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Research

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Cardioprotective Effect of Losartan Alone or in Combination with Remote Ischemic Preconditioning on the Biochemical Changes Induced by Ischemic/Reperfusion Injury in a Mutual Prospective Study with a Clinical and Experimental Animal Arm

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ABSTRACT

Background and Aims: Losartan is an angiotensin receptor II antagonist used clinically to reduce blood pressure and treat hypertension or hypertrophic cardiomyopathy. It is also proposed to suppress cardiac injury following reperfusion. We evaluated the potential effect of Losartan alone and in combination with remote ischemic preconditioning (RIPC), an established treatment, on the biochemical changes induced by ischemic/reperfusion injury (I/R) in both humans and rabbits.

Methods: Thirty consecutive patients undergoing elective percutaneous coronary intervention (PCI) were divided into three groups (10 each): group 1 (control group without any preconditioning), group 2 (patients who were maintained on losartan (50-100 mg /day) for one month before PCI), group 3 (similar to group 2 but PCI was preceded by RIPC). On the other hand, sixty adult male New Zealand white rabbits were divided into 6 groups (10 each): group I (control), group II (sham), group III (I/R as 30 min ischemia followed by 120 min reperfusion), group IV (regular losartan 20 mg/kg for 40 days orally followed by I/R), group V (I/R preceded by RIPC) and group VI (similar to groups IV but I/R was preceded by RIPC). Tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6), nitric oxide (NO), troponin I (cTnI), creatine kinase MB (CK-MB) and C-reactive protein (CRP) were measured in blood for all study groups.

Results: Clinical and experimental parts showed that groups with RIPC combined with losartan pretreatment enhanced cardioprotective effects of RIPC against I/R injury as evidenced by significant reduction ($p<0.001$) in the levels of IL-6 and cTnI; also the level of NO was significantly ($p<0.$ 0.001) increased compared with other groups. However, this effect was not significant regarding the level of TNF-α, CK-MB nor CRP.

Conclusions: Pretreatment with losartan enhances the cardioprotective effects of RIPC in ischemic reperfusion injury.

KEY WORDS: Ischemic reperfusion; Losartan; Remote ischemic preconditioning; Biochemical markers; PCI.

ABBREVIATIONS: RIPC: Remote ischemic preconditioning; cTnI: troponin I; NO: Nitric Oxide; IL-6: Interleukin-6; TNF-α: Tumor Necrosis Factor- α; CRP: C-reactive protein; PCI: Percutaneous Coronary Intervention; CAD: Coronary Artery Disease; ROS: Reactive Oxygen Species; I/R: Ischemic Reperfusion.

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INTRODUCTION

Sudden reperfusion of the ischemic myocardium, initiates a cascade of cellular events that lead to additional cellular damage and death called ischemic reperfusion (I/R) injury.[1] Investigational studies have identified several factors that mediate the harmful effects of I/R injury such as the pH paradox**,** intracellular and mitochondrial Ca^{2+} overload**,** accumulation of reactive oxygen species **(**ROS) due to massive input of oxygen by reperfusion and down regulation of basal nitric oxide (NO) in the cell.[2-4] Percutaneous coronary intervention (PCI) has become the principal coronary revascularization strategy for stable and unstable coronary artery disease (CAD) patients.[5-7] However, (I/R) injury post PCI may represent either an acceleration of the pathological processes initiated during ischemia or new pathophysiological changes that develop after reperfusion. The injury can mediate cell death, microvascular damage, myocardial stunning, and potentially lethal arrhythmias which worsen the clinical outcomes after PCI.[8,9]

Ischemic preconditioning (IPC) concept is now well known[10] where the application of brief episodes of ischemia reperfusion before sustained ischemia creates a condition of adaptive responses of cardiac cells to various kinds of stress induced by I/R.[6] Further research in the area led to the development of remote ischemic preconditioning (RIPC) technique where the application of brief cycles of ischemia reperfusion in organs remote from the tissue of sustained ischemia can reduce the I/R injury in that ischemic tissue on reperfusion.[11] In addition, some drugs which have unique pleotropic effects presented as anti-inflammatory, immunomodulatory and anti-oxidative properties have been proposed to reduce myocardial I/R injury. Examples are statins and angiotensin II receptor blockers (ARBs).[12-14]

In our first report, we evaluated the cardioprotective effects of the statin atorvastatin.[15] This second part of our study aimed to evaluate a possible cardioprotective effect of the angiotensin II receptor blocker, Losartan, alone and in combination with RIPC on patients undergoing PCI and in experimental rabbits which were exposed to I/R injury. The serum levels of the biochemical parameters interleukin-6 (IL-6), Tumor necrosis factor-α (TNF-α), C-reactive protein (CRP), Troponin I (cTnI), nitric oxide (NO), and Creatine kinase MB (CK-MB) were assessed as biochemical markers of cardiac injury and inflammation.

MATERIALS AND METHODS

Clinical Part

Patient recruitment: As presented in details previously,[15] we performed a prospective non-randomized parallel groups comparison at the catheterization laboratory of Assiut University Hospitals (AUH).

Patients between 18 and 80 years of age, scheduled to undergo an elective PCI and able to give an informed consent were eligible for enrollment in the study (Table 1). Elective PCI was defined as any coronary revascularization in a low-risk, haemodynamically stable patient who presents to the facility for a planned PCI or for a coronary angiogram followed by ad hoc PCI. Exclusion criteria were (1) emergency PCI; (2) diabetic patients on regular treatment (3) nicorandil or glibenclamide use; (4) peripheral vascular disease affecting upper limbs; and (5) patients with severe renal impairment or on regular dialysis and (6) patients not taking Losartan 50-100 mg for at least one month pre PCI in patients groups, (7) more than 3 hours time gap between RIPC application and the PCI procedure.

Consecutive patients undergoing elective PCI between September 2014 and April 2015, were invited to participate in the study during their attendance at a routine preadmission clinic. Thirty eligible patients, after obtaining informed consent, were systematically allocated to the study groups according to our daily based allocation technique.

Study design: Patients were divided into three groups (10 patients in each group):

Group 1: Control group which includes patients who were exposed to PCI without any preconditioning.
Group 2: Patients who were maintained on Losartan at a dose 50-100 mg/day for at least one month before undergoing PCI.
Group 3: Patients who were maintained on Losartan at a dose 50-100 mg/day for at least one month before the day of application of PCI technique. At that day, PCI was preceded by RIPC.

The study protocol was approved by the Ethical Committee of Assist Faculty of Medicine and a written informed consent was obtained from all participants. The consent form was designed with an explanation on the purpose and conduction of this research study.

Induction of remote ischemic preconditioning (RIPC): RIPC was induced by inflating a standard 9 inch blood pressure cuff to 200 mmHg on the left upper arm for five minutes (transient ischemia) for three cycles and each was separated by a reperfusion period of five minutes, during which the cuff was kept deflated.[16] The protocol was commenced on the day of PCI procedure and was timed around an hour before the intervention procedure. If the intervention was delayed more than three hours after RIPC, the patient was excluded from the study as the endothelial protection conferred by RIPC disappears after 4 hours of preconditioning stimulus.[16]

PCI was performed using femoral approach in all our patients using 6 French guiding catheters. Participants were pre-treated with aspirin 150 mg and clopidogrel 600 mg orally before the procedure, and intra procedural intravenous heparin bolus 10,000 IU. Glycoprotein IIb/IIIa inhibitors, stent implantation and all other medication was given at the discretion of the primary operator, adhering to best conventional clinical practice. Participants were managed conventionally following PCI, including a period of in-patient observation, with 150 mg/day

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Table 1: Demographic and Clinical Data of Patients used Losartan Alone or in Combination with Remote Ischemic Preconditioning and Control Groups before Percutanious Coronary Intervention.

Variable	Control PCI Group 1 (N=10)	Losr. Group 2 (N=10)	Losr. +RIPC Group 3 (N=10)	*p*
Age (years)	56±10	56±8	57±8	NS
Male Gender, n (%)	6 (60%)	6 (60%)	8 (80%)	NS
Risk factors				
Smoking, n (%)	5 (50%)	7 (70%)	6 (60%)	NS
Hypertension, n (%)	4 (40%)	4 (40%)	5 (50%)	NS
Dyslipidemia, n (%)	3 (30%)	4 (40%)	4 (40%)	NS
BMI, median (IR)	27.1 (25.1-30.2)	26.3 (25-30.1)	26.2 (25 -30.1)	NS
Clinical details				
LVEF, %	59±9	60±10	60±10	NS
Time since the latest angina before PCI<24 h, n (%)	2 (20%)	2 (20%)	3 (30%)	NS
Angina CCS grade III/IV, n (%)	2 (20%)	3 (30%)	2 (20%)	NS
Hemoglobin (mg/dL)	12±2	12±3	12±3	NS
Base-line SCr, (mg/dL)	0.95±0.31	0.98±0.41	0.98±0.39	NS
Medications in last month, n (%)				
Aspirin therapy	10 (100%)	10 (100%)	10 (100%)	NS
Clopidogrel therapy	4 (40%)	6 (60%)	6 (60%)	NS
Losartan 50-100 mg	0	10 (100%)	10 (100%)	NS
Atorvastatin 80 mg	0	0	0	NS
Beta blockers	8 (80%)	9 (90%)	8 (80%)	NS

Data are presented as mean±standard deviation, number (%) of patients or median (interquartile range). BMI: Body Mass Index; CCS: Canadian Cardiology Society; CKD: Chronic Kidney Disease; CABG: Coronary Artery Bypass Graft; Losr.: Losartan; LVEF: Left Ventricular Ejection Fraction; RIPC: Remote Ischemic Preconditioning; NS: Not Significant; PCI: Percutanious Coronary Intervention; SCr: Serum Creatinine.

of aspirin and 75 mg/day of clopidogrel for 1 month in case of a bare-metal stent and for 1 year in case of a drug eluting stent as described in details in our previous report.[15]

Blood sampling and serum preparation: The blood samples were drawn from a blood vessel in the arm immediately before and 24 hr after PCI procedures. ELISA kits were used for determination of serum concentrations of IL-6 (Orgenium Laboratories, Finland), TNF-α (ASSAYPRO, USA), and Troponin-I (Monobind-inc., USA), C- reactive protein serum level was determined by latex agglutination test kit produced by Lab Care Diagnostics, India. Nitric oxide serum level was determined by kit produced by Biodiagnostic, Egypt, which is used for colorimetric determination of nitrite in serum. Creative kinas-MB serum level was determined by kit produced by Chema Diagnostic, Italy which is used for the spectrophotometric quantitative determination of CK- MB inserum or plasmas described in details in our previous report.[15]

Peri-procedural parameters: Without prior knowledge of the study allocation of the participants as described in details in our previous report.[15]

Angiographic parameters: The target vessel characteristics and the final result of PCI (predilation, postdilation, stent diameter, stent length and number) were noted. Angiographic lesion characteristics were classified. Preprocedural and postprocedural assessments of coronary blood flow (Thrombolysis in Myocardial Infarction flow score) were performed as described in details previously.[15] Other angiographic complications occurring during PCI (artery dissection, perforation, or jailed side branch with compromised flow) and contrast dose were noted.

Experimental Part

Animals: Adult male New Zealand white rabbits weighing 2000-2500 grams were purchased from the animal care unit of the Faculty of Medicine, Assiut University and cared for in compliance with the Guide for the Care and Use of Laboratory Animals published by National Institute of Health (NIH) as described in details in our previous report.[15] The experimental animal part of the study was approved by the Pharmacology Department Council of the Faculty followed by approval by the Ethics Committee of Assiut University, Assiut Governorate, Egypt.

Chemicals: Losartan was purchased as amosar tablet 100 mg from Amoun Drug Company, Egypt. The tablets were crushed and grinded by a mortar to form powder for suspensions in distilled water in concentrations 2% for Losartan which given to the rabbits orally (as mentioned below in the experimental design). Urethane 20% was used for anesthesia and it was dissolved in sterile saline before injection.

Experimental design: The rabbits were divided into 10 groups (n=10 each) as follows:

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Group I: Control which took distilled water.
Group II: Sham which was exposed to all surgical procedure except the induction of ischemia.
Group III: Model of myocardial ischemia reperfusion (i.e., I/R group).
Group IV: I/R preceded by remote ischemic preconditioning (RIPC) (i.e., RIPC+ I/R).
Group V: Losartan treated; the rabbits were administered Losartan orally once daily at a dose of 20 mg/kg for 6 weeks[12,13] followed by induction of I/R (i.e., Losartan + I/R).
Groups VI: were exactly similar to groups V in exposure to Losartan and I/R respectively I/R was preceded by RIPC.

Experimental Procedure

Anaesthesia and endotracheal intubation: Was described in details in our previous report.[15]

Induction of I/R injury: Left anterior descending (LAD) coronary artery occlusion and reperfusion were performed according to the method described before.[15]

Induction of RIPC: RIPC was carried out as described before.[15]

Blood sampling and biochemical analysis: As described in our primary report.[15]

Statistical Analysis

Data were expressed as mean±standard error. Statistical analysis was performed by one way ANOVA followed by *post hoc*-Tukey's test to compare more than two groups. In case of comparison of two different groups, unpaired student *t*-test was used. All statistical analyses were performed using Graph Pad Prism (version 5.0, Graph Pad Software Inc., San Diego, California, USA) and significance was considered at $p<0.05$.

RESULTS

Clinical Part

The 3 clinical groups were comparable regarding demographic, clinical and procedural characteristics as presented in Tables 1 and 2. Table 3 showed that patients who received pretreatment with Losartan before undergoing PCI in group 2 and patients who received the drug and exposed to RIPC before doing PCI in group 3 developed significant reduction in serum levels of IL-6, TNF-α and CRP associated with significant increase in serum level of NO compared with corresponding values in patients who were exposed directly to PCI only.The synergistic effects of adding RIPC to Losartan pretreatment in group 3 was more prominent in the form of significant reduction in the values of IL-6 and cTn compared to Losartan group only. On the other

Table 2: Angiographic and Periprocedural Data of Patients used Losartan Alone or in Combination with Remote Ischemic Preconditioning and Control Groups Before Percutaneous Coronary Intervention.

Parameter	Control PCI Group 1 (N=10)	Losr. Group 2 (N=10)	Losr.+RIPC Group 3 (N=10)	*p*
Angiographic parameters				
Target vessel, n (%)				
LM	0	0	0	
LAD	5 (50%)	5 (50%)	4 (40%)	NS
LCx	2 (20%)	2 (20%)	3 (30%)	NS
RCA	3 (30%)	2 (20%)	3 (30%)	NS
Side Branch >2mm, n (%)	4 (40%)	3 (30%)	4 (40%)	NS
CTO, n (%)	1 (10%)	0	1(10%)	NS
Subtotal occlusion, n(%)	2 (20%)	3 (30%)	2 (20%)	NS
Lesion type (AHA/ACC), n (%)				NS
A	4 (40%)	6 (60%)	5 (50%)	
B	4 (37%)	3 (30%)	3 (30%)	
C	2 (20%)	1 (10%)	2 (20%)	
PCI-related parameters				
Pre dilation	4 (40%)	4 (40%)	4 (40%)	NS
Post dilation	8 (80%)	8 (80%)	9 (90%)	NS
Number of DES	4 (40%)	5 (50%)	5 (50%)	NS
Mean number of stents per case	1.9±0.5	1.9±0.7	1.8±0.7	NS
Mean stent diameter per case, mm	3.0±0.7	2.9±0.8	3.0±0.8	NS

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Mean stent length per case, mm	31±17	30±17	30±17	NS
Contrast, mL	150±90	160±80	150± 80	NS
Glycoprotein IIb/IIIa inhibitors	2 (20%)	1 (10%)	1 (10%)	NS
Clinical state during angioplasty				
SBP, mm Hg	125 ± 20	132 ± 15	133 ± 17	NS
DBP, mm Hg	78±12	76±13	76±11	NS
HR, beats per minute	70±14	75±12	75±11	NS
After stent implantation, n (%)				
TIMI flow score 3	9(90%)	10(100%)	10 (100%)	NS

Data are presented as mean±standard deviation, or number (%) of patients. CTO: Chronic Total Occlusion; DBP: Diastolic Blood Pressure; DES: Drug Eluting Stents; HR: Heart Rate; LAD: Left Anterior Descending Artery; LCx: Left Circumflex Artery; LM: Left Main Artery; Losr.: Losartan; NS: Not Significant; PCI: Percutaneous Coronary Intervention; RCA: Right Coronary Artery; RIPC: Remote Ischemic Preconditioning; SBP: Systolic Blood Pressure; TIMI: Thrombolysis in myocardial infarction.

Table 3: Effect of Losartan Alone or Preceded with Remote Ischemic Preconditioning on Serum Levels of Different Biomarkers in Patients Undergoing Percutaneous Coronary Intervention Compared with Control Group.

Parameters	PCI (Group 1)	Losartan+PCI (Group 2)	Losartan +RIPC+PCI (Group 3)	Statistical analysis			
				ANOVA#		Post hoc##	
				F	*p* value	(Tukey's test) a	b
IL-6 (pg/mL)	16.24±0.54	13.65±0.55*	8.86±0.49	51.61	0.002	0.002	0.02
TNF-α (pg/mL)	35.11±0.57	17.5±0.88*	26.17±0.51	53.86	<0.001	0.01	<0.001
CRP (mg/L)	16.80±1.96	8.40±1.83*	8.80±1.93	54.68	<0.001	<0.001	0.31
cTnI (ng/mL)	2.80±0.09	2.50±0.11	1.03±0.09	54.55	0.01	<0.001	<0.001
NO(M mol/L)	46.62±1.29	52.96±0.95*	57.07±0.61	38.00	<0.001	<0.001	<0.001
CK-MB (U/L)	75.79±3.59	73.50±1.19	72.67±1.86	34.52	0.85	0.85	0.47

Results are represented as mean±SE, n=10 for each group.
ANOVA test between the three groups followed by *posthoc* Tukey's test
group 3 *versus*: group 1 [a], group 2 [b]
* Unpaired *t*-test between group 1 *vs*. group 2 Significance at $p<0.05$

hand significant increase in NO production in the group 3 compared to group 1 and 2 ($p<0.001$) (Figure 1). However, no treatment had any effect on levels of CK-MB compared to the control group.

Experimental Part

The administration of Losartan alone for 6 weeks before induction of I/R achieved significant reduction in the serum levels of IL-6, TNF-α and CRP, associated with significant elevation in NO level (Table 4). cTnI and CK-MB however, were unchanged. These findings collectively suggest a possible biochemical cardioprotective effect of Losartan alone against I/R injury.

Table 5 illustrated that the application of RIPC before induction of I/R induced significant reduction of IL-6, TNF-α while NO serum level significantly increased in comparison with their corresponding values in I/R group. Figure 2, showed that the addition of RIPC to Losartan before induction of I/R compared to Losartan only, achieved positive addition to its effect on the serum levels of different biochemical parameters mainly IL-6, TNF-α, CRP, cTnI and NO.

DISCUSSION

The main finding of the present study with its clinical and experimental animal parts, were that Losartan in addition to RIPC have beneficial cardioprotective effect against ischemic reperfusion injury, assessed by measurement of circulating markers. This beneficial effect is defined from the biochemical point of view through reduction of some inflammatory markers associated with cardiac injury like IL-6, TNF-α and CRP associated with increase in NO level.

The changes reported in our study as regards the serum levels of different biochemical parameters in the I/R model compared with control and sham group coincide with I/R injury and previous studies reported similar results.[17,18]

In our study; Losartan pretreatment in animal model of I/Rat a dose of 20 mg/kg/day for 6 weeks before induction of I/R achieved a biochemical cardioprotective effect through reduction of I/R induced elevation of IL-6, TNF-α and CRP. Angiotensin II is described as a potent pro-inflammatory mediator causing up regulation of macrophages to induce inflam-

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Table 4: Effect of I/R Preceded by Daily Administration of Losartan 20 mg/kg for 6 Weeks on the Serum Levels of Different Biochemical Parameters in Comparison with I/R Group of Rabbits.

Parameters	I/R group	I/R preceded by losartan group	*p*-value#
IL-6 (pg/mL)	10.49±0.49	8.46±0.25 [b]	0.0026
TNF-α (pg/mL)	11.79±0.38	4.19±0.50 [b]	<0.0001
CRP (mg/L)	652.8±58.7	341.3±28.2 [b]	0.0002
cTnI (ng/mL)	1.13±0.04	1.12±0.04	0.86
NO (M mol/L)	27.59±0.60	30.59±0.66 [b]	0.004
CK-MB (U/L)	1173±53.3	1159±54.6	0.85

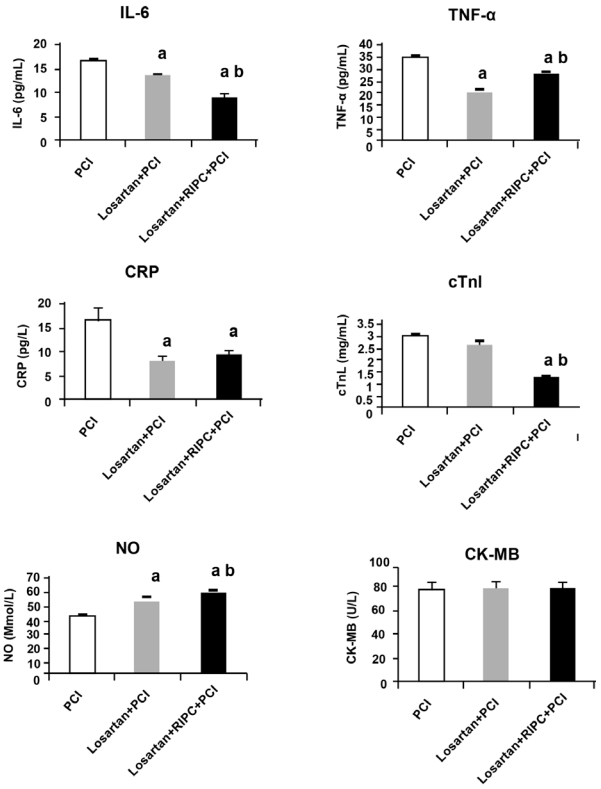
Results are represented as mean±SE, n=10 for each group.
[#] Unpaired *t*-test. Significance at $p<0.05$.[b]: *vs.* I/R group.

Table 5: Effect of I/R Preceded by 3 Cycles of RIPC on the Serum Levels of IL-6, TNF-α, CRP, cTnI, NO and CK-MB in Comparison with I/R Alone on Rabbits.

Parameters	I/R group	I/R preceded by RIPC group	*p*-value#
IL-6 (pg/mL)	10.49±0.49	8.38±0.32[b]	0.0089
TNF-α (pg/mL)	11.79±0.38	7.42±0.51[b]	<0.0001
CRP (mg/L)	652.8±58.7	704.0±64.0	0.58
cTnI (ng/mL)	1.13±0.04	1.13±0.06	0.96
NO (M mol/L)	27.59±0.60	32.54±0.87[b]	0.0003
CK-MB (U/L)	1173±53.3	1214±70.4	0.65

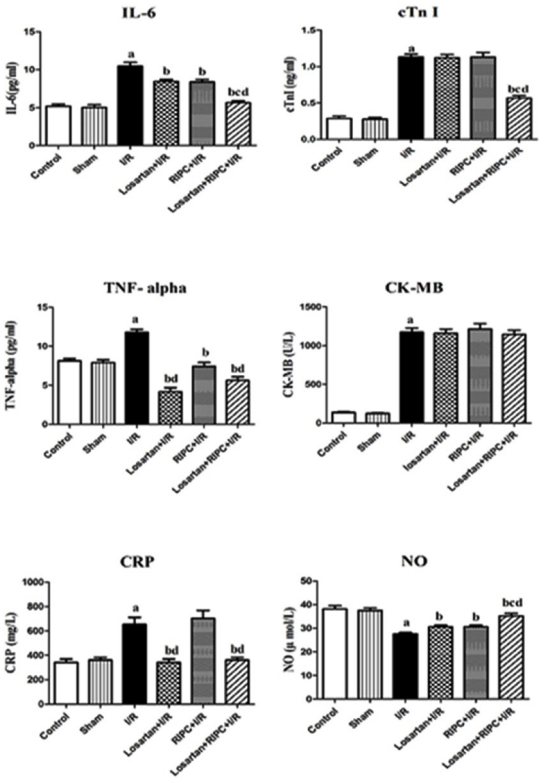
Results are represented as mean ± SE, n=10 for each group.
[#]Unpaired *t*-test. Significance at $p<0.05$.[b]: *vs.* I/R group.

Figure 1: The Effects of Pre-Treatment with Losartan (50-100 mg/day for at Least One Month) only and Plus Remote Ischemic Pre-Conditioning (RIPC) on Serum Levels of Different Biochemical Parameters in Patients undergoing Percutaneous Coronary Intervention (PCI).



IL-6: Interleukin 6, TNF-alpha: Tumor Necrosis Factor-alpha, CRP: C-Reactive Protein, cTnI: Cardiac troponin-I, NO: Nitric Oxide, CK-MB: Creatine kinase-MB. Results are represented as mean±SE, n=10 for each group. ANOVA test between all groups followed by post hocTukey's test. [a]=$p<0.05$ *vs.* PCI, [b]=$p<0.05$ *vs.* Losartan +PCI.

Figure 2: Influence of Remote Ischemic Pre-Conditioning (RIPC) Plus Pre-Treatment with Losartan on Biochemical Parameters in Ischemic/ Reperfusion (I /R) Animal Model.



IL-6: Interleukin 6, TNF-alpha: Tumor Necrosis Factor-alpha, CRP: C-Reactive Protein, cTnI: cardiac troponin-I, NO: Nitric Oxide, CK-MB: Creatine kinase-MB. Results are represented as mean±SE, ANOVA test between all groups: $p<0.05$, followed by post-hocTukey's test.
[a]=$p<0.05$ *vs.* control, [b]=$p<0.05$ *vs.* I/R,[c]=$p<0.05$ *vs.* Losartan+I/R, [d]=$p<0.05$ *vs.* RIPC+I/R

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matory cytokines during various pathological cardiovascular insults.[19] Furthermore, angiotensin II has been found to have a dual effect on macrophages through activation of the mitogen-activated protein kinase (MAPK) pathway and up-regulation of early growth response-1 (Egr-1) gene expression which both master the switch for vascular inflammatory responses.[20,21] So, by blocking Ang II signaling, Losartan reduces the inflammatory response as presented in different studies concerned with heart failure treatment outcomes[22-24] Losartan induced elevation of NO level in the animal model could reflect the ability of this drug to improve endothelial functions. Losartan by inhibiting angiotensin II receptor may decrease the release of ROS, keeping NO which improves the endothelial function.[25]

The elevated CK-MB and cTnI levels in I/R model reflected ischemic injury. Losartan pretreatment in our model did not show any change in either CK-MB or cTnI following I/R. This finding coincides with the findings of a previous study on rat model of I/R, losartan at a dose of 2 mg/kg administered intravenously 10 minutes before induction of ischemia did not affect either cTnI or CK-MB. On the contrary, lowering of CK-MB levels was reported in the effluent liquid of an isolated heart model linked to langend off reperfused apparatus where the heart was pretreated with valsartan before induction of I/R.[26] The discrepancy in losartan effect on CK-MB levels between the two animal models may be ascribed to the difference in study design where our model was *in vivo,* while the other study[26] was *in vitro*. *In vitro* model of I/R allowed direct perfusion of the ang II blocker (valsartan) to the heart while in our study losartan was administered orally, so *in vitro* model may achieve better availability of the drug to the heart than *in vivo* model.

The change in cTnI serum level may require longer duration of reperfusion to be affected by these drugs at a measurable level and in our experiment the reperfusion period was limited by the available conditions for survival of animals; however, in the clinical part the level of cTnI level was significantly reduced in the pretreated group as it was measured 24 hr post PCI which is the peak elevation of troponin post I/R.[27] However, the synergistic effects of Losartan with RIPC compared to Losartan alone were able to reduced cTnI levels.

In this part of the study, the application of RIPC before induction of I/R showed possible beneficial biochemical effect against cardiac injury either in the experimental or clinical parts also as previously reported in our 1st report.[15] It has been reported that application of RIPC before myocardial I/R suppresses the pro-inflammatory gene expression in circulating leukocytes leading to inhibition of cytokine synthesis, leukocyte chemotaxis, adhesion and migration, exocytosis, innate immunity signaling pathways, and apoptosis.[28] Onthe other hand, RIPC induces NOS expression leading to increase in NO levels with subsequent reoxygenation in post ischemic myocardium during reperfusion.[29-30]

It has been reported before that infants undergoing repair of simple congenital heart defects through open heart surgery-related cardiopulmonary bypass developed lowered serum concentrations of IL-6, IL-8, IL-10 and TNF-α when RIPC was induced pre-operatively *via* three cycles of 5 minutes of ischemia followed by 5 minutes reperfusion on the left upper arm using a blood pressure cuff compared with control group who were exposed to that surgery without preceding RIPC.[31] Furthermore, a metaanalysis of randomized clinical trials to establish the cardioprotective effect of RIPC in patient on patients with myocardial infarction who were exposed to RIPC before PCI showed significant reduction in cardiac troponin level but no change in CRP.[32] Also the use of RIPC<2 h before PCI, reduces the incidence of PCI-related MI and improves ischemic symptoms in patients undergoing elective PCI in our previous report on 200 patients undergoing PCI.[33]

In the current study, the additive cardioprotective biochemical effects of RIPC combined with Losartanin the animal model of I/R and Losartanin the clinical part encourages a suggestion to use the RIPC plus pharmacological preconditioning (Losartan in this study) for cardiac patients before doing PCI at least to guard against periprocedure cardiac injury.

CONCLUSIONS

The present study with its clinical and experimental animal parts revealed that pretreatment with Losartan combined with RIPC exert cardioprotective effects in both humans and animal models, reducing both inflammatory responses and myocardial damage.

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CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

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