

Myocardial Preconditioning Potential of Hedgehog Activator Purmorphamine (Smoothened Receptor Agonist) against Ischemia-Reperfusion in Deoxycortisone Acetate Salt-Induced Hypertensive Rat Hearts

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Abstract

Aim: To investigate the myocardial preconditioning potential of hedgehog activator smoothened (SMO) receptor agonist purmorphamine (PUR) against ischemia-reperfusion (I/R) injury in deoxycortisone acetate (DOCA) salt-induced hypertensive rat heart. **Methods:** Hypertension in rats was produced by surgical removal of the kidney and DOCA salt administration for 6 weeks. Isolated rat heart was subjected to 30 min ischemia followed by 120 min of I/R. The heart was subjected to pharmacological preconditioning with the hedgehog (Hh) activator PUR (1 μ M), PUR (2 μ M), and atractyloside (4 μ M), an mPTP opener, in the last episode of reperfusion before I/R. Elevated blood pressure was evaluated through tail-cuff method. Myocardial infarction was assessed in terms of the increase in lactate dehydrogenase, creatinine kinase-muscle/brain, and infarct size through triphenyltetrazolium chloride staining. Further, there were a decrease in the release of nitrite and coronary flow rate. Immunohistochemistry was performed for the assessment of tumor necrosis factor- α level in cardiac tissue. **Results:** Pharmacological preconditioning with PUR significantly attenuated I/R-induced myocardial infarction. However, atractyloside limits the ameliorative preconditioning potential of PUR and confirmed the role of Hh pathway in ischemic preconditioning. **Conclusion:** It may be concluded that the Hh activator PUR (SMO receptor agonist) prevents I/R injury in DOCA salt-induced hypertensive rat heart.

Keywords: Atractyloside, deoxycorticosterone acetate, ischemic preconditioning, mean arterial blood pressure, myocardial ischemia, sonic hedgehog

INTRODUCTION

Hypertension (HTN) is a significant risk factor for various cardiovascular disorders; stroke and millions of people worldwide suffer from the disease resulting in significant morbidity, mortality, and financial burden globally.^[1-3] In 90%–95% of cases, the high blood pressure is due to nonspecific lifestyle and genetic factors.^[4,5]

Myocardial ischemia develops when the coronary blood supply to the myocardium is reduced either in terms of absolute flow rate (low-flow or no-flow ischemia) or relative to increased tissue demand (demand ischemia).^[6] While reperfusion is necessary for tissue survival and it is worth noting that reperfusion itself can also cause tissue damage termed as reperfusion injury.^[7]

Reperfusion injury results in myocyte damage through myocardial stunning, microvascular and endothelial injury, and irreversible cell damage or necrosis termed lethal ischemia-reperfusion (I/R) injury.^[8] This usually occurs in cardiomyocytes that not only have been severely injured by ischemia but also may develop in reversibly injured myocytes.^[9] Ischemic preconditioning (IPC) is defined as

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a powerful endogenous protective phenomenon in which short intermittent cycles of ischemia and reperfusion before subsequent prolonged ischemic insult rendering the myocardium transiently more resistant to deleterious effects of I/R-induced injury.^[10,11] IPC stimulates the generation of several endogenous ligands which binds to their respective G-protein coupled receptors and initiate a signaling cascade, i.e. activation of phosphatidylinositol 3-kinase (PI3K) and phospholipase C.^[12,13]

HTN leads to high-energy demand in cardiac cells.^[14,15] Furthermore, mitochondria mediate cell survival and death (apoptosis), regulate the generation of reactive oxygen species for cellular signaling, modulate intracellular calcium homeostasis, and participate in thermogenesis.^[16] Hedgehog (Hh) proteins belong to a class of morphogen involved in many biological processes during embryonic development; they are relatively silent during normal adult life; although, they may be recruited postnatal in response to tissue injury.^[17] Activation of HH signaling is necessary for coronary development in the embryonic heart and sufficient to promote the formation of new coronary vessels in the adult heart.^[18]

HH signaling pathway could regulate specification, patterning, and growth of cardiac progenitors and vessels.^[19] Hh signaling involves two different types of cells such as cardiomyoblast and the perivascular cell which controls the coronary vein and artery development. Strikingly, during this progress, the epicardium acts as a center of signaling for the wave-like growth of the coronary vasculature. Moreover, the Sonic Hh (Shh)/vascular endothelial growth factor/Notch5 signaling to specific endothelial cells decides its arterial fate by targeting Dll4 and Efnb2. As a morphogenic gene, Hh signaling has been shown to be implicated in cardiac development.^[20] Deletion of Hh may induce several cardiac malformations including ventricular hypoplasia, septation defects, and outflow tract shortening.

Ischemia has been proved to act as a critical trigger for the activities of the Hh pathway in cardiomyoblast cells, neurons, astrocytes, and neural progenitor cells.^[21] It has been believed that inflammation in ischemic myocardium might activate different intracellular signaling elements such as nuclear factor-B, PI3K/Akt (a serine/threonine protein kinase) and K-Ras oncogene, all of which can increase the cellular expression of Hh ligands, including Shh protein, GLI activities, and Hh signaling activation.^[22]

Moreover, several studies showed that the embryonic Hh pathway was reactivated in adult animal models of ischemic injury, including hindlimb ischemia and myocardial infarction.^[21] Accordingly, administration of Hh as a recombinant protein or through gene therapy promotes angiogenesis in ischemic tissues and provides protection from ischemic injury in rodent and large animal models. These studies directly implicate the Hh signaling pathway as a potential therapeutic target for pharmacological angiogenesis and make a compelling case for the potential therapeutic use

of Hh agonists in patients with ischemic heart disease.^[23] Therefore, the present study was designed to investigate the myocardial preconditioning potency of Hh signaling pathway activator purmorphamine (PUR) a smoothened receptor agonist against I/R in deoxycortisone acetate (DOCA) salt-induced hypertensive rat hearts.

METHODS

Experimental animals

Wistar albino rats (180–220 g) of either sex were used in the present study. Animals were housed in the Central Animal House, ISF College of Pharmacy, Moga, India. The animals were kept in polypropylene cages with husk bedding under standard conditions of light and dark cycle with food and water. Animals were acclimatized to laboratory conditions before performing experiments. All the experiments were carried out between 9:00 and 15:00 h. The experimental protocol was reviewed and approved by the Institutional Animal Ethics Committee (IAEC) (ISFCP/IAEC/CPCSEA/Meeting No. 21/2018/Protocol No. 347 and was carried out in accordance with the guidelines of the Indian National Science Academy for the use and care of experimental animals.

Drugs and chemicals

The drug PUR and Atractyloside was obtained from Cayman Chemicals Co.-Pro Lab Marketing Pvt. Ltd. New Delhi, India. Triphenyltetrazolium chloride (TTC) was acquired from CDH Pvt. Ltd., New Delhi, India. The lactate dehydrogenase (LDH), creatine kinase-muscle/brain (CK-MB), estimation kits were purchased from Coral Clinicals System, Goa, India. Unless stated, all other chemicals and biochemical reagent were purchased from Sigma Aldrich which are of the highest analytical grade.

Induction of uninephrectomy

DOCA-Salt-induced HTN-Mineralocorticoid-induced HTN is due to the sodium-retaining properties of the steroid causing increase in plasma and extracellular volume. The hypertensive effect was increased by salt loading and unilateral nephrectomy in rats. Wistar albino rats (180–220 g) were anesthetized with ketamine (60 mg/kg, i.p.) and xylazine (5 mg/kg, i.p.) then a flank incision was given, and the left kidney was removed. The rats were injected twice weekly with 40 mg/kg, s.c. desoxycorticosterone-acetate for 6 weeks. Drinking water was replaced with a 1% NaCl and 0.2% KCl solution. Blood pressure started to rise after 1 week and reached mean arterial blood pressure-volume >160 mmHg after 6 weeks. Then, the animals were sacrificed for isolated heart preparation for Langendorff's apparatus. All the parameters were assessed afterward.

Assessment of hypertension

The rat blood pressure was measured by tail-cuff method (NIBP-BIOPAC MP 100, USA). Rat tail was heated with heater for exactly 3 min. The animal was then placed in restrainer until it got adapted to the given condition. After that,

the tail was inserted into the cuff and pressure with cutoff limit 250 psi is applied on the tail. After getting stable readings, final reading of the mean arterial blood pressure was recorded on BIOPAC [Figure 1].

Experimental protocol

The present study comprises eight groups consisting of six animals ($n = 6$) [Figure 2].

- Group 1: (Sham control; $n = 5$): Isolated normal rat heart preparation was allowed to stabilize for 10 min and then perfused continuously with Krebs–Henseleit buffer solution for 190 min without subjecting them to global ischemia and reperfusion
- Group 2: (Ischemia-reperfusion in normal rat heart; $n = 5$): Isolated normal rat heart preparation was allowed to stabilize for 10 min and was perfused for 40 min. Krebs–Henseleit buffer solution and then, it was subjected to 30 min global ischemia followed by 120 min of reperfusion
- Group 3: (Ischemia-preconditioning in normal rat heart; $n = 5$): Isolated normal rat heart preparation was allowed to stabilize for 10 min and then subjected to four cycles of IPC, each cycle comprised 5 min global ischemia followed by 5 min reperfusion with Krebs–Henseleit solution. Then, the preparation was subjected to 30 min global ischemia followed by 120 min of reperfusion
- Group 4: (IPC in hypertensive rat heart; $n = 5$): Isolated hypertensive rat heart preparation was allowed to stabilize for 10 min and was perfused for 40 min with Krebs–Henseleit buffer solution. Then, it was subjected to 30 min global ischemia followed by 120 min of reperfusion

- Group 5: (IPC in hypertensive rat heart; $n = 5$): Isolated hypertensive rat heart preparation was allowed to stabilize for 10 min and subjected to four cycles of IPC, each cycle comprised 5 min ischemia followed by 5 min reperfusion with Krebs–Henseleit buffer solution. Then, it was subjected to 30 min global ischemia followed by 120 min of reperfusion
- Group 6: (Preconditioning with PUR (1 $\mu\text{M/L}$) in hypertensive rat heart; $n = 6$): Isolated hypertensive rat heart preparation was allowed to stabilize for 10 min and after stabilization hypertensive rat heart was subjected to four cycles of pharmacological preconditioning, each cycle comprised 5 min perfusion with PUR (1 $\mu\text{M/L}$) solution followed by 5 min reperfusion with Krebs–Henseleit buffer solution. Then, the preparation was subjected to 30 min global ischemia followed by 120 min of reperfusion
- Group 7: (Preconditioning with PUR (2 $\mu\text{M/L}$) in hypertensive rat heart; $n = 6$): Isolated hypertensive rat heart preparation was allowed to stabilize for 10 min and after stabilization hypertensive rat heart was subjected to four cycles of pharmacological preconditioning,

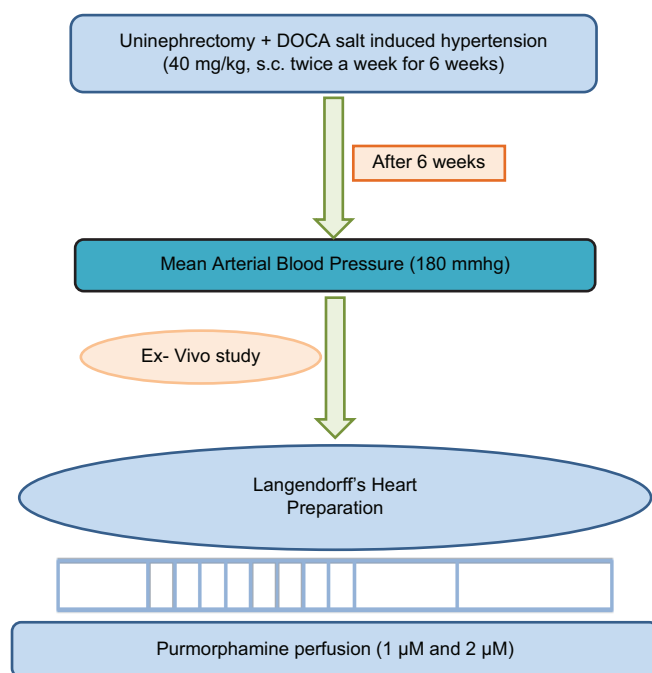


Figure 1: Uninephrectomy and deoxycortisone acetate salt-induced hypertension associated with Langendorff's heart preparation

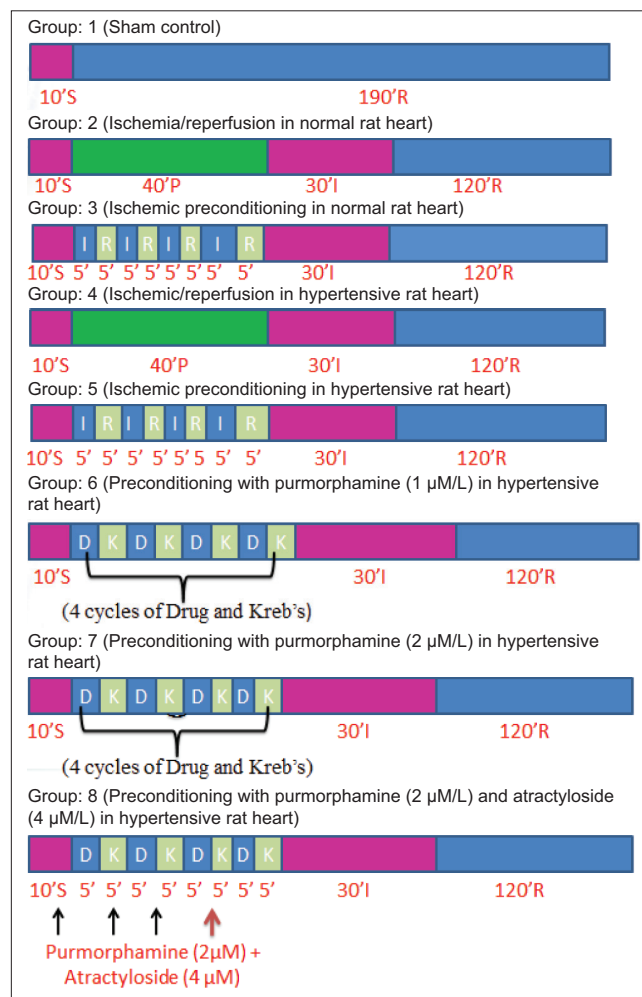


Figure 2: Schematic representation of experimental protocol schedule

each cycle comprised 5 min perfusion with PUR (2 μ M/L) solution followed by 5 min reperfusion with Krebs–Henseleit buffer solution. Then, the preparation was subjected to 30 min global ischemia followed by 120 min of reperfusion

- Group 8: (Preconditioning with PUR (2 μ M/L) and atractyloside (4 μ M) in hypertensive rat heart): Hypertensive rat heart preparation was perfused with atractyloside (4 μ M) during 10 min of stabilization and after stabilization rat heart preparation was subjected to four cycles of pharmacological preconditioning, each cycle comprised 5 min perfusion with PUR (2 μ M/L) solution followed by 5 min reperfusion with Krebs–Henseleit solution. Then, the preparation was subjected to 30 min global ischemia followed by 120 min of reperfusion.

Isolated perfused rat heart

Rats were administered heparin (500 IU/L, i.p.) 20 min before sacrificing the animal by cervical dislocation. The heart was rapidly excised and immediately mounted on Langendorff's apparatus (Digital Langendorff's system, Radnoti LLC, Monrovia, USA). The heart was enclosed by a double walled jacket, the temperature of which was maintained by circulating water heated to 37.8°C. The preparation was retrogradely perfused at constant pressure (By peristaltic pump) with Krebs's Henseleit (K-H) buffer (NaCl 118 mM; KCl 4.7; CaCl₂ 2.5 mM; MgSO₄·7 H₂O 1.2 mM; KH₂PO₄ 1.2 mM; C₆H₁₂O₆ 11 mM), Ph 7.4, bubbles with 95% O₂, and 5% CO₂. Global ischemia was produced for 30 min by closing the inflow of Krebs–Henseleit solution, which was followed by 120 min of reperfusion. Coronary effluent was collected before ischemia, immediately, 5 min and 30 min after reperfusion for the estimation of LDH and CK-MB.^[24]

Ischemic preconditioning

Isolated rat heart was subjected to four brief episodes of ischemia and reperfusion (each episode comprised 5 min ischemia followed by 5 min of reperfusion) after a stabilization period of 10 min. Then, the heart was subjected to 30 min ischemia followed by 120 min of reperfusion.

Assessment of myocardial injury

The extent of myocardial injury was determined by measuring the release of LDH and CK-MB in coronary effluents by using commercially available kits (Coral Clinical System, Goa, India). Values were expressed in international units per liter (IU).

Estimation of lactate dehydrogenase release

LDH was estimated in samples of coronary effluent collected after stabilization and immediately and 30 min after reperfusion by modified International Federation of Clinical Chemistry (IFCC) method using commercially available kit (Coral Clinical System, Goa, India) spectrophotometrically (UV-1700 Spectrophotometer, Shimadzu, Japan) at 340 nm.

Estimation of creatine kinase-muscle/brain release

CK-MB release was estimated in samples of coronary effluent after stabilization and 5 min after reperfusion by modified

IFCC method using commercially available kit (Coral Clinical System, Goa, India) spectrophotometrically (UV-1700 Spectrophotometer, Shimadzu, Japan) at 340 nm.

Assessment of myocardial infarct size

The heart was removed from the Langendorff's apparatus. Both the atria and root of aorta were excised and ventricles were frozen at –20°C. Frozen ventricles were sliced into uniform sections of 1–2-mm thickness. The slices were incubated in 1% TTC for 30 min at 37°C in 0.2 M Tris-chloride buffer (CDH Pvt. Ltd., New Delhi) (prepared by dissolving 7.27 g of Tris (hydroxymethyl) methylamine and 5.27 g of sodium chloride in water, adjusting pH to 7.4, finally diluting up to 1000 ml with distilled water).^[25] TTC is converted to red formazone pigment by NADH and dehydrogenase enzyme, and therefore, the viable cells were stained brick red. The infarcted cell should have lost the enzyme and cofactor and thus remain unstained or dull yellow. The ventricular slices were placed between two glass plates. A transparent plastic grid with 100 squares in 1 cm² was placed above it. The average area of ventricular slice was calculated by counting the number of squares on either side. Similarly, number of square falling over the nonstained dull yellow area was counted. Infarct size was expressed as a percentage of average ventricular volume.

Estimation of tumor necrosis factor-alpha levels

Tumor necrosis factor-alpha (TNF- α) level was estimated by using TNF- α kit (RayBio, Rat TNF-alpha ELISA kit protocol) which uses a microtiter plate reader at 450 nm. Concentrations of TNF- α were calculated from the plotted standard curve. The average for the duplicate readings was calculated for each standard and sample, and the blank values were subtracted. A standard curve was constructed by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis, and the best fit curve was drawn through the points on the graph using regression analysis. The samples which were diluted were multiplied by the dilution factor to get the exact concentration of the unknown samples.

Biochemical assessment of oxidative stress markers

Estimation of serum thiobarbituric acid reactive species level

The quantitative measurement of thiobarbituric acid reactive species (TBARS), an index of lipid peroxidation in the heart was performed according to the method of Ohkawa *et al.* 1979. A volume of 0.2 ml of the supernatant homogenate was pipette out in a test tube, followed by addition of 0.2 ml of 8.1% sodium dodecyl sulfate, 1.5 ml of 30% acetic acid (pH 3.5), and 1.5 ml of 0.8% of thiobarbituric acid and the volume was made up to 4 ml with distilled water. The test tubes were incubated for 1 h at 95°C, then cooled and added 1 ml of distilled water followed by addition of 5 ml of the n-butanol-pyridine mixture (15:1 v/v). The test tubes were centrifuged at 4000 g for 10 min. The absorbance of the developed pink color was measured spectrophotometrically (UV-1700 Spectrophotometer, Shimadzu, Japan) at 532 nm. A standard calibration curve was prepared using 1–10 nM of 1,1,3,3-tetramethoxy propane. The

concentration of TBARS value was expressed as nanomoles per g of wet tissue weight.^[26]

Estimation of nitrite concentration

The accumulation of nitrite in the supernatant, an indicator of the production of nitric oxide (NO), was determined by a colorimetric assay using Greiss reagent (0.1% N-(1-naphthyl ethylenediamine dihydrochloride, 1% sulfanilamide, and 2.5% phosphoric acid) as described by Green *et al.*, 1982. Equal volumes of supernatant and Griess' reagent were mixed, the mixture incubated for 10 min at room temperature in the dark and the absorbance determined at 546 nm spectrophotometrically. The concentration of nitrite in the supernatant was determined from sodium nitrite standard curve and expressed as $\mu\text{mol/mg}$ protein.

Statistical analysis

The results were expressed as a mean $\pm$ standard deviation. The percent (%) infarct size, TNF- α , and TBARS were analyzed using one-way ANOVA followed by Tukey's multiple comparison tests. Nitrite and coronary flow levels were analyzed using two-way ANOVA followed by following Bonferroni. $P < 0.05$ was considered as statistically significant.

RESULTS

Effect of uninephrectomy and deoxycortisone acetate salt on diastolic blood pressure in Wistar rats

Uninephrectomy with DOCA salt administration significantly enhanced the % increase in diastolic blood pressure on the 42nd day (177 ± 75 mmHg) as compared with 0 day (111 ± 53 mmHg) [Figure 3].

Effect of uninephrectomy and deoxycortisone acetate salt on Systolic blood pressure in Wistar rats

Uninephrectomy with DOCA salt administration significantly enhanced the % increase in systolic blood pressure on

the 42nd day (206 ± 25 mmHg) as compared with 0 day (111 ± 78 mmHg) [Figure 4].

Effect of uninephrectomy and deoxycortisone acetate salt on mean arterial blood pressure in Wistar rats

Uninephrectomy with DOCA salt administration significantly enhanced the % increase in mean arterial blood pressure on the 42nd day (186 ± 69 mmHg) as compared with 0 day (103 ± 54 mmHg) [Figure 5].

Effect of purmorphamine on creatine kinase-muscle/brain release in deoxycortisone acetate salt mediated hypertensive rat hearts

CK-MB release was markedly increased in I/R control group as compared to sham control. In IPC treated rat heart, there was a significant decrease in CK-MB release in isolated rat heart as compared to I/R control group. However, the cardioprotective effect of IPC was remarkably abolished in hypertensive rat heart. Moreover, preconditioning with PUR 1 and PUR 2 significantly attenuated the LDH release when compared with normal and hypertensive rat heart. Atractyloside impeded the cardioprotective effect of IPC in normal rat heart and PUR mediated preconditioning in hypertensive rat hearts [Figure 6].

Effect of purmorphamine on lactate dehydrogenase release in deoxycortisone acetate salt-mediated hypertensive rat hearts

LDH release was markedly increased in I/R control group as compared to sham control. In IPC treated rat heart, there was significantly decrease in LDH release in isolated rat heart as compared to I/R control group. However, the cardioprotective effect of IPC was remarkably abolished in hypertensive rat heart. Moreover, preconditioning with PUR 1 and PUR 2 significantly attenuate the LDH release when compared with normal and hypertensive rat heart. Atractyloside impeded the cardioprotective effect of IPC

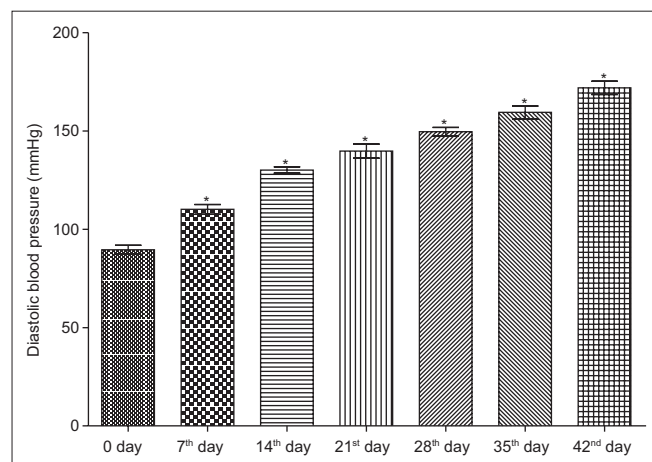


Figure 3: Effect of uninephrectomy and deoxycortisone acetate salt on diastolic blood pressure in Wistar rats. Values were expressed as mean $\pm$ standard deviation ($n = 6$). *signifies $P < 0.05$ as compared to 0 day

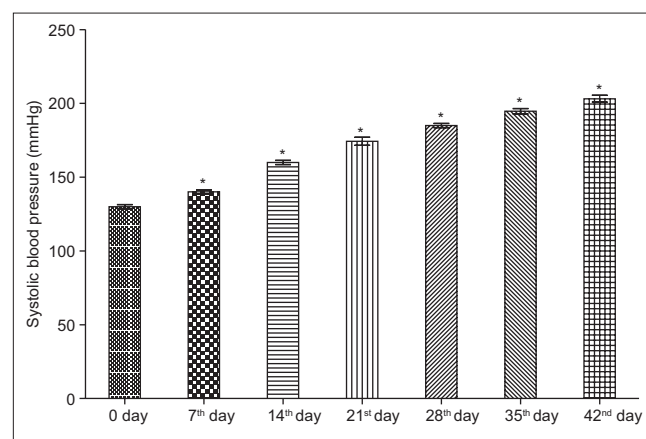


Figure 4: Effect of uninephrectomy and deoxycortisone acetate salt on systolic blood pressure in Wistar rats. Values were expressed as mean $\pm$ standard deviation ($n = 6$). *Signifies $P < 0.05$ as compared to 0 day

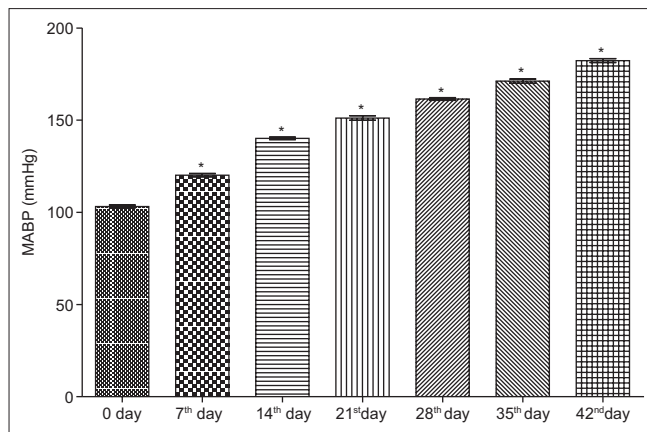


Figure 5: Effect of uninephrectomy and deoxycortisone acetate salt on mean arterial blood pressure in Wistar rats. Values were expressed as mean $\pm$ standard deviation ($n = 6$). *Signifies $P < 0.05$ as compared to 0 day

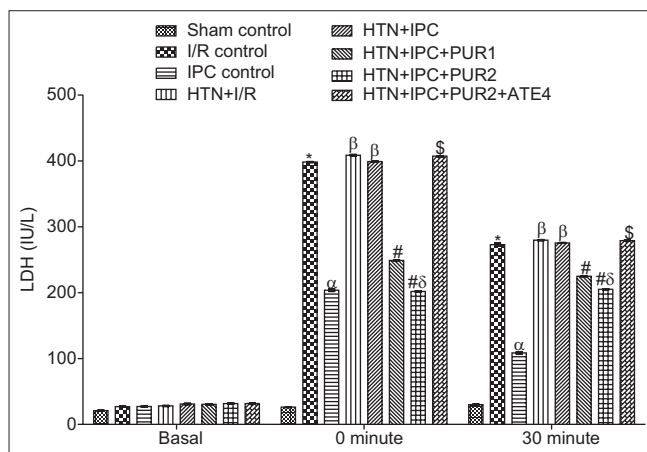


Figure 7: Effect of purmorphamine on LDH release in deoxycortisone acetate mediated hypertensive rat heart. Values were expressed as mean $\pm$ standard deviation ($n = 6$). *Signifies $P < 0.05$ versus sham control; $^αP < 0.05$ versus ischemia-reperfusion control; $^βP < 0.05$ versus IPC control; $^#P < 0.05$ versus HTN + IPC; $^δP < 0.05$ versus HTN + PUR1; $^SP < 0.05$ versus HTN + PUR2

in normal rat heart and PUR mediated preconditioning in hypertensive rat hearts [Figure 7].

Effect of purmorphamine on nitrite release in deoxycortisone acetate salt mediated hypertensive rat hearts

Nitrite release was markedly decreased in I/R control group as compared to sham control. In IPC treated rat heart, there was significantly increase in nitrite release in isolated rat heart as compared to I/R control group. However, the cardioprotective effect of IPC was remarkably abolished in hypertensive rat heart. Moreover, preconditioning with PUR 1 and PUR 2 significantly attenuate the nitrite release when compared with normal and hypertensive rat heart. Atractyloside impeded the cardioprotective effect of IPC in normal rat heart and PUR mediated preconditioning in hypertensive rat hearts [Figure 8].

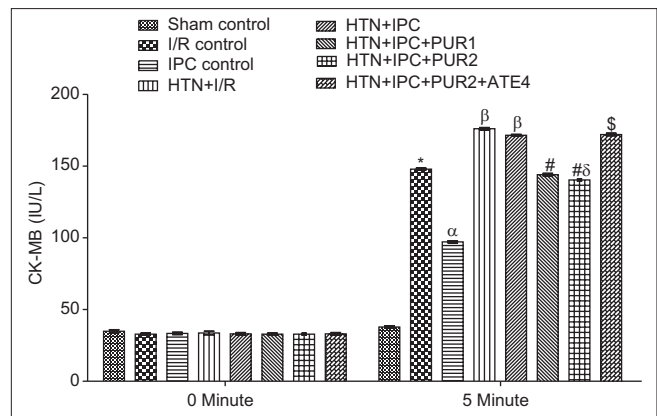


Figure 6: Effect of purmorphamine on creatine kinase-muscle/brain release in deoxycortisone acetate mediated hypertensive rat hearts. Values were expressed as mean $\pm$ standard deviation ($n = 6$). *Signifies $P < 0.05$ versus sham control; $^αP < 0.05$ versus ischemia-reperfusion control; $^βP < 0.05$ versus IPC control; $^#P < 0.05$ versus HTN + IPC; $^SP < 0.05$ versus HTN + PUR1; $^δP < 0.05$ versus HTN + PUR2

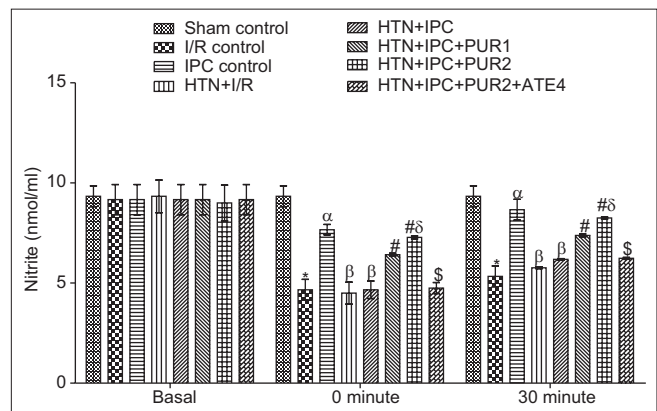


Figure 8: Effect of purmorphamine on nitrite release in deoxycortisone acetate salt-mediated hypertensive rat hearts. Values were expressed as mean $\pm$ standard deviation ($n = 6$). *Signifies $P < 0.05$ versus sham control; $^αP < 0.05$ versus ischemia-reperfusion control; $^βP < 0.05$ versus IPC control; $^#P < 0.05$ versus HTN + IPC; $^δP < 0.05$ versus HTN + PUR1; $^SP < 0.05$ versus HTN + PUR2

Effect of purmorphamine on thiobarbituric acid reactive species in deoxycortisone acetate salt mediated hypertensive rat hearts

TBARS level was markedly increased in I/R control group as compared to sham control. In IPC treated rat heart, there was significantly decrease in TBARS level in isolated rat heart as compared to I/R control group. However, the cardioprotective effect of IPC was remarkably abolished in hypertensive rat heart. Moreover, preconditioning with PUR 1 and PUR 2 significantly attenuate the TBARS level when compared with normal and hypertensive rat heart. Atractyloside impeded the cardioprotective effect of IPC in normal rat heart and PUR-mediated preconditioning in hypertensive rat hearts [Figure 9].

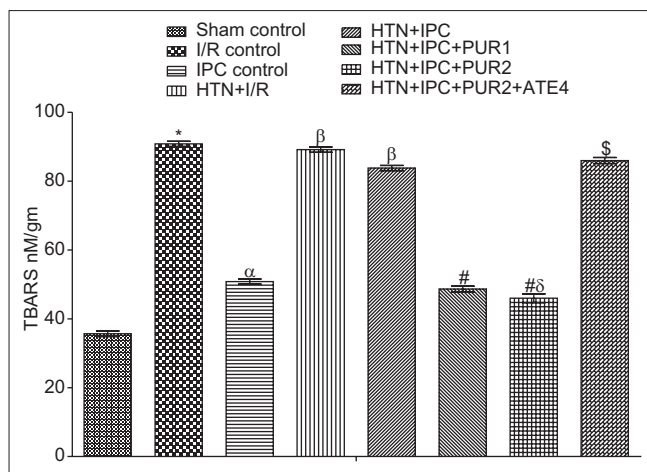


Figure 9: Effect of purmorphamine on TBARS in deoxycortisone acetate salt-mediated hypertensive rat hearts. Values were expressed as mean $\pm$ standard deviation ($n = 6$). *Signifies $P < 0.05$ versus sham control; $^{\alpha}P < 0.05$ versus ischemia-reperfusion control; $^{\beta}P < 0.05$ versus IPC control; $^{\#}P < 0.05$ versus HTN + IPC; $^{\$}P < 0.05$ versus HTN + PUR1; $^{\$}P < 0.05$ versus HTN + PUR2

Effect of purmorphamine on infarct size and triphenyltetrazolium chloride stained in deoxycortisone acetate salt-mediated hypertensive rat hearts

Infarct size was markedly increased in I/R control group as compared to sham control. In IPC treated rat heart, there was significantly decrease in infarct size in isolated rat heart as compared to I/R control group. However, the cardioprotective effect of IPC was remarkably abolished in hypertensive rat heart. Moreover, preconditioning with PUR 1 and PUR 2 significantly attenuate the infarct size when compared with normal and hypertensive rat heart. Atractyloside impeded the cardioprotective effect of IPC in normal rat heart and PUR mediated preconditioning in hypertensive rat hearts [Figure 10a and b].

Effect of purmorphamine on tumor necrosis factor- α in deoxycortisone acetate salt-mediated hypertensive rat hearts

TNF- α level was markedly increased in I/R control group as compared to sham control. In IPC treated rat heart, there was significantly decreased in TNF- α level in isolated rat heart as compared to I/R control group. However, cardioprotective effect of IPC was remarkably abolished in hypertensive rat heart. Moreover, preconditioning with PUR 1 and PUR 2 significantly attenuate the TNF- α level when compared with normal and hypertensive rat heart. Atractyloside impeded the cardioprotective effect of IPC in normal rat heart and PUR mediated preconditioning in hypertensive rat hearts [Figure 11].

Effect of purmorphamine on coronary flow rate in deoxycortisone acetate salt mediated hypertensive rat hearts

The coronary flow rate was markedly decreased in I/R control group as compared to sham control. In IPC treated rat heart,

there was significantly increase in coronary flow rate in isolated rat heart as compared to I/R control group. However, the cardioprotective effect of IPC was remarkably abolished in hypertensive rat heart. Moreover, preconditioning with PUR 1 and PUR 2 significantly attenuate the coronary flow rate when compared with normal and hypertensive rat heart. Atractyloside impeded the cardioprotective effect of IPC in normal rat heart and PUR mediated preconditioning in hypertensive rat hearts [Figure 12].

DISCUSSION

HTN or increased blood pressure is a serious disorder which leads to the excessive workload on cardiac cells.^[27] HTN is responsible for high-mortality rate worldwide. The main factors contributing in the pathogenesis of HTN includes genetics, activation of neurohormonal systems such as the sympathetic nervous system and renin-angiotensin aldosterone system, obesity. Increased stress, sedentary lifestyle, high salt intake, act as extra burden affecting the cardiomyocytes and lead to alterations in the normal functioning of the cellular machinery.^[28]

The HH protein is involved in angiogenesis and cardiovascular development through activation of the ligand-dependent signaling transduction.^[29] Activation of Hh signaling is both necessary for coronary development in the embryonic heart and sufficient to promote the formation of new coronary vessels in the adult heart.^[18] HH pathway is reactivated in adult animal models of ischemic injury, including hindlimb-ischemia and myocardial infarction.^[21] Accordingly, administration of (Hh) as a recombinant protein or via gene therapy promotes angiogenesis in ischemic tissues^[30] and provides protection from ischemic injury in rodent and large animal models.^[21] These studies directly implicate the Hh signaling pathway as a potential therapeutic target for pharmacological angiogenesis and make a compelling case for the potential therapeutic use of HH agonists in patients with ischemic heart disease.

Furthermore, pharmacological preconditioning with PUR improves recovery of function after I/R in both rat and human myocardium.^[31] Therefore, in the present study, pharmacological preconditioning with Hh agonist PUR has been employed to investigate the role of Hh against I/R injury in hypertensive rat heart. In the current study, hypertensive myocardium preconditioning with PUR at two doses 1 μ M/L, 2 μ M/L alone and 2 μ M/L with mPTP opener atractyloside was performed to delineate the pathway of PUR-mediated preconditioning.

In the current research, we performed unilateral nephrectomy along with the administration of the DOCA salt. Uninephrectomy and simultaneous administration of mineralocorticoid DOCA salt induces HTN in the animals after 6 weeks. Isolated rat heart preparations were mounted on Langendorff's apparatus and subjected to I/R injury. Thirty minutes ischemia followed by 120 min reperfusion was able to produce myocardial injury and assessed in terms of increased myocardial infarct size and an

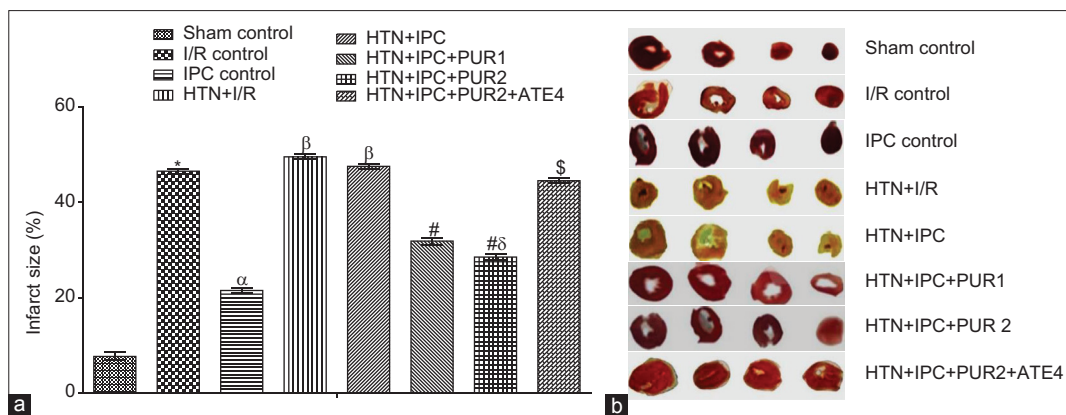


Figure 10: (a) Effect of Purmorphamine on infarct size in deoxycortisone acetate salt-mediated hypertensive rat hearts. Values were expressed as mean $\pm$ standard deviation ($n = 6$). *Signifies $P < 0.05$ versus sham control; $^{\alpha}P < 0.05$ versus ischemia-reperfusion control; $^{\beta}P < 0.05$ versus IPC control; $^{\#}P < 0.05$ versus HTN + IPC; $^{\delta}P < 0.05$ versus HTN + PUR1; $^{\$}P < 0.05$ versus HTN + PUR2. (b) Effect of purmorphamine on infarct size in TTC stained heart sections

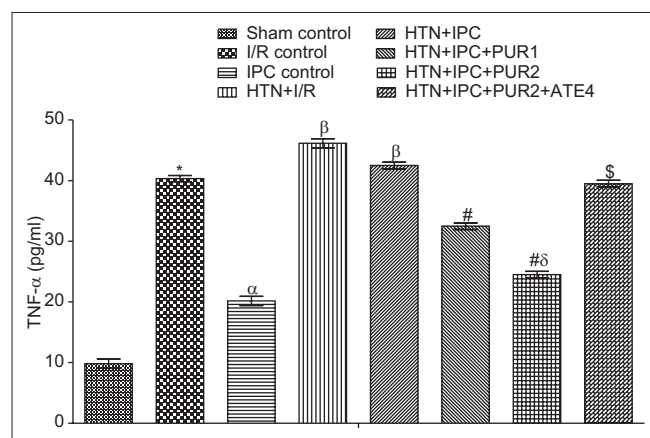


Figure 11: Effect of purmorphamine on TNF- α in deoxycortisone acetate salt mediated hypertensive rat hearts. Values were expressed as mean $\pm$ standard deviation ($n = 6$). *Signifies $P < 0.05$ versus sham control; $^{\alpha}P < 0.05$ versus ischemia-reperfusion control; $^{\beta}P < 0.05$ versus IPC control; $^{\#}P < 0.05$ versus HTN + IPC; $^{\delta}P < 0.05$ versus HTN + PUR1; $^{\$}P < 0.05$ versus HTN + PUR2

elevated release of LDH and CK-MB in the coronary effluent. The increase in lipid peroxidation has been suggested as an indicator of oxidative stress.^[32] IPC limits the complications initiated by I/R injury that was associated in hypertensive rat hearts. IPC induced by four episodes of global ischemia and reperfusion was reported to produce a cardioprotective effect in isolated rat heart preparation.^[22,33] It has been reported that the maximum release of LDH takes place immediately after reperfusion and peak release of CK-MB was after the 5 min of reperfusion. Therefore, samples of coronary effluents were collected at these time intervals to estimate the amount of LDH and CK-MB in the present study.

The infarct size has been assessed macroscopically because good correlation has been reported between macroscopic and microscopic assessment of infarct size. Thus, infarct size was measured macroscopically using TTC staining. All the viable cells contain cofactor NADH and enzyme LDH which

convert TTC stain to red. However, infarcted cells lack LDH enzyme and cofactor NADH and thus remain unstained or dull yellow.^[34]

Furthermore, oxidative stress is also an indicator of cardiac cell injury and measured in terms of TBARS which was noted to be increased as a result of I/R.^[35] TBARS, considered as a final product and marker of hydrogen-free radical metabolism generated during the pathological reactions following during I/R. In this study, PUR significantly reduced the lipid peroxidation.

The study also illustrates that the administration of DOCA salt results in increased blood pressure on the 42nd day when compared to day as well as exhibit marked increase in TNF- α level in ischemic, and hypertensive ischemic rat myocardium, as compared with sham control rats. Myocardial ischemia is sufficient to generate enough TNF- α , leads to myocardial dysfunction.^[36] The role of TNF- α in I/R injury is probably dependent on the absolute levels of TNF- α during I/R period and high release of this cytokine may produce deleterious alterations caused by I/R, and results in decreased cardiac contractility functions and serve as an initiator for the production of inflammatory cytokines such as interleukin-1 (IL-1), IL-2, and IL-6.^[37]

Vascular endothelium releases NO, which stimulates vasorelaxation preserves the integrity of vascular endothelium lining.^[38] DOCA salt mediated HTN results in dysfunction of vascular endothelium. In our results, pharmacological preconditioning with PUR remarkably increased nitrite level as compared with sham and hypertensive I/R group.

In normal and hypertensive rat heart, IPC significantly reduced the I/R-induced myocardial injury in terms of reduction in infarct size, decreased the release of LDH, CK-MB, TNF- α , TBARS, and increased nitrite level and coronary flow rate. Pharmacological preconditioning with PUR (1 μ M L) and PUR (2 μ M L) significantly restored the attenuated cardioprotective effect of IPC in hypertensive rat heart. Preconditioning with PUR for 20 min before ischemia

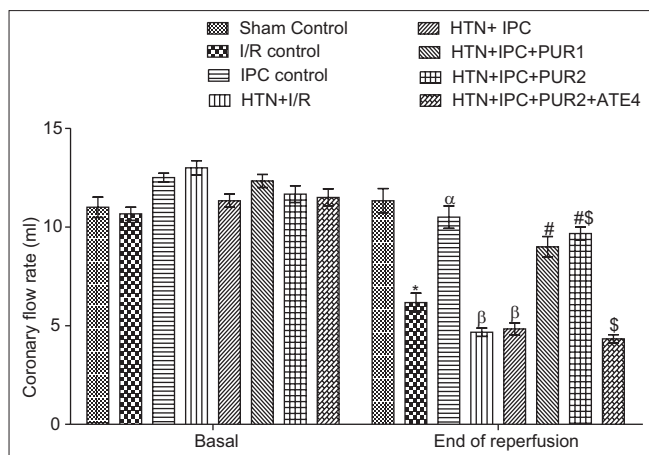


Figure 12: Effect of purmorphamine on coronary flow rate in deoxycortisone acetate salt mediated hypertensive rat hearts. Values were expressed as mean $\pm$ standard deviation ($n = 6$). *Signifies $P < 0.05$ versus Sham Control; $^{\alpha}P < 0.05$ versus ischemia-reperfusion Control; $^{\beta}P < 0.05$ versus IPC control; $^{\#}P < 0.05$ versus HTN + IPC; $^{\$}P < 0.05$ versus HTN + PUR1; $^{\$}P < 0.05$ versus HTN + PUR2

notably reduced myocardial infarct size, LDH release and CK-MB release in normal and hypertensive rat myocardium subjected to I/R injury. Moreover, the levels of TNF- α were also decreased in PUR treated perfused hearts indicating the anti-inflammatory action of this drug in ischemic-reperfused and hypertensive rat heart. It was found that exogenous administration of PUR decreases TBARS level hence controlling the oxidative stress in I/R injured myocardium.

Whereas, atractyloside abolished the preconditioning potential of IPC in normal rat heart was confirmed by increase in LDH, CK-MB, TNF- α , and TBARS levels. Atractyloside, when given in PUR perfused hearts abrogated the cardioprotective potential of preconditioning in hypertensive rat heart that was confirmation regarding the involvement of Hh signaling mediated by target drug PUR.

CONCLUSION

DOCA salt-induced HTN in the animals was characterized by significantly elevated Diastolic blood pressure, systolic blood pressure, and mean arterial blood pressure. Preconditioning with PUR, an Hh agonist significantly decreased myocardial infarct size, release of LDH and CK-MB in coronary effluent, decreased TNF- α , TBARS, and increased nitrite levels and coronary flow rate. Preischemic treatment with atractyloside, an mPTP opener (4 μ M/L) significantly abrogated the cardioprotective potential of PUR in normal as well as hypertensive ischemic perfused rat hearts. Therefore, it can be concluded that Hh pathway has a pivotal role in cardioprotection and stimulation of Hh pathway has preconditioning potential against I/R injury in hypertensive myocardium.

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Conflicts of interest

There are no conflicts of interest.

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