Reproducible and stable surgical hypertension using a smaller renal artery clip model in Wistar rats

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Abstract

Animal models of hypertension are crucial for studying the pathophysiology of diseases and developing new treatments. This study introduces a reproducible technique for inducing surgical renovascular hypertension in rats using a smaller renal artery clip. The surgical groups were compared to the control group and standard drug group to assess the effectiveness of the surgical model and its stability for 21 days. The results revealed a progressive increase in systolic and diastolic blood pressure in the surgical group. Moreover, the heart rate and body weight in the renal hypertension group demonstrated a sustained elevation at the end of 21 days. These findings confirm the efficacy of this surgical model in inducing hypertension. This surgical animal model is helpful for studying physiological parameters for investigating potential therapeutic interventions against hypertensi

Key words: Blood pressure, Surgical model, Renal artery occlusion, Hypertension, Rats

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Introduction

Hypertension is a major risk factor for cardiovascular disease, and it is estimated that 1.13 billion cases of reported hypertension worldwide. Hypertension is characterized by the elevation of blood pressure, which can damage the heart, blood vessels, and other organs. Hypertension has many causes, including genetics, diet, lifestyle, and underlying medical conditions. In some cases, the cause of hypertension is unknown (Cabral A. & Vasquez J., 1991). Animal models of hypertension are essential for understanding the causes and mechanisms of the disease and developing new treatments. One of the models of hypertension is the surgical model, which is induced by clipping the left renal artery in rats. A surgical model of hypertension is induced by clipping the left renal artery in rats. The 2K1C model of hypertension is a surgical model induced by clipping the renal artery in rats. Both kidneys are left intact, but one renal artery is constricted. This results in decreased blood flow to the kidneys, which activates the renin-angiotensin-aldosterone system (RAAS). The RAAS is a system of hormones that regulates blood pressure. When the RAAS is activated, it releases the hormone angiotensin II, which causes the blood vessels to constrict, thereby increasing blood pressure (Hall, J. E., & Guyton, A. C. 2005). The 2K1C model is a more severe model of hypertension than the 1K1C model because the contralateral kidney in the 2K1C model is able to compensate for the decreased blood flow to the clipped kidney. However, over time, the contralateral kidney also begins to fail, and the blood pressure rises even further (Hall, J. E., & Guyton, A. C. 2005). The surgical model is a reliable and reproducible model of hypertension (Touyz, R. M., & Schiffrin, E. L. 2010). A study reported that the mean systolic blood pressure in hypertensive rats was 186.6 mmHg, compared to 153.6 mmHg in sham-operated controls. The surgical model has been used to study various aspects of hypertension, including the role of the kidneys, the RAAS, and the sympathetic nervous system (Lincevicius et al., 2017). The renovascular denervation (RDN) model is considered a study of an invasive surgical model. In this theoretical framework, the neural pathways responsible for innervating the renal organs undergo degeneration. The involvement of the sympathetic nervous system in hypertension has been investigated using the RDN model (Chen, S., et al., 2022). Another surgical model, the renal transplantation model, has also been employed for this purpose. In this study, a renal organ sourced from a hypertension rodent is surgically transferred into a normotensive rodent recipient. The utilization of the renal transplantation model has been employed in the examination of the kidney's involvement in hypertension, as demonstrated by (Lu, H., et al. 2022). The developed surgical model for inducing renovascular hypertension in rats demonstrated a significantly higher overall success rate

than the conventional model, as evidenced by the persistent and substantial increases in both systolic and diastolic blood pressure over 21 days. This innovative approach minimized renal damage and provided a more stable blood pressure profile, enhancing the model's reproducibility across various research investigations. These findings highlight the effectiveness and potential of the surgical model as a superior tool for studying hypertension and assessing therapeutic strategies compared to the traditional approach.

Materials and methods

Animals

Eight to nine weeks-old Wistar male rats 180-220 g were obtained and housed at the animal house facility of Central Food Technological Research Institute (CSIR-CFTRI). Rats were maintained at a relative humidity of 40–70% and temperature of 25 ± 2 °C with a 12 h light to 12 h dark cycle and housed in polypropylene cages. Animals were grouped and were kept on an *ad libitum* AIN-93 M diet (Reeves, 1997) and water for five days of acclimatization and during the experimental period for 21 days (Table 1). The Institutional Animal Ethics Committee has approved (No: CFT/IAEC/320/2023) this experimental protocol in accordance with the guidelines of the Committee for Control and Supervision of Experiments on Animals, Ministry of Fisheries, Animal Husbandry and Dairying, Govt. of India, New Delhi. In this study, animal welfare was prioritized through meticulous adherence to ethical guidelines, ensuring the reproducibility and stability of surgical hypertension in Wistar rats using a smaller renal artery clip model, thereby minimizing potential distress and promoting humane research practices.

Materials

Surgical instruments include surgical scissors, forceps, 2-0 (3 metric) 45mm 3/8 circle non-absorbable and 3.5 metric absorbable surgical sutures. The anesthetic mix used was ketamine (8 ml), xylazine (0.8 ml), and 0.9% saline with a dosage of 0.1ml/100g b.w IP (Figure 1. A) and blood pressure measurement equipment by IITC Life Science, United States (MRBP system) was a non-invasive tail-cuff blood pressure monitor. In this study, silver clips with a diameter of 2 mm were autoclaved. These clips were custom-designed and fabricated at a local jeweler's workshop. Sterile saline solution, povidone-iodine solution 5% (w/v), heating a sterile operating table (surgical platform), and tetracycline 5mg/kg (b/w) as an antibiotic were used for postoperative care. Amlodipine 10mg/kg (b/w) was used as a standard drug. Carprofen injection was used as analgesic.

Tail-cuff pressure measurement

The tail-cuff method is a non-invasive way to measure blood pressure in rats. The method involves placing the rat in a cage on the platform of a tail-cuff machine. The tail of the rat is then passed through the tail-cuff, which is inflated to a pressure slightly below the systolic blood pressure of the rat. The rat is allowed to adapt to the inflated cuff for a few minutes, and then, the systolic and diastolic blood pressure values are recorded for at least 10 cycles for 5 min. The Systolic blood pressure is the peak pressure in the tail-cuff during systole, and the Diastolic blood pressure is the minimum pressure in the tail cuff during diastole (Yang et al., 2023) (Figure 1. A).

Surgical induction of renovascular hypertension

The method was developed with modification of previously reported methods (Lincevicius et al., 2017; Shimoura et al., 2017). The animals were restrained and anesthetized with the cocktail of Ketamine and Xylazine i.p (Figure 1 B ,C). The surgical area was disinfected with povidone-iodine and draped (Figure 2 A), An incision was made on the flank region of the retroperitoneum to expose the left renal artery (Figure 2. B), allowing further manipulation. The kidneys are located in the retroperitoneum (Figure 2. C) and the posterior abdominal wall, by carefully isolating the left renal artery by separating from the vein, nerves, and connective tissues (Figure 2.D). The placement of the clip precisely targeted the abdominal aorta, a sizable artery that supplies blood to the abdominal organs, including the kidneys (Figure 2. E). The clip's exact placement made it possible to restrict the blood flow in the left renal artery, by blocking the renal artery simulating renovascular hypertension. The clip is placed inside and observed for 20 days to heal up to measure further parameters. Further, the incision on the retroperitoneum (Figure 2. F) was carefully sutured with a 2-0 monofilament polyamide sterilized surgical needle and non-absorbable suture on the outer skin (Figure 2. G) to ensure the proper wound healing process. In order to maintain consistent environmental conditions throughout the study period, the rats were housed in individual Cages in a temperature- and humidity-controlled environment, Makrolon III cage (MC) systems with aubiose hemp bedding were used. In (MC) systems, the food was placed in the lid. All the rats had ad libitum access to tap water, provided in plastic bottles with a non-drip nipple. Animals' blood pressure was

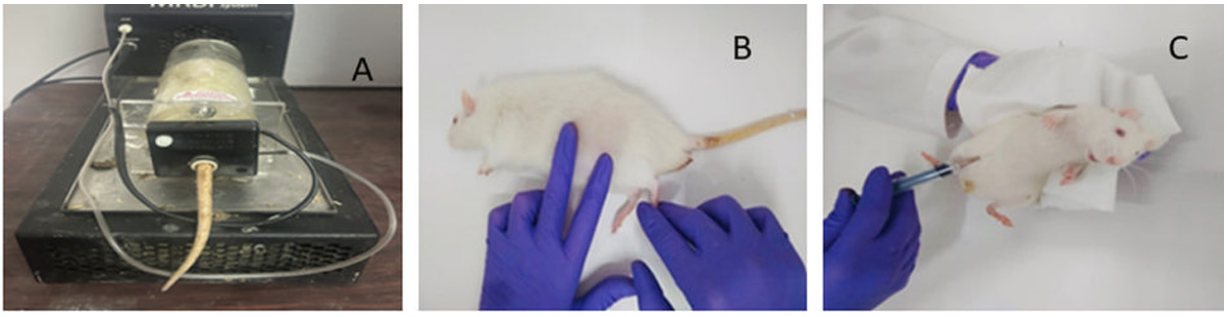

Figure-1 A Monitoring of blood pressure levels in rat before the surgery by MRBP tail cuff machine.

Figure-1 B To keep the rat's body temperature stable, the body is placed on a heating pad further trimming the hairs on the left cranium between the ribs and the left lower limb.

Figure-1 C Injection of ketamine and xylazine intraperitoneally to anaesthetize the rat

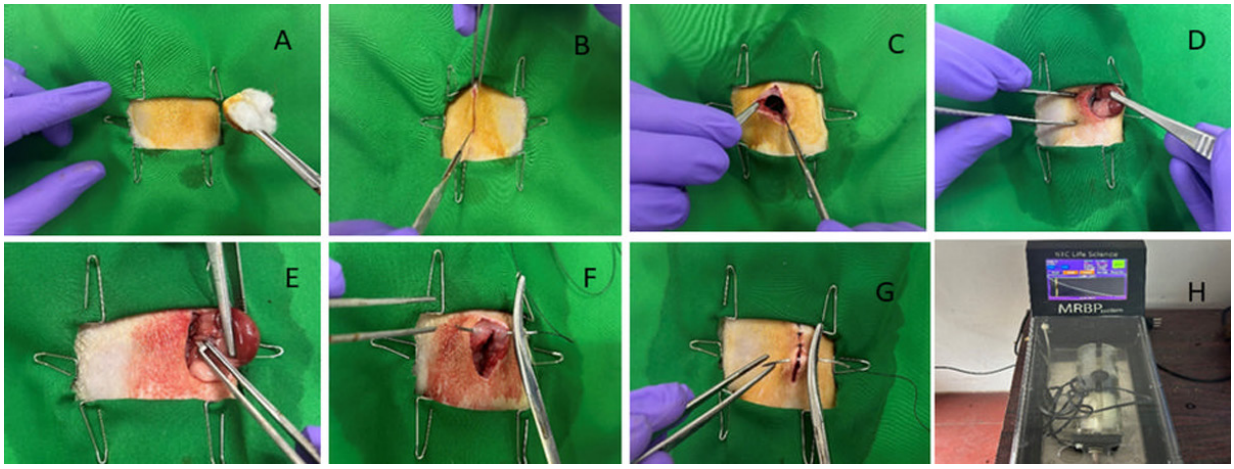

Figure-2 A Povidone-iodine application to the area to disinfect it.

Figure-2 B A 2 cm-long incision in the midline of the left dorsal-ventral side of the abdomen.

Figure-2 C Cut and separate the abdominal muscles apart to reveal the kidneys.

Figure-2 D Location of the kidney in the abdominal cavity and isolating with forceps.

Figure-2 E Location of the left renal artery and securing it with a silver clip.

Figure-2 F Use of absorbable sutures to suture the abdominal muscle.

Figure-2 G Use non-absorbable sutures to suture skin layer.

Figure-2 H Daily monitoring of blood pressure.

Sex	Male Wistar Rat		
Groups	**Non-surgical control rats**	**Standard drug (amlodipine 5mg/kg BW)**	**Surgical model**
No of Animals	3	3	3

Table-1 Demographic Characteristics of the Study Cohort, Including Group Assignment, Sex, and Number of Animals

Group /weeks	Day 1	Day 7	Day 14	Day 21
A .Systolic blood pressure (mmHg)				
Non-surgical control rats	122±2.05	120±3.29	125±2.44	123±2.94
Standard drug (amlodipine 5mg/kg BW)	123±2.51	121±3.51	134±3.51	148±2.56
Surgical model	125±2.86	138±2.44	150±2.94	172±2.86
B .Diastolic blood pressure (mmHg)				
Non-surgical control rats	75±2.70	85±2.86	82±7.22	78±2.05
Standard drug (amlodipine 5mg/kg BW)	87±3.05	97±4.04	105±3.60	114±4.14
Surgical model	82±3.26	95±2.44	110±2.94	124±2.86
Heart rate (BPM)				
Non-surgical control rats	348±2.44	341±2.86	346±2.74	348±4.11
Standard drug (amlodipine 5mg/kg BW)	345±4.61	335±4.62	385±5.03	386±4.16
Surgical model	361±3.68	446±3.68	442±3.26	441±3.68
D.Body weight (g)				
Non-surgical control rats	195±3.24	202±2.86	208±2.44	215±3.26
Standard drug (amlodipine 5mg/kg BW)	195±3.05	203±5.13	217±4.58	225±4.17
Surgical model	198±3.29	215±3.26	222±2.44	230±2.94

Table-2. The effect of renal hypertension in systolic BP (mmHg), diastolic (mmHg), heart Rate (BPM) and body weight (g) in different groups of Wistar rats

monitored for up to 20 days after surgery. Rats were found to have systolic arterial pressures more than 160 mmHg 3 weeks after surgery (Figure 2. H), and after the first 5 days of surgery, Amlodipine 10mg/kg (b/w) and tetracycline 5mg/kg (b/w) were administered by oral gavage. Carprofen (@5 mg/kg body weight) injection was given subcutaneously prior to surgery and once daily for three days to relieve pain during and after the operation. The surgery procedure was minimal and resulted in minimal fluid loss. As a result, these rats met the inclusion criteria for the surgical renovascular hypertension group.

Statistical Analysis

The experiments were conducted in triplicate. The final results of the analysis were provided as the mean values, accompanied by the standard deviation (n=3). ANOVA was employed for statistical analysis using Duncan's multiple range test on the SPSS platform to ascertain the existence of any significant difference ($p<0.05$) among the samples.

Result

Before surgery, systolic blood pressure (SBP) values in the non-surgical control and renal hypertension groups were comparable, with the renal hypertension group having greater SBP than the control group (172±2.86 mmHg vs. 123±2.94 mmHg). The SBP in the renal hypertension group increased significantly to 138±2.44 mmHg in the first week following surgery, while it remained practically stable in the control group (120±3.29 mmHg). During the second week, the SBP difference between the renal hypertension group and the control group increased to 150±2.94 mmHg versus 125±2.44 mmHg.

The SBP in the renal hypertension group climbed to 172±2.86 mmHg in the third week, while the SBP in the control group remained stable at 123±2.94 mmHg. SBP (172±2.86) increased consistently in the renal hypertension group. The SBP in the control group remained constant at 123±2.94 mmHg. The SBP of the renal hypertension group (172±2.86 mmHg) increased consistently from the beginning of the first week to the third week when compared to the SBP of the conventional drug group, amlodipine (1482.56 mmHg) (Table 2 A,).

Diastolic Blood Pressure (DBP) was higher in the renal hypertension group than in the control group before surgery (82±3.26 mmHg vs. 75±2.70 mmHg), however, within the first week following the renal hypertension surgery, DBP in the renal hypertension group was elevated to 95±2.44 mmHg, while it increased to 85±2.86 mmHg in the control group. The DBP in the renal hypertension group ascended to 110±2.94 mmHg in the second week, significantly greater than the DBP in the control group, which was 82±7.22 mmHg. The DBP in the renal hypertension group increased significantly in the third week, reaching 124±2.86 mmHg, while the DBP in the control group remained stable at 78±2.05 mmHg. Comparing the DBP of the renal hypertension group (124±2.86 mmHg) to the DBP of the amlodipine-containing conventional drug group (114±4.14 mmHg), there was a steady increase from the beginning of the first week to the third week (Table 2B).

The heart rate (BPM) in the renal hypertension group was marginally higher than that of the control group (361±3.68 vs. 348±2.44 BPM). In the first week following renal hypertension surgery, the renal hypertension group's heart rate increased to 446±3.68 BPM, whereas the heart rates of the control group decreased to 341±2.86 BPM. Whereas in the second week, the heart rate in the renal hypertension group remained high at 442±3.26 BPM, while it was 346±2.74 BPM in the control group, and in the third week, the heart rate in the renal hypertension group stayed largely constant at 441±3.68 BPM. When compared to the heart rate of the standard drug group (386±4.16 BPM) using amlodipine, there was a constant increase in the renal hypertension group's heart rate (441±3.68 BPM) from the beginning of the first week to the third week (Table 2C).

Before surgery, the renal hypertension group's body weight (198±3.29 g vs 195±3.24 g) was comparable, however after the first week, the renal hypertension group's body weight increased to 215±3.26 g, whereas the control group's body weight increased to 202±2.86 g. The body weight in the renal hypertension group increased to 222±2.44 g in the second week, demonstrating a significant difference from the body weight of 208±2.44 g in the normal control group, and by the third week, the body weight in the renal hypertension group had increased to 230±2.94 g, while the body weight in the control group decreased to 215±3.26 g. There was no difference in body weight between the standard drug group (225±4.17 g), which contained amlodipine., there was an increase in the renal hypertension group's weight (230±2.94 g) from the beginning of the first week to the third week (Table 2D).

Discussion

The importance of the rat as an animal model for cardiovascular research has increased with the development of transgenesis and targeted gene disruption (Philippe Wiesel et al., 1997). The renal hypertension and 1K1C models were developed in rats by (Miksche et al., 1976). These models were extensively utilized to better understand the connection between the renin-angiotensin system, hypertension, and cardiovascular diseases (BH Laragh et al., 1995). The development of renal hypertension hypertensive models in rats utilizing a tiny renal arterial clip is described in the current study. This improved method for inducing renovascular hypertension in rats. This model will be useful for studying the pathophysiology and treatment of renovascular hypertension, as well as for developing new antihypertensive drugs when compared to the earlier studies by (Miksche, L. W. 1976) and (Goldblatt et al, 1934). Our findings are supported by other research that showed a rise in blood pressure when the renal artery was clipped (Philippe Wiesel et al., 1997). Induction of hypertension leads to an increase in systolic and diastolic blood pressure; both SBP and DSP indicate positive results in inducing hypertension, as shown in previous research (Choi, S. H., et al. 2008). In addition to the earlier reported studies, a large body of anecdotal evidence suggests that animals with hypertension can experience a rise in body weight (Zhu, J., et al. 2017). A rise in heart rate was also observed in this method which corroborates which is shown in the previous research (Kim, Y. H., et al. 2009). The induction of hypertension suggests that the renin-angiotensin-aldosterone system (RAAS) may have been activated due to decreased blood supply to the kidneys, as seen by the higher heart rate in the renal hypertension group. The renal hypertension group's increase in body weight raises the possibility that hypertension may have an effect on metabolic functions or fluid retention. The use of scientific terminology like "renovascular hypertension," "renin-angiotensin-aldosterone system," and "systolic blood pressure" enhances

the study's credibility and applicability. The results of the data analysis show a distinct separation between the experimental and control groups, supporting the model's usefulness in the research of hypertension. Two commonly utilized animal models of hypertension are spontaneously hypertensive rats (SHR) and stroke-prone SHR (SHRSP). The animals are born with normal blood pressure, but within the first two to four months of life, they gradually acquire severe hypertension (Francesco Amenta et al., 2003). Okakamoto and Aoki created the SHR strain of spontaneously hypertensive Wistar rats by mating Wistar rats with those with the highest blood pressure (Pinto, Y. M., et al. 1998). The SHR stroke-prone (SHR-SP) sub-strain is a more advanced sub-strain with significantly higher blood pressure and a significant risk of dying from a stroke. However, it takes two to four months for animals in SHR groups to acquire hypertension, but our findings for the renal hypertension group showed that this happened in a period of 3 week and compared to SHR animal models and this also be used as one of the renal hypertension models.

Conclusion

In this study, a novel surgical method employing a smaller renal artery clip was developed and successfully tested to induce renovascular hypertension in rats. Hypertension, a significant risk factor for cardiovascular diseases, necessitates precise animal models for in-depth study and therapeutic innovation. Traditionally, renal hypertension models involved constricting the left renal artery, resulting in renal damage and variable blood pressure profiles, limiting their utility in hypertension research. Our innovative approach mitigated renal injury and generated a stable blood pressure profile, enabling more accurate assessments of hypertension's impact on kidneys and other organs. Notably, this method's constant blood pressure profile enhances precision and reproducibility across diverse research studies. This advancement not only refines hypertension research but also emphasizes the importance of minimizing animal suffering while maximizing scientific insights. Our results provide valuable impetus for ongoing hypertension medication research, potentially alleviating the global burden of cardiovascular diseases.

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Competing interest

The authors declare that there are no non-financial competing interests related to the research presented in this article. All authors have no affiliations with or involvement in any organization or entity with a direct financial interest in the subject matter discussed in the manuscript.

Author Contributions

The work and manuscript preparation were conducted by Syed Abdur Rahman. Sangh Priya provided guidance during the experiment, monitored the progress, and edited the manuscript. Muthukumar conceptualized, planned, and supervised the work, ultimately finalizing the manuscript.

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