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Antisense gene therapy in the long-term control of hypertension

Review Article

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Abbreviations: **RAS**, renin-angiotensin system; **ACE**, angiotensin converting enzyme; **AT_1R**, angiotensin II type 1 receptor; **AT_1R-AS** angiotensin II type 1 receptor antisense; **Ang**, angiotensin; **SHR**, spontaneously hypertensive rat

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Summary

Studies from the last two decades have established that both circulating and tissue renin-angiotensin system (RAS) are important. Their coordinated interaction is essential in the regulation of blood pressure and play a key role in the development, establishment and maintenance of hypertension. Interruption of the RAS pathway, either by preventing the formation of Ang II (i.e. ACE inhibitor) or by blocking its actions at the level of the receptor (i.e. AT_1 receptor antagonists), has been shown to reduce BP and protect against target-organ injury. Since there are problems associated with pharmacological control of high blood pressure, we developed a viral gene delivery approach to target hypertension. It was our intention to try and interrupt the RAS at the genetic level in order to achieve long term control of hypertension and reversal of pathophysiology associated with the disease. In general, delivery of antisense to the AT_1R was able to prevent (for up to 18 months) or reverse the elevated blood pressure, and the alterations in vascular calcium homeostasis, alterations in ion channel activity, and cardiac vascular ultrastructure. These results demonstrate that antisense gene delivery is useful in the long-term treatment of hypertension.

I. Current pharmacological treatment for hypertension

A stepped care regimen, starting with drugs of lowest toxicity and adding drugs from other groups, is often used to manage hypertension. First line therapy is the use of diuretics including the thiazides. If response to the thiazides is inadequate to control the hypertension, a beta-adrenoceptor (β-blocker) would then be added to the regimen. If the antagonist response to the diuretic and the β-blocker is inadequate at tolerated doses then a direct vasodilator (calcium channel blocker) is generally added. Finally, if this combination does not work or is not tolerated an ACE inhibitor is then substituted. In actuality, the ACE inhibitors are widely prescribed drugs of choice and have been beneficial in a wide groups of patients with primary hypertension. The reason for such a success using ACE inhibitors is that they not only attenuate vasoconstriction but have some important vasoprotective effects. These vasoprotective effects include: an antiatherogenic effect, an antiproliferative and antimigratory effect, improves/restores endothelial function, antiplatelet effect, enhances fribrinolysis, and improves arterial compliance (Lonn *et al.*, 1994). Thus it is not surprising that emphasis has been placed in developing a strategy aimed at the RAS.

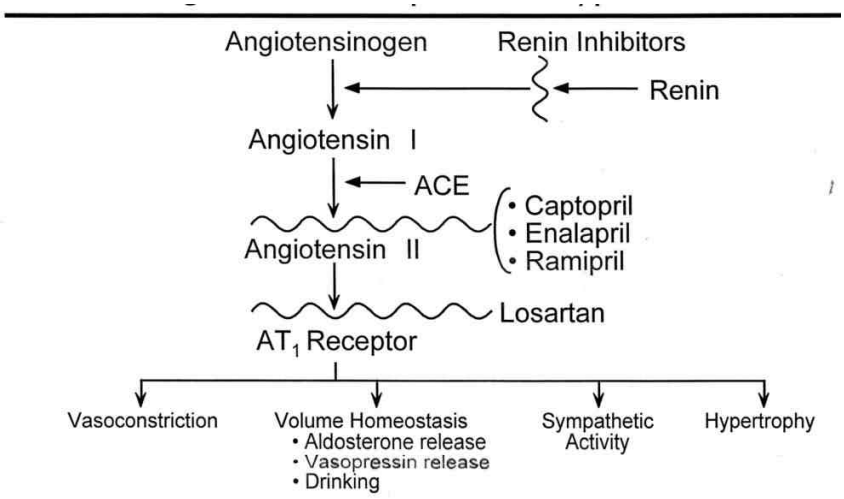

Table 1. Pharmacological therapy for the treatment of angiotensin II-dependent hypertension

II. Renin-angiotensin system and its role in hypertension

Primary human hypertension is characterized by normal cardiac output and an increase in total peripheral resistance (Khalil *et al.*, 1990). Hypertension is one of the most important risk factors for stroke, congestive heart failure, myocardial infarction, end-stage renal diseases and peripheral vascular disease (Stamler *et al.*, 1993; Kang *et al.*, 1994; Whelton 1994). Studies from the last two decades have established that both circulating and tissue renin-angiotensin system (RAS) are important, that their coordinated interaction is essential in the regulation of blood pressure and they play a key role in the development, establishment and maintenance of hypertension (Brunner *et al.*,1993; Whelton 1994; Hsueh *et al.*,1995).

The relevance of the RAS to blood pressure control is further supported by reports that various genes that encode renin, angiotensinogen, angiotensin converting enzyme (ACE), and the angiotensin II type 1 receptor (AT_1R) have been associated with hypertension in both human and animal models (Kurtz *et al.*,1990; Jeunemaitre *et al.*,1992; Bonnardeaux *et al.*, 1994). Interruption of the RAS pathway, either by preventing the formation of Ang II (i.e. ACE inhibitor) or by blocking its actions at the level of the peptide receptor (i.e. AT_1 receptor antagonists), has been shown to reduce BP and protect against target-organ injury (Vogt *et al.*,1993; Kang *et al.*, 1994; Kaneko *et al.*, 1996 and **Table 1**). In fact, blockade of the RAS has become a well-accepted treatment for Ang-dependent hypertension and congestive heart failure (Vogt *et al.*,1993). Since ACE inhibition and AT_1R blockade are standard means to treat hypertension and that AT_1R encoding gene polymorphism is coupled with hypertension in both humans and in animal models of hypertension (Kurtz *et al.*,1990; Jeunemaitre *et al.*,1992; Brunner *et al.*, 1993; Bonnardeaux *et al.*, 1994), it would only appear logical that AT_1R is an important target in the intervention of high blood pressure and hypertension. Although major strides have been made in developing drugs which interfere with either Ang II formation or its action toward the management of Ang-dependent hypertension, there is neither long-term prevention nor a cure for this disease.

There are a number of limitations in the current pharmacological therapy to treat Ang-dependent forms of hypertension as summarized in **Table 2**. ACE inhibitors and AT_1R antagonists must be administered chronically to achieve long term antihypertensive benefits. Required daily dosing and undesirable side effects such as sexual dysfunction, coughing, and lethargy, increased serum Ang II levels (with AT_1R antagonists), and diminish patient compliance. Finally the attenuation or delay of non-hemodynamic pathophysiological impairments with these agents does not entirely reduce the risk to hypertensive patients (de Divitiis *et al.*,1993; Vogt *et al.*,1993). In other words current pharmacological therapies do not cure hypertension; only control the disease.

Pharmacological Agents:

- Administered on a regular basis
- Compliance
- Control hypertension, but not cure it
- Don't always reverse pathophysiological alterations associated with the disease
- Side effects

Table 2: Why administer gene therapy for the treatment of hypertension?

Delivery System	Target	Duration of Action	Reference
Naked DNA	Kallikrein	Months (2)	Xiong *et al., Hypertens.*, 1995
	eNOS	Months (2-3)	Lin *et al., Hypertens.*, 1997
			Christopherson *et al., J.C.I.*, 1997
Adenovirus	ANP	Weeks (5)	Lin *et al., Hum. Gene. Ther.*, 1998
	Kallikrein	Weeks (4)	Yayama *et al., Hypertens.*, 1998
Retrovirus	ANP	Weeks (5)	Lin *et al., Hypertens.*, 1995
	Adrenomedullin	Weeks (8)	Chao *et al., Hypertens. Res.*, 1997

Table 3. Gene therapy and hypertension sense approach.

Delivery System	Target	Duration of Action	Reference
ODN (central/peripheral)	ATG, AT_1R	Days (~7)	Gyurko *et al., Reg. Pep.*, 1993
			Wielbo *et al., Hypertens.*, 1995
			Tomita *et al., Hypertens.*, 1995
AAV (central/peripheral)	AT_1R	Weeks (5)	Phillips *et al., Hypertens.*, 1997
Retrovirus (peripheral)	AT_1R	Months (3-18)	Iyer *et al., P.N.A.S.*, 1996
			Lu *et al., Hypertens.*, 1996
			Martens *et al., P.N.A.S.*, 1998
			Gelband *et al., Hypertens.*, 1999
			Reaves *et al., Biophys. J.*, 1999

Table 4. Gene therapy and hypertension antisense approach.

Therefore to circumvent the above problems associated with pharmacological control of high blood pressure, a number of research groups have used a gene delivery approach to target hypertension (**Tables 3** and **4**). Two approaches have been used to target hypertension using gene therapy; namely a *sense* and an *antisense* approach. Using the sense approach, Dr. Chao and her colleagues have been successful in over expressing genes relevant to vasodilatory effects. The genes have been delivered in hypertensive rats either in the form of naked DNA or by a viral mediated transduction system (**Table 3**). For example, genes encoding kallikrein, ANP, eNOS and adrenomedullin have been successfully delivered and have had short-term reduction in high blood pressure and other beneficial effects on pathophysiological parameters associated with hypertension (Lin*et al.*, 1995; 1997; Xiong *et al*., 1995; Chao *et al.*, 1997; Yayama *et al*., 1998). The laboratories of Phillips and Tomita independently have utilized an antisense oligodeoxynucloetide or naked DNA delivery approach to interrupting the RAS in order to target hypertension (**Table 4**; Gyurko *et al.*, 1993; Wielbo *et al*., 1995; Tomita *et al.*,1995). However, the effects were short lived and did not present a major advance over the traditional pharmacological therapy. Later it was shown that viral delivery systems could extend the duration of antihypertensive action (**Table 4**, Phillips 1997). Although these studies did not produce desired long-term effects and thus were limited in scope, they were highly relevant in setting the stage indicating that a gene therapy strategy hold great potential in the treatment and cure of hypertension.

Our objective has been to extend these concepts and investigate the feasibility of the antisense gene therapy approach in order to achieve long term control of hypertension and reversal of pathophysiology associated with the disease.

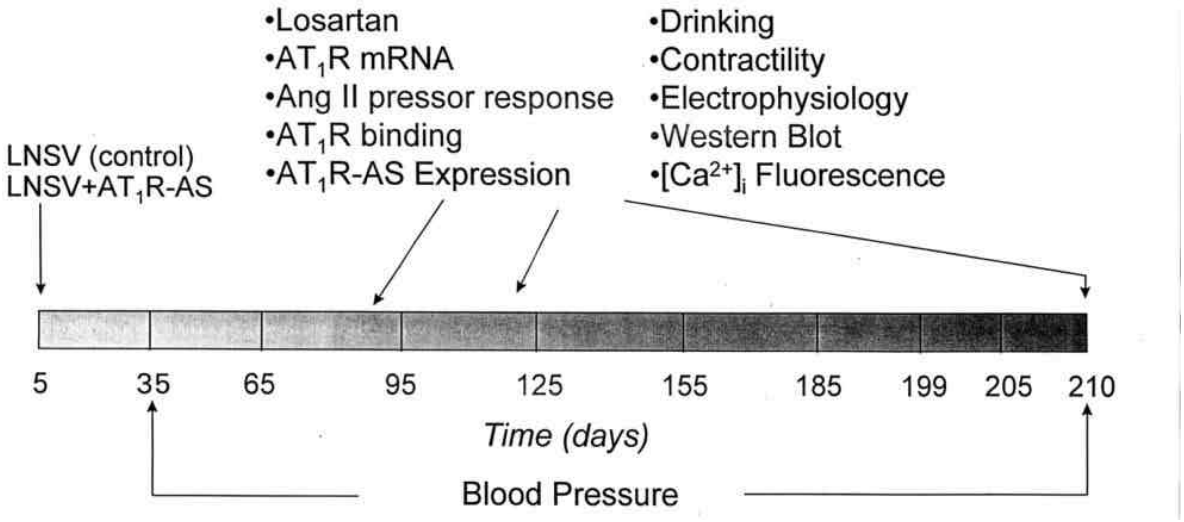

Table 5. Protocol for AT_1-R-AS gene delivery.

III. Retroviral (LNSV)-mediated gene delivery system as a model to study AT_1R-AS therapy

We chose the LNSV retrovirus because of its high infectivity, ability to effectively integrate into cells particularly, in slowly and rapidly diving cells, and its potential for long-term expression of an introduced gene (Lu and Raizada, 1995, Lu *et al.*, 1995). In addition, the vector has been shown to influence non-dividing cells to a limited degree (Murata *et al.*, 1998; personal communication N. Muzyczka, Univ. Florida). These properties, coupled with the fact that significant remodeling occurs during the development and establishment of hypertension, we argued that retroviruses may be an excellent vector for such purposes. With this in hand, our first attempts were to show that retroviral mediated gene delivery of AT_1R-AS *in vitro* would be successful. Astroglial cells in primary cultures were chosen first to demonstrate the efficiency of gene transduction mediated by this vector. The viral particles were able to infect >96% of the cells as evidenced by the detection of AT_1R-AS transcript using RT- *in situ* PCR and Northern analysis. This was associated with a significant decrease in the number of AT_1 receptors and AT_1 receptor-mediated actions in these cells (Lu *et al.*, 1995b). Next, primary neuronal cultures from hypothalamus and brainstem were used since neurons in culture have limited capacity to multiply and since neurons form the SHR show an increased expression of the AT_1R gene, an increased Ang II-dependent norepinephrine (NE) uptake, increased stimulation of mRNA for c-fos and the NE transporter when compared to the normotensive control (Lu *et al.* 1995a, 1995b). Infection of neuronal cultures with the LNSV containing AT_1R-AS resulted in decrease in AT_1R number, an inhibition of AT_1R-mediated stimulation of both c-fos and NE transporter mRNA, as well as NE uptake in the SHR neurons (Lu *et al.*, 1995a, 1995b). These data not only showed the retrovirally mediated delivery of AT_1R-AS could be used to selectively control the actions of Ang II but laid the framework for the *in vivo* studies.

IV. Prevention of the development of high blood pressure and associated pathophysiology using *in vivo* AT_1R-AS gene delivery

The first *in vivo* approach that we used was based on the hypothesis that interruption in the activity of the RAS at a "critical" stage in the development would prevent the onset of high blood pressure and other pathophysiology alterations associated with hypertension on a permanent basis. We used the spontaneously hypertensive rat (SHR) which is the most widely used animal model for studying human primary hypertension. As stated previously pharmacological intervention has been relatively successful in normalizing the elevation in blood pressure associated with hypertension in this model. However, the assumption that reduction of blood pressure will totally reverse hypertension-induced pathophysiological changes remains unclear. The protocol used for AT_1R-AS gene delivery was to give a single intracardiac injection of the antisense into the ventricle of a 5 day old rat (**Table 5**). This route of administration insured that the antisense was delivered through out the periphery .

Indeed, using this route of administration, the AT_1R-AS is expressed in a number of physiologically relevant tissue, including adrenals, heart, mesenteric arteries, kidney, and liver (Iyer *et al.*, 1996). With the knowledge that the AT_1R-AS is expressed in a number of different tissue types we next

investigated whether the AT_1R-AS had any effect on blood pressure and other cardiovascular pathophysiological alterations associated with the SHR (**Figure 1** and **Table 6**). We first reported that AT_1R-AS can prevent the onset of high blood pressure for up to 90 days after a single injection (**Figure 1**, Iyer *et al.,* 1996). We have extended those studies and now show an extension of up to 120 days (Figure 1, Martens *et al.*, 1998), 210 days (**Figure 1**, Gelband *et al.*, 1999) and 18 months (Reaves *et al*., 1999). This prevention of an increase in blood pressure is associated with a decrease in the specific binding of Ang II to the AT_1R (Iyer *et al.*,1996). Similarly the AT_1R-AS gene delivery prevented the Ang II dependent stimulation of blood pressure and the Ang II-stimulated increase in drinking in the SHR (Iyer *et al.*,1996). A number of cardiovascular pathophysiological alterations are exhibited in hypertension. These include altered renal resistance and arteriolar contractile sensitivity to circulating agents (i.e. Ang II and norepinephrine) as well as voltage dependent stimuli (KCl), endothelial dysfunction, increased smooth muscle cell Ca^{2+} current, increased Ca^{2+} release from the sarcoplasmic reticulum of smooth muscle cells, decreased smooth muscle cell voltage-dependent potassium channel (Kv) activity, increased left ventricular to body weight ratios and increased cardiac fibrosis. AT_1R-AS gene delivery prevented all cardiovascular pathophysiological alterations associated with the disease mentioned above but caused no visible inflammatory response (Martens *et al.,* 1998; Gelband *et al.*, 1999).

V. Reversal of the development of high blood pressure and associated pathophysiology using *in vivo* AT_1R-AS gene delivery

Although we have used this gene delivery approach to prevent the development of high blood pressure and cardiovascular pathophysiology in the developing SHR, the ultimate strategy would be the reversal of these actions in the *adult* SHR. Therefore we performed *in vivo* gene delivery studies in the adult SHR to determine if we could reverse the pathophysiology associated with hypertension. A similar protocol was used for gene delivery except the AT_1R-AS was injected into the adult SHR six days in a row instead of a single injection (Gelband *et al.,* 1998). This protocol resulted in a significant lowering of blood pressure for up to 45 days. At day 45 the blood pressure of the SHR treated with AT_1R-AS was similar to the control SHR. In renal resistance arterioles the enhanced contractile response to KCl, norepinephrine, and angiotensin II as well as decreased endothelium-dependent relaxation was reversed in the SHR treated with AT_1R-AS. Finally, the left ventricular weight to body weight ratio, an index of hypertension, was reversed in the adult SHR treated with AT_1R-AS. These results demonstrated the potential use of a similar gene transfer approach for long term *reversal* of hypertension.

VI. Future directions

Is antisense gene therapy targeting the RAS a therapeutic step forward? In short, the answer is yes. It results in the prevention and reversal of the increase in mean blood pressure and the associated pathophysiological impairments in hypertension. It also offers an alternative to the compliance problem and complications of vascular and target-organ injury. Finally, the AT_1R-AS therapy does not produce a significant increase in plasma Ang II levels compared with losartan, the AT_1R antagonist (Lu *et al.,* 1997). Therefore, AT_1R-AS gene delivery and therapy does have prolonged antihypertensive effects without the possible adverse side effects produced by traditional pharmacological therapies.

Yet, there is a still question regarding the method of delivery. Conventional wisdom states that the LNSV retrovirus should only be successful in a population of cells undergoing cell division. Yet we find that there is an effect in the adult SHR. This leads to our first future direction and that is the development of a better viral gene delivery tool. The ideal viral vector should have the following characteristics for its successful use in a long-term reversal of hypertension: (**i**) high titer should be achieved reproducibly and conveniently; (**ii**) chromosome specific integration; (**iii**) long-term expression; (**iv**) cell specificity and (**v**) no immune response. To date the ideal viral vector does not exist, but with genetic engineering it is only a matter of time before it is developed. At the present time the virus of choice may be a lenti or adeno-associated virus (AAV)-based vectors. A lentiviral based vector, for example, has the potential to be highly infective, can integrate into the host genome, has long term expression and little immune response. However, they are poorly defined at the present time. In contrast, AAV vectors are not highly infective but elicit a small immune response.

In order for this approach to be successful for consideration in humans, it needs to demonstrate its effectiveness in many other forms of hypertension. Thus, our alternative direction would be to examine the feasibility of this approach in both non-genetic models of hypertension (such as the two kidney, one-clip Goldblatt model and the DOCA salt model of hypertension) as well as a monogenetic model of hypertension (such as the renin-transgenic rat). Other components of the RAS, such as antisense to ACE and angiotensinogen should also be tested in the prevention/reversal of hypertension. Antisense to ACE is of particular importance since ACE inhibitors have been shown to be beneficial not only as antihypertensive agents but also to play an important role in protecting against myocardial infarction, kidney failure, and the restenosis/remodeling that occurs after balloon injury in angioplasty. The latter would

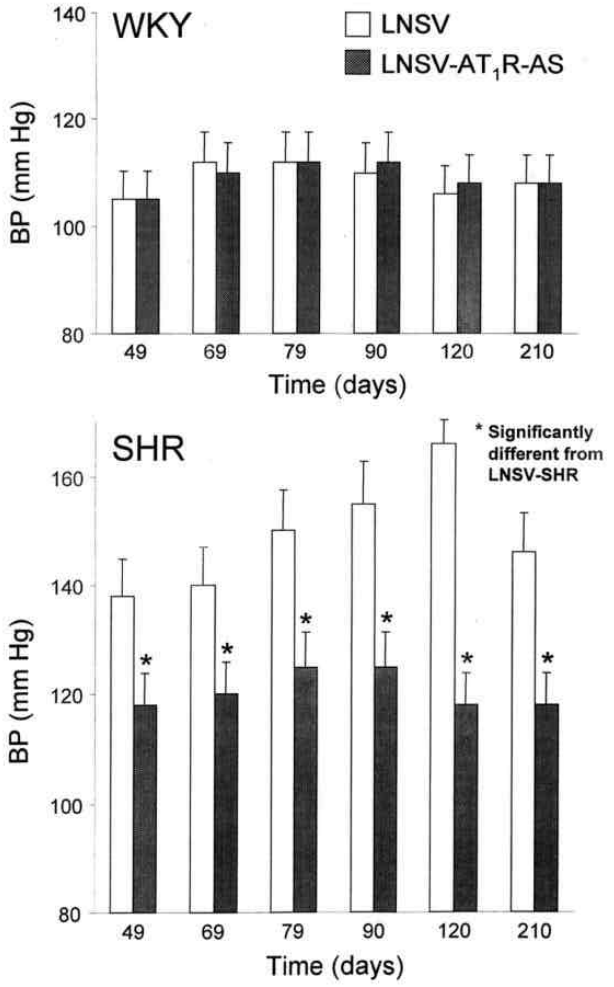

Figure 1. Time course of the change in blood pressure after antisense gene delivery. There is no change in the blood pressure in the control or antisense treated WKY rats. However there is a signifncat decrease in blood pressure in the SHRs that were treated with antisense. P<0.05, n> 8.

be clinically beneficial to those who are not only hypertensive but undergo coronary balloon angioplasty every year. Taken together gene therapy holds promise for a single dose, long-term treatment of hypertension and other potentially lethal cardiovascular disorders.

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	SHR/WKY	SHR + LNSV/WKY	SHR + AT_1R-AS/WKY
Blood Pressure	+ + +	+ + +	no Δ
AT_1R Number	+ + +	+ + +	no Δ
Ang II-Stimulated Drinking	+ + +	+ + +	no Δ
LVW/BW	+ + +	+ + +	no Δ
Cardiac Fibrosis	+ + +	+ + +	no Δ
E-C Coupling	↑ Potency	↑ Potency	no Δ
Endothelium-Dependent Relaxation	↓ Efficacy	↓ Efficacy	no Δ
Ion Channel Activity	↓ Kv current ↑ Ca^{2+} current	↓ Kv current ↑ Ca^{2+} current	no Δ
Basal and Agonist-Stimulated Vascular $[Ca^{2+}]_i$	+ + +	+ + +	no Δ

See Iyer *et al.*, 1996; Martens *et al.*, 1998; Gelband *et al.*, 1999

Table 6. Effect of AT_1-RAS in developing rats

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