REVIEW ARTICLE

Received 5th November 1996
Accepted 17th July 1997
Indian J. Pharm. Sci., 59(5) pp. 215-219

Gene Therapy in Hypertension

RAJESHRI G. KARKI, ANITA A. MEHTA AND R.K. GOYAL*
Dept. of Pharmacology, L.M. College of Pharmacy, Ahmedabad 380 009.

Gene therapy is emerging out as an important trend in the pathophysiology and treatment of hypertension. Several gene targets have been identified in recent years. They include renin, angiotensin converting enzyme, angiotensin receptors, kallikrein, endothelin, nitric oxide, adrenomedullin, glucocorticoid- suppressible hyperaldosteronism and several peptides including atrial natriuretic factor. Clinically, there are several problems before gene therapy is actually available. Once gene therapy becomes a reality, it will be a significant breakthrough in the field of treatment of hypertension.

ADVANCES in molecular and cellular biology have described the proteins that mediate many disease processes, while DNA technology provides ready access to the genes that control these events. The size, complexity and cellular inaccessibility of these proteins make their delivery or modification by conventional pharmacological means impossible. Gene therapy overcomes these barriers by the selective introduction of recombinant DNA into tissues so that the biologically active proteins can be synthesized within the cells whose function is to be altered. Gene therapy is a medical/surgical intervention in which a functional gene is introduced or a defective gene is replaced in a cell of an organism suffering from an acquired or a genetic disease so as to treat or cure a wide variety of diseases. It is a pharmaceutical therapy with DNA as a drug molecule to be targeted to a specific site.

There are two main approaches to gene therapy. *Somatic gene therapy and germ cell therapy.* Somatic gene therapy involves the insertion of a gene into a cell other than a germ cell, viz. hematopoietic stem cells of the bone marrow, hypatocytes (liver cells), keratinocytes (skin cells) and endothelial cells. Genes inserted into cell populations of this type are not passed onto future generations. Germ cell therapy, which has not yet been carried out in humans, involves the injection of genes into germ cells viz. sperm, egg or early embryo. Inserted genes would be distributed among the somatic and germ cells and the genetic change will then be transmitted to future generations. For successful delivery of DNA to host cells, either virus is used as the vector[1,2,3] or the DNA material is inserted directly into the cells by physical (microinjection, electroporation),[4] chemical (eg. calcium phosphate co-precipitate method) or fusion (protoplast[4], RBC ghosts, liposomes[1]) techniques.

Gene therapy was previously thought to be useful for genetic diseases providing the missing protein in a therapeutic fashion. It has also been extended to the correction of acquired diseases like Cancer, AIDS and Viral diseases (Table 1). In this article we have described some of the emerging trends in the pathophysiology and treatment of hypertension with gene as the target.

Gene targeting in hypertension

Essential hypertension is now considered as a genetic disease caused primarily by a combination of quantitative genetic variants that individually have only modest effects. Attempts are now being made

*For correspondence

Table 1 : Disease targets for Gene Therapy

	DISEASE	APPROACHES	OBJECTIVES
	Gene encoding:		
1.	Cancer	Cytokines	Potentiation of antitumor response.
		Tumor suppressor genes	Induction of apoptosis
		Promote immunological recog-inition of tumor	Tumor destroyed by T cells
		Sensitivity genes	Tumor cell destroyed by prodrug derivative
		Oncogene expression inhibited	Induction of apoptosis.
2.	AIDS	Intracellular immunization	Inhibition of HIV replication and its spread
		Use of suicide gene	Elimination of HIV infected cells
		HSV-TK gene transfer	Depletion of HIV infected cells
3.	Cystic Fibrosis	Cystic Fibrosis Transport Regulator	Normalization of camp regulated chloride channel
4.	Familial Emphysema	Alpha-1 antitrypsin	Correction/restoration of alpha-1 antitrypsin levels.
5.	Adenosine Deaminase Deficiency (ADA)	Introduction of ADA gene	Overcome the deficiency
6.	Haemophilia	Factor VIII or IX	Overcome the deficiency
7.	Alzheimers Dementia	Neurotropic Factor	Prevention of neuronal degeneration

to investigate various genes that are involved in the pathophysiology of hypertension. This provides models for study of new drug molecules acting on these targets and thereby control blood pressure, atherosclerosis and hypertrophy. Some of the genetic targets identified recently are as follows:-

(1) Renin-Angiotensin System (RAS)

Renin has long been known as a key enzyme of RAS which plays an important role in the regulation of blood pressure. The renin gene locus has been linked to hypertension in Dahl salt sensitive rats with low renin hypertension. Yu *et al.*[5] have identified a specific renin intron-1 binding protein ($RIBP_1$) that binds to E_2 site. This gene is believed to be involved in the regulation of renin gene expression. Nishimura *et al.*[6] reported that gene expression of renin in hypothalamus does not depend on circulating RAS but is regulated by tissue specific mechanisms. High salt diet stimulates gene expression of renin in hypothalamus which plays an important role in pressure control mechanisms. In yet another study, Holmer *et al.*[7] reported that beta-adrenoceptor activation is one of the most powerful stimuli for renin secretion and renin gene expression *in vivo*, albeit, the time course of activation is substantially different. Further, the ability of adrenergic agonists to stimulate the renin system appears to be dependent on the rate of salt intake.

An insertion (I)/deletion (D) polymorphism of the angiotensin converting enzyme (ACE) gene has been associated as well as an increased risk for

myocardial infarction, left ventricular hypertrophy, and coronary artery disease. Shieh *et al.*[8] reported that in Chinese population,I/D polymorphism of the ACE gene is associated with hypertension. It was however, apparently not associated to insulin resistance in hypertensive subjects. Ishigami *et al.*[9] reported a decrease in D-allele of ACE gene polymorphism in older Japanese patients with hypertension suggesting that D-allele my be associated with mortality and morbidity in the sub-group of patients. Pinto *et al.*[10] found that DD genotype is associated with increased insulin resistance.

Polymorphism of the angotensin II type 1 receptor (AT_1R) gene has been reported in five positions (T^{573}---->C, A^{1062}----> G, A^{1166} ----> C, G^{1517}---> T,A^{1878}---->G).The allelic frequency of C^{1166} polymorphism of AT_1R gene has been reported to be increased in Caucasians with hypertension. Miyamoto et al.[11]reported that C^{1166} polymorphism is also observed in Japanese population.

AT_2 receptors are implicated in growth inhibition, vasorelaxation and apoptosis. Goto *et al.*[12] reported that there is low expression of AT_2 receptors in the kidney of SHRSP (Stroke-prone SH rats) during early development and this may be involved in the pathogenesis of hypertension.

(2) Kallikreins:-

Kallkreins are a family of serine proteases involved in the post- translational processing of polypeptides to their bioactive or inactive forms. They are encoded by a highly conserved multigene family (KLK) in several species, of which thirteen genes have been characterised in the rats but just three genes in the humans.

The tissue kallikrein-kinin system has been implicated in the pathogenesis of hypertension.[13,14] Decreased kallikrein levels have been associated with hypertension, family history of hypertension,increased blood pressure, increased sodium intake and decreased potassium intake in multiple case-control and dietary intervention studies. Recently

Table 2

PROSPECTIVE GENE TARGETS IN HYPERTENSION
Renin intron 1 binding protein
Angiotensin Converting enzyme (D-allele)
Angiotensin II type 1 receptor gene
Acidic fibroblast growth factor
Endothelin converting enzyme I
Kallikrein
Nitric oxide synthase
Adrenomedullin gene
Hybrid CYPII/CYPII gene (Gluco corticoid suppressible hyperaldosteronism)
11--hydroxydehydrogenase type I
Atrial Natriuretic factor A
Atrial Natriuretic factor B
Carditrophin I
Calcium regulated gene
NPrC gene in adipose tissue

Wang *et al.*[15] reported that direct delivery of human kallkrein gene caused a sustained reduction in systolic blood pressure in spontaneously hypertensive rats. Preliminary gene seggregation analysis suggested that a common major gene explained a large percentage of the variation in urinary kallikrein excretion. Reduced urinary kallikrein excretion has been described in a number of genetically hypertensive rats.[16] Low kallikrein may contribute to hypertension and high urinary kallikrein may have protective effects against hypertension. Clinical studies have shown that the blood pressure of hypertensive patients may be temporarily lowered by oral administration of pig pancreatic kallikrein.[17,18]

This is one of the promising venture in gene therapy offering a non-invasive and a potential clinical alternative for treating human hypertensive disease.

(3) Endothelins and nitric oxide:-

Endothelial cells that line the blood vessels are a source of several substances that regulate

vascular smooth muscle (VSM) tone. Recently much attention has been on endothelins and nitric oxide (NO) which are the most potent vasoconstrictors and vasodilator substances released from the endothelium and are responsible not only for the regulation of VSM tone but also atherosclerosis. Genetic alteration of these cells might be useful to alter or prevent the process of atherosclerosis or to provide local delivery of anticoagulants.

A variety of genes have been expressed by *in vivo* gene transfer for the purpose of developing useful clinical applications and for developing models of pathogenic mechanisms. Acidic fibroblast growth factor (FGF-1) when expressed in procine arteries, causes vessel wall thickness (intimal hyperplasia) where as another factor TGF-beta$_1$ when expressed ectopically in the vessel results in extracellular matrix synthesis and thereby thickening of intima.

Maemura *et al.*[19] presented an ET_1 over expressing transgenic mice model. This will be a new experimental model to study the physiological role of ET_1 in hypertension, atherosclerosis and cardiomyopathy.

The conversion of big endothelin precursors to the active mature peptides is catalysed by endothelin converting enzyme-1 (ECE-1) which exists in two isoforms;-1-alpha and -1-beta. Orzechowski et al.[20] reported that ECE-1 alpha is widely expressed in human tissues.

Nitric oxide has also been reported to play an important role in the pathophysiology of various cardiovascular disease. Three types of NO synthase (NOS) as key enzymes for NO have been isolated. Prostagland in D_2 has been reported to reduce NO formation in inducible NOS-induced VSM cells with decrease in NOS mRNA. NO in the brain affects central regulation of cardiovascular responses in rats. Nanbu *et al.*[21] reported that gene expression of constitutive NOS in hypothalamus strongly correlates with systemic hypertension in rats. Attenuated gene expression of constitutive NOS in hypothalamus may be one of the reasons for increased vasopressin secretion and accelerated sympathetic activity in DOCA salt hypertension.

(4) Adrenomedullin:-

It is a potent vasoactive peptide which causes hypotension. It has been reported that adrenomedullin gene delivery causes a long lasting reduction in blood pressure in genetically hypertensive rats[22].

(5) Glucocorticoid suppressible Hyperaldosteronism gene:-

Glucocorticoid suppressible hyperaldosteronism is a dominantly inherited form of hypertension believed to be caused by the presence of a hybrid CYPIIB1/CYPIIB2 gene. Kotelevtsev *et al.*[23] reported a role of 11-B-hydrosydehydrogenase type I (11-0H SDI) in the regulation of glucocorticoid access to glucocorticoid and mineralocorticoid receptors. This enzyme may be involved in glucocorticoid mediated processes specially in enhancement of sensitivity of target tissues to insulin and noradrenaline.

(6) Atrial Natriuretic Factor:

Kawakami *et al.*[24] reported that gene expression of Atrial Natriuretic factor type A (ANF-A) and type B (ANF-B) play an important role in the processes of progression and regression of cardiovascular hypertrophy in zenovascular hypertensive rats.

Besides several other genes have been cloned which elucidates augmented gene expression and thereby cause hypertension and hypertrophy of the heart. Notably among them are cardiotrophin- 1[25], calcium regulated gene[26], alpha$_1$ beta adrenoceptor mRNA in kidney[27] and NPrC gene in adipose tissue[11]. Attempts are also being made to study the genes that may lead to insulin resistance, hyperinsulinaemia and obesity which are some of the most common risk factors in cardiovascular diseases.

The dream of gene therapy, being available clinically, is still far from realization. This is essentially because of potential dangers of the therapy, which include malfunctioning of transplanted genes, transformation of cells into cancerous cells and activation of wrong cells diverting them from vital tasks. Besides, there are dangerous effects and several legal issues. Moreover, where gene therapy of hypertension is concerned, it is a disease with no obvious disturbances to the patient thereby becoming more difficult to convince the patient to go for gene therapy. Of course, if gene therapy turns out to be a real success in other diseases, treatment of hypertension with this therapy may be more beneficial for the prevention of cardiac morbidity and mortality which is a growing concern of health scientists.

REFERENCES

1. Stephen, L.E. and Wilson, J.M., In: Hardman, J.G., Limbird, L.E., Molinoff, P.B., Ruddon,D.R., Gilman, A.G., Eds, Goodman and Gilman's The pharmacological basis of therapeutics, 9th Ed., McGraw Hill, U.S.A, 1995, 77.
2. Berg, P., Cohen, S. and Boyer, H., In: Lehninger, A.L., Nelson, D.L.and Cox, M.M.Eds, Principles of Biochemistry, 2nd Ed., CBS Publishers, New Delhi, 1993, 1006.
3. Vile, R.G. and Russell, S.J., **Br. Med, Bull.,** 1995, 51, 12.
4. Hooley, P., In: Techniques for Engineering genes, *Butterworth-Heinemann Ltd, Great Britain,* BIOTOL, 1993, 83.
5. Yu, H., Morgan, T.O. and Nicolantanio, R.D., **J. Hypert.,** 1996, 14, S16.
6. Nishimura, M., Nanbu, A., Takahashi, H., Iwai, N., Kinoshita, M. and Yoshiimura, M., **J. Hypert.,** 1996, 14, S17.
7. Holmer, S.R., Kramer, B.K., Kaissling, B., Putnik, K., Riegger, A.J.G. and Kurtz, A., **J. Hypert.,** 1996, 14, S18.
8. Shieh, S.M., Jemg, C.Y. and Sheu, W.H.H., **J. Hypert.,** 1996, 14, S32.
9. Ishiigam, T., Umemura, S., Ohno, A., Tamura, K., Yamaguchi, S., Iwamoto, T., Yoshda, K., Hb, K., Watanabe, Y., Ochiai, H., Miyazaki, N. and Ilshii, M., **J. Hypert.,** 1996, 14 S33.
10. Pinto, Y.M., Heesen, W.F., Buiten, A., Graeff, P.A., May, J.F. and Gilst, W.F., **J. Hypert.,** 1996, 14, S31.
11. Miyamoto, Y., Yoshimasa, T., Itoh, H., Igaki, T., Harada, M., Yamashita, J., Chun, T., Doi, K., Ishikawa, M., Hori, Y., Kawahara, K., Ogawa, E., Inoue, M., Masuda, I . and Sato, Y., **J. Hypert.,** 1996, 14, S29.
12. Goto, M., Mukoyama, M., Tanaka, I ., Suga, S., Matsumoto, T., Nakagawa, M., Ishibash, R., Michibata, H., Kasahara, M., Sugawara, A. and Nakao, K., **J. Hypert.,** 1996, 14, S2.
13. Margolius, H.S., **Annu. Rev. Pharmacol. Toxicol.,** 1989, 29, 343.
14. Scili, A.G. and Carretero, O.A., **Kideny Int.,** 1986, 29, 120.
15. Wang, C., Chao,L. and Chao, J., **J. Clin, Invest.,** 1995,95, 1710.
16. Geller, R.G., Margolius, H.S.,Pisano, J.J. and Keiser, H.R., **Circ. Res.,** 1975, 36, 103.
17. Overlack, A., Stumpe, K.O., Kolloch, R., Ressel, C. and Krueck, F., **Hypertension (Dallas),** 1981, 3, 118.
18. Ogawa, K., Ito. T., Ban, M., Mochizuki, M. and Satake, T., **Klin. Wochenschar.,** 1985, 63, 332.
19. Maemura, K., Kurihara, H., Ueda, O., Oda, H., Kodama, T., Suzuki, H., Kumada, M., Ishikawa, T. and Yazaki, Y., **J. Hypert.,** 1996, 14, S2.
20. Orzechowski, H.D., Menzel, S., Kroger, B., Schimdt, M. and Paul, M., **J. Hypert.,** 1996, 14, S22.
21. Nanbu, A., Nishimura, M., Takahashi, H. and Yoshimura, M., **J. Hypert.,** 1996, 14, S16.
22. Chao, J., Jin, L., Lin, F.K., Chao, L., **J. Hypert.,** 1996, 14, S2.
23. Kotelevtsev, Y.V., Sekl, J.R., Edward, C.R.W. and Mullins, J.J., **J. Hypert.,** 1996, 14, S2.
24. Kawakami, H., Okayama, H., Ikeda, S., Shigamatsu, Y., Hamada, M. and Hiwada, K., **J. Hypert.,** 1996, 14, S16.
25. Ishikawa, M., Saito, Y., Miyamoto, Y., Nakagawa, O., Kuwahara, H. and Ogawa, E., **J. Hypert.,** 1996, 14, S24.
26. Jia, H.P., Tremblay, J., Gossard, F., Benishin, C.G., Pang, P.K., Hamet, P. and Lewanczuk, R., **J. Hypert.,** 1996, 14, S25.
27. Yamaguchi, S., Umemura, S., Tamura, K., Hibi, K., Nyui, N., Ishigami, T., Kihara, M., Yabana, M., Takagi, N. and Ishii. M., **J.Hypert.,** 1996, 14, S25.