PRECLINICAL SCREENING MODELS FOR HYPERTENSION IN RODENTS: A REVIEW

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Summary

Coronary heart disease (CHD) is defined as heart disease due to an abnormality of the arteries that supply blood and oxygen to the heart. It is estimated to be the most common cause of death globally by 2020. Hypertension is one of the leading causes of disability, morbidity and mortality among the population. Although the exact cause and mechanism of hypertension is not know and attributed to the complex and multifactorial process, various genetic and environmental factors, such as high sodium intake, cigarette smoking and mental stress etc. are prominently involved. The antihypertensive drug development is a continuous process and success of which largely depend upon selection of suitable animal model. The ideal animal model for hypertension research should have human-like cardiovascular anatomy, hemodynamics, and physiology. The aim of this article is to briefly review the most widely used rodent models of experimental hypertension and recent advances.

Key Words: Experimental hypertension; Coronary heart disease; Angiotensin-converting enzyme

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Introduction

Hypertension is most common cardiovascular disease and is a major public health issue.[1] Recent studied have reported an increasing trend in the prevalence of hypertension in Indian subcontinent. This increase was found to be about 30% in urban population and 10% in rural habitants in last three decades.[2, 3] The uncontrolled hypertension further result in serious life threatening out comes like CHD, and hence effective antihypertensive drug therapy has got mainstay in cardiovascular disease management.[4]

The pathophysiology of hypertension is complex and can be influenced by various parameters like food habits, life style and age etc, the drug therapy need to be modified accordingly. The animal models of hypertension share many features which are common to human hypertension. The antihypertensive drug discovery and its fruitful outcome are largely dependent on selection of suitable animal model during the preclinical study phase.

In general an ideal animal model of hypertension should fulfill the following criteria [5]:

- It should be feasible in small animals.
- It should be able to predict the potential antihypertensive properties of an agent.
- It should consume minimal quantities of compounds.
- It should be simple to perform and uniformly reproducible.
- It should be comparable to some form of human hypertension.

Preclinical Screening Models for Hypertension

The various types of preclinical Screening models of hypertension being used are:

1. Surgically induced hypertension
2. Endocrine hypertension
3. Dietary hypertension
4. Neurogenic hypertension
5. Psychogenic hypertension
6. Chemically induced hypertension
7. AG II infusion induced hypertension
8. Recent advances

Surgically Induced Hypertension:

The Goldblatt et al in 1934 introduced the first animal model of hypertension in dogs evoked by unilateral constriction of renal artery (2K1C model) and was followed by similar demonstration in rats and rabbit in same decade. End organ damage in 2K1C model depends on size and time of clipping and usually including endothelium dysfunction. In dogs, achieving a sustained increase in blood pressure is more difficult because of their pronounced renal autoregulatory capacity. [6]

Renal hypertension is produced by renal artery constriction, which activates peripheral renin angiotensin aldosterone System (RAAS) and sympathetic nervous system. Various methods of inducing renovascular hypertension as described by Goldblatt are:

A) Two Kidney One Clip (2K1C) Hypertension:

In 2K1C model of hypertension the renal artery is constricted on only one side with other artery (or kidney) left untouched. This result in sustained increase in BP due to increased plasma renin activity (PRA), which in turn increases circulating angiotensin-II, a potent vasoconstrictor However, there is no salt and water retention because of the other normal kidney being intact. Thus, the resultant hypertension at this stage is renin-angiotensin dependent**.** Addition to this 2K-1C animals showed high BP, increased serum concentration of PGE_2 and TxB_2, hypertrophy of the unclipped kidneys, but not in the clipped kidneys and NHE-1 and NHE-3 isoforms were increased in both the 2K-1C kidneys, whereas α-actin was increased in the clipped but not in unclipped kidneys. Sodium pump activity was decreased in the clipped kidneys, but remained unchanged in the unclipped kidneys. [7-14] Following general anesthesia with ketamine (1mg/100 g i.p.,), a Goldblatt renovascular hypertension (2K1C) is induced in rodents as follows:A retroperitoneal flank incision is made and the left renal artery is exposed and cleared. Then a U shaped silver clip with a gauge of 0.25 mm is placed around the renal artery and secured in place and the incision is sutured and the animals are returned to their cages. [12, 14]

B) One Kidney One Clip (1K1C) Hypertension:

Constriction of renal artery is done on one side and contralateral kidney is removed. There is increase in BP within few hours due to rapid salt and water retention. [15, 16]

C) Two Kidney Two Clip (2K2C) Hypertension:

The Na^+-deplete 2K2C hypertensive rat is an appropriate model for investigating the action of drugs which are designated for renin-angiotensin system blockade in high-renin patients. Constriction of aorta or both renal arteries is done in 2K2C. There is a patchy ischemic kidney tissue, which secretes renin leading to increased BP. [16]

Endocrine Hypertension:

The most common endocrine model to induce hypertension is administration mineralocorticoids specially deoxycorticosterone acetate (DOCA). Mineralocorticoids cause retention of sodium and water in the body leading to increase blood volume and hence increase the blood pressure. Glucocorticoids can also induce hypertension in rodents, possible mechanism via activation of RAS, but they are less effective than DOCA salt. [17]

A) DOCA-salt Induced Hypertension:

DOCA-salt treated animals, the probable mechanism of induction of hypertension due to retention of sodium and water, which increases circulating blood volume and results in hypertension. Rennin angiotensin system is suppressed in DOCA salt hypertension model, so use of ACE inhibitor or AT1 receptor antagonist should not affect the blood pressure. [17, 18] A role of brain atrial natriuretic peptide (ANP) was also suggested in development of hypertension because of the increased ANP content of some brain nuclei in DOCA–salt hypertensive rats. [19, 20] For induction of hypertension, male Sprague-Dawley rats are anesthetized with sodium pentobarbital (50 mg/kg, i.p.), and the right kidney of each rat was excised through a right flank incision. After a l-week recovery period, these rats are subcutaneously administered DOCA (15 mg/kg) suspended in corn oil, twice weekly upto four weeks and drinking water is replaced by 1% NaCl.[21-25]

B) Adrenal Regeneration Hypertension:

Hypertension is produced in rats by unilateral nephrectomy followed by removal of right adrenal gland and enucleation of left adrenal gland. Drinking water is replaced with 1% saline. Hypertension develops during regeneration of adrenal glands in about 2 weeks [26].

Dietary Hypertension:

A) Increased Salt Intake:

Physiologically, normal kidney has the ability to excrete easily the daily salt load without allowing a marked rise in extracellular volume. Chronic ingestion of excess salt produces hypertension in rats which mimics human hypertension morphologically. High salt intake hypertension has been produced in rats, rabbits and chicks by replacing drinking water with 1 % sodium chloride for 9-12 months. [27]

B) High Fructose Diet:

Several studies have demonstrated that chronic fructose feeding leads to insulin resistance, glucose intolerance, hyperinsulinemia and hypertriglyceridemia in relatively short time in normal rats [28-30]. These metabolic changes lead to essential hypertension. Male Sprague-Dawley rats were fed a fructose-enriched diet that consisted of 21% protein, 5% fat, 60% carbohydrate, 0.49% sodium, and 0.49% potassium for 5 weeks, which produced hyperinsulinemia, hypertension, and hypertriglyceridemia. In this model rats maintained on the above fructose diet upto 7 weeks developed high systolic blood pressure. [31-32]

Neurogenic Hypertension:

Vasodilator and depressor reflexes, originating in the baroreceptor areas of the carotid sinus and aortic arch, Stimulation of the afferent buffer fibers exerts an inhibitory influence on the vasomotor center, and their sectioning leads to a persistent rise in blood pressure. In this way, acute neurogenic hypertension can be induced in dogs[33]. Electrical or chemical stimulation of different areas of brain leads to development of hypertension in rats e.g., electrical stimulation of hypothalamus, glutamate injection into the rostral ventrolateral medulla. [34]

Denervation of Sinoaortic Baroreceptors:

This is the most often used neurogenic model of hypertension. In dogs, cardioaortic nerve is located at the junction of superior laryngeal and vagus nerve and runs in the form of several fine strands. These strands unite and may be traced back as a white band lying within the vagal sheath alongside the cervical sympathetic nerve. Following bilateral vagotomy and carotid sinus denervation, the region is painted with 5% phenol and then alcohol to ensure complete denervation of the carotid sinus. There is sudden increase in BP. The dog is allowed to equilibrate for approximately 30 min and a bolus of the test compound can be given by intravenous

administration. BP returns to normal within about 2 days because the response of vasomotor center to the absent baroreceptor signals fades away, which is called "resetting of baroreceptors". Thus, this is only an acute type of hypertension. In rabbits, right carotid sinus can be removed together with 2 cm segments of the right cervical sympathetic and depressor nerves while the left carotid sinus can be removed later on. In rats, sinoaortic denervation leads to marked and sustained increase in BP, which is comparable to renovascular hypertension or DOCA-induced hypertension. [35]

Psychogenic Hypertension:

It has been reported that elevation of BP resulting from repeated exposure to stressful situation may lead to state of persistent hypertension. [36] The stress induced hypertension is associated with either normal or suppressed plasma rennin activity values, suggesting that the hypertension in these animals is not rennin dependent[37]. Borderline hypertensive rats (BHR) are useful for psychogenic hypertension. BHRs that are exposed to daily sessions of either short (20 min) or long (120 min) duration air-jet stimulation developed hypertension within 2 weeks in comparison to home cage controls. Other types of stress have been applied, such as emotional stimuli, psychosocial stress, immobilization stress and electrical stimuli, but in all cases the results are similar. [38]

Chemically Induced Hypertension:

A) Dexamethasone Induced Hypertension:

Dexamethasone is a synthetic glucocortocoid that is commonly used in clinical practice and that increases blood pressure in rats and in human beings. Chronic dexamethasone treatment increases oxidative stress and systolic blood pressure in rats and reactive oxygen species (ROS) production in human umbilical vein endothelial cells. [39] Dexamethasone hypertension is found to be accompanied by a decrease in serum reactive nitrogen intermediate (NOx) concentration and endothelial nitric oxide synthase (eNOS), mRNA levels in heart, kidney and liver in mice. [40-43] Dexamethasone (20µg/kg/day, in a volume of 1 mL/kg) is administered subcutaneously every day upto 13 days increased SBP from 122 ±5 to 136±3 mm Hg. [39, 41]

B) Cadmium Chloride Induced Hypertension:

Cadmium chloride induced hypertension might be due to the fact that the metal ion might mimic Ca^{2}+ ion as a partial agonist and

produce a direct contractile effect on vascular smooth muscle. [44] Hypertension is produced by chronic administration of Cadmium chloride (1 mg/kg/day, i.p, for 2 weeks). [5]

C) Cyclosporine Induced Hypertension:

Cyclosporine is a macrolide antibiotic induced widespread vasoconstriction of systemic circulation and an increase in arterial blood pressure. [45] Chronic treatment with cyclosporine A (CsA) is associated with the development of arterial hypertension. CsA-induced hypertension is accompanied by decreases in urinary NO_2/NO_3 and increases in $TBXA_2$ suggested that a vasodilator pathway is suppressed and a vasoconstrictor pathway is activated, most likely NO and cyclooxygenase pathway to produce $TBXA_2$, a vasoconstrictor. [46-51] CsA (25 mg/kg) in 1 ml of olive oil, ip injection daily for 7 days cause hypertension in Sprague–Dawley rats. [46]

D) L-NAME Induced Hypertension:

Nitric oxide (NO) has a role in many cellular and cardiovascular phenomena, including the regulation of vascular smooth muscle tone. The chronic inhibition of NO biosynthesis by the oral administration of non- selective NO synthase (NOS) inhibitor N^{ω}-nitro-L-arginine-methyl ester (L-NAME) results in hypertensive cardiomyopathy in rats. This model is characterized by sustained increase in mean arterial pressure and a decrease in heart rate, a reduction in cardiac output, and changes in myocardial contractility, histological alterations consisting of extensive area of myocardial fibrosis, necrosis and increase in cardiac collagen levels. [52- 55] In this model L-NAME is given up to 4 weeks by oral routes (40mg/Kg) at the corresponding volume of 1 ml/kg body weight to induce hypertension. Blood pressure rise to increase after 2 week & these systolic Blood pressure values is reached between 170 & 190 mm of Hg after 4 weeks. [52]

Hypertension Induced by AGII Infusion:

AGII-induced hypertension is associated with a cardiac hypertrophy and fibrosis and activates pathways involved in oxidative stress in the heart, the vessel wall and the brain.[56, 57] The continuous delivery of high doses of AGII (0.7 mg/kg/ day) by means of osmotic pumps can rapidly induce an increase in blood pressure of around 45 mmHg. [5]

Recent advances:

The most common cause of hypertension in humans is essential hypertension, in which multiple genes contribute to the individual phenotype, each by diverse allele effects, penetrance, and contributions. As a result, no single genetic defect can explain development of essential hypertension in humans. [58] The decoding of the human and mouse genomes allowed generation of transgenic or gene-targeted models suitable for studying hypertension. The phenotype-driven experimental approach takes advantage of the natural variation among inbred strains and crosses to find quantitative traits and determine which genes are responsible. In contrast, in the genotype-driven approach, a known gene is studied with genetics-based interventions (overexpression or ablation). [59, 60] Gene function in hypertension is most often studied with gene overexpression (e.g., transgenic) or deletion (knockout), usually related to candidate systems involved in regulation of vascular tone, renal physiology, and/or electrolyte and fluid homeostasis, and several experimental models of Genetic hypertension have been developed *(Table 1)*.

"Table 1: *Various experimental models of Genetic hypertension"*

Phenotype-driven	**Genotype-driven**
Spontaneously hypertensive rat (SHR) SHR-stroke prone Dahl salt-sensitive rat Genetically hypertensive rat Sabra model Lyon hypertensive rat Obesity-related	Renin-angiotensin system Sympathetic NS Atrial natriuretic peptide Nitric oxide Endothelin Neuropeptide Y Vasopressin Prostaglandin Kallikrein-kinin Vasopressin

Gene-targeting approaches in rodents can be performed for four main aims:

(i) Studying the role of a single gene through its disruption or overexpression;

(ii) Analyzing the contribution of genes that transfer a susceptibility to end-organ damage;
(iii) Evaluating the effects of naturally occurring gene variants to regulate blood pressure and
(iv) Comparing biological effects of human versus murine genes to blood pressure control in humanized mice.

A) RAS (Renin-angiotensin System):

Association of gene polymorphism of the AT1 receptor, angiotensinogen, or ACE with hypertension has been controversial and likely interacts with comorbid conditions. Both transgenic and knockout mouse models of various components of the RAS have been constructed. Mice may have 2 subtypes of the gene for AT1 (AT-1a and AT-1b) and the REN gene (Ren-1d and Ren-2d). Overexpression of the rat angiotensinogen gene in mice, without or with concomitant overexpression of rat renin, leads to development of high blood pressure. Indeed, blood pressures in mice carrying various numbers of copies of the angiotensinogen gene are predictable and increase at approximately 8 mm Hg per gene copy, whereas conversely mice completely knocked-out for this gene are hypotensive. Similarly, ACE knockout mice are also hypotensive, especially males and mice selectively deficient of the vascular rather than proximal tubular enzyme. [58]

B) Other Genetic Models:

Genetic hypertension in rats may be accompanied by a defect in renal prostaglandin catabolism. Nevertheless, gene-targeted mutants for either the PGI_2 or the thromboxane-A_2 receptors, and for cyclooxygenase-1 and cyclooxygenase-2, have normal blood pressures. On the other hand, mice with targeted disruption of the PGE2 receptor display salt-sensitive hypertension, which implies a role for PGE2 in salt excretion, but regulation of blood pressure by PGE_2 is influenced by its receptor expression, sex, and genetic background. [58, 59]

Conclusion

With recent advances of molecular genetics, the rodents have become the human's best friend, helping to study the mechanisms of many diseases. Genetic models of hypertension were especially successful in rodents and have encouraged studies in human population with the candidate gene approach, as well as the

development of new classes of drugs to decrease blood pressure and target organ injury. Importantly, nongenetic approaches have complemented the investigation of the effects of secondary hypertension on end-organ injury in a larger variety of animal models, thereby enriching our understanding of the pathophysiology of this disease. In addition, experimental models of hypertension associated with comorbidities common in clinical practice that accentuate development of hypertension and/or target organ injury may provide closer simulation of the human disease. The next few years will see very rapidly progress in the extent of our knowledge on the genetic basis of hypertension, and we can wish that new therapeutic strategies will be born from this research.

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