Research Paper

Solid plexiglass clips to induce reproducible renal hypertension in the rat

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Received: 11.7.2005
Revised: 29.9.2005
Accepted: 10.10.2005

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ABSTRACT

Objective: The objective of this study was to induce reproducible renal hypertension in rats using plexiglass clips, and to compare it with that induced by silver clips.
Materials and Methods: Saw blades (0.21-0.22 mm thick) were used to make clips (4 x 2 x 2 mm) from a piece of 2-mm thick plexiglass. Rats were subjected to sham-operation or placement of plexiglass or silver clips around left renal artery, and 4 weeks later their mean blood pressure (MBP, mmHg), heart rate (HR, bpm), and heart weight (HW), left kidney weight (LKW), right kidney weight (RKW) and body weight (BW, g) were determined. The RKW, LKW and HW were calculated as a percentage of body weight.
Results: Four weeks after sham-operation or placement of clips around renal artery, MBP, HW and RKW were significantly higher and LKW was significantly lower in left renal artery-clipped rats using plexiglass or silver clips than sham-operated ones. There was also no significant differnce among the values of HR or BW from the 3 groups. Moreover, there was no significant difference among MBP, HR, LKW, RKW or BW from sham-operated or renal artery-clipped rats.
Conclusion: The findings suggest that placement of solid plexiglass clips around left renal artery resulted in hypertension comparable to that induced by silver clips.

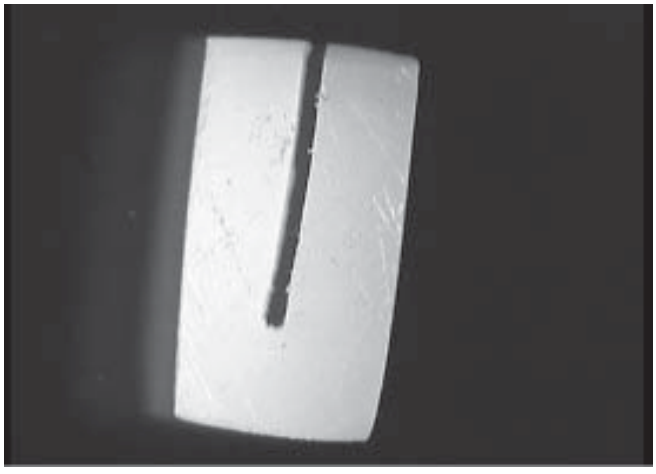
KEY WORDS: Renal hypertension, two-kideny, one-clip, plexiglass clips, silver clips, rat.

Introduction

The two-kidney, one-clip renovascular hypertension was first described by Goldblatt *et al*,[1] who demonstrated that partial constriction of the renal artery with an adjustable silver clip in dogs led to persistent hypertension. Since then hypertension in rats has been achieved by clamping renal artery by adjustable silver clips. The adjustable silver clips suffer from certain disadvantages, like variations in silver flexibility leading to poor fabrication and less uniformity in adjustable clips compared to fixed-size silver clip[1] and procuring difficulties from far away manufacturers. Therefore the present study was undertaken with the objective to induce renal hypertension by clips made from inexpensive and easy-to-obtain material like polymethyl methacrylate (plexiglass).

Clips with an internal diameter of 0.20-0.21 mm, was made from a piece of 2 mm thick plexiglass. Using a saw blade (0.21-0.22 mm thick), 3 mm deep slits were made. Clips were then cut and separated in rectangular pieces ([4×2×2 mm] Figure 1). The internal diameter of silver clips (a courtesy of Dr. Mohammad Ali Sharifi, Iran University of Medical Sciences, Tehran, Iran) was 0.2 mm. Three groups ($n=6$ each) of male Sprague-Dawley rats (200-250 g) consisting of a (1) sham, (2) a renal artery (left renal artery) clipped with plexiglass clips and (3) a renal artery clipped with silver clips were included for the study. Under xylosine (8 mg/kg) and ketamine (60 mg/kg) anesthesia, (i.p.) animals were subjected to sham surgery or placement of clips around left renal artery. Briefly a 2 cm incision was made in the left flank and left kidney was exposed. Then the left renal artery was separated from left renal vein and surrounding tissues. Afterwards, a clip was placed around renal artery as close as possible to the aorta. After recovery from anesthesia, each animal was housed in a single cage. Four weeks after the surgical procedure, (MBP) systolic and diastolic pressures and heart rate (HR) were measured under thiobutabarbital Inactin (100 mg/kg) anesthesia. Afterwards, they were sacrificed by a bolus of anesthetic thiobutabarbital and the weights of heart (HW), left kidney (LKW) and right kidney (RKW) were determined and were normalized to body weight. Mean blood pressure (MBP) was calculated as diastolic pressure

Figure 1: A plexiglass clip with a magnification of 10x



plus one third of pulse pressure. The data, presented as Mean ± SD, were analyzed using one way analysis of variance followed by Tukey test. $P \leq 0.05$ was considered significant.

There was no mortality in any of the groups. Body weights on the day of operation (BWO) or on the day of experiment (BWE) of sham-operated rats (SOR), renal artery clamped rats with plexiglass clips (RACRPC) and renal artery clamped rats with silver clips (RACRSC) were not significantly different. Moreover, there was no significant difference in the HR among the groups. The RACRPC and RACRSC groups had significantly higher MBP, HW and RKW and a significantly lower LKW. There was no significant difference in the values of HR, MBP, HW, RKW, LKW, BWO or BWE between RACRPC and RACRSC groups [Table 1].

The cost and time to purchase the adjustable silver clips from abroad, along with the difficultly in reproducibility and consistency of hypertension induced by this type of clips[2,3] prompted us to try to induce hypertension by easy-to-get means. We did try to make solid silver clips using a silver bar. However, the use of solid silver clips were with many limitations. Firstly, the hardness or softness of silver bars depended on the carat of silver in the alloy and ranged from very soft to very hard. Obtaining the most appropriate alloy with a favorable hardness required repeated visits to the silversmith, which did not seem justified in terms of the time spent. Secondly, we found sawing of the silver bar quite difficult, which was limted by the hardness of the silver bar. Thirdly, a solid silver clip was found to be much heavier than a solid plexiglass clip with an identical size. Consequently, we were not sure that such a heaviness would not lead to falling off of clips placed around renal artery or would not lead to failure. We therefore decided to make clips using something other than silver bar. We found that plexiglass clips with the above-mentioned dimensions could induce hypertension in which values of MBP, HW, RKW and LKW were comparable to those in hypertension induced by silver clips.[4] The fabrication of solid plexiglass clips is cheaper by cost and can be done in any pharmacology or physiology lab using simple and much less sophisticated equipments. Low cost and reproducibility of plexiglass clip-induced hypertension provide a favorable opportunity to induce experimental renal hypertension and for preclinical evaluation of new antihypertensives.

Table 1

Values of heart rate (HR, beat/min), mean arterial pressure (MAP, mmHg), heart weight (% 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 day (BWE, g) of sham-operated and left renal artery clipped rats using plexiglass or sliver clips at 4 weeks after sham-operation or renal artery clipping

	Sham	*Plexiglass clip*	*Silver clip*
HR (beats/min)	362 ± 32	412 ± 23	426 ± 18
MAP (mmHg)	103 ± 7	173 ± 3*	172 ± 7*
HW (% of body weight)	0.28 ± 0.01	0.38 ± 0.01*	0.39 ± 0.04*
LKW (% of body weight)	0.37 ± 0.02	0.27 ± 0.02*	0.28 ± 0.02*
RKW (% of body weight)	0.37 ± 0.01	0.46 ± 0.03*	0.49 ± 0.02*
BWO (g)	233 ± 9	240 ± 10	229 ± 12
BWE (g)	289 ± 20	271 ± 16	272 ± 11

Values (n=6) are as Mean ± SD, *Denotes significantly different from sham-operated group

Acknowledgements

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

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Source of Support: Vice Presidency in Research Affairs, Shiraz University of Medical Sciences. **Conflict of Interest:** Nil.