GSC Biological and Pharmaceutical Sciences
eISSN: 2581-3250 CODEN (USA): GBPSC2
Cross Ref DOI: 10.30574/gscbps
Journal homepage: https://gsconlinepress.com/journals/gscbps/

(REVIEW ARTICLE)

Check for updates

Review on *in vivo* and *in vitro* experimental model of anti-hypertensive agent

Shubham Purushottam Jaybhaye *, Samiksha Dnyaneshwar Ajmire, Sachin Ramesh Baringe, Mahesh Bhanudas Narkhede, Pavan Prabhakar Chinchole and Pratik Sunil Kapse

Department of Pharmacology, Dr. Rajendra Gode College of Pharmacy, Malkapur, Maharashtra, India 443101.

GSC Biological and Pharmaceutical Sciences, 2022, 19(03), 127–132

Publication history: Received on 02 May 2022; revised on 04 June 2022; accepted on 06 June 2022

Article DOI: https://doi.org/10.30574/gscbps.2022.19.3.0222

Abstract

We aimed to evaluate the use of specific antihypertensive drugs and drug classes, as well as combinations in patients treated with 3 or more drugs classified as having or not resistant hypertension (RH), controlled or uncontrolled RH and true versus white-coat RH. From the Spanish ABPM Registry, we identified 21238 patients treated with 3 (14264) or more (6974) antihypertensive drugs of different classes. Among patients treated with 3 drugs we compared those with controlled (<140/90 mmHg; No RH) or uncontrolled (RH) office BP. In patients treated with 4 or more drugs we compared controlled versus uncontrolled RH. Hypertension continues to be an important public health concern because of its associated morbidity, mortality and economic impact on the society. It is a significant risk factor for cardiovascular, cerebrovascular and renal complications. It has been estimated that by 2025, 1.56 billion individuals will have hypertension. The increasing prevalence of hypertension and the continually increasing expense of its treatment influence the prescribing patterns among physicians and compliance to the treatment by the patients. A careful selection of drug therapy along with close follow-up offers the best prospect to reduce the burden of morbidity and mortality in hypertension. This article provides an overview of drugs in the management of patients with hypertension.

Keywords: Antihypertensive; Cerebrovascular; Renal Complications; ABPM Registry

1. Introduction

Hypertension is a major contributing factor for cardiovascular disease (CVD) and renal diseases that can increase the risks of comorbidities such as myocardial infarction, stroke and heart failure (HF) [1]. Studies have revealed that risk factors such as obesity and genetic factors can influence the occurrence and development of hypertension [2,3]. In addition, complicated regulatory networks, including the renin-angiotensin-aldosterone system (RAAS), the nervous system and arterial remodelling [4-6] also affect the progression of hypertension. Because blood pressure (BP) is difficult to control, the priority is finding drug targets to effectively control and manage BP in the hypertensive population. In this review, we primarily describe the classical and new drug targets used in hypertension therapy. Hypertension is the most common chronic disease in the world and produces substantial morbidity and mortality. However, in the majority of individuals, the precise cause of elevated blood pressure (BP) cannot be determined. Risk factors for primary (formerly called essential) hypertension include advancing age, obesity, high dietary NaCl consumption, and low dietary potassium intake, although these appear to contribute to, but not cause, hypertension. Renin-sodium profiling has been used to classify primary hypertension, suggesting that the phenotype is highly variable, but treatment remains largely empirical and influenced by race and comorbid disease. A number of hypertensive subtypes also exist, and although they may make up only a small fraction of the cases of hypertension, they can nonetheless be relatively common, given the broad prevalence of hypertension itself. Malignant hypertension is related to, but pathophysiological distinct from, primary hypertension, as is the syndrome of preeclampsia. Secondary causes may involve the renal vasculature, endocrine organs, and kidney and may be involved in up to 20% of cases of resistant

* Corresponding author: Shubham Purushottam Jaybhaye
Department of Pharmacology Dr. Rajendra Gode College of Pharmacy, Malkapur, Maharashtra, India 443101.

hypertension. Finally, an increasing number of drugs used to treat cancer and other conditions are now recognized as causing hypertension, which is often severe. Genetic forms of hypertension with mendelian inheritance are rare but have helped to identify important BP-regulating pathways. Over the past 20 years, some the most important scientific breakthroughs have emanated from the discovery of the basis of rare subtypes of human hypertension. Among these are the solution of nearly all the monogenic causes of hypertension; identification of discrete somatic mutations that cause primary aldosteronism; the discovery that polymorphisms in the APOL1 gene underlie some racial disparities in hypertensive kidney disease; the discovery that placental insufficiency generates placental growth factor and sFlt-1 (soluble fms-like tyrosine kinase-1), factors that mark and contribute to preeclampsia; and finally, the recognition that certain anticancer drugs commonly cause hypertension by impairing the function of the vascular endothelium and the glomerulus.

The initial animal models of hypertension to be developed involved constriction of renal arteries (Goldblatt kidney) or parenchyma (Page kidney); the pathophysiology closely mimicked their human analogs. However, renovascular hypertension and Page kidney represent only a small fraction of human hypertension. Most experimental studies of hypertension using animals have therefore focused on understanding the mechanisms of primary hypertension.

Although excellent animal models with good human fidelity have been developed for many of these rare causes of hypertension,1,2 models of primary hypertension have been more difficult to develop, largely because the causes of the human disorder are unclear. Of National Institutes of Health–sponsored hypertension research, studies using Ang II (angiotensin II) infusion make up a disproportionate share (nearly 50%) [7] Only 4% of studies focus on aging and 4% focus on DOCA (deoxycorticosterone acetate)–salt hypertension (which itself does not model primary aldosteronism). Thus, an important unmet need is to develop better animal models that more closely mimic the discrete hypertensive syndromes that now populate the clinic such as primary aldosteronism. A corollary would be that the portfolio of hypertension research might more closely mimic the spectrum of human hypertension.

A second important unmet need is to resolve ongoing controversies concerning pathogenesis. Proponents for individual pathways, including the primacy of the nervous system, kidney, and vasculature in the development of hypertension, typically focused on their own views and interests, often independently of considerations of heritability, environmental exposure, and developmental programming. Despite >50 years of work, there is no consensus integrating this range of contributing causative factors. This persistent lack of convergence slows bona fide progress and can limit the impact of the field. Addressing this unmet need will require that we bring together diverse teams with competing views who are committed to this common goal.

2. Utility and Validity of Animal Models of Hypertension

Across a range of human diseases, including hypertension, animal models have been useful for unravelling disease pathogenesis by providing incisive experimental strategies not possible in human studies. In hypertension, the utility of animal models for improving the understanding of the pathogenesis, prevention, and treatment of hypertension and its comorbidities depends on their validity for representing human forms of hypertension, including responses to therapy, and the quality of studies in those models. Recently, the utility of animal studies in translational medical research has come under increasing scrutiny because of low study reproducibility and problems such as bias, poor experimental design and execution, analytical and logical errors, and incomplete reporting. [7-11]. Published recommendations on ways to mitigate these issues should be considered for any studies using animal models. It should be noted that >1000 scientific journals have endorsed guidelines designed to improve the reporting of animal experiments. Nonetheless, these guidelines should be applied cautiously because excessive regulation may also hinder studies in animals.

2.1. *In vivo* Models

Induced Reno vascular

2.1.1. Harry Goldblatt and the discovery of renin

In 1934, pathologist Harry Goldblatt established the first animal model of hypertension. This model provided researchers with the tools to delineate the renin-angiotensin system of blood pressure control and, eventually, to design enzyme inhibitors for the treatment of chronic hypertension. As early as the mid–19th century, physicians had noted that patients with kidney disease and those with long-term high blood pressure (hypertension) both had enlarged heart muscles. The first clue to this kidney–hypertension connection came from physiologists Robert Degerstedt and Per Bergman who injected kidney extracts into rabbits, thus triggering a dramatic rise in blood pressure. In a prophetic

explanation, they suggested that the kidneys produced a soluble protein they called it renin that triggered a rise in blood pressure. [12].

2.1.2. Renin in human hypertension

Simple theory would predict high renin levels in patients with hypertension, but instead, says Laragh, "renin levels are all over the place." Thus, despite an understanding of the renin angiotensin system in animals, there was still doubt about its role in humans. The development of ACE inhibitors helped explain this seeming inconsistency. Laragh and colleagues injected the first such inhibitor into patients and found a drop in blood pressure, but only in patients with high levels of circulating renin. In other studies, Laragh showed that hypertension not caused by increased renin results from insufficient salt excretion by the kidney. The work of Laragh and others thus revealed that hypertension is not a single mechanistic process, but still confirmed Goldblatt's original idea: production of renin by the kidney regulates blood pressure. [12-13]. Evidence from animal studies demonstrates that the renin-angiotensin (ANG II) system and sodium retention play major roles in experimental renovascular hypertension (RVH). Two basic models have been described. In the first, one-clip two-kidney Goldblatt hypertension, the ischemic kidney secretes renin, which leads to increased ANG II formation and hence elevation of blood pressure (BP). As BP rises, sodium excretion by the intact contralateral kidney increases (pressure natriuresis); therefore, there is no sodium retention. In the second, one-clip one-kidney Goldblatt hypertension, the contralateral kidney is removed. In this case the pressure natriuresis can no longer occur, and sodium retention occurs. The ensuing expansion of plasma volume inhibits renin secretion, so that in this model the renin level is normal or low. Following the clipping of the renal artery, renal blood flow and pressure are maintained distal to the stenosis by an ANG II-mediated vasoconstriction. This acts preferentially on the efferent glomerular arterioles, so that the ratio of pre glomerular to post glomerular resistance is reduced, which helps to maintain glomerular filtration despite the reduced renal perfusion pressure. In the contralateral kidney the afferent arteriolar resistance is increased, probably as a direct result of exposure to the higher intra renal arterial pressure.

2.2. *In vitro* models

2.2.1. Monocrotaline induced Pulmonary Hypertension

Most studies of the mechanisms of hypertensive pulmonary vascular remodeling are in animal models. The two most commonly used are hypoxia- and monocrotaline-induced pulmonary hypertension in rats, although transgenic mice are increasingly being used. While the rat models have provided important groundwork for the current use of Ca2+ channel blockers, PGI2 analogy, ET-1 receptor antagonists, inhaled NO, and phosphodiesterase inhibitors in the clinical treatment of PAH, and continue to provide new insights into the myriad cellular and molecular mechanisms involved in regulation of pulmonary vascular tone and structure, it should be emphasized that neither develops the obstructive neointimal lesions found in severe PAH. In fact, if the hypertensive lungs of chronically hypoxic or monocrotaline-injected rats are vasodilated and fixed under positive intravascular pressure, it is observed that the adventitial and medial thickening of small muscular PAs causes little, if any, reduction in lumen area. Thus, it appears that much of the muscular PA medial hypertrophy and luminal narrowing typically reported in hypoxia- and monocrotaline-induced hypertensive rat lungs fixed without maximal vasodilation is due to vasoconstriction (vasospasm), rather than to structural inward remodeling of the vascular wall. Recent studies showing marked reversal of established pulmonary hypertension in either chronically hypoxic or monocrotaline-injected rats by acute administration of Rho-kinase inhibitors, such as Y-27632 and fasudil, supports the idea that sustained vasoconstriction, rather than structural medial thickening of either muscular Pas or neo muscularized pulmonary arterioles, is a major cause of increased pulmonary vascular resistance in these models. This is not to say that the remodelling plays no role in the increased resistance, but rather that its apparent contribution is exaggerated in Pas that are not vasodilated before fixation. It is noteworthy that in studies of vascular remodelling in systemic hypertension, it has long been appreciated that the direct contribution of vascular structure to luminal narrowing needs to be determined under conditions of complete lack of vascular tone, that is, under maximal vasodilation [14]

2.2.2. ACE Inhibition in Guenia Pig ileum

Studies indicate that ACE inhibitors and some Ang metabolites increase B2R functions as allosteric enhancers by inducing a conformational change in ACE. This is transmitted to B2Rs via heterodimerization with ACE on the plasma membrane of cells. ACE inhibitors are also agonists of the B1R, at a Zn-binding sequence on the second extracellular loop that differs from the orthosteric binding site of the des-Arg-kinin peptide ligands. Thus, ACE inhibitors act as direct allosteric B1R agonists. When ACE inhibitors enhance B2R and B1R signalling, they augment NO production. Enhancement of B2R signalling activates endothelial NO synthase, yielding a short burst of NO; activation of B1Rs results in a prolonged high output of NO by inducible NO synthase. These actions, outside inhibiting peptide hydrolysis, may contribute to the pleiotropic therapeutic effects of ACE inhibitors in various cardiovascular disorders. ACE inhibitors can enhance both B2 and B1R signalling. Blocking kinin inactivation by ACE raises the concentration of intact B2R

agonists, which are also the substrates of CPN and -M. This can generate more des-Arg-kinin B1R agonists (Figure). The successful use of antagonists of the Ang II type 1 receptor (AT1R) for many of the same indications as ACE inhibitors does not prove ACE inhibitors work only through reducing Ang II as there are complex interrelationships among Ang II, BK and their receptors. Ang II has 2 receptors, AT1R and AT2R. AT1R is blocked by drugs such as losartan, which can shift Ang II actions to the AT2R. This switching of receptors further counteracts AT1R effects because it leads to the release of mediators such as nitric oxide (NO) and is attributed partially to release of BK to activate B2Rs, a form of "crosstalk."[28] Intricate Ang II and kinin receptor interrelationships were also indicated in animal experiments where both kinin B1 and B2Rs share in the favourable cardiovascular effects of AT1R blockade [15-17].

2.2.3. Alpha 2-adrenoceptors and endothelium-dependent relaxation in canine large arteries

The carotid, coronary, renal, mesenteric and femoral arteries of the dog were precontracted with the thromboxane mimetic after ensuring that the resting conditions were comparable from the Laplace relationship. In the presence of prazosin (1 microM) and propranolol (3 microM), noradrenaline (NA) relaxed the arteries in the order coronary greater than carotid greater than femoral greater than renal = mesenteric. When maximum relaxation to nitroglycerin (10 microM) was taken to be 100% the maximum relaxation to noradrenaline in each artery was: coronary 70%; carotid 34%; femoral 19%; renal 7% and mesenteric 2%. In endothelium-intact arteries UK14304 mimicked the relaxation responses to NA and idazoxan shifted the curves to both agonists to the right, consistent with an alpha 2-adrenoceptor classification. Substance P relaxed the arteries in the same order as for NA but showed higher efficacy i.e.: coronary 100%; carotid 80%; femoral 71% renal 49%; and mesenteric 41%. Removal of the endothelium abolished the relaxation to NA. We conclude that endotheliumdependent relaxation to NA and substance P varies greatly across 5 large arteries of the dog. This may indicate that endothelium-derived relaxing factor (EDRF) release is site-dependent or that the efficacy of EDRF on smooth muscle varies; being greatest in the coronary and weakest in the renal and mesenteric arteries [18-19].

2.2.4. Beta adrenoreceptors activity

Renal a-Adrenergic Receptors

The majority of studies on a-adrenergic receptors in hypertension have been performed in the kidney. Most investigators have described an increased density of renal adrenergic receptors in SHR when compared with Wistar-Kyoto (WKY) rats, although two studies did not detect significant changes. This alteration appears to be specific for SHR and has not been described in other models of genetic hypertension such as the MHS,24 the SBH,19 or the DS rat54 when compared with their respective control strains. The increase in renal adrenergic Peripheral Adrenergic Receptors 109 receptor in SHR precedes the elevation of blood pressure. 48 Animal models of acquired hypertension have unchanged or decreased renal adrenergic receptors[20-29]. Similarly, many studies have demonstrated an elevated density of renal a2-adrenergic receptors in SHR. Such increases have also been found in two other models of genetic hypertension, the DS54-58 and the SBH rat [20-29].

3. Conclusion

As mentioned above, morphological changes in the organs depend probably on rat breeding, genetic factors and even interspecies differences. A multitude of different animal hypertension models enables scientists to choose the one that is most suitable for their specific purposes. Nevertheless, an appropriate approach to the interpretation of the obtained results is necessary. Unfortunately, it is often impossible to relate the results obtained in animal hypertension models to those obtained in humans with hypertension. Moreover, it has been shown that very often the selection of a given model significantly affects the obtained results. Therefore, before starting an experiment, scientists should know the exact specification of a given animal model and then they should consider its relevance to the planned objectives. It seems to be reasonable to select more than one model for the experiment because such an approach makes it possible to obtain more complete and comparable results.

Compliance with ethical standards

Acknowledgments

The authors are thankful to Dr. Prashant K. Deshmukh, Principal, Dr. Rajendra Gode College of Pharmacy, Malkapur M.S., and India-443101 for providing the research facilities and encouragement.

Disclosure of conflict of interest

The authors declare that there is no conflict of interests regarding the publication of this article.

References

[1] K Rahimi, CA Emdin, S MacMahon. The epidemiology of blood pressure and its worldwide management Circ. Res. 2015; 116: 925-936.

[2] JE Hall, JM do Carmo, AA da Silva, Z Wang, ME Hal.l Obesity-induced hypertension: interaction of neurohumoral and renal mechanisms Circ Res. 2015; 116: 991-1006.

[3] S Padmanabhan, M Caulfield, AF Dominiczak. Genetic and molecular aspects of hypertension Circ. Res. 2015; 116: 937-959.

[4] L Te Riet, JH van Esch, AJ Roks, AH van den Meiracker, AH Danser Hypertension: renin-angiotensin-aldosterone system alterations Circ. Res. 2015; 116: 960-975

[5] G Grassi, A Mark, M Esler. The sympathetic nervous system alterations in human hypertension Circ. Res. 2015; 116: 976-990.

[6] S Laurent, P Boutouyrie. The structural factor of hypertension: large and small artery alterations Circ. Res. 2015; 116: 1007-1021.

[7] Reproducibility Issues in Research with Animals and Animal Models: Workshop in Brief. Washington, DC: National Academies Press; 2015:8.

[8] Sjoberg EA. Logical fallacies in animal model research. Behav Brain Funct. 2017;13:3. doi: 10.1186/s12993-017-0121-8

[9] Zeiss CJ, Johnson LK. Bridging the gap between reproducibility and translation: data resources and approaches. ILAR J. 2017;58:1–3. doi: 10.1093/ilar/ilx017

[10] Avey MT, Moher D, Sullivan KJ, Fergusson D, Griffin G, Grimshaw JM, Hutton B, Lalu MM, Macleod M, Marshall J, Mei SH, Rudnicki M, Stewart DJ, Turgeon AF, McIntyre L; Canadian Critical Care Translational Biology Group. The devil is in the details: incomplete reporting in preclinical animal research. PLoS One. 2016;11:e0166733. doi: 10.1371/journal.pone.0166733

[11] Reichlin TS, Vogt L, Würbel H. The researchers' view of scientific rigor-survey on the conduct and reporting of in vivo research. PLoS One. 2016;11:e0165999. doi: 10.1371/journal.pone.0165999)

[12] Tigerstedt, R., and P. Bergmann. 1898.Skand. Arch. Physiol. 8:223–271.)

[13] Gavras, H., H.R. Brunner, J.H. Laragh, J.E. Sealey, I. Gavras, and R.A. Vukovich. 1974.N. Engl. J. Med. 291:817–821.8. Laragh, J.H. 1973. Am. J. Med. 55:261–274.)

[14] (I.F. McMurtry, ... P.L. Jones, in Encyclopedia of Respiratory Medicine, 2006)

[15] Ignjatovic T, Stanisavljevic S, Brovkovych V, Skidgel RA, Erdös EG. Kinin B1 receptors stimulate nitric oxide production in endothelial cells: signaling pathways activated by angiotensin I-converting enzyme inhibitors and peptide ligands. Mol Pharmacol 2004;66:1310–1316.

[16] Ignjatovic T, Tan F, Brovkovych V, Skidgel RA, Erdös EG. Novel mode of action of angiotensin I converting enzyme inhibitors. Direct activation of bradykinin B1 receptor. J Biol Chem2002;277:16847–16852.

[17] Stanisavljevic S, Ignjatovic T, Deddish PA, Brovkovych V, Zhang K, Erdös EG, Skidgel RA. Angiotensin I-converting enzyme inhibitors block protein kinase C epsilon by activating bradykinin B1 receptors in human endothelial cells. J Pharmacol Exp Ther 2006;316:1153–1158.

[18] . Cocks TM, Angus JA. Endothelium-dependent relaxation of coronary arteries by noradrenaline and serotonin. Nature. 1983 Oct 13;305(5935):627–630.

[19] Furchgott RF. The role of endothelium in the responses of vascular smooth muscle to drugs. Annu Rev Pharmacol Toxicol. 1984;24:175–197.

[20] Yamada S, Yamamura HI, Roeske WR: Alterations in central and peripheral adrenergic receptors in deoxycorticosterone/salt hypertensive rats. Life Sci 1980-,27:2405-2416

[21] Parini A, Diop L, Dausse J-P, Ben Ishay D: Sabra rats as a model to differentiate between Na+and GTP regulation of orradrenoceptor densities. Eur J Pharmacol 1985;112:97-104

[22] Diop L, Parini A, Dausse JP, Ben-Ishay D: Cerebral andm renal a-adrenoceptors in Sabra hypertensive (SBH) and normotensive (SBN) rats: Effects of high-sodium diet /Cardiovasc Pharmacol 1984;6{suppl 5):S742-S747

[23] Parini A, Diop L, Ferrari P, Picotti G, Finardi G, Dausse J-P:Cerebral and renal alpha-adrenoceptors in Milan hyperten-sive rat strain. / Hypertens 1986;4(suppl 3):S213-S215

[24] Michel MC, Kanczik R, Khamssi M, Knorr A, Siegl H, Beckeringh JJ, Brodde O-E: a- and /3-adrenoceptors inhypertension: I. Cardiac and renal oi, /3i, and ft-adrenoceptors in rat models of acquired hypertension. /Cardiovasc Pharmacol 1989;13:421-431

[25] Sanchez A, Vidal MJ, Martinez-Sierra R, Saiz J: Ontogeny of renal alpha-1 and alpha-2 adrenoceptors in the spontane-ously hypertensive rat. / Pharmacol Exp Ther 1986;237:972-979

[26] Saiz J, Lara B, Torres A, Sanchez A: Hypertensinogenic factors and renal a-adrenoceptors in young SHR and WKY rats. Life Sci 1987;41:2261-2268

[27] Yamada S, Nakamoto M, Hayashi M, Tomita T, Hayashi E: Tubular and glomerular adrenoceptors in stroke-prone spontaneously hypertensive rats. / Hypertens 1986;4(suppl3):S2O9-S211

[28] Jeffries WB, Yang E, Pettinger WA: Renal a,-adrenergic receptor response coupling in spontaneously hypertensive rats. Hypertension 1988;12:8O-88

[29] McCaughran JA Jr, Juno CJ, O'Malley E, Rosenthal M: The ontogeny of renal ct\- and oradrenoceptors in the Dahl rat model of experimental hypertension. JAuton Nerv Syst 1986;17:1-20.