Title: Remote preconditioning by aortic constriction: affords cardioprotection as classical or other remote ischemic preconditioning? Role of iNOS

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Abstract:

Dose remote preconditioning by aortic constriction (RPAC) affords cardioprotection similar to classical or other remote ischemic preconditioning stimulus? Moreover study was also designed to investigate role of inducible nitric oxide synthase in remote preconditioning by aortic constriction. There are sufficient evidences that "ischemic preconditioning" has surgical applications and afford clinically relevant cardioprotection. Transient occlusion of circumflex artery, renal artery, limb artery or mesenteric artery preconditions the myocardium against ischemia reperfusion injury in case of ischemic heart disease leading to myocardial infraction. Here abdominal aorta was selected to produce RPAC. Four episodes of Ischemia-reperfusion of 5 min each to abdominal aorta produced RPAC by assessment of infract size, LDH and CK. These studies suggest RPAC produced acute (FWOP) and delayed (SWOP) cardioprotective effect. RPAC demonstrated a significant decrease in Ischemia-reperfusion induced release of LDH, CK and extent of myocardial infract size. L-NAME (10 mg/Kg i.v.), Aminoguanidine (150 mg/Kg s.c.), Aminoguanidine (300 mg/Kg s.c.), S-methyl isothiourea (3 mg/Kg i.v.), 1400W (1 mg/Kg i.v.) administered 10 min. before global ischemia reperfusion produced no marked effect. Aminoguanidine (150 mg/Kg s.c.), Aminoguanidine (300 mg/Kg s.c.), S-methyl isothiourea (3 mg/Kg i.v.), 1400W (1 mg/Kg i.v.) pretreatment after RPAC produced no significant effect on acute RPAC induced decrease in LDH, CK and infract size, whereas L-NAME (10 mg/Kg i.v.) increased RPAC induced decrease in LDH, CK and infract size. Most interesting observation is in delayed RPAC, where all NOS inhibitors pretreatment attenuate RPAC induced decrease in LDH, CK and infract size. In conclusions, "Remote preconditioning by aortic constriction" (RPAC) affords cardioprotection similar to classical or other remote ischemic preconditioning stimulus. Moreover, late or delayed phase of RPAC has been mediated by inducible nitric oxide synthase (iNOS) whereas it has not involved in acute RPAC.

Key words: Remote preconditioning by aortic constriction (RPAC), L-NAME, Aminoguanidine, S-methyl isothiourea, 1400W, NOS inhibitors, Creatine phosphokinase (CK), Lactate dehydrogenase (LDH), Inducible Nitric Oxide Synthase (iNOS), Cardioprotection.

Introduction

Myocardial Infract size is an established determinant of clinical complications and patient survival in an event of acute coronary occlusion. Ischemic heart disease leading to myocardial infarction is considered to be one of the major causes of cardiovascular morbidity and mortality. Revascularization of ischemic heart with thrombolytic agents, angioplasty or saphenous vein grafting is primary requirement. Moreover, a delay to institute reperfusion deprives most of its beneficial effects as a direct function of time. Thus attention has been focused to understand the adaptive mechanisms that will make the myocardium more resistant to ischemia of longer duration and to restore its viability on reperfusion. Repeated short episodes of ischemia-reperfusion have been demonstrated to make the myocardium transiently more resistant to the deleterious effects of subsequent and prolonged ischemic insult. This paradoxical form of myocardial adaptation has been termed as ischemic preconditioning which was reported to provide protection against infract size and arrhythmias and improving post ischemic contractile function. Thus ischemic preconditioning has surgical application and affords clinically relevant cardio protection. Preconditioning has been reported to protect the myocardium even in diseased states such as hypertrophy and diabetic myocardium [1-3].

Ischemic preconditioning is acquired by transient ischemic stress in the same tissue or organ[4]. Short occlusions of renal artery[4] or mesenteric artery[5,6] or limb artery[7,8,9], also precondition that myocardium against ischemia reperfusion injury. Ischemic stress in the remote regions termed as "remote preconditioning". The cardio protective effects of ischemic preconditioning are biphasic. An early effect which lasts for 1-2 h and delayed effect which appears after 24 h. However, the delayed effect of remote preconditioning is not yet investigated [5].

The antiarrythmic effect of classical ischemic preconditioning involves activation of soluble guanylate cyclase through NO and subsequent elevation of cGMP [10,11,12]. In contrast to limited involvement of NO in classical ischemic preconditioning, substantial evidence implicates it in delayed ischemic preconditioning [13,14] . NO can act as a trigger as well as mediator of delayed phase of ischemic preconditioning. Delayed cardioprotective effect ischemic preconditioning is accompanied by increased activity of Nuclear factor Kappa-B (NF-κβ) [15] which may in turn induce the expression of iNOS. It is interesting to note that selective iNOS inhibitors such as

aminoguaidine and S-methyl-isothiourea, when administered before sustained ischemia abolish the preconditioning – induced delayed cardioprotection against stunning [8] and infraction [16,17]. Moreover administration of endotoxin and nontoxic derivative MLA confers preconditioning like delayed cardioprotective through iNOS induction[18,19]. Role of inducible nitric oxide synthase by intestinal ischaemia induce late preconditioning against myocardial infarction[20].

The present study is designed to investigate the acute and delayed cardioprotective effect of remote aortic preconditioning. Moreover, it is also envisaged to study the role of inducible nitric oxide synthase (iNOS) in cardioprotective effect of remote preconditioning by aortic constriction.

Materials and methods:

Animals

Wistar albino rats (100-300 g) of either sex were employed in the present study. They are fed on standard laboratory chow (Kisan Feeds Ltd., New Delhi, India) and had free access to tap water ad libitum. All the experimental protocols were performed according to Animals Ethical Committee, Gyan Vihar School of Pharmacy, and Protocol No. 42.

Remote aortic preconditioning

Rats were anesthetized with thiopental sodium (25 mg Kg^{-1} i.v.). A 2 cm long incision was given on the abdomen. Lower portion of abdominal aorta was isolated and the suture (numbered 5/0) was passed beneath it away from the origin of renal arteries. Aorta was occluded by tying a shoe lace knot and knot was untied from reperfusion. Aorta was occluded for 5 min and was reperfused for 5 min. Four such episodes were used to produce preconditioning [21]. In case animals were to be used after 24h of aortic preconditioning the abdomen was sutured in layers and animals were allowed to recover.

Global ischemia and reperfusion in isolated rat heart

Heparin (500IU, i.p.) was administered about 20 min. before sacrificing the animal. Heart was rapidly excised and immediately mounted on Langendorff's apparatus[22]. Aorta was retrogradely perfused at a constant pressure of 70 mmHg with Kreb's Henseleit buffer (NaC1 118mM; kc1 4.7 mM; $CaCl_2$ 2.5mM; $MgSO_4.7H_2O$ 1.2mM; $NaHCO_3$ 25 mM; Kh_2PO_4 1.2mM; $C_6H_{12}O_6$ 11mM) pH 7.4, maintained AT 37^OC bubbled with 95% O_2 and 5% CO_2. Flow rate was maintained between 6-9 ml/min using Hoffman's screw. The heart was enclosed by a double walled jacket, the temperature of which was maintained by circulating water heated to 37^OC.

Global ischaemia was produced for 30 min by blocking the in-flow Kreb's buffer. It was followed by reperfusion for 120 min. ECG (BPL CARDIAART 108T-DIGI, New Delhi, India) was monitored using two silver electrodes fixed at left ventricular apex and right auricle. ECG was recorded immediately after stabilization, 5 min, 15 min and 30 min during ischemia and immediately, 5 min, 15 min, 30 min, 60 min and 120 min after reperfusion. Coronary effluent was collected at same time intervals during reperfusion for LDH and CK estimation.

Infract size measurement

Heart was removed from Langendorff's apparatus. Both the auricles and the root of aorta were exercised, and ventricles were kept overnight at $4^{O}C$. Frozen ventricles were sliced into uniform sections of 2-3 mm thickness. The slices were incubated in 1% triphenyltetrazolium chloride (TTC) at $37^{O}C$ in 0.2 M Tris buffer (pH 7.4) for 20 min. TTC is converted to red formazone pigment by NADH and dehydrogenase enzyme and therefore, stained the viable cells deep red. The infracted cells have lost the enzyme and cofactor and thus remained unstained or dull yellow. The ventricular slices were placed between two glass plates. A transparent plastic grid with 100 squares in 1 cm^2 was placed above it. Average area of each ventricular slice was calculated by counting the number of squares on either side. Similarly, number of squares falling over non-stained dull yellow area was also counted. Infracted area was expressed as a percentage of total ventricular area. Whole of ventricular slices were weighed. Infracted dull yellow part was dissected and weighed. Infract size was expressed as a percentage of total ventricular weight.

Estimation of Lactate dehydrogenase (LDH)

LDH was estimated in coronary effluent by 2,4-DNPH method[23].

Principle

LDH catalyses the following reaction:

Lactate + NAD ⟷ Pyruvate + NADH

The pyruvate so formed is coupled with 2,4-dinitrophenylhydrazine (2,4-DNPH) to give corresponding hydrazone which gives brown colour in alkaline medium. The intensity of this colour is proportional to the amount of LDH activity and is measured spetrophotometrically at 440 nm.[Figure 1]

Estimation of Creatine phosphokinase (CPK)

CPK was measured in the coronary effluent by modified method of Hughes[24].

Principle

CPK catalyses the following reaction:

Creatine phosphate + ADP ⟷ Creatine + ATP

At pH 7.4, CPK catalyses the forward reaction. The creatine so formed, reacts with diacetyl and –naphthol in alkaline medium to give pink colour. The intensity of this colour is proportional to enzyme activity and is measured spectrophotometrically at 520 nm. Mg^{2+} and cysteine are added as activators. P-Chloromercuribenzoate stops the reaction by inactivating the enzyme.

Experimental Protocol

Twenty four groups of Wistar albino rats were employed in the present study.

Remote Aortic Preconditioning Induced Acute or First Window of Protection (FWOP): First window of protection (FWOP) was observed immediately after remote aortic preconditioning.

Group 1 (FWOP Control Group; n=6)

Rats were subjected to surgical procedure for aortic isolation but aorta was not occluded. Hearts were excised 40 min after sham operation. Isolated hearts were perfused on Langendorff's apparatus and were subjected to global ischaemia for 30 min followed by reperfusion for 120 min.

Group II (FWOP Remote Preconditioning by Aortic Constriction Group; n = 6)

Rats were subjected to remote preconditioning by aortic constriction as described earlier. Hearts were excised immediately after last episode of reperfusion, perfused on Langendorff's apparatus and were subjected to global ischaemia for 30 min followed by reperfusion for 120 min.

Group III (FWOP L-NAME (10mg/kg i.v.) [25] Treated Control Group; n=6)

Rats were administered 10 mg/kg of L-NAME i.v., 10 min before excising the heart for Langendorff's perfusion. Rest of the protocol was same as described in group I.

Group IV (FWOP L-NAME (10mg/kg i.v.) Treated Remote Preconditioning by Aortic Constriction Group; n=6)

L-NAME (10 mg/kg i.v.) was administered during last episode of reperfusion during remote preconditioning by aortic constriction i.e. 10 min before isolating the heart for Langendorff's perfusion. Rest of the protocol was same as described in group I.

Group V (FWOP Aminoguanidine (150mg/kg i.v.) [26,27] Treated Control Group; n=6)

Rats were administered 150 mg/kg of aminoguanidine i.v. 10 min before excising the heart for Langendorff's perfusion. Rest of the protocol was same as described in group I.

Group VI (FWOP Aminoguanidine (150mg/kg i.v.) Treated Remote Preconditioning by Aortic Constriction Group; n=6)

Aminoguanidine (150 mg/kg i.v.) was administered during last episode of reperfusion during remote preconditioning by aortic constriction i.e. 10 min before isolating the heart for Langendorff's perfusion. Rest of the protocol was same as described in group I.

Group VII (FWOP Aminoguanidine (300mg/kg s.c.)[28] Treated Control Group; n=6)

Rats were administered 300 mg/kg of aminoguanidine i.v. 10 min before excising the heart for Langendorff's perfusion. Rest of the protocol was same as described in group I.

Group VIII (FWOP Aminoguanidine (300mg/kg i.v.) Treated Remote Preconditioning by Aortic Constriction Group; n=6)

Aminoguanidine (300 mg/kg i.v.) was administered during last episode of reperfusion during remote preconditioning by aortic constriction i.e. 10 min before isolating the heart for Langendorff's perfusion. Rest of the protocol was same as described in group I.

Group IX (FWOP S-methyl isothiourea (3mg/kg i.v.)[28] Treated Control Group; n=6)

Rats were administered S-methyl isothiourea (3mg/kg i.v.) 10 min before excising the heart for Langendorff's perfusion. Rest of the protocol was same as described in group I.

Group X (FWOP S-methyl isothiourea (3mg/kg i.v.) Treated Remote Preconditioning by Aortic Constriction Group; n=6)

S-methyl isothiourea (3mg/kg i.v.) was administered during last episode of reperfusion during remote preconditioning by aortic constriction i.e. 10 min before isolating the heart for Langendorff's perfusion. Rest of the protocol was same as described in group I.

Group XI (FWOP 1400W (1mg/kg i.v)[25] Treated Control Group; n=6)

Rats were administered 1400W (1 mg/kg i.v.) 10 min before excising the heart for Langendorff's perfusion. Rest of the protocol was same as described in group I.

Group XII (FWOP 1400W (1mg/kg i.v.) Treated Remote Preconditioning by Aortic Constriction Group; n=6)

1400W (1 mg/kg i.v.) was administered during last episode of reperfusion during remote preconditioning by aortic constriction i.e. 10 min before isolating the heart for Langendorff's perfusion. Rest of the protocol was same as described in group I.

Remote Aortic Preconditioning Induced Delayed or Second Window of Protection (SWOP): Second window of protection (SWOP) was observed 24h after remote aortic preconditioning.

Group XIII (SWOP Control Group; n=6)

Rats were subjected to surgical procedure for aortic isolation but aorta was not occluded. Hearts were excised 24h after sham operation. Rest of the protocol was same as in group I.

Group XIV (SWOP Remote Preconditioning by Aortic Constriction Group; n=6)

Rats were subjected to remote preconditioning by aortic constriction and hearts were excised 24h after remote aortic preconditioning. Rest of the protocol was same in group I.

Group XV (SWOP L-NAME (10 mg/kg i.v.) Treated Control Group; n=6)

Rats were subjected to same protocol as described in group XIV except that L-NAME (10 mg/kg i.v.) was administered 10 min before excising the heart for Langendorff's perfusion.

Group XVI (SWOP L-NAME (10 mg/kg i.v.) Treated Remote Preconditioning by Aortic Constriction Group; n=6)

Rats were subjected to same protocol as described in-group XIV except that L-NAME (10 mg/kg i.v.) was administered 10 min before excising the heart for Langendorff's perfusion.

Group XVII (SWOP Aminoguanidine (150 mg/kg i.v.) Treated Control Group; n=6)

Rats were subjected to same protocol as described in group XI except that aminoguanidine chloride (150 mg/kg i.v.) was administered 10 min before excising the heart for Langendorff's perfusion.

Group XVIII (SWOP Aminoguanidine(150 mg/kg i.v.) Treated Remote Preconditioning by Aortic Constriction Group; n=6)

Rats were subjected to same protocol as described in-group XIV except that aminoguanidine (150 mg/kg i.v.) was administered 10 min before excising the heart for Langendorff's perfusion.

Group XIX (SWOP Aminoguanidine (300 mg/kg i.v.) Treated Control Group; n=6)

Rats were subjected to same protocol as described in group XIII except that aminoguanidine chloride (300 mg/kg i.v.) was administered 10 min before excising the heart for Langendorff's perfusion.

Group XX (SWOP Aminoguanidine (300 mg/kg i.v.) Treated Remote Preconditioning by Aortic Constriction Group; n=6)

Rats were subjected to same protocol as described in-group XIV except that aminoguanidine (300 mg/kg i.v.) was administered 10 min before excising the heart for Langendorff's perfusion.

Group XXI (SWOP S-methyl isothiourea (3mg/kg i.v.) Treated Control Group; n=6)

Rats were subjected to same protocol as described in group XIII except that S-methyl isothiourea (3mg/kg i.v.) was administered 10 min before excising the heart for Langendorff's perfusion.

Group XXII (SWOP S-methyl isothiourea (3mg/kg i.v.) Treated Remote Preconditioning by Aortic Constriction Group; n=6)

Rats were subjected to same protocol as described in-group XIV except that S-methyl isothiourea (3mg/kg i.v.) was administered 10 min before excising the heart for Langendorff's perfusion.

Group XXIII (SWOP 1400W (1mg/kg i.v.) Treated Control Group; n=6)

Rats were subjected to same protocol as described in group XIII except that 1400W (1mg/kg i.v.) was administered 10 min before excising the heart for Langendorff's perfusion.

Group XXIV (SWOP 1400W (1mg/kg i.v.) Treated Remote Preconditioning by Aortic Constriction Group; n=6)

Rats were subjected to same protocol as described in-group XIV except that 1400W (1mg/kg i.v.) was administered 10 min before excising the heart for Langendorff's perfusion.

Drugs and chemicals

Aminoguanidine hydrogen carbonate (Lancaster Chemicals, Madras, India), 1400W ([N-(3-(aminomethyl)benzyl)acetamidine]sulfate) (a specific iNOS inhibitor) was from Acros Organics (Noisy le Grand, France), dissolved in distilled water immediately before use, S-methylthiourea sulfate (Sigma-Aldrich USA) L-NAME (nitro-L-arginine-methylester) (non-specific iNOS inhibitor) (Sigma-Aldrich USA), Tris buffer was prepared by adding 50 ml of 0.2 M Tris (CDH chemicals, N.Delhi, India) in 32.5 ml of 0.2 M HCl and volume was made up to 200 ml with distilled water. All other reagents used in the study were analar grade of Qualigens (Glaxo, Mumbai, India)

Statistical analysis

Values for enzymatic data and infract-size were expressed as mean ± S.E.M. Statistical significance was calculated using one way analysis of variance. Dunnett's test and student t test were employed as post-hoc tests for comparison with control group and for multiple comparisons between groups respectively. A value of $p < 0.05$ is considered to be statistically significant. Sigma10 software was used for statistical analysis.

Results

Effect of remote aortic preconditioning and NOS (Nitric Oxide Synthase) Inhibitors pretreatment on coronary flow rate and heart rate

Global ischaemia for 30 min produced a marked decrease in coronary flow rate [Table 1] and heart rate [Table 2] and this decrease persisted for the entire 120 min of reperfusion. NOS (Nitric Oxide Synthase) Inhibitors **[**L-NAME(10 mg/Kg i.v.), Aminoguanidine(150 mg/Kg s.c.), Aminoguanidine (300 mg/Kg s.c.), S-methyl isothiourea (3 mg/Kg i.v.), 1400W (1 mg/Kg i.v.)] pretreatment and remote aortic preconditioning produced no significant change in flow rate [Table 1] and heart rate [Table 2].

Effect of NOS (Nitric Oxide Synthase) Inhibitors pretreatment on ischemia/reperfusion-induced myocardial injury

The peak increase in release of LDH in coronary effluent of isolated rat heart subjected to global ischaemia and reperfusion was noted immediately and 30 min after reperfusion [Table 3], whereas peak increase in release of CK was noted after 5min of reperfusion. L-NAME (10 mg/Kg i.v.), Aminoguanidine (150 mg/Kg s.c.), Aminoguanidine (300 mg/Kg s.c.), S-methyl isothiourea (3 mg/Kg i.v.), 1400W (1 mg/Kg i.v.) administered 10 min before removing the heart for ischaemia/reperfusion study using Langendorff's apparatus produced no marked effect on ischaemia/reperfusion-induced in release of LDH [Table 3] CK [Table 5] and myocardial infract size [Table 7].

Acute (FWOP) and Delayed (SWOP) effects of remote preconditioning by aortic constriction (RPAC) on ischemia/reperfusion-induced myocardial injury

Rat heart isolated immediately (acute/FWOP) or 24 h (delayed/SWOP) after remote preconditioning by aortic constriction (RPAC) demonstrated a significant decrease in ischaemia/reperfusion induced release of LDH [Table 3,4] CK [Table 5,6] and extent of myocardial infract size [Table 7,8]. Remote preconditioning by aortic constriction (RPAC) produced acute (FWOP) and delayed (SWOP) cardioprotective effect.

Effect of NOS (Nitric Oxide Synthase) Inhibitors on Acute (FWOP) and Delayed (SWOP) remote preconditioning by aortic constriction (RPAC) in ischemia/reperfusion-induced myocardial injury

In rat heart isolated immediately after remote preconditioning by aortic constriction (Acute/FWOP) and pretreatment with Aminoguanidine(150 mg/Kg s.c.), Aminoguanidine (300 mg/Kg s.c.), S-methyl isothiourea (3 mg/Kg i.v.), 1400W (1 mg/Kg i.v.) produced no significant effect on preconditioning induced decrease in LDH [Table 3], CK [Table 5] release and myocardial infract size [Table 7]. L-NAME (10 mg/Kg i.v.) administration increase release of LDH [Table 3] CK [Table 3] in coronary effluent and also increased myocardial infract size [Table 5] in Acute/FWOP remote preconditioning by aortic constriction.

On the other hand, administration of L-NAME(10 mg/Kg i.v.), Aminoguanidine(150 mg/Kg s.c.), Aminoguanidine (300 mg/Kg s.c.), S-methyl isothiourea (3 mg/Kg i.v.), 1400W (1 mg/Kg i.v.) 24 hr after subjecting rat heart to remote aortic preconditioning (delayed/SWOP), attenuated remote preconditioning by aortic constriction (RPAC) induced decrease in LDH [Table 4], CK [Table 6] release in coronary effluent and myocardial infract size [Table 8].

Discussion

In the present study, four episodes of occlusion of aorta followed by reperfusion markedly protected the rat heart against sustained ischemia and reperfusion induced myocardial injury. The observed acute and delayed cardio protective effect of remote preconditioning by aortic constriction has been supported by our earlier observation noted with remote renal preconditioning [4,5,6]. It has been clarified that the has no limitation of this experimental condition, as ex vivo experimental results could be directly extrapolated into clinical setting as well as in vivo animal condition supported by findings by D.J. Hausenloy et al., 2007; D.J. Hausenloy et al., 2010 and H**.**E**.** Bøtker et al., 2010 [7,8,9]. In the present study the delayed cardio protective effect of remote by aortic constriction is attenuated with aminoguanidine, L-NAME, S-methyl isothiourea and 1400W (NOS inhibitors). Aminoguanidine is reported to attenuate endotoxin-induced delayed cardio protection [19].

1400W, a selective iNOS inhibitor, was used to determine whether this isozyme was involved in the cardio-protective mechanism. 1400W was chosen because, because of its higher selectivity for iNOS (5000 times more selective for iNOS than for eNOS) [25]. A specific role of iNOS-derived NO as a mediator of delayed cardioprotection has also been reported for ischaemic preconditioning (IP). Thus, iNOS induction shown to be necessary for the development of delayed protection conferred by IP in anaesthetised rabbit models of myocardial infarction and

stunning[25]. Vegh et al. have also demonstrated that iNOS inhibition prevents the development of delayed preconditioning against arrhythmias, in the dog. Using iNOS knockout mice, Guo et al. have shown that targeted disruption of the iNOS gene completely abrogates the infarct-sparing effect of late IP, demonstrating that the activity of iNOS is indispensable for this cardioprotective phenomenon to occur [25]. Further more, iNOS appears to be a final mediator of several other forms of delayed myocardial preconditioning, such as that induced by NO donors, endotoxin derivatives and exercise. Although recent studies suggest that adenosine A1 receptor agonist-induced cardioprotection occurs independently of either early generation of NO or induction of iNOS, an implication of iNOS has also been shown in this form of pharmacological preconditioning[25].

Recent evidence has shown that the cardioprotection afforded by the late phase of ischemic preconditioning (PC) is mediated by upregulation of inducible nitric oxide synthase (iNOS). However, the specific cardiac cell type(s) that express(es) iNOS in response to ischemic PC remains unknown. Thus, mice underwent a sequence of six cycles of 4-min coronary occlusion/4-min reperfusion, which induces late PC, and tissue samples were collected at serial times for measurement of mRNA (Northern) and protein levels (Western). In addition, whole heart samples were cryosectioned for *in situ* hybridization and immunohistochemistry. The steady-state levels of iNOS mRNA in the ischemic regions started to increase at 1 h after ischemic PC, peaked at 3 h (201±31% of sham, n=5 P<0.01) and remained elevated at 24 h (177±22% of sham, n=5 P<0.01). In accordance with these data, iNOS protein expression was increased at 24 h (219±41% of sham, n=5 P<0.01). In contrast, neither endothelial nitric oxide synthase (eNOS) mRNA levels nor its protein expression changed at any time-point[26].

Administration of aminoguanidine (300 mg/ kg, s.c.) or S-methylisothiourea sulfate (3 mg/ kg, i.v.), both relative inducible NO synthase inhibitors, 60 or 30 min before sustained myocardial ischemia not only abolished the late preconditioning afforded by intestinal ischemia, but also inhibited the ability of intestinal ischemia preconditioning to significantly reduce neutrophil infiltration. A change in inducible NO synthase activity was not observed in normal myocardium 24 h after intestinal ischemia, but 30 min of coronary occlusion significantly increased the inducible NO synthase activity in the preconditioned group, which was abolished by

aminoguanidine or S-methylisothiourea sulfate. In conclusion, above data provide pharmacological evidence that induction of inducible nitric oxide synthase, following intestinal ischemia, is associated with increased myocardial tolerance to infarction 24 h later [20]. The same is confirmed in the present study that aminoguanidine (300 mg/ kg, s.c.) or S-methylisothiourea sulfate (3 mg/ kg, i.v.), both relative inducible NO synthase inhibitors, abolished the late preconditioning afforded by remote preconditioning by aortic constriction.

Aminoguanidine in the dose employed is reported to inhibit selectively iNOS [27,28]. The late phase of ischemic preconditioning is a delayed adaptive response that renders the heart relatively resistant to sustained ischemia and reperfusion. NO is identified as a initial signal for triggering the late cardio protective effect of classical ischemic preconditioning [15,16,29]. Activation of pro-inflammatory mediators such as cytokines and inducible nitric oxide synthase (iNOS) have been shown to contribute to myocardial injury after ischemia and reperfusion [30-32]. Up regulation of iNOS may account to precondition the heart by brief ischemic stress. Different induction of mRNA for inducible nitric oxide synthase in rat smooth muscle cells in culture and in aortic strips has been found. One of the transcription factors that could activate gene expression in response to ischemic preconditioning is the nuclear factor κβ (NF-κβ). This oxidant sensitive transcription factor plays a critical role in the immediate – early activation of multitude of genes encoding signaling and defense proteins expressed in the response to various stressful situations and therefore appears to be a general mediator of cellular responses to stress[33-35]. It is well established that the 5' flanking region of iNOS gene contains a consenus sequence that NF-κβ and that the activation of NF- κβ is a central mechanism controlling the induction of iNOS in several cell types including cardiac myocytes [31,36-38]. Therefore, it may be possible that the noted delayed cardio protective effect remote preconditioning by aortic constriction may be due to upregulation of iNOS due to shear stress exerted on myocardium by aortic occlusions. This contention is supported by the results of the present study because aminoguanidine, a selective iNOS inhibitor, attenuated the delayed cardio protective effect of remote aortic preconditioning[27,28]. Our results are supported by observations, which implicate the role of iNOS in delayed cardio protective effect of classical ischemic preconditioning [15,16,19]. It is further supported that pacing induced delayed protection against arrythmias is attenuated by aminoguanidine, an inhibitor of nitric oxide synthase[39]. Delayed or Second window

preconditioning induced by adenosine A1 receptor activation is independent of early generation of nitric oxide or late induction of inducible nitric oxide synthase [40].

Induction of iNOS requires a lag phase. Acute cardio protective effect of remote preconditioning by aortic constriction was observed immediately after the preconditioning stimulus. Therefore the involvement of iNOS in it, may be remote because induction of iNOS require some lag time [26,27]. Moreover, this contention is further supported by our results which have demonstrated that aminoguanidine, S-methyl isothiourea, 1400W in the dosage employed has produced no notable effect on acute cardio-protective effect of remote preconditioning afforded by aortic constriction. Therefore, it may be possible that the acute cardio-protective effect of remote preconditioning by aortic constriction may be mediated through the activation of eNOS may be as result of shear stress [32,41] and confirmed by present study because L-NAME, a non-specific NOS inhibitor abolished acute cardioprotective effect produced by remote preconditioning by aortic constriction.

Over the past decade, an enormous number of studies (>100) have focused on the role of nitric oxide (NO) in myocardial ischemia. It is important to distinguish the function of NO in unstressed (non-preconditioned) myocardium from its function in preconditioned myocardium (i.e. myocardium that has shifted to a defensive phenotype in response to stress). The time has come to translate this enormous body of experimental evidence into clinically useful therapies by harnessing the cytoprotective properties of NO [42-46].

Preconditioning is in experimental studies the most powerful mode of cardio protection known. The signal transduction pathways involve a variety of trigger substances, mediators, receptors, and effectors. The studies of preconditioning in cardiac surgery provide conflicting results but the majority of studies show that ischemic preconditioning is an effective adjunct to myocardial protection. However, ischemic preconditioning with repeated clamping of the aorta will never get widespread use. If the “preconditioning response” is to be exploited in cardiac surgery, targeting the underlying molecular mechanisms must provide easily applicable techniques or drugs, which are shown in large scale clinical studies to be beneficial [47]. Remote ischemic preconditioning increases the tolerance of the myocardium to ischemia, reduces ischemic chest

discomfort during coronary balloon occlusion, and reduces the prevalence of cardiac troponin I [cTnI] release after elective PCI [48]. The data add to the growing number of studies suggesting that remote ischemic preconditioning is a safe, effective, noninvasive, and cost-effective strategy for reducing ischemic cardiac damage in settings where myocardial ischemic damage is expected [49].

Conclusions

On the basis of the above results and discussion, it can be concluded that remote preconditioning by aortic constriction has produced acute and delayed cardioprotective effect as found earlier with classical and other remote preconditioning stimulus. Recent evidence has shown that the cardioprotection afforded by the late phase of ischemic preconditioning (PC) is mediated by inducible nitric oxide synthase (iNOS). The steady-state levels of iNOS mRNA in the ischemic regions started to increase at 1 h after ischemic PC, peaked at 3 h and remained elevated at 24 h. In accordance with these data, iNOS protein expression was increased at 24 h [26]. In the present study iNOS has been involved in the delayed cardio-protective effect afforded by remote preconditioning by aortic constriction as supported by above findings. On the other hand activation of iNOS may not participate in acute cardio-protective effect of remote preconditioning by aortic constriction.

Conflict of interest

There is no conflict of interest.

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Table 1: Acute Effect (FWOP) and delayed effect (SWOP) of remote preconditioning by aortic constriction (RPAC) and and NOS inhibitors Pretreatment on Coronary Flow Rate (ml/min) in Isolated Rat Heart Subjected to Global Ischaemia (30 min) and Reperfusion (120 min).

Groups	BASAL	Imm.R.	5 min R.	15 min.R.	30 min.R.	60 min.R.	120 min.R.
(I) Sham (FWOP)	8.40±0.13	4.40*±0.24	5.32*±0.05	4.98*±0.18	4.16*±0.42	2.90*±0.48	1.24*±0.5
(II) RAPC (FWOP)	8.22±0.30	4.36*±0.37	4.94*±0.29	4.30*±0.50	4.92*±0.67	2.58*±0.56	1.96*±0.41
(III) L-NAME (10 mg/Kg i.v) pretreatment immediately after Sham operation (FWOP)	10.38±1.53	7.04*±0.72	4.92*±0.72	4.04*±0.71	3.92*±0.73	2.85*±0.40	2.49*±0.51
(IV) L-NAME (10 mg/Kg i.v) pretreatment immediately after RPAC (FWOP)	9.73±0.49	5.98*±0.49	4.82*±1.06	3.93*±1.12	3.53*±0.20	3.19*±0.61	2.63*±0.24
(V) Aminoguanidine (150 mg/Kg s.c) pretreatment immediately after Sham operation (FWOP)	8.06±0.63	4.78*±0.79	3.98*±0.75	3.70*±0.70	2.58*±0.65	2.02*±0.47	1.66*±0.42
(VI) Aminoguanidine (150 mg/Kg s.c) pretreatment immediately after RPAC (FWOP)	9.66±0.96	6.86*±1.19	6.46*±1.06	6.00*±0.71	5.20*±1.10	4.30*±0.74	3.46*±0.46
(VII) Aminoguanidine (300 mg/Kg s.c) pretreatment immediately after Sham operation (FWOP)	9.51±0.35	5.79*±0.62	5.96*±0.41	5.06*±1.25	4.17*±0.83	4.06*±0.62	3.24*±0.82
(VIII) Aminoguanidine (300 mg/Kg s.c) pretreatment immediately after RPAC (FWOP)	11.23±0.59	5.29*±0.45	4.02*±0.37	3.95*±0.48	2.79*±0.63	2.86*±0.94	2.71*±0.93
(IX) S-methyl isothiourea (3 mg/Kg i.v) pretreatment immediately after Sham operation (FWOP)	9.67±0.28	6.19*±1.02	5.39*±1.02	5.26*±0.63	3.82*±0.63	3.72*±0.49	3.43*±0.59
(X) S-methyl isothiourea (3 mg/Kg i.v)pretreatment immediately after RPAC (FWOP)	9.52±0.69	7.03*±0.54	6.08*±0.97	5.49*±0.37	4.94*±0.47	4.62*±0.92	3.49*±0.80
(XI) 1400W (1 mg/Kg i.v) pretreatment immediately after Sham operation (FWOP)	11.03±0.63	7.84*±0.81	3.83*±0.49	3.94*±1.05	2.68*±0.64	2.40*±0.67	1.97*±0.62
(XII) 1400W (1 mg/Kg i.v)pretreatment immediately after RPAC (FWOP)	9.97±0.96	5.86*±1.30	4.60*±0.28	3.98*±0.52	2.80*±0.27	2.73*±0.31	1.29*±0.53
(XIII) Sham (SWOP)	8.20±0.12	4.84*±1.22	5.26*±0.95	4.04*±1.34	2.06*±0.52	2.62*±0.28	1.02*±0.17
(XIV) RAPC (SWOP)	8.18±0.20	4.76*±0.93	5.20*±0.78	4.40*±1.09	4.06*±1.25	2.46*±1.04	1.30*±0.30
(XV) L-NAME (10 mg/Kg i.v) pretreatment immediately after Sham operation (SWOP)	10.46±0.64	6.04*±0.58	7.32*±0.30	4.78*±1.21	3.86*±0.94	3.04*±1.02	3.02*±0.41
(XVI) L-NAME (10 mg/Kg i.v) pretreatment 24 hr. after RAPC	11.92±0.28	5.49*±0.82	5.60*±0.25	3.93*±0.72	3.86*±0.63	2.97*±0.79	2.08*±0.53
(XVII) Aminoguanidine (150 mg/Kg s.c) pretreatment immediately after Sham operation (SWOP)	9.42±0.25	5.12*±0.49	4.82*±0.46	3.92*±0.52	2.80*±0.40	2.08*±0.44	1.28*±0.10
(XVIII) Aminoguanidine (150 mg/Kg s.c) pretreatment 24 hr. after RAPC	10.96±0.60	7.26*±0.92	7.68*±1.27	6.48*±1.18	4.20*±1.00	3.98*±0.89	2.18*±0.61
(XIX) Aminoguanidine (300 mg/Kg s.c) pretreatment immediately after Sham operation (SWOP)	10.80±1.27	7.90*±1.03	7.79*±0.22	5.42*±0.23	4.54*±0.74	3.64*±0.62	3.50*±0.83
(XX) Aminoguanidine (300 mg/Kg s.c) pretreatment immediately after RPAC (SWOP)	11.82±0.44	5.92*±0.39	5.62*±1.20	5.39*±0.85	3.03*±0.59	2.91*±0.29	2.84*±0.67
(XXI) S-methyl isothiourea (3 mg/Kg i.v) pretreatment immediately after Sham operation (SWOP)	9.07±0.73	4.97*±0.85	4.69*±0.82	4.49*±0.46	4.06*±0.52	3.05*±0.85	2.94*±1.03
(XXII) S-methyl isothiourea (3 mg/Kg i.v)pretreatment immediately after RPAC (SWOP)	10.57±0.59	5.70*±0.31	5.62*±0.73	5.20*±0.72	3.98*±0.83	2.74*±0.94	2.79*±0.47
(XXIII) 1400W (1 mg/Kg i.v) pretreatment immediately after Sham operation (SWOP)	9.73±1.34	6.39*±0.76	6.17*±0.68	4.92*±0.64	2.99*±0.95	2.82*±0.42	1.74*±0.29
(XXIV) 1400W (1 mg/Kg i.v)pretreatment immediately after RPAC (SWOP)	8.94±1.42	5.89*±1.07	5.18*±0.87	4.05*±0.77	3.97*±1.18	3.32*±0.79	2.59*±1.02

Vales are mean ± SEM (n=6). Coronary flow rate was measured after stabilization (basal), immediately (Imm.R), 5 (5min.R), 15 (15 min.R), 30 (30 min. R.), 60 (60 min. R.) and 120 (120 min. R.) min after reperfusion (R). *P<0.05 vs Basal, Sham denotes sham operated.

Table 2: Acute Effect (FWOP) and delayed effect (SWOP) of remote preconditioning by aortic constriction (RPAC) and NOS inhibitors Pretreatment on Heart Rate (beats/min) in isolated Rat Heart Subjected to Global Ischaemia (30 min) and Reperfusion (120 min).

Groups	BASAL	5 min R.	15 min.R.	30 min.R.	60 min.R.	120 min.R.
(I) Sham (FWOP)	205±30	144*±20	174*±20	146*±15	132*±10	126*±15
(II) RAPC (FWOP)	228±35	120*±30	180*±30	144*±30	156*±30	114*±19

(III) L-NAME (10 mg/Kg i.v) pretreatment immediately after Sham operation (FWOP)	226±18	146*±31	164*±08	152*±13	143*±22	111*±25
(IV) L-NAME (10 mg/Kg i.v) pretreatment immediately after RPAC (FWOP)	207±22	122*±13	178*±26	143*±28	140*±07	119*±32
(V) Aminoguanidine (150 mg/Kg) pretreatment immediately after Sham operation (FWOP)	182±12	124*±23	142*±15	136*±15	138*±20	124*±20
(VI) Aminoguanidine (150 mg/Kg) pretreatment immediately after RAPC (FWOP)	216±26	120*±19	114*±17	132*±08	120*±10	112*±10
(VII) Aminoguanidine (300 mg/Kg) pretreatment immediately after Sham operation (FWOP)	178±23	136*±22	159*±18	153*±26	134*±06	125*±13
(VIII) Aminoguanidine (300 mg/Kg) pretreatment immediately after RAPC (FWOP)	207±24	148*±24	136*±25	132*±14	122*±16	118*±19
(IX) S-methyl isothiourea (3 mg/Kg i.v) pretreatment immediately after Sham operation (FWOP)	182±19	147*±26	177*±28	141*±28	132*±24	131*±20
(X) S-methyl isothiourea (3 mg/Kg i.v.)pretreatment immediately after Sham operation (FWOP)	221±33	149*±31	168*±32	168*±21	152*±27	141*±28
(XI) 1400W (1 mg/Kg i.v) pretreatment immediately after Sham operation (FWOP)	233±21	130*±19	188*±25	171*±23	139*±22	102*±24
(XII) 1400W (1 mg/Kg i.v)pretreatment immediately after RPAC (FWOP)	194±16	127*±12	152*±34	166*±11	127*±30	126*±18
(XIII) Sham (SWOP)	228±30	120*±20	138*±10	139*±20	126*±14	113*±12
(XIV) RAPC (SWOP)	252±30	126*±15	180*±20	120*±08	114*±15	125*±10
(XV) L-NAME (10 mg/Kg i.v) pretreatment immediately after Sham operation (SWOP)	203±16	147*±31	179*±18	152*±13	133*±24	108*±16
(XVI) L-NAME (10 mg/Kg i.v) pretreatment immediately after RPAC (SWOP)	225±18	132*±24	158*±30	138*±20	143*±19	122*±21
(XVII) Aminoguanidine (150 mg/Kg s.c.) pretreatment 24 hr. after Sham operation	218±09	140*±12	146*±11	134*±05	123*±08	96*±13
(XVIII) Aminoguanidine (150 mg/Kg s.c.) pretreatment 24 hr. after RAPC	234±11	122*±16	152*±16	140*±11	144*±15	118*±07
(XIX) Aminoguanidine (300 mg/Kg s.c.) pretreatment immediately after Sham operation (SWOP)	223±15	146*±17	183*±27	159*±16	149*±07	123*±19
(XX) Aminoguanidine (300 mg/Kg s.c.) pretreatment immediately after RAPC (SWOP)	193±23	135*±21	177*±15	148*±19	113*±12	116*±07
(XXI) S-methyl isothiourea (3 mg/Kg i.v) pretreatment immediately after Sham operation (SWOP)	202±08	149*±29	169*±14	161*±27	151*±23	121*±14
(XXII) S-methyl isothiourea (3 mg/Kg i.v)pretreatment immediately after Sham operation (SWOP)	189±26	138*±06	168*±22	182*±06	147*±15	128*±10
(XXIII) 1400W (1 mg/Kg i.v) pretreatment immediately after Sham operation (SWOP)	253±07	128*±31	176*±25	173*±15	128*±19	108*±12
(XXIV) 1400W (1 mg/Kg i.v)pretreatment immediately after RPAC (SWOP)	199±28	142*±22	149*±17	141*±27	132*±06	126*±22

Vales are mean ± SEM (N=5). Heart rate was measured after stabilization (basal), 5 (5 min.R.), 15 (15 min. R.), 30 (30 min.R.), 60 (60 min. R.) and 120 (120 min. R.) min after reperfucion (R).
*P<0.05 vs Basal, Sham denotes sham operated.

Table - 3: Acute Effect (FWOP) of Remote Preconditioning by Aortic Constriction and NOS inhibitors Pretreatment on Lactate Dehydrogenase (LDH) Release in coronary Effluent of Isolated Rat Heart Subjected to Global Ischaemia (30 min) followed by Reperfusion (120 min).

L-NAME (10 mg/Kg i.v) pretreatment (FWOP)	Basal	Sham	197 ± 6.653
		RAPC	98.5 ± 4.976*
		L-NAME pretreatment in Sham	180.833 ± 3.419
		L-NAME pretreatment in RAPC	179.5 ± 0.582*
	Immediate Reperfusion	Sham	1310.167 ± 22.511*
		RAPC	336 ± 10.906*
		L-NAME pretreatment in Sham	807.833 ± 10.406
		L-NAME pretreatment in RAPC	808.031 ± 4.379*
	30 minute Reperfusion	Sham	999 ± 34.438
		RAPC	207.833 ± 10.134*
		L-NAME pretreatment in Sham	902.833 ± 10.613
		L-NAME pretreatment in RAPC	896.243 ± 7.892*
Aminoguanidine (150 mg/Kg s.c.) pretreatment (FWOP)	Basal	Sham	197 ± 6.653
		RAPC	98.5 ± 4.976*
		Aminoguanidine(150 mg/Kg s.c.) pretreatment in Sham	180.833 ± 3.419
		Aminoguanidine(150 mg/Kg s.c.) pretreatment in RAPC	83.3 ± 0.601*
	Immediate Reperfusion	Sham	1310.167 ± 22.511*

		RAPC	336 ± 10.906*
		Aminoguanidine (150 mg/Kg s.c.)pretreatment in Sham	807.833 ± 10.406
		Aminoguanidine (150 mg/Kg s.c.)pretreatment in RAPC	347.333 ± 5.690*
	30 minute Reperfusion	Sham	999 ± 34.438
		RAPC	207.833 ± 10.134*
		Aminoguanidine(150 mg/Kg s.c.) pretreatment in Sham	902.833 ± 10.613
		Aminoguanidine(150 mg/Kg s.c.) pretreatment in RAPC	231.333 ± 6.448*
Aminoguanidine (300 mg/Kg s.c.) pretreatment (FWOP)	Basal	Sham	216.947 ± 2.756
		RAPC	106.621 ± 8.796*
		Aminoguanidine(300 mg/Kg s.c.) pretreatment in Sham	188.639 ± 12.137
		Aminoguanidine(300 mg/Kg s.c.) pretreatment in RAPC	198.723 ± 7.105
	Immediate Reperfusion	Sham	1402.172 ± 25.347*
		RAPC	342.926 ± 14.488*
		Aminoguanidine (300 mg/Kg s.c.) pretreatment in Sham	827.833 ± 17.786
		Aminoguanidine (300 mg/Kg s.c.) pretreatment in RAPC	346.342 ± 6.780*
	30 minute Reperfusion	Sham	1062.415 ± 24.368*
		RAPC	219.623 ± 15.462*
		Aminoguanidine (300 mg/Kg s.c.) pretreatment in Sham	1102.833 ± 15.134
		Aminoguanidine (300 mg/Kg s.c.) pretreatment in RAPC	230.372 ± 7.822*
S-methyl isothiourea (3 mg/Kg i.v) pretreatment FWOP)	Basal	Sham	239.452 ± 17.531
		RAPC	111.133 ± 12.673*
		S-methyl isothiourea pretreatment in Sham	179.937 ± 14.762
		S-methyl isothiourea pretreatment in RAPC	211.385 ± 14.178
	Immediate Reperfusion	Sham	1382.194 ± 23.848*
		RAPC	337.963 ± 19.863*
		S-methyl isothiourea pretreatment in Sham	836.623 ± 27.637
		S-methyl isothiourea pretreatment in RAPC	352.262 ± 13.840*
	30 minute Reperfusion	Sham	1069.415 ± 24.368*
		RAPC	223.377 ± 18.682*
		S-methyl isothiourea pretreatment in Sham	1104.373 ± 24.345
		S-methyl isothiourea pretreatment in RAPC	226.252 ± 17.223*
1400W (1 mg/Kg i.v) pretreatment (FWOP)	Basal	Sham	239.452 ± 17.531
		RAPC	111.133 ± 12.673*
		1400W pretreatment in Sham	185.357 ± 10.652
		1400W pretreatment in RAPC	223.539 ± 18.768
	Immediate Reperfusion	Sham	1382.194 ± 23.848*
		RAPC	337.963 ± 19.863*
		1400W pretreatment in Sham	836.623 ± 27.637
		1400W pretreatment in RAPC	352.262 ± 13.840*
	30 minute Reperfusion	Sham	1069.415 ± 24.368*
		RAPC	223.377 ± 18.682*
		1400W pretreatment in Sham	1131.468 ± 4.485
		1400W pretreatment in RAPC	231.723 ± 22.846*

LDH was estimated after stabilisation (Basal), immediately (Imm) and 30 min (30') after reperfusion, sham denoted that rats were not subjected to remote preconditioning by aortic constriction and RPAC denoted that rats were subjected to remote preconditioning by aortic constriction. Values are mean ± S.E.M. of six experiments. *= $p<0.05$ vs Sham as compared to its respective basal value.

Table - 4: Acute Effect (SWOP) of Remote Preconditioning by Aortic Constriction and NOS inhibitors Pretreatment on Lactate Dehydrogenase (LDH) Release in coronary Effluent of Isolated Rat Heart Subjected to Global Ischaemia (30 min) followed by Reperfusion (120 min).

L-NAME (10 mg/Kg i.v) pretreatment (SWOP)	Basal	Sham	207.294 ± 0.420
		RAPC	83.667 ± 0.529*
		L-NAME pretreatment in Sham	196 ± 1.358
		L-NAME pretreatment in RAPC	198.667 ± 0.831
	Immediate Reperfusion	Sham	902.583 ± 15.290*
		RAPC	131.806 ± 5.333*
		L-NAME pretreatment in Sham	821.463 ± 13.472
		L-NAME pretreatment in RAPC	792.762 ± 7.036
	30 minute Reperfusion	Sham	204.343 ± 5.533*
		RAPC	92.427 ± 1.587*
		L-NAME pretreatment in Sham	187.385 ± 6.333
		L-NAME pretreatment in RAPC	190.563 ± 3.416
Aminoguanidine (150 mg/Kg s.c.) pretreatment (SWOP)	Basal	Sham	199.667 ± 0.760
		RAPC	89.667 ± 0.667*
		Aminoguanidine(150 mg/Kg s.c.) pretreatment in Sham	193 ± 1.065
		Aminoguanidine(150 mg/Kg s.c.) pretreatment in RAPC	183.667 ± 0.333
	Immediate Reperfusion	Sham	852.5 ± 12.529*
		RAPC	101.333 ± 5.806*
		Aminoguanidine (150 mg/Kg s.c.)pretreatment in Sham	803.333 ± 11.427
		Aminoguanidine (150 mg/Kg s.c.)pretreatment in RAPC	783.667 ± 5.596
	30 minute Reperfusion	Sham	198.333 ± 8.815*
		RAPC	80.333 ± 1.358*
		Aminoguanidine(150 mg/Kg s.c.) pretreatment in Sham	177.5 ± 4.233
		Aminoguanidine(150 mg/Kg s.c.) pretreatment in RAPC	182.5 ± 2.141
Aminoguanidine (300 mg/Kg s.c.) pretreatment (SWOP)	Basal	Sham	207.294 ± 0.420
		RAPC	83.667 ± 0.529*
		Aminoguanidine(300 mg/Kg s.c.) pretreatment in Sham	196 ± 1.358
		Aminoguanidine(300 mg/Kg s.c.) pretreatment in RAPC	198.667 ± 0.831
	Immediate Reperfusion	Sham	902.583 ± 15.290*
		RAPC	131.806 ± 5.333*
		Aminoguanidine (300 mg/Kg s.c.) pretreatment in Sham	821.463 ± 13.472
		Aminoguanidine (300 mg/Kg s.c.) pretreatment in RAPC	792.762 ± 7.036
	30 minute Reperfusion	Sham	204.343 ± 5.533*
		RAPC	92.427 ± 1.587*
		Aminoguanidine (300 mg/Kg s.c.) pretreatment in Sham	187.385 ± 6.333
		Aminoguanidine (300 mg/Kg s.c.) pretreatment in RAPC	190.563 ± 3.416
S-methyl isothiourea (3 mg/Kg i.v) pretreatment (SWOP)	Basal	Sham	214.954 ± 14.230
		RAPC	102.340 ± 12.425*
		S-methyl isothiourea pretreatment in Sham	176 ± 11.538
		S-methyl isothiourea pretreatment in RAPC	189.727 ± 10.127
	Immediate Reperfusion	Sham	1002.583 ± 14.231*
		RAPC	124.318 ± 4.395*
		S-methyl isothiourea pretreatment in Sham	848.634 ± 11.437
		S-methyl isothiourea pretreatment in RAPC	824.527 ± 17.327
	30 minute Reperfusion	Sham	192.463 ± 13.334*
		RAPC	98.241 ± 13.552*

		S-methyl isothiourea pretreatment in Sham	192.331 ± 6.459
		S-methyl isothiourea pretreatment in RPAC	188.693 ± 12.156
1400W (1 mg/Kg i.v) pretreatment (SWOP)	Basal	Sham	207.294 ± 0.420
		RPAC	83.667 ± 0.529*
		1400W pretreatment in Sham	204.593 ± 1.871
		1400W pretreatment in RPAC	213.429 ± 0.361
	Immediate Reperfusion	Sham	902.583 ± 15.290*
		RPAC	131.806 ± 5.333*
		1400W pretreatment in Sham	816.361 ± 14.792
		1400W pretreatment in RPAC	832.642 ± 16.386
	30 minute Reperfusion	Sham	204.343 ± 5.533*
		RPAC	92.427 ± 1.587*
		1400W pretreatment in Sham	203.865 ± 19.592
		1400W pretreatment in RPAC	225.724 ± 12.426

LDH was estimated after stabilisation (Basal), immediately (Imm) and 30 min (30') after reperfusion, sham denoted that rats were not subjected to remote preconditioning by aortic constriction and RPAC denoted that rats were subjected to remote preconditioning by aortic constriction. Values are mean ± S.E.M. of six experiments. *= $p<0.05$ vs Sham as compared to its respective basal value.

Table 5: Acute Effect (FWOP) of Remote Preconditioning by Aortic Constriction and NOS inhibitors Pretreatment on Creatine Kinase (CK) Release in coronary Effluent of Isolated Rat Heart Subjected to Global Ischaemia (30 min) followed by Reperfusion (120 min).

L-NAME (10 mg/Kg i.v) pretreatment (FWOP)	Basal	Sham	20.167 ± 0.601
		RPAC	2.167 ± 1.078*
		L-NAME pretreatment in Sham	22.333 ± 1.116
		L-NAME pretreatment in RPAC	23.927 ± 0.475
	5 minute Reperfusion	Sham	140.5 ± 4.015*
		RPAC	30.5 ± 2.172*
		L-NAME pretreatment in Sham	126.5 ± 3.731
		L-NAME pretreatment in RPAC	133.5 ± 2.232
Aminoguanidine (150 mg/Kg s.c.) pretreatment (FWOP)	Basal	Sham	20.167 ± 0.601
		RPAC	2.167 ± 1.078*
		Aminoguanidine(150 mg/Kg s.c.) pretreatment in Sham	22.333 ± 1.116
		Aminoguanidine(150 mg/Kg s.c.) pretreatment in RPAC	07 ± 0.683*
	5 minute Reperfusion	Sham	140.5 ± 4.015*
		RPAC	30.5 ± 2.172*
		Aminoguanidine (150 mg/Kg s.c.)pretreatment in Sham	126.5 ± 3.731
		Aminoguanidine (150 mg/Kg s.c.)pretreatment in RPAC	43.5 ± 2.232
Aminoguanidine (300 mg/Kg s.c.) pretreatment (FWOP)	Basal	Sham	20.167 ± 0.601
		RPAC	2.167 ± 1.078*
		Aminoguanidine(300 mg/Kg s.c.) pretreatment in Sham	22.333 ± 1.116
		Aminoguanidine(300 mg/Kg s.c.) pretreatment in RPAC	07 ± 0.683*
	5 minute Reperfusion	Sham	140.5 ± 4.015*
		RPAC	30.5 ± 2.172*
		Aminoguanidine (300 mg/Kg s.c.) pretreatment in Sham	126.5 ± 3.731
		Aminoguanidine (300 mg/Kg s.c.) pretreatment in RPAC	59.5 ± 2.232*
S-methyl isothiourea (3 mg/Kg i.v) pretreatment (FWOP)	Basal	Sham	22.627 ± 0.652

		RPAC	3.263 ± 1.738*
		S-methyl isothiourea pretreatment in Sham	24.333 ± 1.346
		S-methyl isothiourea pretreatment in RPAC	08.934 ± 0.832*
	5 minute Reperfusion	Sham	152.563 ± 3.515*
		RPAC	33.725 ± 4.259*
		S-methyl isothiourea pretreatment in Sham	134.5 ± 4.318
		S-methyl isothiourea pretreatment in RPAC	36.5 ± 3.542
1400W (1 mg/Kg i.v) pretreatment (FWOP)	Basal	Sham	20.167 ± 0.601
		RPAC	2.167 ± 1.078*
		1400W pretreatment in Sham	22.333 ± 1.116
		1400W pretreatment in RPAC	07 ± 0.683*
	5 minute Reperfusion	Sham	140.529 ± 4.015*
		RPAC	63.084 ± 2.172*
		1400W pretreatment in Sham	126.821 ± 3.731
		1400W pretreatment in RPAC	67.284 ± 2.232

LDH was estimated after stabilisation (Basal), immediately (Imm) and 30 min (30') after reperfusion, sham denoted that rats were not subjected to remote preconditioning by aortic constriction and RPAC denoted that rats were subjected to remote preconditioning by aortic constriction. Values are mean ± S.E.M. of six experiments. *= $p<0.05$ vs Sham as compared to its respective basal value.

Table 6: Delayed Effect (SWOP) of Remote Preconditioning by Aortic Constriction and NOS inhibitors Pretreatment on Creatine Kinase (CK) Release in coronary Effluent of Isolated Rat Heart Subjected to Global Ischaemia (30 min) followed by Reperfusion (120 min).

L-NAME (10 mg/Kg i.v) pretreatment (FWOP)	Basal	Sham	40.167 ± 2.937
		RPAC	18.333 ± 2.565*
		L-NAME pretreatment in Sham	38.167 ± 2.428
		L-NAME pretreatment in RPAC	23.421 ± 1.751*
	5 minute Reperfusion	Sham	233.5 ± 19.155*
		RPAC	43.5 ± 2.012*
		L-NAME pretreatment in Sham	203 ± 9.856
		L-NAME pretreatment in RPAC	121.647 ± 12.246*
Aminoguanidine (150 mg/Kg s.c.) pretreatment (FWOP)	Basal	Sham	40.167 ± 2.937
		RPAC	18.333 ± 2.565*
		Aminoguanidine(150 mg/Kg s.c.) pretreatment in Sham	38.167 ± 2.428
		Aminoguanidine(150 mg/Kg s.c.) pretreatment in RPAC	36 ± 1.751
	5 minute Reperfusion	Sham	233.5 ± 19.155*
		RPAC	43.5 ± 2.012*
		Aminoguanidine (150 mg/Kg s.c.)pretreatment in Sham	203 ± 9.856
		Aminoguanidine (150 mg/Kg s.c.)pretreatment in RPAC	237.167 ± 3.842
Aminoguanidine (300 mg/Kg s.c.) pretreatment (FWOP)	Basal	Sham	40.167 ± 2.937
		RPAC	18.333 ± 2.565*
		Aminoguanidine(300 mg/Kg s.c.) pretreatment in Sham	38.167 ± 2.428
		Aminoguanidine(300 mg/Kg s.c.) pretreatment in RPAC	36 ± 1.751
	5 minute Reperfusion	Sham	233.5 ± 19.155*
		RPAC	43.5 ± 2.012*
		Aminoguanidine (300 mg/Kg s.c.) pretreatment in Sham	203 ± 9.856
		Aminoguanidine (300 mg/Kg s.c.) pretreatment in RPAC	237.167 ± 3.842

S-methyl isothiourea (3 mg/Kg i.v) pretreatment (FWOP)	Basal	Sham	43.617 ± 3.657
		RPAC	17.426 ± 4.585*
		S-methyl isothiourea pretreatment in Sham	40.524 ± 3.258
		S-methyl isothiourea pretreatment in RPAC	39.469 ± 2.541
	5 minute Reperfusion	Sham	236.5 ± 9.553*
		RPAC	45.483 ± 3.126*
		S-methyl isothiourea pretreatment in Sham	205.930 ± 10.567
		S-methyl isothiourea pretreatment in RPAC	229.167 ± 5.835
1400W (1 mg/Kg i.v) pretreatment (FWOP)	Basal	Sham	40.167 ± 2.937
		RPAC	18.333 ± 2.565*
		1400W pretreatment in Sham	37.616 ± 2.428
		1400W pretreatment in RPAC	36.83 ± 1.751
	5 minute Reperfusion	Sham	233.5 ± 19.155*
		RPAC	43.5 ± 2.012*
		1400W pretreatment in Sham	213 ± 9.856
		1400W pretreatment in RPAC	226.167 ± 2.242

LDH was estimated after stabilisation (Basal), immediately (Imm) and 30 min (30') after reperfusion, sham denoted that rats were not subjected to remote preconditioning by aortic constriction and RPAC denoted that rats were subjected to remote preconditioning by aortic constriction. Values are mean ± S.E.M. of six experiments. *= $p<0.05$ vs Sham as compared to its respective basal value.

Table-7: Acute Effect of Remote Preconditioning by Aortic Constriction and NOS inhibitors Pretreatment on Myocardial Infarct Size in Isolated Rat Heart Subjected to Global Ischaemia (30 min) followed by Reperfusion (120 min):

L-NAME (10 mg/Kg i.v) pretreatment (FWOP)	% Infract By Volume	Sham	41.167 ± 1.662
		RPAC	24.934 ± 1.238*
		Aminoguanidine pretreatment in Sham	45.5 ± 1.979
		Aminoguanidine pretreatment in RPAC	46.167 ± 0.872
	% Infract By Weight	Sham	42.5 ± 2.592
		RPAC	27.833 ± 1.662*
		Aminoguanidine pretreatment in Sham	47 ± 1.414
		Aminoguanidine pretreatment in RPAC	46.667 ± 1.308
Aminoguanidine (150 mg/Kg s.c.) pretreatment (FWOP)	% Infract By Volume	Sham	41.167 ± 1.662
		RPAC	24 ± 1.238*
		Aminoguanidine pretreatment in Sham	45.5 ± 1.979
		Aminoguanidine pretreatment in RPAC	30.167 ± 0.872*
	% Infract By Weight	Sham	42.5 ± 2.592
		RPAC	27.833 ± 1.662*
		Aminoguanidine pretreatment in Sham	47 ± 1.414
		Aminoguanidine pretreatment in RPAC	27.667 ± 1.308*
Aminoguanidine (300 mg/Kg s.c.) pretreatment (FWOP)	% Infract By Volume	Sham	42.274 ± 3.732
		RPAC	23.429 ± 2.368*
		Aminoguanidine pretreatment in Sham	46.425 ± 2.079
		Aminoguanidine pretreatment in RPAC	30.290 ± 0.977*
	% Infract By Weight	Sham	42.578 ± 2.392
		RPAC	28.333 ± 1.766*
		Aminoguanidine pretreatment in Sham	47.472 ± 1.741
		Aminoguanidine pretreatment in RPAC	26.733 ± 2.468*
S-methyl isothiourea (3 mg/Kg	% Infract By Volume	Sham	40.333 ± 4.724

i.v) pretreatment (FWOP)		RPAC	24.728 ± 2.383*
		Aminoguanidine pretreatment in Sham	45.541 ± 2.799
		Aminoguanidine pretreatment in RAPC	31.673 ± 1.726*
	% Infract By Weight	Sham	42.500 ± 3.924
		RAPC	26.833 ± 2.674*
		Aminoguanidine pretreatment in Sham	47.000 ± 2.414
		Aminoguanidine pretreatment in RPAC	26.667 ± 2.338*
1400W (1 mg/Kg i.v) pretreatment (FWOP)	% Infract By Volume	Sham	43.67 ± 1.662
		RAPC	24.859 ± 1.238*
		Aminoguanidine pretreatment in Sham	45.5 ± 1.979
		Aminoguanidine pretreatment in RPAC	44.167 ± 0.872
	% Infract By Weight	Sham	42.5 ± 2.592
		RPAC	27.833 ± 1.662*
		Aminoguanidine pretreatment in Sham	47 ± 1.414
		Aminoguanidine pretreatment in RPAC	48.667 ± 1.308

Sham denoted that rats were not subjected to remote remote preconditioning by aortic constriction and RPAC denoted that rats were subjected to remote preconditioning by aortic constriction. Values are mean ± S.E.M. of six experiments. * = $p<0.05$ vs Sham

Table-8: Delayed Effect of Remote Preconditioning by Aortic Constriction and NOS inhibitors Pretreatment on Myocardial Infarct Size in Isolated Rat Heart Subjected to Global Ischaemia (30 min) followed by Reperfusion (120 min):

L-NAME (10 mg/Kg i.v) pretreatment (SWOP)	% Infract By Volume	Sham	45.167 ± 1.424
		RPAC	24.5 ± 0.764*
		L-NAME pretreatment in Sham	45 ± 1.238 [a]
		L-NAME pretreatment in RPAC	45.333 ± 1.430 [a]
	% Infract By Weight	Sham	45 ± 0.966
		RPAC	24.167 ± 0.872*
		L-NAME pretreatment in Sham	45.333 ± 1.116 [a]
		L-NAME pretreatment in RPAC	47 ± 1.461
Aminoguanidine (150 mg/Kg s.c.) pretreatment (SWOP)	% Infract By Volume	Sham	45.167 ± 1.424
		RPAC	24.5 ± 0.764 [a]
		Aminoguanidine (150 mg/Kg s.c.) pretreatment in Sham	45 ± 1.238
		Aminoguanidine (150 mg/Kg s.c.) pretreatment in RPAC	45.333 ± 1.430
	% Infract By Weight	Sham	45 ± 0.966
		RPAC	24.167 ± 0.872*
		Aminoguanidine (150 mg/Kg s.c.) pretreatment in Sham	45.333 ± 1.116
		Aminoguanidine (150 mg/Kg s.c.) pretreatment in RPAC	47 ± 1.461
Aminoguanidine (300 mg/Kg s.c.) pretreatment (SWOP)	% Infract By Volume	Sham	44.675 ± 2.434
		RPAC	24.568 ± 1.767*
		Aminoguanidine (300 mg/Kg s.c.) pretreatment in Sham	45.783 ± 1.253
		Aminoguanidine (300 mg/Kg s.c.) pretreatment in RPAC	46.232 ± 1.630
	% Infract By Weight	Sham	45.024 ± 1.667
		RPAC	24.667 ± 0.762*
		Aminoguanidine (300 mg/Kg s.c.) pretreatment in Sham	45.933 ± 3.121
		Aminoguanidine (300 mg/Kg s.c.) pretreatment in RPAC	47.672 ± 2.346
S-methyl isothiourea (3 mg/Kg i.v) pretreatment (SWOP)	% Infract By Volume	Sham	48.528 ± 2.246
		RPAC	24.558 ± 1.674*

		S-methyl isothiourea pretreatment in Sham	45.000 ± 2.283
		S-methyl isothiourea pretreatment in RPAC	45.533 ± 4.436
	% Infract By Weight	Sham	45.824 ± 0.966
		RPAC	23.783 ± 0.724*
		S-methyl isothiourea pretreatment in Sham	45.333 ± 1.116
		S-methyl isothiourea pretreatment in RPAC	47.000 ± 4.244
1400W (1 mg/Kg i.v) pretreatment (SWOP)	% Infract By Volume	Sham	45.167 ± 1.424
		RPAC	24.5 ± 0.764*
		1400W pretreatment in Sham	45 ± 1.238
		1400W pretreatment in RPAC	45.333 ± 1.430
	% Infract By Weight	Sham	45 ± 0.966
		RPAC	24.167 ± 0.872*
		1400W pretreatment in Sham	45.333 ± 1.116
		1400W pretreatment in RPAC	47 ± 1.461

Sham denoted that rats were not subjected to remote preconditioning by aortic constriction and RPAC denoted that rats were subjected to remote preconditioning by aortic constriction. Values are mean ± S.E.M. of six experiments. * = $p<0.05$ vs Sham