypertensive medication for more than 2 weeks [8]. Therefore, improving patients' compliance should be a primary goal for any new antihypertensive agents. The creation of a vaccine against

hypertension promises to eliminate the problems associated with conventional pharmacological agents, including non-compliance with daily dosing schedules, undesirable side effects and drug-drug interactions, and irregular, short-acting and ineffective diurnal blood pressure control.

2. Target of vaccine

Unlike vaccines generated against bacteria and viruses, a specific target antigen, anti-hypertension vaccine is complicated by the multi-factorial etiology of hypertension. The renin–angiotensin–aldosterone system (RAAS) is probably the most important regulator of systemic blood pressure and believed to be a major factor in hypertension onset. The RAAS cascade begins with the biosynthesis of the glycoprotein enzyme, renin, in the juxtaglomerular cells of the renal afferent arterioles. Renin cleaves angiotensinogen to form the decapeptide angiotensin-I (AngI). AngI is then further cleaved by angiotensin converting enzyme (ACE), a dipeptidyl carboxypeptidase, to produce an octapeptide, angiotensin-II (AngII). AngII can then bind to angiotensin type 1 (AT-1) receptors and type 2 (AT-2) receptors to initiate a myriad of downstream signalling pathways.

The effects of the AT-1 receptor include vasoconstriction, aldosterone synthesis and secretion, increased vasopressin secretion, cardiac hypertrophy, augmentation of peripheral noradrenergic activity, vascular smooth muscle cell proliferation, decreased renal blood flow, renal renin inhibition and renal tubular sodium reuptake. AT-2 receptors are more abundant in the fetus and neonate, suggesting a role in embryonic development and a possible vasodilatation effect. ACE inhibitors (captopril, enalapril, benazepril, ramipril andtrandolapril), AngII receptor blockers (irbesartan, candesartan, valsartan and losartan) and direct renin inhibitors (aliskiren) have all successfully been used to reduce high blood pressure. Both ACE inhibitors and AngII receptor blockers have been on the market for many years, and there is evidence to support their benefits beyond blood pressure control. In particular, such agents have been demonstrated to improve the ejection fraction in patients with congestive heart failure [9,10], to reduce the levels of urine protein in diabetic patients [11,12] and prevent recurrent strokes [13]. Vaccines targeting renin, AngI, AngII and AT-1 receptors are currently under development (Figure 2), only two of which are inhuman clinical trial phase, PMD3117 (AngI vaccine) and CYT006-AngQb (AngII vaccine).

Not all primary hypertension is expected to respond well to RAAS blockade therapy. Low-renin-level hypertension, a sub-group of primary hypertension, comprises nearly 25% of all primary hypertension cases and is found more frequently in blacks and in the elderly [14]. The etiology of increased blood pressure in this group is probably due to a salt-sensitive phenotype [15]. Interestingly, Aliskiren (a direct renin inhibitor) failed to lower blood pressure in patients who have low renin levels [16], indicating that a single

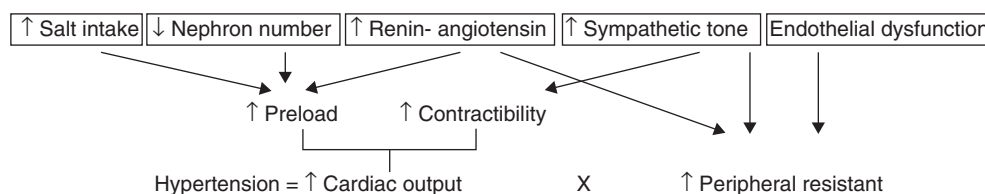


Figure 1. Schematic pathophysiology of primary hypertension.

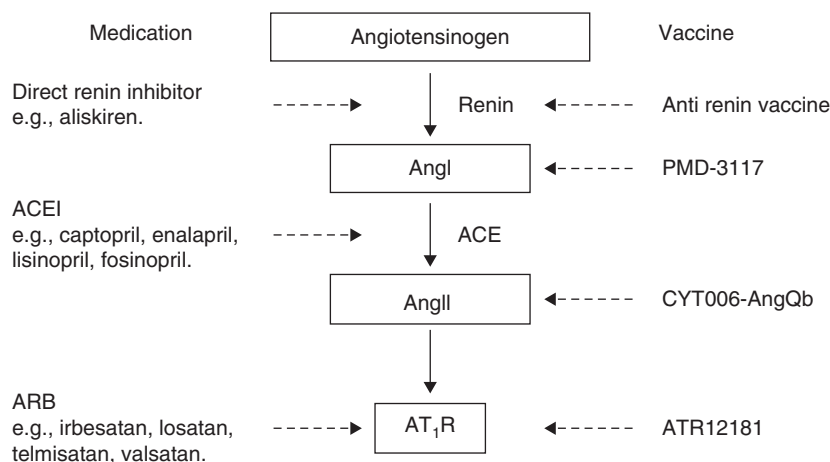


Figure 2. Schematic representation of the classic renin-angiotensin system with common oral medication and vaccines blocking specific targets. The inhibitory actions are indicated by dashed lines with arrows.

targeted vaccine approach may not be suitably effective in all hypertension sufferers.

3. Renin vaccine

Renin was first identified in 1898 by Tigerstedt and Bergman. In 1951, Goldblatt and colleagues conducted the first human study on renin vaccine by injecting hog renin into human subjects. Patients with primary hypertension developed antibodies against renin, but failed to achieve reduced blood pressure [17]. This result was later explained by *in vitro* studies that revealed that anti-hog renin antibodies were unable to cross-react with human renin and suggested that renin molecules are species-specific. Between 1950 and 1980, active and passive immunization against renin was extensively investigated in hopes of identifying a human species-specific therapeutic (Tables 1 and 2).

Before the development of the monoclonal antibody technique in 1980, many problems existed due to the impurity of renin; however, most of the studies were carried out in renal vascular hypertension animal models and showed success in blood pressure reduction [18,19]. In terms of safety, though, investigators particularly looked for consequent onset of autoimmune diseases but found no immune complexes in renal biopsy.

Michel *et al.* [20] decided to add adjuvant to the renin vaccine and obtained a more profound clinical response. Mixtures of purified human renin with either Freund's adjuvant, water in oil emulsion, or dead mycobacteria were injected into marmosets. The marmosets developed high titres of anti-renin antibodies and presented with a significant reduction in blood pressure. However, 1 – 4 months after immunization, the animals became sick and died within another month. This phenomenon was characterized as autoimmune disease by the presence of immunoglobulin that co-localized with renin in the afferent arterioles, cellular inflammatory proliferation around the intrarenal artery and interstitial nephritis. The same group then repeated the study in a spontaneously hypertensive rat (SHR) model but chose mouse renin, which shares 80% homology with rat renin, in the presence of Freund's adjuvant. Vaccine injection resulted in reduced blood pressure, a high titre of anti-renin antibodies, total inhibition of the plasma renin activity and a significant decrease in aldosterone secretion. Unfortunately, these animals presented with autoimmune disease of the kidneys. In response to the secondary effects found in these studies, no further research on renin vaccine has been carried out and reported in the literature.

Table 1. Passive transfer of renin antibodies.

Ref.	Specificity of immunogenic renin	Antibody	Species of model	Experimental model	Blood pressure effect
Wakerlin (1958) [55]	Hog (crude kidney cortex extract)	Dog (polyclonal)	Dog	Renovascular hypertension (1K, 1C)	-40 mmHg
Frank (1963) [18]	Human (semipurified)	Dog (polyclonal)	Monkey (macaca)	Renovascular hypertension	-40 mmHg
	Hog (semipurified)	Dog (polyclonal)	Monkey (macaca)	Normotensive Renovascular hypertension	-8 mmHg 0 mmHg
Weiser <i>et al.</i> (1969) [56]	Hog (semipurified)	Dog (polyclonal)	Rat	Renovascular hypertension (2K, 1C)	0 mmHg
Hill <i>et al.</i> (1970) [57]	Hog (semipurified)	Dog (polyclonal)	Dog	Renovascular hypertension (1K, 1C)	-40 mmHg
Romero <i>et al.</i> (1973) [58]	Hog (semipurified)	Dog (polyclonal)	Rabbit	Renovascular hypertension (2K, 1C) (1K, 1C)	-35 mmHg 0 mmHg
Skeggs <i>et al.</i> (1975) [59]	Hog (semipurified)	Rabbit (polyclonal)	Rabbit	Renovascular hypertension (1K, 1C)	0 mmHg
Slater <i>et al.</i> (1984) [60]	Human (purified)	Rabbit (polyclonal)	Primate (macaca)	Renovascular hypertension (acute 1K, 1C)	-25 mmHg
				Sodium-replete	-35 mmHg
				Sodium-depleted	0 mmHg
				Normotensive	-11 mmHg
Michel <i>et al.</i> (1987) [20]	Human (purified)	Rabbit (polyclonal)	Primate (marmoset)	Sodium-replete	0 mmHg
				Sodium-depleted	-15 mmHg
Wood <i>et al.</i> (1986) [61]	Human (purified)	Mouse (monoclonal)	Primate (marmoset)	Normal salt-intake	-30 mmHg
				Salt-depleted	-20 mmHg

1K,1C: One kidney, one clip; 2K,1C: Two kidneys, one clip.
This table is modified from Michel JB *et al.*, Journal of Hypertension 1989 [65].

Table 2. Active immunization against renin.

Ref.	Specificity of immunogenic renin	Species of model	Experimental model	Blood pressure effect
Godblatt <i>et al.</i> (1951) [17]	Hog	Human	Essential hypertension	0 mmHg
Wakerlin <i>et al.</i> (1953) [55]	Hog	Dog	Renovascular hypertension (1K,1C)	-40 mmHg
Katz <i>et al.</i> (1957) [62]	Hog	Dog	Essential hypertension	-40 mmHg
			Pyelonephritic hypertension	-20 mmHg
			Normotension	-8 mmHg
Helmer <i>et al.</i> (1958) [63]	Hog	Human	Essential hypertension	0 mmHg
			Renovascular hypertension	-40 mmHg
			Renovascular hypertension (2K,2C)	-50 mmHg
Frank (1963) [18]	Human	Primate (macaca)	Renovascular hypertension (1K,1C)	-30 mmHg
			Primate (macaca)	0 mmHg
Deodhar <i>et al.</i> (1964) [64]	Dog	Dog	Renovascular hypertension	-40 mmHg
Skeggs <i>et al.</i> (1975) [59]	Dog	Rabbit	Renovascular hypertension (1K,1C)	-40 mmHg
Michel <i>et al.</i> (1987) [20]	Human	Primate (marmoset)	Normotensive (normal salt intake)	-40 mmHg

1K,1C: One kidney, one clip; 2K,2C: Two kidneys, two clips.
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4. AngI vaccine

Unlike renin, AngI and AngII are very small peptide molecules, composed respectively of only ten and eight amino acids. Investigators believe that the smaller sizes will be less likely to stimulate autoimmune diseases.

In 1989, Reade *et al.* immunized SHRs with an AngI vaccine coupled with *Limulus polyphemus* hemocyanin (LPH). Despite the successful induction of AngI antibody at high titre, the weekly measurement of arterial pressure did not reveal any reduction during the six month study period [21].

Gardiner *et al.* immunized normotensive rats against AngI with a vaccine (PMD-2850) that consisted of an AngI analogue conjugated with a tetanus toxoid (TT) carrier protein, in the presence of aluminum hydroxide (AIOH). Active immunization with PMD-2850 on days 0, 21, and 42 significantly suppressed responses to exogenous AngI on day 63 and had no response to AngII administration.

Downham *et al.* compared AngI vaccines between two carriers, the TT and keyhole limpet haemocyanin (KLH) vaccine (PMD-3117) [22]. Because TT is a common antigen and most adults have already been exposed to it, it may exhibit limited effectiveness in a vaccine [23]. Studies in rats have shown that both vaccines induced an equivalent immune response and inhibition of the pressor effects to exogenous AngI. In humans neither vaccine caused an anti-AngI-IgG response from the first dose, but a response was mounted after the second dose. An anti-carrier protein IgG response was stimulated by the highest dose of AngI-TT conjugate vaccine (100 µg AngI) that was eliminated at lower doses (25 or 50 µg AngI). AngI-KLH conjugate vaccine resulted in anti-carrier protein IgG at all the tested doses (25, 50 and 100 µg AngI). The authors of this study concluded that KLH appeared to be a suitable alternative to TT as a carrier protein for AngI.

Brown *et al.* continue to work on the AngI-vaccine (PMD-3117) in a Phase IIa clinical trial [24]. Primary hypertension patients (n = 27) who had already exhibited responsiveness to an ACEI or angiotensin receptor blocker (ARB) were randomly assigned to receive three or four injections of PMD-3117 or AIOH (placebo) over a 6 week period. According to the published protocol, patients will stop ACEI or ARB for 2 weeks before starting the vaccine and then re-start the medication and continue on it for a period of 6 weeks. The results, thus far, have indicated that anti-(AngI) antibody titre rose after the second injection in both PMD-3117 plus ACEI or ARB groups, and the titre peaked on day 64. Median half-life was determined to be 85 days (95% CI, 44 and 153). The vaccination did not influence blood pressure. However, PMD-3117-treated groups presented with higher levels of plasma renin compared with the AIOH group following withdrawal of ACEI or ARB at day 64 (p = 0.033), probably an effect of the negative feedback pathway between AngII to renin. At 42 days after the first injection, aldosterone excretion was found to be decreased in PMD-3117-treated subjects to

6% (95% CI, 1 and 31%) of values recorded for patients that received AIOH (p = 0.012).

The authors concluded that the biochemical measurements provide evidence of renin system blockage, but higher titres will be required to achieve a decrease in blood pressure. This will require a better adjuvant than AIOH. To this end, a new formulation of PMD-3117 has been developed, and the 'Covaccine HT' adjuvant clinical trial (NCT00702221) has been initiated and results are expected in 2010 [25].

5. AngII vaccine

The first attempts at active immunization against AngII were carried out in rats and rabbits and coupled synthetic AngII to bovine serum albumin and emulsified AngII in Freund's adjuvant. Johnston *et al.* concluded from these studies that although immunization against AngII showed a greatly reduced response to exogenous AngII, it played no direct role in the production or maintenance of experimental renal hypertension [26]. Similar results were recorded by Macdonald *et al.* in 1970 [27].

In 2007, Cytos Biotechnology developed an AngII-specific vaccine (CYT006-AngQb) composed of an AngII peptide with an N-terminal Cys-Gly-Gly extension that is covalently coupled to virus-like particles (VLP) derived from the coat protein of the bacteriophage Qb (Figure 3). This approach is based on the belief that conjugating antigens to the surface of the VLP highly repetitive structure will lead to a strong B-cell response against self antigens; this new technology may help overcome the 'self' tolerance limitations of immunization against AngII [28,29].

In addition, potent T helper epitopes are provided by the carrier, which is of great importance since tolerance or 'ignorance' at the B cell level can more easily be overcome than tolerance at the T cell level; T cells against self antigens are often severely anergized or deleted [30,31].

VLPs are rods or icosahedral supra-molecular structures. VLPs have diameters in the range of 25 – 100 nm. The outer shells have coat proteins from virus (bacteriophage Qb was used in this vaccine), antigenically indistinguishable between VLP and the virus from which they were derived. Inside they have bacterial RNA that lacks replicative genetic information, not an infectious molecule.

The genes encoding VLPs can be made from many sources, including animal viruses, plant viruses and bacteriophages. These genes can be recombinantly expressed into hosts, including, bacteria, yeast and mammalian cells. Because the repetitive surface is identical to a virus without the ability to infect, VLPs can induce strong immune responses that make them highly effective vaccines [32].

In the Phase I, randomized, placebo-controlled, double-blind clinical trial (NCT00500786) of CYT-006-AngQb (100 µg sc; n = 12) compared with placebo (n = 4), no significant changes were observed in normotensive blood pressure in the treatment group [33].

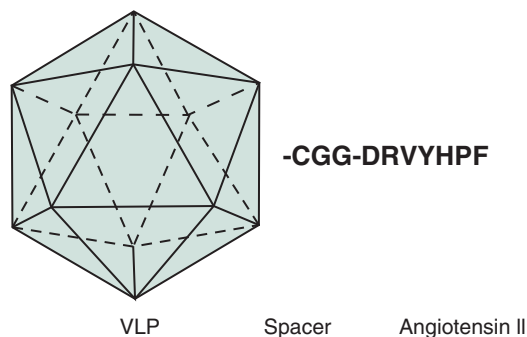


Figure 3. Structure of the AngQb vaccine molecule. The modified angiotensin II peptide is composed of the amino acid sequence of angiotensin 1 – 8 octapeptide (angiotensin II) fused at its N-terminus to a spacer sequence containing a cysteine to permit directional conjugation to the Qb virus-like particle (VLP).

A Phase IIa randomized double-blind multicentre study was conducted in 2005 [34]. The study population was 72 patients with mild-to-moderate hypertension. They were randomly assigned to get high dose (300 µg), low dose (100 µg) CYT006-AngQb or placebo at weeks 0, 4, and 12. The 24-h ambulatory blood pressure was measured at weeks 0 and 14. Both CYT006-AngQb groups had high IgG titers against angiotensin AngII after only one injection. The antibody response was strongly boosted after the second injection, and reached peak levels of response about two weeks after the third injection. The angiotensin AngII-specific IgG response in the group that received the high dose was much higher than that of the group that received the low dose.

The average half-life after the third injection was determined to be 17 weeks. There was no change observed in the concentration of immune complexes containing C1 and C3 or the level of complement factor C3a, which suggests that there was little to no immune complex deposition. There was, however, a significant reduction in the early-morning blood pressure surge, as compared with placebo, in the 300 µg group; the change was measured at -25 mmHg in systolic blood pressure and -13 mmHg in diastolic blood pressure (-9/-4 mmHg on average/24 h). Most of the reported side effects were transient and mild, including local injection-site reactions. This trial was the first, to our knowledge, to show that vaccination against a vasoactive endogenous substance can effectively reduce blood pressure in human beings. Interestingly, the drop in blood pressure was most pronounced in the early morning, when the RAAS is most active and when most stroke and cardiovascular events occur [35]. The most recent data from the same investigator examined administration of the vaccine more frequently (at weeks 0, 2, 4, 6, and 10). This modification was expected to induce higher antibody titres and, thereby, a stronger blood pressure reduction. Surprisingly, the result showed on average a fivefold higher antibody titre but only -2.3/-0.4 mmHg

blood pressure reductions as compared with the first study. In order to understand this discrepancy, the researchers analyzed the biochemical properties of the induced antibody responses and found that the antibody affinities to AngII were significantly lower in the second study than in the first ($p < 0.001$). In addition, the amount of AngII that was found to be sequestered in the blood of vaccinated individuals was approximately 33% lower in the second study. The individual changes in daytime ambulatory blood pressure correlated with the individual antibody affinities ($p = 0.10$) and, in particular, with measures for the off-rates, which describe how long AngII is bound to the antibodies ($p = 0.006$). Taken together, these results indicated that patients whose antibodies had a higher affinity and which bound AngII for a longer period of time experienced a more profound blood pressure reduction. However, no statistically significant correlation was found between antibody titres and blood pressure reductions ($p = 0.47$). Cytos Biotechnology has announced their intent to prospectively test this hypothesis using higher dosages.

6. AT-1 receptor vaccine

Zhu *et al.* immunized SHR with a peptide-based vaccine made of a seven-amino-acid, (AFHYESR) sequence from the second extracellular loop of rat AT-1A receptor (ATR12181) [36]. The carrier protein was a TT complex in combination with Freund's adjuvant. Repeated vaccinations (on weeks 0, 4, 8, 12, 16, 24, 32, 40 and 52) were administered and an observational study commenced at week 64. The animals exhibited marked induction of anti-ATR12181 antibodies and had systolic blood pressure lowered by 17 mmHg, decreased cardiac hypertrophy and attenuation of kidney injuries. There were no signs of autoimmune diseases in the biopsied sections of heart and kidney [37]. Because Freund's adjuvant is not safe for use in humans, the next phase of this study will focus on the use of ATR12181 in combination with VLP in human subjects.

There is a risk of an agonist effect from AT1 antibodies. AT1 receptors belong to the family of seven-transmembrane receptors, with three extracellular loops in the N-terminus. In normal physiology, the AngII molecule will bind to the AT1 receptor at multiple sites in order to generate an agonist conformational change that causes receptor activation [38]. The receptor can be blocked by occupation of an intramembrane site that overlaps with the space occupied by the agonist or by inducing conformational changes that prevent agonist binding.

Agonistic AT1 antibodies exist in certain transgenic mice. They are defined by the epitope on the receptor's second extracellular loop, but no specific antigens have been identified. These types of agonistic AT1 antibodies are now believed to be the cause of preeclampsia in humans [39]. Mice with antibodies against ATR12181 (amino acid positions 181 – 187 in the second extracellular loop) did not develop hypertension or extra-cardiac organ damage, indicating that antibody

against ATR12181 did not display agonistic characteristics [40]. The different antibody effects of agonist/antagonist are probably due to different antigen sites being used on the AT1 receptor.

7. Safety issues

Renin vaccine-induced immune-complex diseases, such as vasculitis or glomerulonephritis, have raised concerns about the safety and feasibility of this type of therapeutic approach. Neither CYT006-AngQb or PMD-3117 showed immune complex in various tissues biopsied from experimental animal subjects, and caused secondary reactions only at the injection site in humans. ATR12181 did not stimulate any autoimmune diseases in animal subjects.

Both CYT006-AngQb and ATR12181 rely on VLP in the vaccine composition. The use of VLP is a relatively new technology and only a few VLP-based vaccines are currently on used in the market, including Gardasil and Cervavax. These particular vaccines have been heavily promoted by the pharmaceutical industry and are used to prevent human papilloma virus (HPV) infection. Both have maintained good safety profiles since their introduction in 2006 [41]. There are currently at least 14 VLP vaccines in development for a wide variety of diseases, including breast cancer, prostate cancer, obesity and Alzheimer's disease [42].

Before any vaccine is allowed on the market, stringent analyses are carried out to determine toxicity; to this end, five categories of potential toxicity have been described [43] included i) Direct toxicity of the vaccine; ii) Toxicity linked to the pharmacodynamic of the vaccine; iii) Toxicity from exacerbation of pre-existing condition; iv) Toxicity of contaminants and impurities of the vaccine; v) Adverse reactions due to the interaction between the various vaccine components. This review will only discuss the first two aspects, which are more relevant to our topics. The first is the direct toxicity to subjects and the second is toxicity resulting from subject's reaction to the vaccine.

The direct toxicity is dependent on the composition of the vaccine. In general, vaccines have three principal components: the target-antigen, the cross-linking moiety and the adjuvant or protein molecule that aids in presenting the antigen to antibody (VLP, KLH, AIOH). The following issues are of particular interest to hypertension vaccines: Target-antigen, AngI or AngII, are known to induce high blood pressure. However, their use in vaccines did not result in decreased blood pressure, probably due to insufficient dosages and antigen being hindered by binding to antibodies.

Cross-linker, *m*-maleimidobenzoyl-*N*-hydroxysulphosuccinimide ester (MBS) and Succinimidyl-6-[(β -maleimidopropionamido) hexanoate (SMPH) were used in PMD3117 and CYT006-AngQb, respectively. These hetero-bifunctional chemical cross-linkers are highly reactive compounds used for covalently conjugating the antigen to carrier protein or VLP. Both molecules are very labile in aqueous solution and

the low concentrations used in vaccine manufacture contribute negligible toxic potential.

VLPs are 30 nm icosahedrons composed of 180 coat protein monomers and host bacteria RNA. In the case of CYT006-AngQb, the RNA is from *Escherichia coli*. Each coat protein has two cysteines that are connected via disulphide bridge with another coat protein to form pentamers and hexamers. The coat protein has no known enzymatic or toxic properties and, from a toxicological perspective, is essentially inert.

E. coli RNA, however, is not an inert molecule. Single-stranded RNA (ssRNA) is the natural ligand for Toll-like receptors 7 and 8 (TLR7/8) [44]. Engagement of these receptors upregulates costimulatory molecules and cytokines, resulting in induction of effective immunity. We have limited data on effects of ssRNA in VLPs, but there are some ssRNA analogues available for use. Imiquimod, Resiquimod, and Loxoribine were developed as general immunomodulators for use in antiviral and cancer therapies [45]. Toxicology programs indicate a high degree of safety with no target organ toxicity other than that attributed to exaggerated pharmacological activity of IFN- α . The toxic effects of this RNA are expected to be of little significance due to the low dose administered and the fact that the molecules have been surrounded by the coat protein.

AIOH is frequently included in immuno-drug formulations to boost B cell responses (as discussed above and in [46]). Its inclusion in vaccines is believed to have no impact on safety [45,46]. Side effects attributed to the use of aluminum in vaccines are limited to local reactions such as swelling, indurations, erythema and cutaneous nodules [47].

KLH is used primarily to induce immune response in humans and has no known toxic properties [48].

The second potential toxicity is the reaction of the subject's body to the vaccine. This is a more complicated phenomenon and is dynamic between individuals. The following issues are of particular interest to hypertension vaccines:

Considering the intended (therapeutic) pharmacological action of the vaccine. The toxicity achieved from neutralizing the action of ATI or ATII by antibodies is not expected to be different from that of any other oral ACEI or ARBs which are generally accepted as safe. Because these vaccines do not interfere with ACE, they are not expected to induce angioedema – the most serious adverse effect of ACEI. High potassium levels and increasing serum creatinine are the most common side effects reported in chronic kidney disease patients. Unfortunately, the vaccines introduced thus far have a long half life and there is no effective way to halt their action in the event of an adverse reaction.

Turning to the risk of agonist effect from AT-1 antibody. Agonist effect will have an undesirable side effect from the simulation of the RAAS pathway. Preeclampsia is now believed to have its pathogenesis from the agonist effect of AT-1 antibody. Early studies have confirmed that the AT-1 antibody bound to the second extracellular loop of receptor [39,49].

Further study was done to locate specific amino acid location in second extracellular loop. Antibodies to specific amino acid locations were tested, VFFIEN (169 – 174), ENTINT (173 – 178), ITVCAF (177 – 182), AFHYESQ (181 – 187) and QNSTLPI (187 – 193). Only antibody to AFHYESQ inhibited the increased beating rate of neonatal rat cardiomyocyte compared with 15 – 25% increase for the others [50]. It is unclear why slightly changing the binding location yield an opposite result. One explanation for this difference is that AT-1 antibody at AFHYESQ can interfere with receptor activation by occupying an intramembrane site that overlaps with the space occupied by the agonist or by inducing conformational changes that prevent agonist binding [38]. AT-1 vaccine was made from antibody against AFHYESQ amino acid and showed no development of hypertension and extra cardiac end organ damage [49].

Immune complex deposition has occurred in subjects administered the renin vaccine. In normal situations, the antigen-antibody complexes are removed by mononuclear phagocytes of the reticuloendothelial system. However, in certain abnormal situations, persistent immune complexes may be deposited in tissues and organs. This can result in activation of complement and effector cells, which cause inflammation and tissue damage. The propensity for immune complexes to form, deposit and cause disease is influenced by antigen valency and concentration, antibody class, concentration and affinity, the concentration and size of the complex, and the complement status of the individual [51]. Immuno-drugs that target larger polypeptide antigens are more likely to induce immune complexes. Renin is a 406-amino acid peptide but AngI, AngII and ATR 12181 are substantially smaller at 10, 8 and 7 amino acid peptides, respectively. The chance of inducing antibodies that recognize two epitopes within these small amino acid peptides is very low given the number of residues typically involved in antigen-antibody recognition and area of contact [52]. In theory, short peptides will preclude the simultaneous binding of two antibodies to one peptide and thus thwart the formation of immune complexes.

There is a possibility that antibodies induced from the vaccine could target other peptides/cells in body that are not the intended targets. This unintentional cross-reactivity may lead to severe biological consequences. The use of *in silico*, *in vitro* or *in vivo* methods to assess whether antibodies generated against a particular epitope will cross-react and result in toxic effects is not well established [53]. There is no known cross-reactivity for any of the hypertension vaccines at this time.

The ability of autoreactive CD4 and CD8 T cells to contribute to pathology is well documented in human diseases, such as multiple sclerosis and type 1 diabetes. There has long been an association of infection and molecular mimicry with T-cell-mediated autoimmune disease. Some examples are *Streptococci* and rheumatic fever, B3 Coxsackieviruses and myocarditis, and *Borrelia burgdorferi* and arthritis.

AN1792, a vaccine developed for Alzheimer's disease, has shown evidence of the presence of unintended autoreactive T cells. Meningoencephalitis occurred in 18 of 298 treated subjects and the inflammatory response was attributed to A β -specific T cells [54]. It is crucial that the vaccine be specific to the B cell because the responses are transient and reversible, and should not continuously stimulate antibody production. T cells respond to peptide epitopes presented on MHC class I or class II molecules. The minimal length of a peptide that can bind to MHC class I (recognized by cytotoxic T lymphocyte) is eight amino acids, and MHC class II molecules (recognized by T_H cells) is 10 – 12 amino acids. Peptides less than eight amino acids long are unable to induce T cell responses. For the AngI and AngII vaccines, the risk of inducing autoreactive T cells is small due to the fact that the target-antigen is only seven to eight amino acids long. Preclinical studies and clinical trials have found no evidence of autoreactive T cell pathology.

8. Conclusions

Many attempts have been made to generate an effective vaccine to block RAAS. Renin vaccine is the earliest vaccine that effectively reduced blood pressure in animal models, but it also led to the onset of various autoimmune diseases. Vaccines against AngI, AngII and AT-1 were developed in hopes of overcoming these particular shortcomings of the original RAAS-targeted vaccines. The main difference in the newer vaccine approaches is that they are based on smaller target antigens, which, in theory, should not stimulate such a profound autoimmune response. The initial results with these vaccines in animal models are promising. Currently, CYT006-AngQb (AngII vaccine) is the only effective vaccine tested in humans that effectively reduces blood pressure (-9/-4 mmHg). It also has a relatively long half-life of 4 months. Subsequent studies showed that the affinity, and not the titres, of the antibodies is the important factor that determines the effectiveness of the vaccine. PMD-3117 (AngI vaccine) was unable to reduce blood pressure in human subjects. Scientists are now focusing on improving the associated adjuvant molecules to overcome this problem. The CYT006-AngQb vaccine employed VLP as its adjuvant while PMD-3117 used KLH. The target antigens, as well as the choice of adjuvants, are likely to be the determining factors for the effectiveness of the vaccine.

It is important to note that even if we can achieve complete blockage of RAAS, we still cannot treat everyone with hypertension using only a single-vaccine approach because of the multi-factorial pathogenesis of hypertension. Nonetheless, it will be a tremendous help in improving patient compliance for at least a sub-population of hypertension sufferers.

The number of subjects treated with these vaccines is still small. In addition, the long-term safety has yet to be established and requires continuing non-clinical studies, widespread clinical testing and post-market evaluations.

9. Expert opinion

An effective vaccine for hypertension will be a breakthrough in hypertension management. At this time, the main objective of these vaccines is to improve patient compliance. These vaccines are in the RAAS blockade class, along with oral ACEI, ARB and direct renin inhibitors. Theoretically (by the law of mass action, $A + B \rightleftharpoons A' + B'$), these vaccines should not be superior to the current oral RAAS blockade medications (providing patients have good compliance). The subgroup of patients with low renin-hypertension is likely to have a limited response to these vaccines. Physicians may be able to use these vaccines alone or in combination with other classes of hypertension medications. CYT006-AngQb (AngII vaccine at dose 300 µg) is the only vaccine that is effective in humans for reducing average blood pressure by -9/-4 mmHg; unfortunately, the increase in frequency of the vaccine only lowers blood pressure by -2.3/-0.4 mmHg. It is unclear why the second study has lower antibody affinities, although it is possible that an as yet unidentified host factor may play a role. The third study that will use higher doses and more frequent vaccination may provide a clear answer as to the most effective vaccine regimen. As mentioned above, effective vaccines will rely on the host to produce the appropriate antibodies to RAAS. Immunocompromised patients may, thus, have less blood pressure reduction effects.

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Declaration of interest

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Vaccines in the management of hypertension

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