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Effects of Antihypertensive Drugs on Specific Harmonic Indices of the Pulse Waveform in Normotensive Wistar Kyoto Rats

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Abstract

We used a self-comparison method and harmonic analysis to compare the blood pressure wave before and after the injection of antihypertensive drugs (atenolol, captopril, and losartan) in normotensive Wistar Kyoto rats. Systolic and diastolic blood pressures decreased significantly after the intraperitoneal injection of drugs. Atenolol significantly reduced all the harmonic proportions of the pulse wave, while captopril and losartan significantly increased the first and fourth harmonic proportions. These findings are the same as those reported for human subjects and confirm that harmonic analysis of the pressure pulse is a useful method to study the efficacy of antihypertensive drugs.

Keywords: animal model, harmonic analysis, captopril, losartan, atenolol, Wistar Kyoto rats

INTRODUCTION

The World Health Organization has estimated that 17.1 million people died as a result of cardiovascular diseases in 2004, which represents 29% of all global deaths in that year. High blood pressure (hypertension) is one of the most important preventable causes of premature death worldwide (1).

The radial or brachial pressure which has high correlation to the central aortic pressure (2–4) is usually used as a noninvasive index to estimate the cardiovascular risk for the convenience of clinical measurements. Since the pressure pulse wave is an overall representation of the circulation system, the alteration of the cardiac function and the arterial system will change the waveform of the pressure pulse (5). Therefore, the pulse waveform analysis could provide more information to study the hypertension and to evaluate the effect of therapies (6).

The development of antihypertensive drugs is costly in terms of money, manpower, and time, and it is thus imperative to determine an appropriate animal model for the development of hypertensive drugs (7). The spontaneously hypertensive rat (SHR) has thus far been considered to be the animal model (8) It has been shown that the renal system of SHRs differs from that of normotensive rats (9–11).

In a preliminary study, harmonic analysis of the pulse wave was used to study the changes induced by captopril on Wistar Kyoto (WKY) rats (12). Compared to the changes in response to the antihypertensive drug found in human subjects (13), these changes are similar to that found in WKY rats both in blood pressure and in the harmonic proportions.

In this study, a harmonic-based method (14,15) was used to analyze the pulse spectrum in the frequency domain, using captopril, an angiotensin-I-converting-enzyme inhibitor (ACEi), and losartan, an angiotensin II receptor blocker (ARB). A β_1-adrenergic receptor blocking agent, atenolol, which exerts its antihypertensive effects mainly on the cardiac output, was studied for comparison. It was expected that the results obtained using WKY rats would be similar to those found previously for human subjects.

METHODS

Animals and Drugs

This study was conducted on male WKY rats aged 6–8 weeks, weighing 240–350 g. The WKY rats were provided by the Experimental Animal Center of National Taiwan University. All experiments conformed to the "Guidelines for the Care and Use of Laboratory Animals" published by the U.S. National Institutes of Health.

Urethane, heparin, captopril, losartan, and atenolol were purchased from Sigma Chemicals (St. Louis, MO, USA). The drugs, which were all dissolved in saline, were administered at the following doses: 2 mL of urethane at 1.32 g/kg, 2 mL of heparin at 1.5 g/L, 3 mL of captopril at 2.5 mg/kg, 2 mL of losartan at 5 mg/kg,

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and 4 mL of atenolol at 5 mg/kg. These doses were transferred to the equivalent dose to be used by rats (that is normalized by body weight then times 6 (16)) according to the standard doses of human hypertension patients (captopril at 25 mg, losartan at 50 mg, and atenolol at 50 mg). These standard doses had given a clear response for change in the harmonic proportions as well as blood pressure on the WKY rats which were similar to those of humans. Therefore, only one dose was used in this study.

Experimental Setup and Procedures

The experimental setup is shown in Figure 1 (17). The rats were anesthetized with urethane and placed on an operating table, the environment around which was maintained at a temperature of 32 ± 2°C. The tail artery was cannulated via an opening made in the tail about 1 cm from the anus, with an intravenous catheter (22-gauge, 0.9 × 25 mm^2 needle; Angiocath Plus, Becton–Dickinson, Seoul, Korea) and flushed with saline and heparin. The cannula was connected to a pressure transducer (P10EZ, Ohmeda, Singapore, Singapore) so that the components of the blood pressure pulse could be measured.

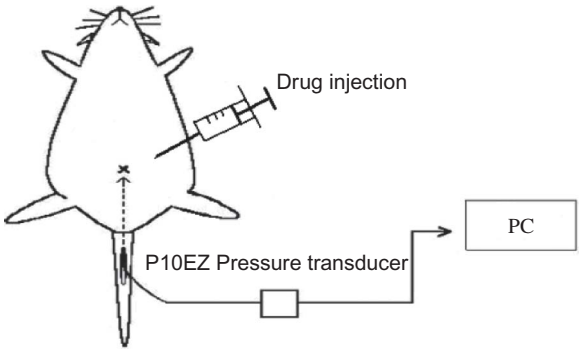

Figure 1. Experiment setup.

After completion of the surgery, the rats were allowed to rest quietly for 120–180 minutes to stabilize their condition. The signal received by the pressure transducer was sent to a preamplifier (Universal Amplifier, Gould Instrument Systems, Valley View, OH, USA) and then to an A/D card (PCI-9111, ADLink, New Taipei City, Taiwan) at a sampling rate of 4096 Hz. A 2-second pulse sequence was acquired every minute; 20 data sequences were recorded and averaged as control data before administering an intraperitoneal injection of the test drug (captopril, losartan, or atenolol). The data sequences were sampled until the end of the experiment. After the experiments were completed, the rats were killed by an overdose of urethane.

Data Analysis

The flowchart of data processing and pulse waveform are shown in Figure 2. Each 2-second pulse sequence contained 12–15 pulses, depending on the heart rate (HR) of the individual rat. Each pulse in the sequence was separated by the two neighboring lowest points. The pulse waveform was adjusted slightly with the slope to make the start and the end point at the same height. The adjusted data were Fourier transformed into the frequency domain and averaged every 10 minutes. Heart beat is a repeated signal with period T, φn is the phase of nth harmonics (where $n = 1 - 5$), the pulse ($P(t)$) can be decomposed into harmonics as the following (18):

$$P(t) = A0 + \sum_{n=1}^{N} An \cos\left(\frac{2\pi n}{T}t + \phi n\right) \qquad (1)$$

In this study, the focus was only on the change in the first five harmonics and the harmonic proportions (Cn, where $n = 1 - 5$) instead of the amplitude (An, where $n = 1 - 5$) were computed. Cn was defined as

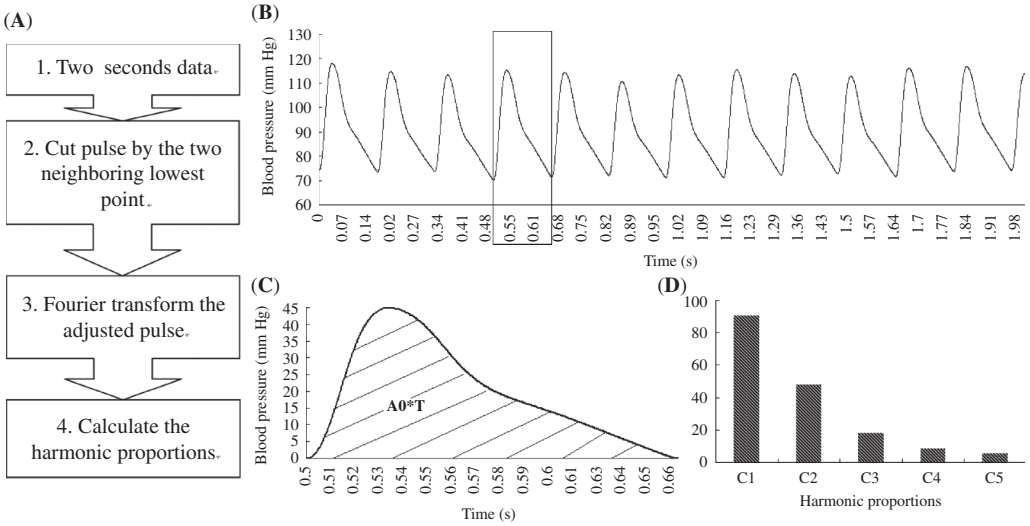

Figure 2. (**A**) Flowchart of data processing. (**B**) A typical 2-second pulse sequence data. (**C**) A typical pulse waveform which was separated by the two neighboring lowest points. (**D**) A typical harmonic analysis of the pulse waveform.

$Cn = An/A0$, where $A0$ is the mean pulse pressure value or the total area under one pulse wave/T as shown in Figure 2C.

The pulse waveform of the 20 minutes of data recorded before each injection was used as control data. The effect of the drugs on the pressure wave index, Cn, is presented as the percentage change, $\%C^{Cn}$, in the experiment between the drug and control conditions as follows:

$$(\%C^{Cn}) = 100 \times \frac{Cn,\text{test} - Cn,\text{cont}}{Cn,\text{cont}} \qquad (2)$$

where Cn,cont is the Cn of the control and Cn,test is the Cn of the test pulse waveform.

Statistical Analysis

Indices such as systolic blood pressure (SBP), diastolic blood pressure (DBP), and HR are reported as mean ± SEM values. Student t test was used for statistical comparisons. The level of statistical significance was set at $P < .05$.

RESULTS

The averaged HR, DBP, and SBP data in each time period for atenolol, losartan, and captopril are shown in Figure 3. Diastolic blood pressure and SBP decreased significantly in response to all of the tested drugs during all of the measured time periods. Atenolol effected a significant decrease in HR, while neither captopril nor losartan had any significant effect on this parameter.

The percentage changes in $\%C^{Cn}$ values of the pulse spectrum between the drug and control data are shown in Figure 4. Captopril caused a stable and significant increase in C1, and increases in C3, C4, and C5 between 20 and 40 minutes following injection, but no significant change in C2. Similarly, losartan caused significant increases in C1, C3, C4, and C5 between 20 and 40 minutes following injection, but no significant change in C2. By contrast, atenolol caused a significant decrease in all of the measured $\%C^{Cn}$ values.

DISCUSSION

The results of this study suggest that normotensive WKY rats represent a useful animal model for distinguishing between antihypertensive drugs with different mechanisms of action. Using the self-comparison method, and the short procedure and simple harmonic analysis, this animal model could be useful for the rapid screening of potential new antihypertensive drugs during the early stages of their development.

Captopril is an ACEi, which may dilate the peripheral arteries and is used to treat hypertension (19,20). Losartan is an ARB that targets the angiotensin II receptor without blocking other hormone receptors or metabolic pathways involved in cardiovascular regulation (21). Atenolol is a β_1-adrenergic receptor blocking agent and has long been considered to be a first-line drug in the treatment of hypertension (22). In humans, atenolol reduces the HR, cardiac output, and blood pressure without dilating the blood vessels (23).

The effects of these three antihypertensive drugs on human subject were discussed and analyzed by pulse wave analysis (24). In previous studies of the authors on antihypertensive drugs in human subjects, the drugs, which are more effective in reducing the central aortic

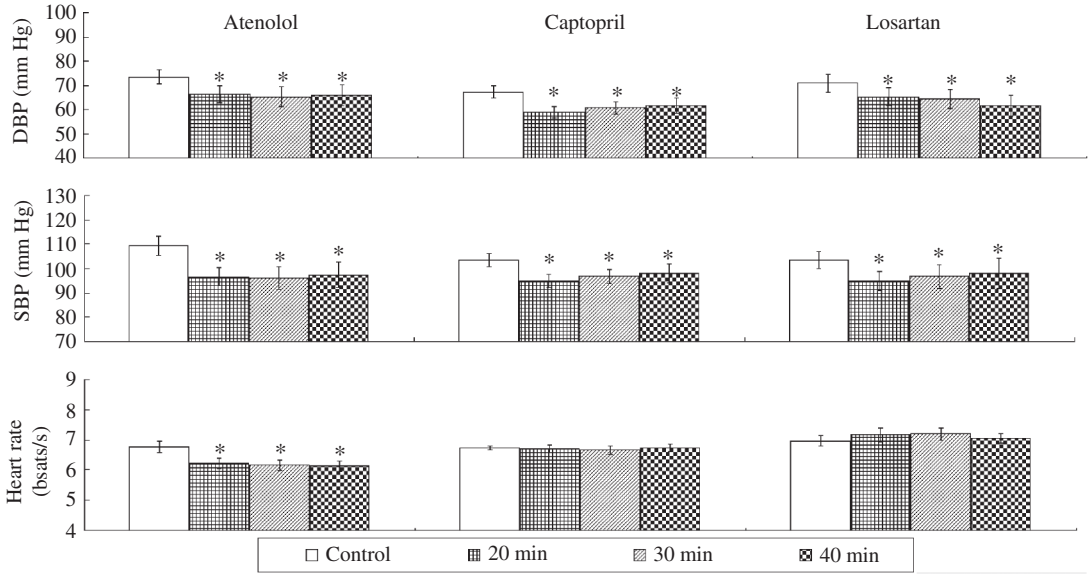

Figure 3. Effect of atenolol (left, $n = 6$), captopril (middle, $n = 8$), and losartan (right, $n = 6$) on DBP, SBP, and HR. Error bars indicate SEM. Abbreviations: DBP – diastolic blood pressure; SBP – systolic blood pressure; HR – heart rate.
*$P < .05$ compared with control.

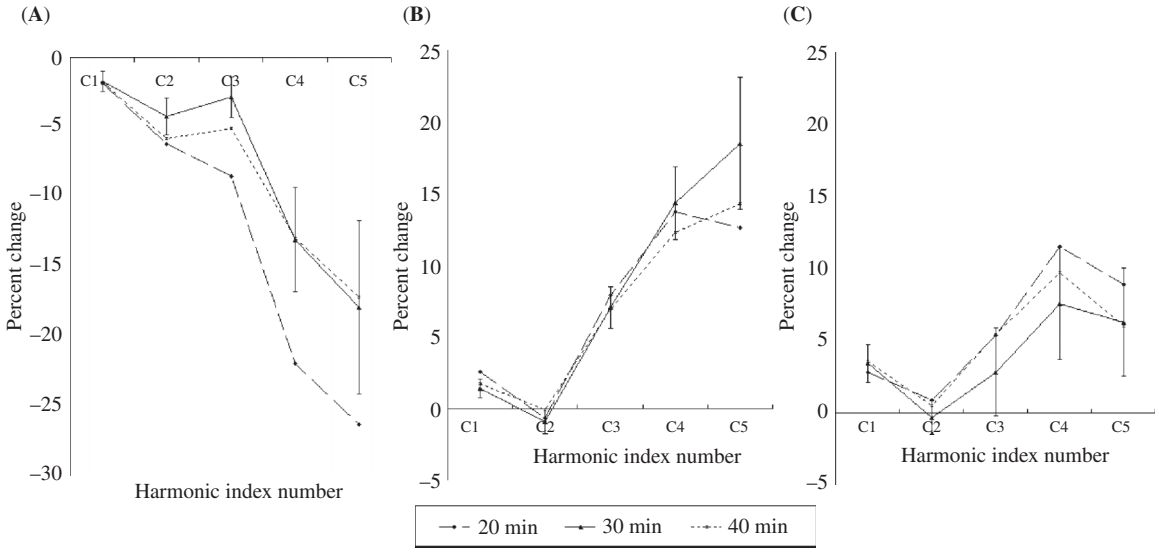

Figure 4. Percent changes in the pulse spectrum caused by (**A**) atenolol ($n = 6$), (**B**) captopril ($n = 8$), and (**C**) losartan ($n = 6$).

or carotid pressure (e.g., ramipril, losartan, dilevalol, isradipine, and nitroglycerin), all increased the harmonic proportion of C4 of the pulse pressure wave, but exerted inconsistent effects on the other harmonics, whereas the C4-increasing effect was not seen following treatment with the β-blocker atenolol (13). These human results are consistent with the results of the animal study presented here.

Normotensive rats are used as a control for comparison with hypertensive rats for all kinds of studies (25,26). In this study the self-comparison method on normotensive WKY rats was used to study the effects of antihypertensive drugs and found significant changes in the harmonic proportions. The self-comparison method evaluates the change from the same rat before and after a drug intervention, avoiding individual variations that can be greater than the effect of the drug itself. This may make this method supersensitive to detect the effect of drugs.

The augmentation index (AIx), an analog signal, is the most widespread pulse waveform analysis method currently utilized to describe the characteristic changes in the pulse waveform with aging (27,28), diseases (29), and vasoactive drugs (30). Atenolol exerts a small decreasing effect or even an increasing effect (4,31,32) on the AIx and decreased C4 in this study. The effect of atenolol on the harmonic proportions resembles the effects of vasopressin and angiotensin II (17). These may explain why atenolol lowers the blood pressure but does not improve the cardiovascular risk (33). On the contrary, for losartan, AIx-decreasing effect is seen in the trial (31) while C4-increasing effect is seen in this study. The harmonic analysis, a digital signal, could be an easier and a more consistent way of representing the AIx (13). It may also be related to the health condition of the internal organs and has been used to study Chinese herbs, acupuncture, and so on (13).

In conclusion, harmonic analysis study of the changes in the pulse wave in response to the antihypertensive drug and the self-comparison method avoid the individual variations that can be greater than the effect of the drug itself. The results found by this method on normotensive WKY rats are the same as those reported for human subjects. These findings confirm that harmonic analysis of the pressure pulse is a useful method to study the efficacy of antihypertensive drugs.

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Declaration of interest: The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

REFERENCES

[1] World Health Organization. Global Health Risks: Mortality and Burden of Disease Attributable to Selected Major Risks. WHO Library Cataloguing-in-Publication Data. Geneva: WHO, 2009.

[2] Kikuya M, Ohkubo T, Asayama K, et al. Ambulatory blood pressure and 10-year risk of cardiovascular and noncardiovascular mortality: the Ohasama study. Hypertension 2005; 45(2):240–245.

[3] Domanski MJ, Davis BR, Pfeffer MA, Kastantin M, Mitchell GF. Isolated systolic hypertension: prognostic information provided by pulse pressure. Hypertension 1999; 34:375–380.

[4] Hirata K, Vlachopoulos C, Adji A, O'Rourke MF. Benefits from angiotensin-converting enzyme inhibitor beyond blood pressure lowering: beyond blood pressure or beyond the brachial artery? J Hypertens 2005; 23(3):551–556. Erratum in: J Hypertens 2005; 23(4):903–904.

[5] McDonald DA. Blood Flow in Arteries, 2nd ed. London: Edward Arnold, 1974.

[6] O'Rourke MF, Adji A. An updated clinical primer on large artery mechanics: implications of pulse waveform analysis and arterial tonometry. Curr Opin Cardiol 2005; 20:275–281.
[7] Frohlich ED, Pfeffer MA, Pfeffer JM. Systemic hemodynamics and cardiac function in spontaneously hypertensive rats: similarities with essential hypertension. In: Strauer BE, ed. *The Heart in Hypertension*. Berlin and New York: Springer, 1981, 53–71.
[8] Okamoto K, Aoki K. Development of a strain of spontaneously hypertensive rats. Jpn Circ J 1963; 27:282–293.
[9] Skov K, Mulvany MJ. Structure of renal afferent arterioles in the pathogenesis of hypertension. Acta Physiol Scand 2004; 181:397–405.
[10] Anderson WP, Kett MM, Stevenson KM, Edgley AJ, Denton KM, Fitzgerald SM. Renovascular hypertension: structural changes in the renal vasculature. Hypertension 2000; 36:648–652.
[11] Chao PT, Jan MY, Hsiu H, et al. Evaluating microcirculation by pulsatile laser Doppler signal. Phys Med Biol 2006; 51: 845–854.
[12] Wang SH, Hsu TL, Jan MY, Wang WK, Wang Lin YY. Effects of captopril on specific harmonic indexes of the peripheral pressure pulse waveform. Proceedings of the 4th International Conference on Bioinformatics and Biomedical Engineering, Chengdu, China, June 18–20, 2010.
[13] Wang Lin YY, Hsu TL, Jan MY, Wang WK. Review: theory and applications of the harmonic analysis of arterial pressure pulse waves. J Med Biol Eng 2010; 30(3):125–131.
[14] Wang Lin YY, Chang SL, Wu YE, Hsu TL, Wang WK. Resonance. The missing phenomenon in hemodynamics. Circ Res 1991; 69:246–249.
[15] Wang Lin YY, Sze WK, Bau JG, et al. The ventricular-arterial coupling system can be analyzed by the eigenwave modes of the whole arterial system. Appl Phys Lett 2008; 92:153901.
[16] Barnes CD, Eltherington LG. Drug Dosage in Laboratory Animals: A Handbook. Berkeley, CA: University of California Press, 1966.
[17] Hsu TL, Chao PT, Hsiu H, et al. Organ-specific ligation-induced changes in harmonic components of the pulse spectrum and regional vasoconstrictor selectivity in Wistar rats. Exp Physiol 2006; 91:163–170.
[18] O'Neil PV. Advanced Engineering Mathematics. Toronto: Thomson, 2007.
[19] Levine TB, Franciosa JA, Cohn JN. Acute and long-term response to an oral converting-enzyme inhibitor, captopril, in congestive heart failure. Circulation 1980; 62:35–41.
[20] Hirooka Y, Imaizumi T, Masaki H, et al. Captopril improves impaired endothelium-dependent vasodilation in hypertensive patients. Hypertension 1992; 20:175–180.
[21] Velasquez MT. Angiotensin II, receptor blocker: a new class of antihypertensive drugs. Arch Fam Med 1996; 5: 351–356.
[22] Guidelines Committee. 2003 European Society of Hypertension—European Society of Cardiology guidelines for the management of arterial hypertension. J Hypertens 2003; 21:1011–1053.
[23] Buckingham RE, Hamilton TC. Comparison of the antihypertensive response to beta-adrenoceptor blocking drugs in intact and adrenal-demedullated spontaneously hypertensive rats. Br J Pharmacol 1980; 68:667–676.
[24] Wilkinson IB, McEniery CM, Cockcroft JR. Comment: atenolol and cardiovascular risk: an issue close to the heart. Lancet 2006; 367:627–629.
[25] Antonaccio MJ, Rubin B, Horovitz ZP. Effect of captopril in animal models of hypertension. Clin Exp Hypertens 1980; 2(3 & 4):613–637.
[26] Bolterman RJ, Manriquez MC, Clara Ortiz Ruiz M, Juncos LA, Romero JC. Effects of captopril on the renin angiotensin system, oxidative stress, and endothelin in normal and hypertensive rats. Hypertension 2005; 46(Part 2): 943–947.
[27] Kohara K, Tabara Y, Oshiumi A, et al. Radial augmentation index: a useful and easily obtainable parameter for vascular aging. Am J Hypertens 2005; 18:11S–14S.
[28] Kelly R, Hayward C, Avolio A, O'Rourke M. Nonivasive determination of age-related changes in the human arterial pulse. Circulation 1989; 80:1652–1659.
[29] London GM, Blacher J, Pannier B, et al. Arterial wave reflections and survival in end stage renal failure. Hypertension 2001; 38:434–438.
[30] Kelly PP, Millasseau SC, Ritter JM, Chowienczyk PJ. Vasoactive drugs influence aortic augmentation index independently of pulse-wave velocity in healthy men. Hypertension 2001; 37:1429–1433.
[31] Nichols WW. Clinical measurement of arterial stiffness obtained from noninvasive pressure waveforms. Am J Hypertens 2005; 18:3S–10S.
[32] O'Rourk MF, Kelly RP, Avolio AP, Hayward C. Effects of arterial dilator agents on central aortic systolic pressure and on left ventricular hydraulic load. Am J Cardiol 1989; 63: 38I–44I.
[33] The CAFE investigators, for the Anglo-Scandinavian Cardiac Outcomes Trial (ASCOT) investigators. Differential impact of blood pressure-lowering drugs on central aortic pressure and clinical outcomes: principal results of the Conduit Artery Function Evaluation (CAFÉ) Study. Circulation 2006; 113(9):1213–1225. DOI: 10.1161/CIRCULATIONAHA.105.595496.