Iranian Journal of Veterinary Research, University of Shiraz, Vol. 8, No. 2, Ser. No. 19, 2007

Short Paper

Chronology of blood pressure changes in renal hypertension induced by solid plexiglass clips in the rat

Nekooeian, A. A.* **and Mashhoodi, T.**

Department of Pharmacology, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran

***Correspondence:** A. A. Nekooeian, Department of Pharmacology, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran. E-mail: nekooeiana@sums.ac.ir

(Received 6 Nov 2005; revised version 18 Jan 2006; accepted 18 Mar 2006)

Summary

The objective of this study was to determine the chronology of changes in blood pressure in renal hypertension induced by solid plexiglass clips in rats. Saw blades with the thickness of 0.21-0.22 mm were used to make clips sized 4 × 2 × 2 mm from a piece of 2-mm thick plexiglass. Rats were subjected to sham-operation or left renal arterial clipping, and 1, 2, 3 and 4 weeks later blood pressure, and heart and kidneys weights were determined. Relative to those of sham-operated rats, mean blood pressure of left renal artery-clipped rats were significantly higher at week 1 through 4 after clipping. Left renal artery clipping was also associated with significant increases in heart and right kidney weights and significant decrease in left kidney weight. The findings suggest that clipping of left renal artery using solid plexiglass clips resulted in changes in blood pressure, heart and left and right kidneys weights similar to reported changes in hypertension induced by silver clips.

Key words: Two-kidney, One-clip, Plexiglass clips, Hypertension, Rat

Introduction

We owe much of our understanding about the physiopathology and treatment of hypertension to animal models. Hypertension has been induced genetically or experimentally in animals. The genetic models of hypertension include spontaneously hypertensive rats, stroke-prone hypertensive rats, Dahl salt-sensitive rats, Milan hypertensive and Lyon hypertensive rats (Kivilghn *et al*., 1997). The common experimentally-induced animal models of hypertension include two-kidney, one-clip; one-kidney, one-clip; remnant kidney or 5/6 nephrectomy; deoxy-corticosterone acetate (DOCA)/salt, fructose-induced (Kivilghn *et al*., 1997) and L-NAME-induced (Ribeiro *et al*., 1992) hypertension.

Rat model of two-kidney, one-clip hypertension is one of the most-used animal model, and has been widely used for the evaluation of antihypertensive drugs, especially those affecting renin angiotensin system (Brown and Sernia, 1994). Two-kidney, one-clip hypertension was first described by Goldblatt *et al*. 1934, who demonstrated that dogs developed persistent hypertension following renal ischaemia produced by partial constriction of renal artery with a flexible silver clip. Afterwards, Leenen and de Jong (1971) as well as Brooks and Muirhead (1971) developed solid silver clips. In a series of elegant experiments, Leenen and de Jong (1971) showed that internal diameter of solid silver clips determined the degree of renal artery stenosis and therefore, allowed some control over the degree of hypertension. Since then, rat model of renal hypertension has been induced using adjustable silver clips made by scientific research instrument companies. After being placed around renal artery, the size of the gap of adjustable silver clip is set using an automotive spark-plug gap setter.

The adjustable silver clips have three disadvantages. Firstly, they can not be made

easily. The softness or hardness of the clips depends on the silver alloy, which takes a range of very soft to very hard. The issue of softness and flexibility is important, since if not proper, it may result in the improper placement around renal artery, or in the change of clips' gap size, which affects the consistency of increase in blood pressure. Secondly, hypertension produced by these of clips is less uniformly successful than that produce by fixed size silver clips (Dussaule *et al*., 1986; Kivilghn *et al*., 1997). Thirdly, the process of purchasing adjustable silver clips from abroad is time consuming and expensive for many countries including Iran. In a previous report we showed that partial constriction of left renal artery with a solid plexiglass clip did result in hypertension comparable to that induced by flexible silver clips (Nekooeian and Mashhoodi, 2007). The objective of the present study was to validate plexiglass clip-induced renal hypertension further by investigating the chronology of changes in blood pressure during four weeks after clipping of left renal artery in rats.

Materials and Methods

Construction of plexiglass clips

The clips were made from 2-mm thick plexiglass. In a piece of plexiglass 3-mm deep slits were made using saw blades with the thickness of 0.21-0.22 mm. Then the clips were cut and separated to have a rectangular shape and a size of 4 × 2 × 2 mm.

Experimental design

Male Sprague-Dawley rat weighing 200-250 gram were allocated to 8 groups (n = 5 per each). Groups 1, 3, 5 and 7 were left renal artery-clipped rats, and groups 2, 4, 6 and 8 were sham-operated rats used as controls.

Experimental protocol

Animals were anaesthetized with intraperitoneal injections of 8 mg/kg xylazine (Alfasan, Holland) and 60 mg/kg ketamine (Alfasan, Holland). Through a left flank incision, the left kidneys were exposed, left renal arteries were separated from renal veins and surrounding tissues. Afterwards, plexiglass clips were applied on the exposed renal arteries as close as possible to the aorta. Once placed over the renal artery, the clip was turned so that the slit opening faced the abdomen ensuring that the renal artery remain within the clip. Care was taken that the renal artery was placed at the depth of the clip slit, and that the flow of blood was visible downstream the clip. The right kidney was not disturbed. Then anitibiotic powder (Cicatrine®, Wellcome, Canada) was applied to the site of incision, and abdominal wall and skin were sutured using absorbable chromic catgut (Supa, Iran) and silk (Supa, Iran) suture material, respectively. Sham-operated animals were subjected to the same procedure, but a clip was not applied to the left renal artery. Animals were then recovered from anaesthesia and were kept two per cages for 12 hrs light 12 hrs dark cycle with food and water ad libitum.

At 1, 2, 3 or 4 weeks after sham operation or placement of clips around left renal artery, animals were anaesthetized with inactin (100 mg/kg). The right jagular vein was cannulated with PE50 catheters (Polyethylene Tubing, Becton Dickinson, USA) for supplying anaesthetic as needed, and the left external carotid artery were cannulated for the measurement of blood pressure. The left external carotid artery was connected to a Gould Statham pressure transducer (Model P23D), connected to a Grass polygraph (Model 7P1D). After surgery, animals were allowed to recuperate for 30 min, and then a measurement of blood pressure and heart rate was performed. Afterwards, they were sacrificed by a bolus of anaesthetic, and the weights of heart, and left and right kidneys were determined.

Calculations and data analysis

Mean blood pressure was calculated as diastolic pressure plus one third of pulse pressure. Heart rate was calculated from the upstroke of pulse pressure, when the Grass physiograph was run at a speed of 10 mm/sec. The weights of heart, and left and right kidneys were normalized to body weight. Data, presented as mean ± SEM, were analysed using unpaired t-test at the

probability of making type one error (P-value) of equal or less than 0.05.

Results

The weights of animals in the sham-operated and renal artery-clipped rats were not significantly different on the days of operation or days of experiments. Moreover, there was no significant difference between the heart rates of sham-operated and renal artery-clipped rats at 1, 2, 3, and 4 weeks after sham operation or left renal artery clipping (Table 1).

Relative to sham operation, left renal artery clipping did result in a statistically significant ($P \leq 0.05$) increase in mean blood pressure and a significant ($P \leq 0.05$) decrease in left kidney weight (adjusted for body weight) at 1, 2, 3 and 4 weeks after sham operation or clipping (Table 1). Moreover, there was no significant difference in the heart weights (adjustd for body weight) of sham-operated and renal artery-clipped rats at 1 and 2 weeks after the operation. However, it was significantly higher in renal artery-clipped rats at 3 and 4 weeks after the operation. In addition, the right kidney weights (adjusted for body weight) were not significantly different between sham-operated and renal artery-clipped rats at 1 and 2 weeks after the operation, whereas, it was significantly higher in renal artery-clipped rats at 3 and 4 weeks after clipping (Table 1).

Discussion

The cost and time necessary for purchasing the adjustable silver clips from abroad, along with the difficulty in reproducibility and consistency of hypertension induced by these clips (Dussaule *et al*., 1986; Kivilghn *et al*., 1997) due to flexibility (Brooks and Muirhead, 1971), prompted us to try to induce hypertension by easy-to-get means. Using saw blades, as mentioned in the method section, helped us to construct clips with dimensions of 4 × 2 × 2 mm and an internal diameter of 0.21-0.22 mm, and a depth of slit of 3 mm. In a previous report, we did demonstrate that the placement of such clips around left renal artery did result in hypertension comparable to that induced by silver clips (Nekooeian and Mashhoodi, 2007). In order to further aunthenticate the plexiglass clip-induced renal hypertension, the present study did examine the chronology of blood pressure changes in the first 4 weeks after clipping, and compared it with the changes reported by others using silver clips.

Application of plexiglass clips to left renal arteries, without disturbing right renal artery, was associated with an increase in mean blood pressure in all animals, which was detectable from the first week after clipping. The increase in mean blood pressure in the present model at 1, 2 and 3 weeks after clipping are comparable with those who reported by Chen *et al*. (1988), Braam *et al*. (1995) and Ludek *et al*. (1999), respectively. Majority of the studies on the rat model of two-kidney, one clip hypertension have used a 4-week duration for the stablishment of hypertension. The mean arterial pressure at 4 weeks after left

Table 1: Heart rate (HR, beat/min), mean arterial pressure (MAP, mmHg), heart weight (HW, % body weight), left kidney weight (LKW, % body weight), right kidney weight (RKW, % body weight), body weight on the day of operation (BWO, g) and body weight on the day of experiment (BWE, g) of sham-operated (Sham) and left renal artery-clipped (LRAC) rats at 1, 2, 3, 4 and 5 weeks after sham operation or renal artery clipping

	Week 1		Week 2		Week 3		Week 4	
	Sham	LRAC	Sham	LRAC	Sham	LRAC	Sham	LRAC
HR	422±14	434±12	446±12	449±13	414±14	416±12	362±32	414±23
MAP	112±6	136±2*	106±4	141±3*	103±3	155±3*	103±7	175±3*
HW	0.29±0.01	0.33±0.03	0.32±0.01	0.34±0.03	0.28±0.01	0.39±0.01*	0.28±0.01	0.40±0.01*
LKW	0.41±0.03	0.33±0.02*	0.39±0.03	0.28±0.02*	0.34±0.04	0.26±0.01*	0.37±0.02	0.30±0.02*
RKW	0.42±0.03	0.47±0.04	0.40±0.02	0.45±0.04	0.33±0.03	0.45±0.03*	0.37±0.01	0.48±0.03*
BWO	233±7	234±10	217±5	229±7	228±12	225±11	233±9	245±10
BWE	233±6	244±10	250±7	242±7	282±27	244±14	289±20	276±16

Values are mean ± SEM (n = 5); *Significant ($P \leq 0.05$) difference from values from sham-operated rats

renal artery clipping reported in the present study lies well within the range of those reported by earlier studies (Beierwaltes *et al*., 1995; Melaragno and Fink, 1996; Amiri and Garcia, 1997; Sigmon and Beierwaltes, 1998; Yoshida *et al*., 1998; Al-Qattan *et al*., 1999; Leckie *et al*., 2000; Dobrian *et al*., 2001; Ocaranza *et al*., 2002).

In agreement with earlier studies (Leenen and de Jong, 1971; Nicoletti *et al*., 1996; Hocher *et al*., 2000; Ocaranza *et al*., 2002) using silver clips, the renal hypertension induced by solid plexiglass clips was associated with an increase in heart weight adjusted for body weight at 3 and 4 weeks after clipping, indicating cardiac hypertrophy. The present model of plexiglass clip-induced hypertension was also associated with a significantly lower left kidney weight, which was due to hypoperfusion. Similar reductions in left kidney weight was reported in previous studies using adjustable silver clips (Haefliger *et al*., 1999; Hocher *et al*., 2000; Palmer *et al*., 2003). Moreover, similar to earlier studies (Hocher *et al*., 2000; Palmer *et al*., 2003), the present model of hypertension was accompanied by hypertrophy of the right kidneys, which might be due to compensatory reflexes and/or hyperperfusion.

Plexiglass is a trade name for polymethyl methacrylate. As far as the literature is concerned, there is no published study on the toxicity of plexiglass. Therefore, it would be interesting to examine as to wether plexiglass is associated with toxicity. However, we have found no statistically significant differences between the mean blood pressure, or the weights of heart, left or right kidney from rats whose hypertension had been induced using plexiglass or silver clips (Nekooeian and Mashhoodi, 2007).

The findings of the present study indicate that placement of plexiglass clips on left renal arteries resulted in a reproducible renal hypertension associated with cardiac and right kidney hypertrophy, and left kidney hypotrophy similar to those reported for renal hypertension induced by silver clips. The use of solid plexiglass clip to induce renal hypertension is of minimum costs, and can be done in any research lab using simple and much less sophisticated equipments.

Acknowledgement

This work was supported by Vice Presidency in Rresearch, Shiraz University of Medical Sciences.

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