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Research article

Development of pharmacological screening method for evaluation of effect of drug on elevated pulse pressure and arterial stiffness

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ABSTRACT

Introduction: Elevated pulse pressure (PP) and amplification of arterial stiffness (AS) are responsible for various cardiovascular disease and deaths. Numerous investigations have identified that different antihypertensive agents influence PP and AS differently. None of the previous studies described any reliable animal model particularly to screen drugs having effects on PP and AS. In present study, we developed an animal model to screen such drugs particularly affecting PP and AS.

Methods: Elevation of PP and amplification of AS were induced in rats by uninephrectomy along with high salt intake (NaCl 4% w/v) for a period of six weeks, and weekly changes in body weight, PP, systolic, diastolic, mean pressure and pulse wave velocity (PWV) were estimated. After six weeks, collagen elastin ratio of aortic segment was estimated. Histomorphometry of abdominal aortic section of rats was done using trinocular microscope.

Results: After six weeks, uninephrectomized rats that were kept on high salt drinking water shown significant increase ($P < 0.001$) in MAP, PP and PWV indicates that hypertension along with elevated PP developed in rats, and increase in collagen/elastin ratio ($P < 0.001$) as well as PWV as compared to normal rats indicates the increase in AS.

Conclusion: The development of condition of hypertension in conjunction with increase in PP and AS in rats can be used as *in-vivo* screening model to determine the potency of drugs for the treatment of hypertension or other cardiovascular diseases associated with high PP and AS.

Pulse pressure (PP) is a biomarker of cardiovascular condition and risk of cardiovascular disease increases when PP is higher than 60 mmHg (Selvaraj et al., 2016; Yildiran et al., 2010). Hypertension with elevated PP causes more arterial damage compared to high blood pressure with normal PP (Dheaa & Al-Omerm, 2010), and outcome is directly proportional to the arterial stiffness (AS), persistently increases with age, and hence increases the risk of cardiovascular events (Fleg & Strait, 2012; Janic, Lunder, & Sabovic, 2014). It has been reported that for each 10 mmHg rise in PP, mortality risk increases by 21%, with a high risk of fatal strokes, intracranial hemorrhage, target organ damage, myocardial damage and cardiovascular mortality (Dolan et al., 2005; Fagard et al., 2008; Rim, 2008; Sultana & Pati, 2014). It may not be always true that a patient having high PP (PP ≥ 60) is hypertensive and a hypertensive patient (BP = 150/110 mmHg) has high PP, for example 130/70 mmHg is not assumed as hypertensive but there is high value of PP, and these patients have high risk of cardiovascular diseases (García-Palmieriemail et al., 2005; Matricciani et al., 2013).

Large artery stiffness is the condition of vascular aging and is estimated by measuring the pulse wave velocity (PWV) (Alfie, Galarza, & Waisman, 2005; Gupta & Bodakhe, 2013; Janic et al., 2014; Wagenseil & Mecham, 2012). AS is a general term that commonly describes distensibility, compliance and elasticity of the arterial vascular system (Stoner, Young, & Fryer, 2012; Tan, Butlin, Liu, Ng, & Avolio, 2012). Generally, in elderly hypertension, central systolic pressure, PP and PWV increases, and therefore increase in AS (Nichols, 2005).

Passive mechanical properties of the large elastic arteries are largely conferred by the extracellular matrix (ECM) components (elastin and collagen) in the arterial wall, while smooth muscle cells (SMCs) contribute a large amount to the mechanical behavior of small muscular arteries, a lesser amount of to large elastic arteries (Faury et al., 1999; González et al., 2005; Kielty, Sherratt, & Shuttleworth, 2002). Elastic fibers are degraded and fragmented with age and disease, leads to increase in arterial wall stiffness (Greenwald, 2007). Numerous studies have been carried out by researchers to discover a drug that can help to maintain normal PP and to regain the elasticity of stiffened arteries in patients having these anomalies (Dheaa & Al-Omerm, 2010; Steppan,

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Barodka, Berkowitz, & Nyhan, 2011). However, due to lack of suitable animal screening model, it is difficult to identify such molecules. In the present study, an attempt has been made to develop an animal model for the assessment of molecules that can reduce elevated PP and AS effectively and independently using Sprague Dawley rats.

It has been reported that reduction in nephron number by uninephrectomy (Un_X) and chronic salt loading during young age after complete nephrogenesis causes salt-sensitive hypertension (Carlstrom, Sallstrom, Skott, Larsson, & Persson, 2007). In the present study, levation in PP and hardening of arteries in rats were achieved by Un_X followed by administration of high salt containing drinking water. Un_X helps to increase the systolic and diastolic pressure and causes salt-sensitive hypertension (Jung et al., 2009), and chronic administration of high salt (NaCl 4%) in diet or solution induces hypertension with stiffening of vascular muscles (Carlstrom et al., 2007).

1. Material and methods

1.1. Animal

Sprague Dawley rats (180 ± 30 g) of either sex were procured from Shree Farms, Bhandara, Maharashtra, India (CPCSEA registration no. 1231/B/08). The rats were acclimatized for seven days, housed in solid-bottomed polypropylene cages and kept under standard husbandry conditions. The rats were fed with standard diet and water *ad libitum*. Experiments were designed and conducted in accordance with the ethical norms approved by Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India and Institutional Animal Ethics Committee (IAEC) of Institute of Pharmaceutical Sciences, Guru Ghasidas Vishwavidyalaya, Bilaspur (994/a/GO/06/CPCSEA).

1.2. Drugs and chemicals

Sodium Chloride (Hi-Media) and Ethanol (Jiangsu Huaxi International, China) procured were of AR grade. Ketamine (Ketmin50, Themis Medicare Ltd) and Xylazine (Rompun, Bayer) were purchased from local medical store.

1.3. Induction of hypertension with elevated PP and stiffened arteries

Rats were randomly selected and divided into four groups (Table 1), group-I rats (normal) were kept on standard diet with normal drinking water, group-II rats (HS) were kept with high salt drinking water. Rats of group-III (Un_X) and IV (Un_X + HS) were fasted overnight, next day, they were prepared for Un_X. After recovery, rats were housed in separate cages and fed with standard diet and water *ad libitum*, the drinking water was replaced with high salt containing drinking water (NaCl 4% w/v) for group-IV rats.

1.3.1. Surgical procedure for uninephrectomy

Before surgery, the rats were weighed and administered one dose of oxytetracycline (30 mg/kg, p.o.), fasted overnight to facilitate surgical access to kidney. Rats were anesthetized using administration of ketamine (60 mg/kg, i.p.) and xylazine (8 mg/kg, i.p.). Once anesthetized, fur on the back was shaved from the sternum to the pelvic area and laid down on its back on a heat pad (maintained at 37 °C), skin was disinfected with alcohol (70% v/v). An incision was made from 10 mm below the sternum to 10 mm above the genitals. Located the left kidney and exposed the renal capsule. The surrounding fats were removed from the renal pelvis to expose the renal artery, silk thread (no. 04) was passed under the artery and tied off with three knots and similarly ureter was also tied off. Kidney was dissected out with the help of fine scissor and the vascular stump was placed back into the abdomen. Absorbable suture no. 05 (PGA- polyglycolic acid sutures) was used to stitch the muscle and skin layer in a continuous stitch, cleaned with alcohol 70%, applied antiseptic ointment (Betadine) and placed on heat pad till the recovery from anesthesia (Gai et al., 2014; Gupta, Vyawahare, Kagathara, Patil, & Pujari, 2009; van Koppen, Verhaar, Bongartz, & Joles, 2013).

Table 1
Division of rats into group as per the treatment scheduled.

Group	Symbol	Treatment	Duration (weeks)
I	Normal	Normal drinking water	6
II	HS	High salt water (NaCl 2% w/v)	6
III	Un_X	Uninephrectomy + normal drinking water	6
IV	Un_X + HS	Uninephrectomy + high salt water (NaCl 2% w/v)	6

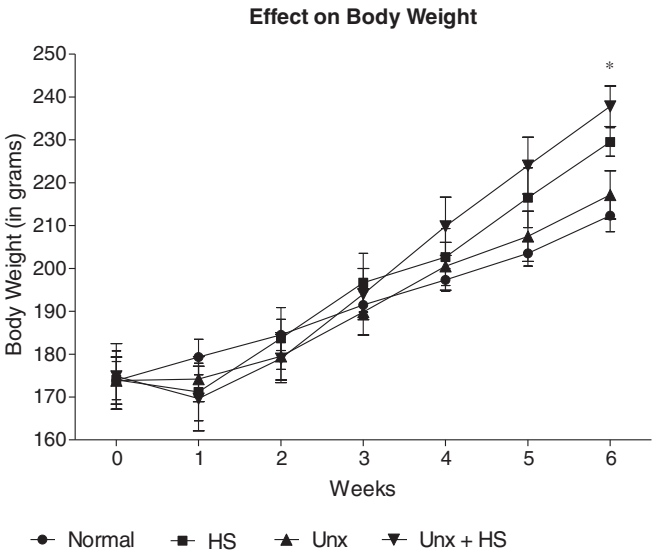

Fig. 1. Weekly changes in the body weight of rats. Data are expressed as mean ± SEM (n = 6) and analyzed by two-way ANOVA followed by Bonferroni post test. *P < 0.05, **P < 0.01 and ***P < 0.001 as compared to normal.

1.3.2. Estimation of PP and AS

Systolic, diastolic and mean arterial pressures were determined by tail cuff method using CODA-8 NIBP System (Kent Scientific Inc., USA). Rats were restrained using restrainer and kept on warming chamber maintained at temperature 35° ± 2 °C, tail of rats inserted in cuffs and recorded blood pressure parameters. PP was calculated by subtracting the DBP to SBP. AS was estimated using pulse wave analysis method using Biopac Data Acquisition System (MP 35).

1.3.3. Estimation of collagen/elastin ratio

Collagen/elastin ratio was determined after quantitative assay of elastin and collagen in abdominal arteries of rats. The quantity of elastin in the aorta was estimated by using a procedure from a previously described method (Wolinsky, 1972) with some modifications. The abdominal artery of length 2 cm was dissected, cleaned the blood contents and surrounding adipose tissue. Opened the blood vessel with the help of fine scissor and recorded the length and width by using a grid in the eyepiece. The arterial segment was agitated in the solution of acetone/diethyl ether (1:1 v/v) for delipidation, dried at room temperature and recorded dry weight using digital weighing balance (precision: 0.01 mg). The dried substance was gently agitated for 24 h in sodium dodecyl sulphate (0.3%) solution for the extraction of cell proteins. After 24 h, washed arterial segment three times in distilled water, and extracted in 2 ml of 0.1 M NaOH in boiling water bath for 15 min to solubilize the extracellular proteins except elastin. The