Original Research

Two useful methods for evaluating antihypertensive drugs in conscious freely moving rats[1]

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KEY WORDS amlodipine; atenolol; blood pressure; nifedipine; nitrendipine; spontaneously hypertensive rat

ABSTRACT

AIM: Computerized analysis of blood pressure in conscious freely moving rats is a sound technique for physiological and pharmacological studies. The present work, based on this technique, was designed to introduce two useful methods for the evaluation of antihypertensive drugs in conscious spontaneously hypertensive rat (SHR). They were the directly intragastric administration of drugs and modified probability sum test for evaluating the synergism of the combination of two drugs. **METHODS AND RESULTS:** (1) Directly intragastric administration was used in conscious rats. A catheter was inserted into stomach immediately after arterial catheter insertion. Three days after operation, blood pressure was recorded and drug might be given intragastically via the gastric catheter. (2) Modified probability sum test was used to evaluate the synergism of two drugs. The formula was: $q=P_{A+B}/(P_A+P_B-P_A\times P_B)$. With this method, it was obtained: q=1.32 for the effects of the combination of atenolol and nitrendipine (20 mg/kg+10 mg/kg) on systolic blood pressure; q=1.41 for the effects of the combination of atenolol and amlodipine (10 mg/kg+1 mg/kg) on systolic blood pressure. **CONCLUSION:** The two methods introduced by the present work will be important and useful for antihypertensive drug evaluation in conscious freely moving rats.

INTRODUCTION

Since the beginning of 1980s, a computerized technique has been developed for measuring blood pressure (BP) for a long time (more than 24 h) in conscious freely moving rats[1-5]. With this technique one can study the cardiaovascular parameters in a physiological condition in rats and avoid the influences of anesthesia and stress. This technique is used not only for physiological studies but also for pharmacological studies including the evaluation of antihypertensive drugs. However, some limitations may be obviously seen with this technique. For example, we can observe the acute effect of an antihypertensive drug on BP only by an intravenous administration, while the clinically used administration is often oral. Can we administer a drug intragastricly without violence as does traditionally by gavage through mouth? A combination of two drugs is often required in the treatment of hypertension. Can we find a valid and relative simple method to judge whether this combination is synergic?

To answer these questions, the present work was designed to introduce two useful methods for antihypertensive-drug evaluation in conscious freely moving rats.

METHODS AND RESULTS

Animals Spontaneously hypertensive rats of either

[1] Project supported by the High Tech Research and Development (863) program (2002 AA2Z346C), and the National Natural Science Foundation of China (39670831 and 30070871).
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Received 2003-05-06 Accepted 2003-09-26

sex weighing 200-240 g with an age of 16 weeks were provided by the animal center of our university. The animals were housed in controlled conditions (temperature: 22 ºC±2 ºC and lighting: 8:00-20:00) and received tap water *ad libitum*. All surgical and experimental procedures were in accordance with institutional animal care guidelines.

Blood pressure measurement in conscious freely moving rats Systolic BP (SBP), diastolic BP (DBP) and heart period (HP) were continuously recorded using previously described technique[6-8]. Briefly, rats were anesthetized with a combination of ketamine (40 mg/kg) and diazepam (6 mg/kg). A floating polyethylene catheter was inserted into the lower abdominal aorta via the left femoral artery for BP measurement, and another catheter, when necessary, was placed into the left femoral vein for intravenous injection. The catheters were exteriorized through the interscapular skin. After a 2-d recovery period, the animals were placed for BP recording in individual cylindrical cages containing food and water. The aortic catheter was connected to a BP transducer via a rotating swivel that allowed the animals to move freely in the cage. After about 14-h habituation, the BP signal was digitized by a microcomputer. SBP, DBP, and HP values from every heartbeat were determined on line. The mean values of these parameters during a period of 24 h were calculated and served as SBP, DBP, and HP.

Method 1: intragastric drug administration in conscious freely moving rats Rat was anesthetized and a catheter was inserted into lower abdominal aorta for BP measurement as mentioned above. Immediately after the insertion of arterial catheter, an incision was made as small as possible on the left upper abdominal wall. A marker line was found on the stomach. In anatomy, this marker line is from the bottom of the esophagus to the greater curvature of the stomach, and divides the stomach into two parts. The left part is almost white, with thin gastric wall relatively lack of blood vessels. The right part is almost red, with thick gastric wall full of blood vessels. Just left the juncture at which the greater curvature and the marker line cross, a small stab was performed with the sharp tip of the scissors, a catheter (PE50) was inserted into the stomach, and a purse-string suture was used to fix the gastric catheter. The catheter was then tunneled subcutaneously, and exteriorized, together with arterial catheter, through the interscapular skin. These procedures have three advantages: 1) The stabbing at the above described position is easier to do than that at the right part of thick gastric wall; 2) ensuring no bleeding and minimal injury on the stomach; 3) ensuring the stomach in normal physiological position and no angle formation in the gastric catheter. Three days after catheterization, BP was recorded and drug could be administered via gastric catheter without stress.

Example 1 Fig 1 shows the BP tracing of a SHR. It received a dose of nifedipine (25 mg/kg, iv), BP decreased immediately after intravenous injection. Maximal decrease in SBP was found about 25 min after drug administration and the blood pressure returned to control level 2 h after the injection (upper panel of Fig 1). About 2 h after the recovery of BP, the same rat was given a dose of nifedipine (50 mg/kg) by ig with the proposed method. It was found that BP decreased 10 min after drug administration and the maximal effect was found about 40 min after drug. BP returned to baseline 3 h after nifedipine administration (lower panel of Fig 1).

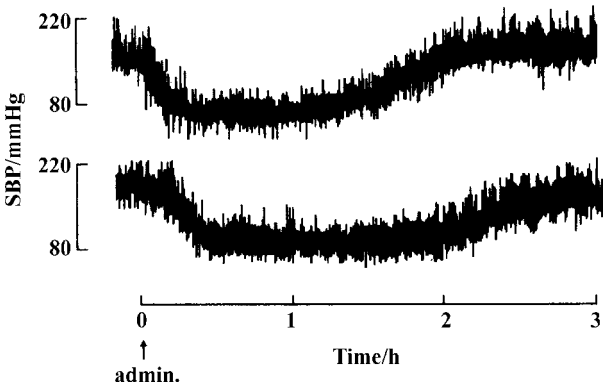

Fig 1. Systolic blood pressure (SBP) tracing of a spontaneously hypertensive rat recorded before and after nifedipine administration. Upper panel: nifedipine (25 mg/kg) was give intravenously (iv); lower panel: nifedipine (50 mg/kg) was given intragastracally (ig). Note: it is SBP tracing, but not pulsatile BP. The thick tracing is due to the time compress. The same for Fig 2.

Example 2 Fig 2 shows the BP tracings of 2 SHR recorded during a period of 25 h. One rat received a single dose of the combination of atenolol and amlodipine (10 mg/kg+1 mg/kg) and another was served as control. It was found that BP decreased about 30 min after drug administration. The antihypertensive effect of the drug was persistent during the 24 h (Fig 2).

Mothod 2: Modified probability sum test for evaluating the synergism of 2 drugs The modified probability sum test[9] was abbreviated as *q* test in the

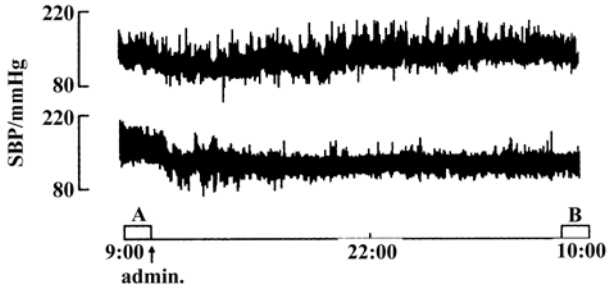

Fig 2. Blood pressure tracing of 2 spontaneously hypertensive rats recorded during a period of 25 h. One rat (lower panel) received a single dose of the combination of atenolol and amlodipine (10 mg/kg+1 mg/kg) and another rat (upper panel) received vehicle.

present work and its formula was: $q=P_{A+B}/(P_A+P_B-P_A\times P_B)$. A and B indicate drug A and drug B; P was the probability or response rate. When $q<0.85$, the combination is antagonistic; when $q>1.15$, it was synergic.

Example 1 Thirty SHR were divided into 3 groups. They were received a single dose of atenolol (20 mg/kg), nitrendipine (10 mg/kg) and the combination (20 mg/kg+10 mg/kg) respectively. Drugs were administered by gavage. SBP was measured by tail-cuff indirect method before and 6 h after the drug administration. Rat with a decrease in SBP>20 mmHg was defined as responder and with a decrease in SBP<20 mmHg as non-responder. It was found that response rate in SBP was 2/10 in atenolol-treated rats, 6/10 in nitrendipne-treated rats and 9/10 in the combination group. According to the above-mentioned formula, $q=0.9/(0.2+0.6-0.2\times0.6)=0.9/0.68=1.32$. This combination was synergic.

Example 2 Sixty-four SHR were divided into 8 groups with 8 rats in each group. BP was monitored continuously with a computerized technique in freely moving rats for more than 25 h. The real time for BP recording was between 9:00 (d 1) and 10:00 (d 2). Drugs were administered at 10:00 (d 1). The mean values obtained during two periods of one hour (9:00-10:00 d 1 and d 2) were taken for calculating the drug effects. Rat with a decrease in SBP>20 mmHg was defined as responder and with a decrease in SBP< 20 mmHg as non-responder. The drugs used in this study were atenolol, amlodipine alone, or in combination at different doses. The response rate was presented in Tab 1. With these results, we have: (1) for 10:0.5, $q=(3/8)/[(2/8)+(2/8)-(2/8)(2/8)]=0.86$; (2) for 10:1, $q=(6/8)/[(2/8)+(3/8)-(2/8)(3/8)]=1.41$; (3) for 10:2, $q=(6/8)/[(2/8)+(5/8)-(2/8)(5/8)]=1.04$. It was concluded that there existed a significant synergism of atenolol and amlodipine on 24 h blood pressure control when the combination was 10:1.

Tab 1. The effects of atenolol, amlodipine alone, and in combination on systolic blood pressure (SBP) in conscious spontaneously hypertensive rats.

Drug	Dose/mg·kg^{-1}	Responder	Non-responder	Probability
Ate	10	2	6	2/8
Aml	0.5	2	6	2/8
Aml	1	3	5	3/8
Aml	2	5	3	5/8
Ate+Aml	10+0.5	3	5	3/8
Ate+Aml	10+1	6	2	6/8
Ate+Aml	10+2	6	2	6/8

Ate indicates atenolol; Aml, amlodipine; responder, rat with a decrease in SBP>20 mmHg; non-responder, rat with a decrease in SBP <20 mmHg.

Example 3 Twenty-four SHR with a SBP between 175-190 mmHg were used. Three days after catheter insertion, BP was monitored for a period of 24 h 10 min after drug administration in conscious freely moving rats with abovementioned methods. The mean values of SBP over 24 h were as follows: 171±16 mmHg in rats treated with atenolol 10 mg/kg, n=8; 171±11 mmHg in rats treated with amlodipine 1 mg/kg, n=8; 155±20 mmHg in rats treated with the combination of atenolol and amlodipine (10+1 mmHg/kg, n=8). Rat with a SBP<160 mmHg was defined as responder and ≥160 mmHg as non-responder. There were 2, 1, and 4 responder rats in the groups treated with single dose of atenolol, amlodipine and the combination, respectively. The q value calculated with abovementioned formula was 1.45. It was synergic.

DISCUSSION

Rats are widely used in cardiovascular researches and SHR is the most frequently used animal model in hypertension researches. It is well known that anesthesia could influence the observation of the depressor effect of an antihypertensive drug[10,11]. Generally, anesthesia potentiates the hypotension of a drug. It was found that the effect of ketanserin was markedly exaggerated by anesthesia[10]. Therefore the study of BP in conscious rats is preferable and computerized BP analysis technique and more recently the telemetry technique have

been adopted for rats. The present work introduced two useful methods for evaluating antihypertensive drugs in conscious rats. These methods will make the above-mentioned techniques more practical and sound in studies of antihypertensive drugs.

Drug administration is the most common problem. Only intravenous injection was available for observing the acute effect of a drug in conscious rats when their BP was directly monitored. The use of intragastric administration with present method made the drug administration similar to clinical practice. It is for the first time to show an immediate effect of oral nifedipine on SBP in conscious rat (Fig 1). However, it should be noted that the oral drug effect in conscious rats is rapider than that in clinical practice, because the drug is in liquid for rats and in solid tablet or capsule for patients.

It is not an easy job to judge whether a combination of two drugs is synergic or not. The probability sum test (q test) was first proposed for this use by Jin in 1980[9]. In the present work, we used this test for evaluating the possible synergism of two antihypertensives in rats. q test used qualitative parameter. As SBP is a quantitative parameter, we should transform it into qualitative one. Therefore, in the present work rat with a decrease in SBP>20 mmHg was defined as responder and with a decrease in SBP<20 mmHg as non-responder. Of course, this arbitrary criterion might be subjected for modification. If no changes in BP available, the absolute values might be also used. As in the example 3, rat with a SBP recorded over 24 h <160 mmHg was defined as responder and ≥160 mmHg as non-responder. Furthermore, the q test could be also used to judge the possible synergism of two drugs on organ protection. One of the parameters, such as organ damage score, left ventricular hypertrophy index, glomerulosclerosis score, might be used[12-14]. For example, organ damage score in SHR control was 10.0±2.0. In rats treated with long-term atenolol, nitrendipine or the combination of atenolol and nitrendipine, an organ damage score <8.0 was defined as effective and >8.0 as ineffective.

In the present work, the SBP value of 24th h after drug was taken for calculating the effectiveness of the drugs. If we took the SBP value of 8th or 12th h after drug, the result might be different. The selection of the value of 24th h after drug was based the consideration of 24-h control of blood pressure. Therefore, the aim of the test should be previously fixed with consideration of clinical significance.

In conclusion, the two methods introduced by the present work will be important and useful for antihypertensive-drug evaluation in conscious freely moving rats.

ACKNOWLEDGEMENTS We wish to express our gratitude to Prof Zheng-jun JIN for his kind and important helps in the design of q test.

REFERENCES

1 Bunag RD. Measurement of blood pressure in rats. In: de Jong W, editor. Elsevier Science BV, Amsterdam; Handbook of hypertension. Experimental and genetic models of hypertension. 1984. p 1-12.
2 Fluckiger JP, Sonnay M, Boillat N, Atkinson J. Attenuation of the baroreceptor reflex by general anesthetic agents in the normotensive rat. Eur J Pharmacol 1985; 109: 105-9.
3 Su DF, Cerutti C, Barres C, Vincent M, Sassard J. Blood pressure and baroreflex sensitivity in conscious hypertensive rats of Lyon strain. Am J Physiol 1986; 251: H1111-7.
4 Jacob HJ, Alper RH, Brody J. Lability of arterial pressure after baroreceptor denervation is not pressure dependent. Hypertension 1989; 14: 501-10.
5 Shan ZZ, Dai SM, Su DF. Relationship between baroreceptor reflex function and end-organ damage in spontaneously hypertensive rats. Am J Physiol 1999; 277: H1200-6.
6 Su DF, Chen L, Kong XB, Cheng Y. Determination of arterial baroreflex-blood pressure control in conscious rats. Acta Pharmacol Sin 2002; 23: 103-9.
7 Miao CY, Shen FM, Su DF. Blood pressure variability is increased in genetic hypertension and L-NAME-induced hypertension. Acta Pharmacol Sin 2001; 22: 137-40.
8 Miao CY, Xie HH, Wang JJ, Su DF. Candesartan inhibits sinoaortic denervation-induced cardiovascular hypertrophy in rats. Acta Phamacol Sin 2002; 23: 713-20.
9 Jin ZJ. Addition in drug combination. Acta Phamacol Sin 1980; 1: 70-6.
10 Su DF, Yang YC, Xie JE. Effects of ketanserin on blood pressure and heart rate in sinoaortic denervated rats. Eur J Pharmacol 1992; 214: 89-91.
11 Su DF, Miao CY. Arterial baroreflex function in conscious rats. Acta Pharmacol Sin 2002; 23: 673-9.
12 Parati G, Pomidossi G, Albini F, Malaspina D, Mancia G. Relationship of 24-hour blood pressure mean and variability to severity of target-organ damage in hypertension. J Hypertens 1987; 5: 93-8.
13 Lu ZA, Xie HH, Xu LP, Yin AF, Miao CY, Su DF. Restoration of arterial baroreflex function contributes to organ protection in spontaneously hypertensive rats treated with long-term hydrochlorothiazide mixture. Clin Exp Pharmacol Physiol 2003; 30: 49-54.
14 Xie HH, Miao CY, Liu JG, Su DF. Importance of blood pressure variability in the organ protection in spontaneously hypertensive rats treated with combination of nitrendipine and atenolol. Acta Pharmacol Sin 2002; 23: 1199-204.