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Review

Vaccine for hypertension: Modulating the renin–angiotensin system

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Abstract

Hypertension, which is one of the most common diseases afflicting mankind, is associated to increased morbidity, mortality and cost to society. Cardiovascular disease is the leading cause of death all around the world and hypertension is the most common reversible risk factor for cardiovascular diseases. The renin–angiotensin system (RAS) commands an important role in the regulation of blood pressure, and so, at present, has been a target for clinical control by drugs acting on the system. Despite the fact that effective drugs are available, only about one out of three people has their blood pressure successfully controlled, and the blame goes to the undesirable side effects and the poor oral drug compliance. Keeping in mind the increasing incidence of hypertension and the patients' inconsistency for the polypharmacy, immunization against renin and the angiotensins, although with less success, had been attempted in the past. More recently, immunization against angiotensin-I with PMD-3117 vaccine, angiotensin-II with CYT006-AngQb vaccine and targeting angiotensin-II type 1A receptor with ATR12181 vaccine have provided optimism in the development of a hypertension vaccine. AngQb vaccine has proved to become the first vaccine ever to lower (−9/−4 mm Hg) blood pressure in human beings. Vaccine could induce long lasting effects with a dosing interval of months, increasing patient acceptability and compliance and thus a better control of high blood pressure. Our objective will be to focus on the importance of the RAS and to explore the extent of safety, efficacy and the future implications of vaccine against the RAS.

Keywords: Auto-immune; Angiotensin-II; Immunization; Virus-like particle

1. Introduction

Cardiovascular disease (CVD) is the leading cause of death all around the world, and hypertension is the most common reversible risk factor for CVD. Hypertension significantly increases the risk of developing disorders such as coronary artery disease, stroke, arrhythmia, heart failure, abnormal renal function and many other complications associated with structural damage to the cardiovascular system. The prevalence of hypertension in the adult population was estimated to be 26.4% in 2000 and is projected to be 29.2% by 2025. The estimated total number of adults with hypertension in 2000 was 972 million; 333 million in economically developed and 639 million in economically developing countries. Study predicts that the number would rise by 60% to a total number of 1.56 billion by 2025 [1]. The prevalence of hypertension varies between 15 and 35% in urban adult population in Asia and while the prevalence and mortality due to CVD is rapidly declining in most developed countries, it is in sharp contrast rising in developing countries [2]. Despite the increasing incidence in number of people with hypertension, Control rates are below 30% in most countries, and thus, only one out of three treated for hypertension is under control [3]. Treatment of hypertension is based on lifestyle interventions and drug therapy. Today we have drugs that are highly effective at lowering blood pressure with minimal side effects. However, both approaches require patient adherence to be effective. Poor compliance is common for both approaches and is the

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main reason for inadequate blood-pressure control. If vaccination against high blood pressure were safe and effective in the long run, it might solve many problems of non-compliance. Besides resolving the compliance problem, the patients will not have to encounter the undesirable side effects of the drugs, would gain smooth, prolonged and progressive onset of action, increased diurnal control of blood pressure and reduction in drug interaction associated with conventional polypharmacy.

The *renin–angiotensin system* (RAS) is a coordinated hormonal cascade in the control of cardiovascular, renal, and adrenal function that governs fluid and electrolyte balance and plays an imminent role in the regulation of arterial blood pressure [4]. Since the discovery of renin as a pressor substance in 1898, the RAS has been extensively studied [5]. The classical enzymatic RAS cascade begins with the biosynthesis of the glycoprotein enzyme, renin, in the juxtaglomerular cells of the renal afferent arterioles. In the classical RAS, renin has no biological activity but cleaves angiotensinogen, the only known precursor protein for angiotensin peptides, to form the decapeptide, angiotensin-I (AngI) (Fig. 1). Angiotensinogen synthesized in the liver provides the majority of systemic circulating angiotensin peptides, but angiotensinogen is also synthesized and constitutively released in other tissues including the heart, vasculature, kidneys, and adipose tissue. AngI is then further cleaved by angiotensin converting enzyme (ACE), a dipeptidyl carboxypeptidase, which is widely present on the endothelial cells of many vascular beds including the lungs, to produce the octapeptide angiotensin-II (AngII), the physiologically active component of the system. AngII is degraded within seconds by peptidases, collectively termed angiotensinases, at different amino acid sites to form fragments, mainly Ang-III and Ang-IV respectively [6,7]. The

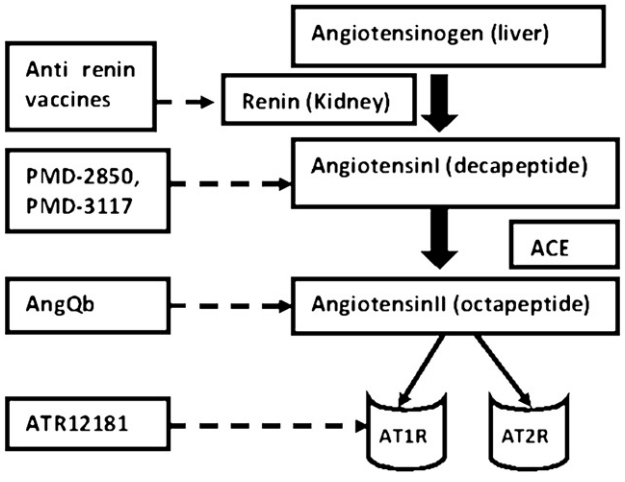

Fig. 1. Schematic representation of the classic renin–angiotensin system and vaccines blocking at their specific targets. The inhibitory actions of the vaccines are depicted in dashed lines with arrows. ACE = angiotensin converting enzyme, AT1R, AT2R = angII-type 1 and type 2 receptors respectively.

action of AngII results from its binding to its specific receptors, type 1 (AT-1) receptors and type 2 (AT-2) receptors. Despite belonging to the same receptor family, the AT-1 and AT-2 receptor subtypes differ markedly in their signaling cascades and biologic activities. The AT-1 receptors are found in vascular and many other tissues and are almost certainly the receptors that transduce AngII mediated cardiovascular actions. The AT-2 binding Sites are much more abundant in fetal and neonatal than in adult tissue, suggesting some role in development. Results suggest that AT2 receptor expression parallels the suppression of vascular smooth muscle cell growth, the reverse of AT-1 receptor [6,7]. Two subtypes of AT-1 receptor, AT-1A and AT-1B, have been identified in mice and the results for their involvement in blood pressure regulation have been mixed. Although these receptor subtypes are pharmacologically indistinguishable and are thought to signal identically it is generally accepted that humans express only one type of AT-1 receptor [8,9].

1.1. The role of angiotensin-II

Angiotensin-II, the octapeptide, is the principle mediator of the pathophysiological actions of RAS via the activation of specific AngII receptors. Virtually all of the known regulatory actions of AngII on blood pressure and osmoregulation have been attributed to the AT-1 receptor. These include potent vasoconstriction, aldosterone and vasopressin release, renal tubular sodium reabsorption, and decreased renal blood flow [10].

Data suggest a direct growth effect of AngII on the left ventricle and indicate a role for the RAS in the *cardiac hypertrophy* that develops in response to pressure overload. The increase in left ventricular mass was completely prevented in animals fed with ACE-inhibitor after abdominal aorta constriction [11]. Regression of the hypertrophic heart despite persistent pressure overload and improved survival in rats has been demonstrated by blocking the RAS [12]. Yet in another study, mice developed myocardial hypertrophy independently from the presence of hypertension, demonstrating that local AngII production is important in mediating the hypertrophic response in vivo [13]. Intratissue formation of AngII plays a critical role in cardiovascular remodeling [14].

For *atherosclerosis*, vascular inflammation is an independent risk factor. AngII has significant pro-inflammatory actions in the vascular wall, inducing the production of reactive oxygen species, inflammatory cytokines, and adhesion molecules, which contributes to the migration of inflammatory cells into tissue injury. AngII is also chemotactic and mitogenic factor for mononuclear cells [15,16]. AngII increases receptor density and sensitivity for other factors that modulate growth of vascular smooth muscle, such as fibroblast growth factor, transforming growth factor beta-1, platelet-derived growth factor, and insulin-like growth factors suggesting an indirect autocrine mechanism for the growth response of vascular smooth muscle to AngII [17]. Through these actions, AngII

augments vascular inflammation, induces endothelial dysfunction and thus atherosclerosis. The proatherogenic property of AngII is especially potent in the presence of hyperlipedemia, and independent of hypertension [15,18].

It has also been suggested that AngII promotes *thrombosis*. AngII increases plasminogen activator inhibitor type1 in rat smooth muscle cells and in humans in vivo and thus alters the fibrinolytic balance [15,19]. This raises the possibility that AngII is prothrombotic, with all of the implications for thrombin, is related in acceleration of atherosclerosis.

It has been demonstrated that *nuclear oncogenes* (c-myc, c-jun, and c-fos) are induced in the vascular walls in the hours immediately after injury. Increased expression of nuclear oncogenes (c-fos and c-jun) was induced by treatment with AngII in vascular smooth muscle cells and cardiomyocytes, which significantly increased the rate of protein synthesis and the size of cardiomyocytes. On the other hand, pre-treatment with AngII blocker (valsartan) before AngII treatment, was able to inhibit all the above effects [20,21]. These results suggest the invaluable role of AngII inhibition at the time of acute cardiovascular injury (e.g. Myocardial infarction) to limit the hyperplasic response.

Thus, AngII is an excellent target for pharmacological or immunological intervention, which could be accomplished by blocking the RAS at its major components. Not only does it play a pivotal role in both the acute and chronic regulation of systemic arterial pressure, but it also is an important modulator of cardiovascular structure and function and may be specifically involved in disease progression. Modification of AngII effect, may therefore serve a dual purpose. Not only will blood pressure reduction occur with less stretch, stress, and turbulence of the vascular wall, but there will also be less stimulation, less inflammation, less thrombosis and less oncogenic expression, either directly or indirectly, for restructuring and remodeling of the cardiovascular tree.

1.2. Drugs blocking the RAS

After the discovery of the first orally active ACE-inhibitor, captopril, in 1977 [22], this class of drug has not only proved to become a cornerstone in the treatment of hypertension [23,24] but has also proved effective to decrease mortality in congestive heart failure and left ventricular dysfunction after myocardial infarction, and also in delaying the progression of diabetic and non-diabetic nephropathy [25]. But despite all these novel effects, ACE is not the preferred target to block the renin–angiotensin system because it also cleaves peptides other than AngI, and this lack of specificity has been the source of some of the most frequent side effects of ACE inhibitors, such as cough, angio-oedema [29]. Thus in search of more specific blockage in the RAS, AngII receptor antagonist (ARB), losartan, was introduced in 1988 [26]. Drugs of this class have been significantly successful to lower the frequency of cough, angio-edema and first-dose hypotension, although some cases of angio-edema have been reported [27]. Numbers of large outcome trials have demonstrated that, AngII receptor antagonist has enabled to improve significantly the tolerability profile of this group of drugs while maintaining a high clinical efficacy [28]. Yet still moving ahead, aliskiren, the first drug of the new class 'renin inhibitor' was introduced in 2003 [29], as to catalyze the rate-limiting step of the RAS, which represents the optimal target for RAS inhibition. Unlike ACE inhibitors and ARBs, which indirectly increase plasma renin activity (PRA), aliskiren directly inhibits renin to decrease PRA, leading to reduced AngI and AngII levels [30]. Its antihypertensive potency is approximately equivalent to that of ARBs, ACE inhibitors, and diuretics and after abrupt withdrawal, persistent BP reduction and prolonged suppression of plasma renin activity is observed [31]. Data on its morbidity and mortality benefits from long-term clinical trials are needed to determine if aliskiren can join ACE inhibitors and ARBs as a preferred agent. However, even with these well tolerated and efficacious oral drugs, poor compliance is a significant constraint in delivering effective treatment for chronic conditions like hypertension. Thus, modulation of RAS function by active immunization may have advantages over the current therapy.

1.3. Trials for hypertension prevention

Studies show that, administration of ACE-inhibitor for a brief period of time in their early age in spontaneously hypertensive rats (SHRs) prevented the subsequent development of hypertension and cardiac hypertrophy in their later life [32,33]. Result of similar studies, with temporary treatment in young human subjects with high familial risks or pre-hypertension showed mixed results. The TROPHY Study indicated an absolute reduction of 26.8% in new onset of hypertension whose effect could extend to up to 2 years after the discontinuation of treatment [34]. Skov et al. concluded that temporary treatment did not delay the onset of hypertension beyond the active period but there was remarkable decrement in systolic and diastolic blood pressure during the active treatment with the ARBs [35]. Although both studies showed marked decrease in renal vascular resistance, filtration fraction and left ventricular mass index, neither study demonstrated a convincing clinical argument on the short term treatment of the high risk subjects for its useful persistent effects.

2. Immunization of the RAS

Vaccination (Latin: vacca = cow) and immunization, whose early forms were developed in ancient India and China as early as 200 B.C. [36], in common speech, generally have the same colloquial meaning. The first ground breaking human vaccine, by Dr. Edward Jenner in 1796 [37], was derived from the benign cowpox virus, which provided immunity to small pox.

Table 1
Active immunization against renin.

Author	Specificity of renin	Studied subject	Experimental model	BP response	Comments
Goldblatt et al. [38]	Hog renin	Human	Essential HTN	No fall in BP	Produced antirenin antibodies
Michel et al. (1987) [43]	Human	Primate (marmosets)	Normotensive primates	Significant fall in BP	Auto-immune disease specific to kidney
Michel et al. (1989) [44]	Mouse	SHRs	Hypertensive rats	Significant fall in BP	Auto-immune disease specific to kidney

Abbreviations: SHR—spontaneously hypertensive rat, BP—blood pressure.

2.1. Renin immunization

It was only in 1951 when Goldblatt [38] first attempted the active immunization against human renin (Table 1). The administration of heterologous (hog) renin in man produced antirenin antibody, but it was ineffective against human renin and no fall in blood pressure in hypertensive patients was noted [38]. Subsequently, active and passive immunization against renin has been extensively investigated [39,40]. In these early studies, active immunization against renin was the target and several species, particularly using hog renin in dogs and human renin in primates have been studied with successful results [39,41]. However, interpretation of these early studies was limited by the impurity of the immunogen, absence of hormonal assays throughout the experiment and problems of species-specificity. As in all cases, semipurified kidney extracts containing predominantly renin, without adjuvants were used, these experiments therefore did not induce immunological memory [42].

Then after, in the late 1980s, active immunization against renin was carried out utilizing the newer immunological tools. This time Michel et al. [43] carried out the active immunization with pure human renin and Freund's adjuvant, in normotensive marmosets on a normal salt intake. Immunization against renin in marmosets was associated with a high titre of renin antibodies, complete inhibition of endogenous plasma renin activity and a decrease in blood pressure. But they all presented an auto-immune disease specific to the kidney. This auto-immune disease was characterized by the presence of immunoglobulins co-localized with renin in the afferent arterioles, the presence of a cellular inflammatory proliferation around the intrarenal arterial tree and an interstitial nephritis. Some years later, another similar study was performed by the same team [44] in spontaneously hypertensive rats (SHRs) using pure mouse submandibular gland renin which shares 80% homology with the rat renin. Immunization with mouse submandibular gland renin in SHR, adjuvanted with Freund's adjuvant, induced a normalization of blood pressure associated with a high titre of antirenin antibodies, total inhibition of the plasma renin activity and a significant decrease in aldosterone secretion. Unfortunately, these animals also presented an

Table 2
Active immunization against the angiotensins.

Author (date)	Immunization against	Vaccine formulation	Study subject	BP Response	Comments
Johnston et al. (1970) [45]	AngII	Bovine serum albumin/ Freund's adjuvant	Hypertensive Rats and rabbits	No response	Greatly reduced response to AngII
Graham et al. (1970) [46]	Ang II	Adsorbed into carbon	Rabbits	No response	Reduced BP response to renin and AngII
Oates et al. (1974) [47]	AngI and/or AngII	Bovine serum albumin/ Freund's adjuvant	Rats	No response	
Reade et al. (1989) [48]	Ang I	Gluta/LPH, Carbo/LPH	SHRs	No BP response	High level of antibody titre
Smits et al. (1999) [49]	AngI	–	SHRs	Not significant	11 mm Hg decrease in Diastolic BP during the sleeping period
Gardiner et al. (2000) [50]	AngI (PMD 2850)	TT/ALOH	Male, Sprague–Dawley rats	Reduced response to AngI but non with AngII	Antibody cross reacted with angiotensinogen
Dowman et al. (2003) [52]	AngI (PMD-2850, PMD-3117)	TT/ALOH, KLH/ ALOH	Rats Human (healthy)	Reduced pressor effects to AngI but non with AngII in rats. No response in human.	KLH was as effective as TT
Brown et al. (2004) [53]	AngI (PMD 3117)	KLH/ALOH	Human	No response	Tolerated, safe, half-life of 100 days
Zhu et al. (2006) [55]	AngII-type 1A receptor (ATR12181)	TT/Freund's adjuvant	SHRs	Significant decrease in BP	Target organ protection, no auto-immune disease detected.
Ambuhl et al. (2006) [59]	AngII (AngQb)	VLP/ALOH	SHRs, human (n=12).	Significant BP decrease in Rats.	Highly immunogenic, tolerated and safe in both rats and human.
Tissot et al. (2008) [60]	AngII (AngQb)	VLP/ALOH	Human (n=72)	Significant decrease in MBP.	Safe, tolerable, and long half-life (4 months).

Abbreviations: Ang = angiotensin, ALOH = aluminum hydroxide, MBP = mean blood pressure, LPH = limulus polyphemus haemocyanin, KLH = keyhole limpet haemocyanin, TT = tetanus toxoid, VLP = virus-like particle, AngQb = simple name given for CYT006-AngQb by the authors.

auto-immune disease of the kidney. This safety concern, thus, then after, discouraged any further research on renin immunization.

2.2. Angiotensin-II immunization

On the other side, it was in 1970 when Johnston et al. [45] attempted the first active immunization against angiotensin-II on rats and rabbits (Table 2). For better immunization, synthetic AngII was coupled to Bovine serum albumin and emulsified in Freund's adjuvant. In the series of experiments he performed, he concluded 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. Graham et al. in 1970 [46] immunized rabbits against AngII and confirmed the specific absence of pressor response to high doses of renin and AngII after immunization. But there was no evidence of modification in established hypertension. Immunization against AngII and combined AngI plus AngII in rats was studied by Oates et al. [47], in which he confirmed that those immunized against equal parts AngI and AngII were found to develop antibody titre 6–15 times greater for AngI than for AngII. The sustained AngI and/or AngII immunity did not alter blood pressure. They also ruled out the involvement of AngII produced within the arteriolar walls from circulating AngI, close to receptor sites, in blood pressure control, stating that AngI immunity during their study should have blocked the mechanism of action.

2.3. Angiotensin-I immunization

Owing the fact that immunization against AngII was unable to alter blood pressure, Reade et al. [48] attempted *immunization against AngI* in SHRs resulting in high levels of antibodies against AngI. Elisa showed a good maturation of the immune system with a sharp elevation of the IgG1 ang IgG2 alpha isotypes after 2 or 3 injections, but still failed to reveal any fall in blood pressure during the 6 months time of immunization. Smits et al. [49], showed no significant decrease in mean blood pressure in immunized SHR and the maintenance of the response to AngII antagonism. The only significant effect was an 11 mm Hg decrease in diastolic blood pressure during the sleeping period in immunized animals. Later Gardiner et al. [50] immunized rats against AngI with an effective vaccine (PMD-2850) which consisted of an AngI analogue conjugated with a tetanus toxoid (TT) carrier protein adjuvanted with aluminum hydroxide (ALOH). Active immunization with PMD-2850 on days 0, 21, and 42, significantly suppressed responses to exogenous AngI on day 63 but had no response to AngII administration. During the active immunization anti-angiotensin antibody titre increased by 32,100 fold, and unexpectedly, these antibodies also cross reacted with angiotensinogen. It was proposed that if PMD-2850 produces antibodies which react with endogenous angiotensinogen and suppress the ability of endogenous AngI, then it would be expected to be more effective than previous active immunizations.

Clinical use of conjugate vaccine containing Tetanus toxoid (TT) may have limited application due to epitopic suppression [51]. Epitopic suppression results when a subject is vaccinated with a compound to which they have been previously exposed. TT is a common immunogen in man. Epitopic suppression is due to antigenic competition between an expanded population of carrier specific B-cell clones, and the unexpanded population of naive, peptide analogue-specific B-cell clones. Thus, the possibility of epitopic suppression limits the use of common immunogens as peptide carriers for novel conjugate vaccines, due to the difficulty of predicting the effectiveness of a response following vaccination.

Downham et al. [52], assessed the efficacy of keyhole limpet haemocyanin (KLH) an alternative peptide carrier protein conjugated AngI vaccine (PMD-3117) to the earlier TT conjugated vaccine (PMD-2850) [50] to immunize rats, and, subsequently, healthy human volunteers (single dose clinical trial: phase Ia). A two-dose clinical trial (phase Ib) was initiated using an AngI–KLH conjugate vaccine. The degree of inhibition of the pressor response to either AngI or AngII was assessed as part of this second clinical trial.

Active immunization of rats with the novel immunogen consisting of AngI conjugated with either TT or KLH demonstrated equivalent anti-Ang-I IgG titres and shift in the dose-response to exogenous AngI challenge. Conversely, no shift in the dose-response to exogenous AngII challenge was observed with either AngI–carrier protein conjugate vaccine treatment in rats. No anti-Ang-I IgG induction was detected in humans given single doses of AngI–TT or AngI–KLH conjugate vaccine. A two-dose immunization with AI–KLH conjugate vaccine of healthy, male, human volunteers caused production of anti-AngI IgG molecules. At 21 days after the second AngI–KLH conjugate vaccination (50 μg) the range of anti-AI IgG titres obtained was 2381–18,651.No statistically significant effect of treatment was found with either AngI or AngII challenge on mean blood pressure (MBP), but it was encouraging that for the study subject showing the largest anti-AngI IgG induction there was a shift of up to two-fold in the challenge dose of exogenous AngI and AngII required to cause a 15-mm Hg rise in DBP. Study showed that KLH provides a suitable alternative to TT as a peptide carrier protein in a novel conjugate vaccine for suppressing AngI responses, in rats. For future clinical efficiency, further studies with AI–KLH conjugate vaccine to improve the anti-AngI IgG titres and evaluate blood pressure lowering effect in patients with essential hypertension were purposed.

Giving continuation to the phase I trial by Downham et al. [52], Brown et al. [53] tested the anti-AngI vaccine (PMD-3117) in a phase IIa clinical trial to hypertensive subjects. One group of 8 patients received three doses of 100 μg of vaccine at 21 days intervals, whereas a second group of 8 patients received the same (100 μg) dose of vaccine on four

occasions at 14 day intervals. 24 patients completed all scheduled injections. Vaccine was well tolerated except for some slight swelling and erythema around the injection site. No patient showed a rise in serum creatinine outside the normal range. Significant antibody induction was detectable after the second injection with both regimes, with no significant difference between the two regimes and also with a prolonged half-life of approx 100 days. Vaccination blunted the fall in plasma renin following withdrawal of ACE-I or ARB and aldosterone excretion was also decreased. However, the vaccine had no effect on Blood pressure, and it was assumed that the titre generated by the formulation of the vaccine was too small to provide sufficient AngI inhibition. Therefore, a new formulation of PMD-3117 has been developed by incorporating with the 'covaccine HT' adjuvant [54], clinical trial has been initiated and results are expected in 2009.

2.4. Virus-like particle (VLP) based vaccine

In China, researchers are focusing a target further along the RAS and have developed a Virus-like particle (VLP)-based antihypertensive vaccine, ATR12181, which utilizes a peptide from the extracellular portion of *AngII-type 1A* (*AT1A*) receptor.

In the preclinical study by Zhu et al. [55,56], 6 SHRs were immunized against the peptide from rat AT1A receptor by repeated subcutaneous injections of ATR12181(peptide-TT-Freund's adjuvant) and observed for 64 weeks to assess its long-term efficacy, safety and its blood pressure lowering ability. Repeated vaccinations resulted in the induction of anti-ATR12181 antibodies (1:2560–1:10,240). A 17 mm Hg reduction of systolic blood pressure was observed in vaccinated SHRs at the 64th week. However, the level of BP did not reach target level (140/90 mm Hg). Significant attenuation of Left ventricular (LV) hypertrophy and reduction of LV fibrosis was demonstrated. Damages of glomerulus and interstitial fibrosis in kidneys were attenuated in vaccinated group, compared to Control group. Microscopic examination of sections from heart, kidneys, lungs, brain, and liver of the animals did not reveal any signs of auto-immune disease, and expression of the nuclear oncogenes c-fos and c-jun in heart and kidney were decreased in comparison to the control group. The long-term results of vaccine ATR12181 in SHRs seemed to be encouraging in all aspects, from BP lowering, target organ remodeling and proving its efficacy and safety, and thus, have provided some new light on the new target AngII-type 1A receptor for future clinical utility.

To break the rules that immunization against AngII is unable to lower blood pressure, a new immunization technology that conjugates antigens to the surface of the highly repetitive structure of *virus-like particles* (VLPs), leading to strong B-cell response against self antigens, was utilized. VLP conjugated with either a peptide or a hapten has been tested in clinical trials and have been shown to be well tolerated and highly immunogenic, with a 100% responder rate [57,58].

Ambuhl et al. [59] utilized this vaccination strategy against a self-antigen in which a peptide derived from AngII was covalently conjugated to VLP, and used to immunize SHRs and also human volunteers.VLP derived from the RNA bacteriophages Qb and AP205 were used in this study. Vaccine for the clinical trial comprised the modified AngII peptide covalently linked to the VLP Qb, termed *CYT006-AngQb* (AngQb). Rats were injected subcutaneously on days 0, 14 and 28 with 400 μg AngQb, or VLP control formulated in aluminum hydroxide. The AngQb vaccine was found to be highly immunogenic in SHRs and inclusion of the adjuvant, aluminum hydroxide, increased the antibody titre by two-fold. By day 30, a difference of 21 mm Hg of blood pressure was observed in comparison with the VLP control. AngQb immunization yielded a consistent reduction in blood pressure lasting more than 35 days after the last boost. On day 35, a nine fold increase in the total AngII concentration was observed in the AngQb group versus the VLP group. In particular, no signs of inflammation were detected in the kidney, indicating no inflammatory immune complex deposition. All human volunteers ($n=12$) receiving single dose subcutaneous injection of AngQb in aluminum hydroxide (100ug) responded with high IgG titres against AngII within 2 weeks of immunization. Titres peaked on week 3 and declined with an average half-life of 19 days. Concentration of immune complexes measured at base line and 7 and 14 days of immunization showed no changes. Fourteen out of 16 subjects showed local adverse events such erythema, edema, pain and indurations at the injection site, all of mild intensity. And as expected no significant changes occurred in blood pressure in these healthy volunteers. This small clinical study was successful to show that AngQb vaccine was highly immunogenic, induced no signs of inflammation or immune complex formation and was tolerable. These positive results of the VLP-conjugated AngII vaccine increased the likelihood that therapeutically relevant reduction in blood pressure would be possible in human hypertensive subjects in the following phase II trial.

With all the hopeful spirit gained from the VLP-conjugated AngII vaccine, AngQb, in the phase I trial [59], Tissot et al. [60] conducted a multicenter, double-blind, randomized and placebo-controlled *phase IIa trial*. 72 patients with mild-to-moderate hypertension were assigned to receive injections of either AngQb 100ug or 300ug, or placebo, adjuvanted with aluminum hydroxide, in week 0, 4, and 12. All volunteers receiving AngQb responded with high IgG titres against AngII after only one injection and the antibody response was strongly boosted after the second injection. The 300 μg dose induced a significantly higher AngII specific IgG response than did the 100 μg dose and the average half-life after the third injection was 17 weeks. No changes in the mean level of complexes containing C1 and C3, and the level of complement factor C3a were recorded, thus excluding the possibility of immune complex

deposition. The vaccine was not associated with any serious adverse effects. The most reported side effects were mild, transient reactions at the injection site and mild influenza-like symptoms. In the 300 μg group, the difference from placebo in the change from baseline in mean ambulatory BP at week 14 was −9·0/−4·0 mm Hg. There was a significant reduction of the early-morning BP surge compared with placebo in the 300 μg group, with a change at 0800 h of −25/−13 mm Hg. Change from supine to standing position did not lead to orthostatic hypotension.

This trial is the first to show that vaccination against a vasoactive endogenous substance can reduce blood pressure in human beings. Interestingly, the drop in BP was pronounced in the early morning, when the RAS is most active and when most stroke and cardiovascular events occur [61].Plasma concentrations of oral drugs are characterized by a steep rise with subsequent peaks and troughs, while the level of anti-AngII antibodies increases comparatively slowly over days without much fluctuation [60]. The 4 months half-life after the third injection is compatible with a treatment regimen of a few injections per year. Optimization of the immunization regimen with shorter dosing intervals and increasing dose is expected to lead to higher antibody titres and consequently to a more robust antihypertensive effect. But concerns of safety issue remains as repeated stimulation of the immune system by booster doses of an endogenous peptide linked to a virus-like particle can cause auto-immune disease. A phase II trial in 300 patients [62] with Alzheimer's disease of vaccination against an endogenous peptide was stopped for auto-immune reason in 2001. Whether continuous inhibition of AngII for several months without the ability to quickly reverse inhibition is safe or does this inhibition play any detrimental role in situations of volume depletion, trauma or shock are some concerns related to this mode of therapy [63]. Because most AngII is synthesized locally in tissues [64], whether this antibody can diffuse from the circulation into the extra-vascular space in concentrations high enough to block the actions of local AngII and whether it has organ protective effect remains an interesting question.

The present trial was, however, small and exploratory, and the authors point out, larger studies are under way to prove the efficacy and safety of antibodies against AngII in patients with hypertension. Nevertheless, the results of this new biotherapy for hypertension are intriguing and promising; the positive outcome of this study is therefore an important milestone in our quest to expand the use of vaccines into today's most widespread chronic diseases.

3. Conclusion

The 60 years journey, from Goldblatt et al. to Tissot et al. has finally given us the vaccine, AngQb, which has successfully lowered BP in hypertensive humans. The RAS has been extensively studied and AngII activation has been nailed as the culprit for most of the cardiovascular functional and structural deterioration. In the early times, renin was the main target and heterologous renin was used as antigen to inhibit the RAS. Although antirenin vaccines were successful in lowering blood pressure, with a high antibody titre and complete inhibition of endogenous plasma in primates and rats, they presented with organ-specific auto-immune disease.

The safety concerns thus diverted the researchers to focus on better targets inside the RAS. Immunization against angII was attempted in rats and rabbits. To increase the immunogenic property, AngII peptide was conjugated to a protein carrier molecule and also adjuvanted, but no effect on blood pressure was reported. Relying on the fact that, AngI is an intermediate peptide between angiotensinogen and Ang II, with no specific site of production and no specific receptor, thus presenting a limited immunopathological risk, Immunizations against AngI was tested. However, several preclinical and clinical studies were unsuccessful at convincingly lowering blood pressure in SHRs and humans. One reason might be that the predominant site of conversion of AngI into Ang II is the tissue interstitium and moreover, the half-life of AngI is short, so its presence is transitory within the tissue; therefore plasma antibodies to AngI do not diffuse sufficiently into the interstitium to efficiently block AngI conversion at the tissue level [65]. Researchers have hoped to overcome this problem by increasing the antibody titre in forthcoming studies. But it has been hypothesized that, if antibodies succeed in gaining access to AngI and thus in blocking its action in the tissue interstitium, the immunized animals would develop an auto-immune disease. Alternate theory states that the location of ACE on the endothelial cell surface may render it more difficult for anti-AngI antibodies to intercept AngI before its cleavage to AngII [59]. In contrast, the AT1R is located on the smooth muscle cell surface, making it easier for anti-AngII antibodies to sequester AngII. Whatever the reason, the anti-AngI vaccine PMD-3117 in its phase IIa trial was well tolerated, safe, with half-life of 100 days and authors assume that increasing the titre by modification in its formulation would lower the BP in the forthcoming trials.

Latest technological advancement in vaccine preparation, by conjugating to a Virus-like particle (VLP) has proved to become a gateway to success. A 64 weeks study in SHRs in china, on a VLP-based vaccine, ATR12181, against the AngII-type 1A receptor has proved to reduce BP by 17 mm Hg. It has also reversed remodeling of target organs meanwhile proving to be safe without any signs of auto-immune disease. Further studies to evaluate its clinical efficacy and safety are essential to prove the validity of this novel target immunization. On the other hand, VLP-based vaccine, AngQb, against AngII, has become the first vaccine to lower (−9/−4 mm Hg) mean-24 h BP in hypertensive humans, with a long half-life of 4 months and reversible antibody response. AngQb vaccine did not lead to any uncontrolled immune stimulation, confirming the good clinical tolerability and safety profile. Reduction in the early-morning BP surge by −25/−13 mm Hg was worth

noting as a beneficial property, that oral drug do not possess. Confirmation of safety is the priority and should be addressed by further clinical assessment in phase IIb and phase III. Poor compliance is the cause that only 30% out of those treated for hypertension are under control. If vaccination against high blood pressure were safe and effective in the long run, it would increase the control rate of hypertension and thus ease the burden of cardiovascular morbidity and mortality in our society.

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The authors of this manuscript have certified that they comply with the Principles of Ethical Publishing in the International Journal of Cardiology [66].

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