LITERATURE REVIEW
2.0 LITERATURE REVIEW

2.1 Aspirin-ACE Inhibitor Interaction

Aspirin and angiotensin-converting enzyme (ACE) inhibitors are widely used in combination to treat a wide spectrum of cardiac disorders. Aspirin through inhibition of platelet aggregation has become a cornerstone in the treatment of acute coronary syndromes including myocardial infarction. On the basis of the results of several trials, aspirin has been widely recommended for both primary and secondary prevention. ISIS-2 demonstrated the benefit of aspirin in the first 35 days after myocardial infarction (ISIS-2 Collaborative group 1988; Baigent et al. 1998) and the Antiplatelet Trialists have reported benefit of long term aspirin use in the post myocardial infarction population (Antiplatelet Trialists’ Collaboration 1994).

In addition to their antihypertensive effect, ACE inhibitors through a number of actions including improved hemodynamics centered on systemic vasodilation, enhanced renal perfusion and function and arrhythmia suppression among others, have been shown to reduce morbidity and mortality in patients with heart failure as well as in patients after myocardial infarction (MI) with left ventricular dysfunction (The SOLVD Investigators 1991; Cohn et al. 1991; Consensus Trial Study Group 1987; Pfeffer et al. 1992).

However, several lines of evidence drawn from mechanistic considerations, animal studies and patient trials have challenged the acceptance of the simultaneous use of these therapies but evidence of harm or benefit with aspirin in patients with heart failure remains far from conclusive.

2.1.1 Theory behind the Aspirin and ACE Inhibitor Interaction

The exact mechanism of aspirin-ACE inhibitor interaction is unknown. One proposed theory relates to the counteractive pharmacodynamics of ACE inhibitors and aspirin.
ACE inhibitors attenuate the formation of angiotensin II and inhibit the degradation of kinins thus potentiating the biologic effects of kinins and enhancing production of prostaglandins. The biological effects of kinins are diverse and include the regulation of vascular remodeling, modulation of neuroendocrine activation (esp. adrenergic) and the response to tissue injury or stress (Linz et al. 1995a). Many of these effects are mediated by nitric oxide, prostaglandins or other autocrine-paracrine factors. Kinins and prostaglandins also appear to play an important role as counter regulatory agents in the pathophysiologic features of heart failure (Dzau et al. 1984). Thus aspirin, which will block prostaglandin production, and ACE inhibitors, which tend to increase prostaglandins have opposite effects. However the physiological sequel of the balance that is established between these agents was unknown and consequently the interaction of aspirin and ACE inhibitors has been the subject of investigations in both animal models and clinical trials.
Drug interaction between COX 1, COX 2 and ACE inhibitor

*Aspirin resistance and genetic polymorphism needs alternative treatment

(+): Stimulation; (-) Inhibition; COX 1 = cyclooxygenase 1; COX 2 = cyclooxygenase 2
PLA$_2$ = Phospholipase A$_2$; AA = Arachidonic Acid; PGI$_2$ = Prostaglandin I$_2$
2.1.2 Animal Models of Aspirin-ACE Inhibitor Interaction

The aspirin and ACE inhibitor interaction has been investigated in several different animal models with inconclusive results. Multiple *in vivo* and *ex vivo* experiments have demonstrated an adverse effect of aspirin on ACE inhibitor induced vasorelaxation. In isolated canine femoral artery segments aspirin partially blocked the endothelium-dependent relaxations by the ACE inhibitor captopril (Moroi et al. 1994). However, in a canine pacing model of heart failure, four days of low-dose aspirin (325 mg/day) had no measurable effect on the hemodynamic response to acute administration of enalaprilat (Evans et al. 1995).

Using rat models, other investigators have shown that aspirin administration can reduce the beneficial effects of ACE inhibition on post-MI ventricular remodeling (Stauss et al. 1994).

However a dog model of ischemia/reperfusion injury showed that aspirin did not affect the attenuation of myocardial stunning by ramiprilat (Rose et al. 1996).

2.1.3 Clinical Studies of Aspirin-ACE Inhibitor Interaction

The effects of the combination of aspirin and ACE inhibitors on the hemodynamics, renal function, pulmonary dynamics, and outcomes have also been examined in clinical studies.

Hall et al. (1992) performed a double blind, randomized, crossover study in 18 patients with ejection fractions less than 40% using pulmonary artery catheters to measure changes in central hemodynamics in response to acutely administered enalapril when given before, concomitant to or the day after a 350 mg dose of aspirin. Prior or concomitant treatment with aspirin abolished the enalapril induced decrease in left ventricular filling pressures, systemic and vascular resistances, and the increase in cardiac output.
Spaulding et al. (1998) also assessed the acute hemodynamic response to enalapril in 20 patients with stable but severe heart failure [New York Heart Association (NYHA) class II or IV] and ejection fraction < 35%. Patients were randomized to ticlopidine (500 mg), an antiplatelet agent that does not interact with prostaglandin synthesis, or aspirin (325 mg). Hemodynamic evaluation performed after 7 days of antiplatelet agents on the effects of enalapril revealed a significant reduction in systemic vascular resistance only in the ticlopidine group.

Guazzi and colleagues (1997a, b, 1999) demonstrated in a series of studies that aspirin interferes with the enalapril-induced improvements in pulmonary function tests and exercise tolerance in a cohort of patients with NYHA class II–III heart failure. Aspirin had no effect on changes in pulmonary function associated with either hydralazine-isosorbide dinitrate or losartan.

Other trials have not demonstrated a significant interaction effect of aspirin and ACE inhibitors on hemodynamics. In a randomized double blind, crossover design study of 13 patients in NYHA class II to IV with a mean left ventricular ejection fraction of 21%, there was no significant difference in the hemodynamic effect of acute administration of captopril (25 mg) in the presence or absence of acute concomitant aspirin (236 mg), despite significant reductions with aspirin administration in circulatory prostaglandin and thromboxane B\textsubscript{2} concentration (van Wijngaarden et al. 1994).

In a double blind study; 26 patients with mild to moderate hypertension receiving enalapril 20 mg twice a day and 26 patients with severe hypertension receiving enalapril 20 mg twice a day with both atenolol and long acting nifedipine were randomly assigned and crossed over to receive 5 days each of aspirin 100 mg/day, aspirin 300 mg/day and placebo. Aspirin 100 mg exerted no significant effect on blood pressure in either group when combined with enalapril. However, 58% of patients with mild to moderate hypertension and 50% of those with severe hypertension experienced greater than 20%
attenuations in mean arterial pressure with the 300 mg aspirin-enalapril combination compared with enalapril alone (Guazzi et al. 1998).

2.1.3.1 Evidence from Large Clinical Trials

2.1.3.1.1 Studies Supporting the Aspirin-ACE Inhibitor Interaction

 Studies of Left Ventricular Dysfunction (SOLVD)

SOLVD randomized 6797 patients with left ventricular systolic dysfunction (LVEF <35%) to receive either placebo or enalapril and showed reduced mortality and hospitalization for heart failure in enalapril treated patients (SOLVD Investigators 1991, 1992).

Subgroup analysis of SOLVD (Alkhadra et al. 1998) comparing event rates between 3017 patients receiving and 3495 not receiving antiplatelet agent (>95% receiving aspirin) showed mortality rates of 18.2% and 28.5% respectively at a mean follow up of 3-3.5 yrs. However, antiplatelet agents were of benefit primarily in patients taking placebo as opposed to those taking enalapril.

The combination of enalapril with an antiplatelet agent was not associated with a mortality benefit (1.00 hazard ratio 95% CI: 0.85-1.17) whereas combining placebo with an antiplatelet agent was (0.68 hazard ratio, 95% CI: 0.85-0.80). Similarly, the mortality benefit of enalapril was primarily in patients not taking antiplatelet agents (0.77 hazard ratio, 95% CI 0.67-0.87) as opposed to those who were (1.10 hazard ratio 95% CI: 0.93-1.30).

The Second Co-operative New Scandinavian Enalapril Survival Study (CONSENSUS II)

In a subgroup analysis of the Second Co-operative New Scandinavian Enalapril Survival Study (CONSENSUS II) involving 3044 patients with acute myocardial infarction, Nguyen et al. (1997) revealed that in the patients
taking aspirin at baseline, those randomized to receive enalaprilat had a 23% increase in 6-month mortality compared with placebo. Of the patients not taking aspirin at baseline those randomized to receive enalaprilat had a 14% decrease in 6-month mortality compared with placebo \( (p=0.047 \text{ for the aspirin enalaprilat interaction}) \). The risk of non fatal major events (myocardial infarction, worsening congestive heart failure) at 6 months was 8% less in patients taking enalapril-aspirin versus placebo-aspirin and 23% less in patients taking enalapril without aspirin compared with placebo without aspirin.

**Global Utilization of Streptokinase and Tissue Plasminogen Activator for Occluded Coronary Arteries (GUSTO I) and EPILOG trial (Evaluation in Percutaneous Transluminal Coronary Angioplasty to Improve Long-term Outcome with Abciximab Glycoprotein IIb/IIIa blocker)**

In a combined retrospective analysis from GUSTO I and EPILOG trial, Peterson et al. (2000) examined that the combined use of aspirin and ACE inhibitor was associated with increased mortality compared with patients treated with aspirin alone (3.3% vs 1.6% \( P<0.001 \) for GUSTO I and 3.7% vs. 1.2%; \( P<0.001 \) for EPILOG).

**The Acute Infarction Ramipril Efficacy (AIRE)**

The AIRE Study Investigators (1993) compared all-cause mortality in 1986 patients with acute myocardial infarction complicated by clinical evidence of heart failure that were randomized to receive either ramipril or placebo. At mean follow up of 15 months, subgroup analysis showed, for all cause mortality compared with placebo, relative hazard of 0.63 (95% CI: 0.43-0.95) in patients receiving ramipril alone and 0.78 (95% CI: 0.61-0.97) in those receiving ramipril-aspirin at randomization.
GISSI-3 study (1994) investigated the effect of lisinopril, transdermal nitroglycerin, and the combination of these two drugs versus standard treatment in patients with acute myocardial infarction. A subgroup analysis of 2790 diabetic patients receiving lisinopril with or without transdermal nitroglycerin showed, for six week mortality compared with control, an estimated odds ratio (OR) of 0.50 with lisinopril alone (99% CI: 0.27-0.90) versus 0.75 (99% CI: 0.50 – 1.1) with lisinopril-aspirin. A comparison of lisinopril with and without aspirin however, showed absolute mortality rates in favour of aspirin administration, 7.7% and 13.5% respectively (Zuanetti et al. 1997).

2.1.3.1.2 Studies Refuting the Aspirin- ACE inhibitor Interaction

The Captopril And Thrombolysis Study (CATS)

The CATS study (Oosterga et al. 1998) compared the infarct size and left ventricular end diastolic volume (LVEDV) after acute myocardial infarction in patients receiving captopril or placebo after streptokinase infusion with or without aspirin. Acute infarct size was comparable between patients taking and those not taking aspirin at baseline. The LVEDV at one year was also similar between patients receiving aspirin plus placebo and those receiving aspirin plus captopril. Thus low dose aspirin (80 or 100 mg) did not attenuate the short term or long term effect of ACE inhibitor after acute myocardial infarction.

The Fourth International Study of Infarct Survival (ISIS-4)

A subgroup analysis of Fourth International Study of Infarct survival (ISIS-4 Collaborative Group 1995) compared captopril with or without oral isosorbide mononitrate or intravenous magnesium, with placebo in 58,050 patients with acute myocardial infarction; 94% of patients of which were receiving
antiplatelet therapy at study entry. The mortality rate for patients receiving captopril-antiplatelet therapy was 6.7% vs. 7.2% for that receiving placebo-antiplatelet therapy. The mortality rate was 16.1% in patients receiving captopril without an antiplatelet agent.

The Heart Outcomes Prevention Evaluation (HOPE)

The HOPE Investigators (2000) did not demonstrate any negative ACE-inhibitor-aspirin interaction. Among 4645 patients treated with ramipril, 75% were receiving aspirin or other antiplatelet agents. Treatment with ramipril substantially lowered the risk of death, heart attack, stroke, coronary revascularization, heart failure and complications related to diabetes mellitus among patients who had evidence of vascular disease or diabetes plus one other cardiovascular risk factor. These benefits were observed whether or not patients were also taking aspirin or other antiplatelet agents.

The Benzafibrate Infarction Prevention (BIP) Trial

In BIP trial, mortality data of 11,575 patients with coronary artery disease were analyzed (Leor et al. 1999). A total of 1247 patients were receiving ACE inhibitor, out of which 50% were on aspirin. The five years mortality was lower among patients on ACE inhibitors and aspirin than patients on ACE inhibitors alone (19% vs. 27%). Cardiovascular mortality was also less in patients taking the combination (12% vs. 18%).

The Survival and Ventricular Enlargement (SAVE) trial

The SAVE trial was designed to study patients with left ventricular dysfunction (ejection fraction <40%). A subgroup analysis (Pfeffer et al. 1992) revealed that patients treated with captopril and aspirin had significantly less mortality compared with patients taking captopril alone (death from all causes, 16.6% vs. 26.0%).
Drug interaction studies between NSAIDs and drugs used in cardiovascular disorders

The Hypertension Optimal Treatment (HOT) study

The HOT study randomly assigned 18,790 hypertensive patients to receive felodipine 5-10 mg/d with aspirin 75mg/d or placebo (Hansson et al. 1998). Patients whose blood pressure was not adequately controlled with felodipine received either an ACE inhibitor (41%) or a beta blocker (28%) in addition to felodipine. At a mean follow up of 3.8 years, aspirin treatment resulted in significant reductions in major cardiovascular events by 15% (p= 0.03) and all myocardial infarction by 36% (P=0.002).

This trial indicated that, in terms of cardiovascular morbidity, low dose aspirin was not detrimental and may even be beneficial in hypertensive patients taking felodipine alone or with an ACE inhibitor or beta blocker.

2.2 COX-2 Inhibitors and the Cardiovascular System

Non-steroidal anti-inflammatory drugs (NSAIDs) are widely used for the treatment of joint inflammation and musculoskeletal injury. Specific COX-2 inhibitors offer promising benefits over older NSAIDs with regard to gastrointestinal safety while maintaining analgesic and anti-inflammatory efficacy. Rofecoxib, the most specific COX-2 inhibitor among the first generation of class was approved by the United States Food and Drug Administration (FDA) in May 1999, accounted for $ 2.5 billion in world wide sales in 2003. This is the first prescription drug since 2001 to be taken off the market for safety reasons. On September 30, 2004, Merck and Co. voluntarily withdrew rofecoxib from the market due to increased risk of cardiovascular events associated with the drug.

There are several other COX-2 inhibitors similar to rofecoxib on the market including celecoxib (celebrex) and a second generation of these agents with improved COX-2 selectivity including valdecoxib (Bextra), etoricoxib (Arcoxia), lumiracoxib (Prexige), parecoxib (Dynastat), deracoxib, tiracoxib and cimieoxib.

The development of COX-2 inhibitors as anti-inflammatory agents without gastric toxicity is based on the premise that COX-1 predominates in the gastric mucosa
and yields protective prostaglandins, whereas COX-2 is induced in inflammation and leads to pain, swelling and discomfort. However, selective COX-2 inhibitors decrease vascular prostacyclin (PGI₂) production and may affect the balance between prothrombotic and antithrombotic eicosanoids (Schmedtje et al. 1997). Unlike the platelet inhibition afforded by COX-1 inhibitors, COX-2 inhibitors do not share this salutary antithrombotic property. In contrast by decreasing vasodilatory and antiaggregatory PGI₂ production, COX-2 antagonists may shift the balance in favour of prothrombotic eicosanoids (e.g. ThromboxaneA₂) and may lead to increased cardiovascular thrombotic events (Belton et al. 2000).

Two major randomized trials, the Vioxx Gastrointestinal Outcomes Research Study (VIGOR) and the Celecoxib Long-Term Arthritis Safety Study (CLASS) have generated data on the trade-off between cardiovascular and gastrointestinal effects of these drugs. The results from VIGOR (Bombardier et al. 2000) showed that the relative risk of developing a confirmed adjudicated thrombotic cardiovascular event (myocardial infarction, unstable angina, cardiac thrombus, resuscitated cardiac arrest, sudden or unexplained death, ischemic stroke and transient ischemic attack) with rofecoxib treatment compared with naproxen was 2.38 (95%, CI: 1.39-4.00). The incidence of myocardial infarction was lower among patients in the naproxen group than among those in the rofecoxib group (0.1% vs. 0.4%). There was no significant difference in cardiovascular event (myocardial infarction, stroke and death) rates between celecoxib and nonsteroidal anti-inflammatory agents in CLASS (Silverstein et al. 2000). The annualized myocardial infarction rates for COX-2 inhibitors in both VIGOR and CLASS were significantly higher than that in the placebo group in a meta analysis of 23,407 patients in primary prevention trials (0.52%); 0.74% with rofecoxib and 0.8% with celecoxib compared with the placebo group (Mukherjee et al. 2001).

**APPROVe trial**: a long-term prospective, randomized placebo-controlled, double blind multicenter clinical trial to test rofecoxib in adenomatous polyposis prevention provided a more comprehensive picture of the cardiovascular safety profile of rofecoxib and led to the withdrawal of Vioxx by Merck. In 2600 patients,
rofecoxib 25 mg was compared with placebo in the prevention of recurrence of adenomatous polyps of the large bowel in patients with a history of colorectal adenomas. In total, 25 patients receiving placebo and 45 receiving rofecoxib demonstrated thromboembolic events (Davies et al. 2004). There were three absolute event rates per 400 patient years in the placebo group vs. six events per 400 patient years in the rofecoxib treatment group. These increased risk of confirmed serious thromboembolic events including heart attack and stroke appeared statistically evident at 18 months of chronic dosing.

2.2.1 Coxibs and Hypertension

The relative contributions of COX-1 and COX-2 isoforms in the production of prostaglandins that are important for blood pressure regulation are not known. Data from clinical trials of coxibs indicate that these agents have similar effects on blood pressure as traditional NSAIDs.

In trials of rofecoxib in patients with osteoarthritis, the incidence of hypertension reported as an adverse event by study investigators was 2.8% among patients treated with 12.5 mg/d dose and 4.0% among those treated with 25 mg/d. These incidences were similar to those observed with comparator NSAIDs – 2.9% with ibuprofen and 1.6% with diclofenac. In the VIGOR trial, the supratherapeutic dose of rofecoxib was associated with a 9.7% incidence of hypertension, compared with 5.5% in the naproxen group.

A potential caveat of these data is that hypertension was reported by investigators and blood pressure was objectively recorded only in a subset of patients. There is a great interpatient variability in terms of blood pressure response to therapy with rofecoxib. Most patients experience small changes in blood pressure and only a very small percentage have significant blood pressure changes. Among 699 patients enrolled in the osteoarthritis trials who had recorded blood pressure measurements and who were treated with rofecoxib, the mean changes from baseline in systolic and diastolic blood pressure were < 3 mm Hg and < 1 mm Hg respectively (FDA Advisory committee 2001).
However, there is limited information from prospective clinical trials addressing the blood pressure effects of coxibs, and more comparative data are needed.

Relatively minor decreases in diastolic blood pressure (5 to 6 mm Hg) have been shown to increase the risk of cardiovascular and cerebrovascular events by 15% and 67% respectively (Collins et al. 1990). Therefore, any potential effects of coxibs on blood pressure need to be clearly defined.

2.2.2 COX-2 Inhibitors and Vascular Physiology

Two prostanoids have major physiologic roles in the vasculature: TXA$_2$ and PGI$_2$ (prostacyclin). TXA$_2$ promotes platelet activation, vasoconstriction and smooth muscle proliferation. TXA$_2$ is mainly produced in platelets and its formation is increased upon platelet activation. Increased excretion of TXA$_2$ metabolites has been reported in patients with unstable angina during episodes of chest pain and has been associated with higher risk of major vascular events in patients with peripheral arterial obstructive disease. Prostacyclin is a vasodilator and a potent inhibitor of platelet aggregation. It is produced by macrovascular endothelial cells and is the major product of arachidonic acid metabolism when these cells are cultured in vitro (Caughey et al. 2001). In patients with unstable angina, prostacyclin synthesis is increased during episodes of chest pain and parallels increases in the synthesis of TXA$_2$ (Fitzgerald et al. 1986). Therefore, animal model and clinical data support the notion that prostacyclin mediates a local compensatory response limiting consequences of platelet activation.

Of the two COX isoforms only COX-1 is expressed in platelets (Patrignani et al. 1999). Therefore only those COX inhibitors which inhibit this isoform can inhibit synthesis of TXA$_2$ and have cardioprotective effects.

Both COX isoforms can be expressed in vascular endothelium. In unstimulated cultured endothelial cells, only COX-1 is detectable and TXA$_2$ is the predominant arachidonic acid metabolite produced (Bustos et al. 1997). Activation of endothelial cells by inflammatory stimuli or shear stress leads to expression of
COX-2 (Bustos et al. 1997; Okahara et al. 1998). As the overall COX activity of the endothelial cells increases, all prostanoids are produced at a higher rate. Kinetic activities of TX synthase, PGI synthase, and PGE synthase, the enzymes involved in metabolism of arachidonic acid downstream from the COX, have different kinetic properties and are saturated at different amounts of the substrate. Formation of TXA₂ in endothelial cells plateaus early, whereas prostacyclin and PGE₂ synthesis are increased to a greater degree (Caughey et al. 2001).

Studies in healthy volunteers show that treatment with COX-2 inhibitors decreases systemic production of prostacyclin with no effects on platelet-derived TXA₂ synthesis (McAdams et al. 1999). Therefore, under normal physiologic conditions, COX-2 seems to be induced in the vascular endothelium and appears to be the major determinant of systemic prostacyclin synthesis. In addition, the expression of COX-2 is increased in atherosclerotic plaques (Schonbeck et al. 1999).

2.3 Sodium Hydrogen Exchange and its role in Hypertension

The Na⁺/H⁺ exchanger (NHE) is an important extrusion system which participates in the regulation of cytosolic pH and cell volume under normal physiological conditions. The NHE represents one of the key mechanisms for restoring intracellular pH (pHᵢ) following ischemia induced acidosis by extruding protons concomitantly with Na⁺ influx in an electroneutral process. At present 6 NHE isoforms have been identified (termed NHE 1 to NHE 6) with the NHE 1 subtype representing the ubiquitous isoform (Karmazyn et al. 2001).

NHE 1 is predominantly localized at the intercalated disk region of atrial and ventricular myocytes in close proximity to the gap junction protein, connexin 43, and to a lesser extent along the transverse tubular system (Petrecca et al. 1999). NHE 2 is expressed in many tissues including intestine, kidney and adrenal gland (Tse et al. 1993). NHE 3 is most prominent in the kidney and gastrointestinal tract (Orlowski et al. 1992). NHE 4 occurs in stomach and NHE 5 is present in brain and testes (Wakabayashi et al. 1997). NHE 6 is present on mitochondrial membrane.
(Numuta et al. 1998). Based on its localization it has been suggested to participate in the regulation of mitochondrial calcium through Na\(^+\)/H\(^+\) and subsequent Na\(^+\)/Ca\(^{2+}\) exchange. NHE 1 to NHE 5 share approximately 34 to 60% amino acid homology whereas NHE 6 shares only 20% homology with the other isoforms.

NHE 1, which is the only isoform expressed on plasma membrane of cardiomyocytes, has been demonstrated to be involved in the pathobiology of several cardiovascular disorders.

NHE 1 activation is postulated to contribute to myocardial injury by Ca\(^{2+}\) overload. When NHE is activated, the simultaneous entry of Na\(^+\) during NHE activation probably represents an important route for increasing intracellular Na\(^+\) concentrations during various conditions. In the ischemic cell particularly, the activation of NHE by intracellular proton generation and the resultant entry of Na\(^+\) results in a potentially disastrous consequence as the excess Na\(^+\) can not be extruded because of depressed Na\(^+\)/K\(^+\) adenosine triphosphate (ATP)ase activity. As a result, the reduction in the transmembrane Na\(^+\) gradient will result in increased intracellular Ca\(^{2+}\) overload and cell death.

Several amiloride analogs nonselectively inhibit all isoforms of NHE and have been demonstrated to confer cardioprotection against ischemia-reperfusion injury in a number of experimental models with infarctions, contractility, enzyme release and arrhythmias as endpoint (Bugge and Ytrehus 1995; Meng et al. 1993; Sharma and Singh 2000).

To date there is much evidence to prove the hypothesis of widespread alterations in cell membrane structure and functions in primary hypertension. An enhancement of sodium proton exchanger activity is a frequently observed ion transport abnormality expressed in circulating cells from a subset of patients with essential hypertension (Rosskopf et al. 1993). Such alterations in membrane transport of ions have also been shown from lymphocytes (Feig et al. 1987) vascular smooth muscle cells (Berk et al. 1989) and erythrocytes of spontaneously hypertensive rats (Orlov et al. 1989).
Only marginal information is available regarding the effect of antihypertensive treatment on NHE activity in peripheral cells of hypertensive subjects. NHE activity is maintained during antihypertensive treatment despite significant reduction in blood pressure (Rosskopf et al. 1992) while quinapril is reported to restore the noted increase in NHE activity in lymphocytes isolated from hypertensive patients (Fortuno et al. 1997).

2.4 Echocardiography

Echocardiography is a noninvasive diagnostic technique that utilizes high frequency ultrasound (2.0-7.0 MHz) to evaluate structural, functional, and hemodynamic status of the cardiovascular system.

Two-dimensional echocardiography is able to visualize the heart directly in real time using ultrasound and provides instantaneous assessment of the myocardium, valves, pericardium, and great vessels. In this, the imaging is performed from multiple acoustic windows, viz. parasternal, apical, subcostal and suprasternal, using different transducer rotations so that the entire heart and great vessels can be displayed in real time and in various two-dimensional planes. Two-dimensional echocardiography is an ideal imaging modality for assessing left ventricular size and function. A qualitative assessment of the cavity size of the ventricle and systolic function can be made directly from the two-dimensional image by experienced observers.

M-mode echocardiography (one dimensional echocardiogram) is derived from the two-dimensional image for objective measurement of chamber size and function. M-mode is utilized for the measurement of dimension and is essential for the display of subtle motion abnormalities of specific cardiac structures. Quantitative assessment of left ventricular size and function can be made by M-mode echocardiography, (measuring systolic and diastolic dimensions of the short axis of the left ventricle) or quantitative two-dimensional echocardiography.
The presence or absence of regional wall motion abnormalities can be visually assessed by examining endocardial motion as well as wall thickening. M-mode and two-dimensional echocardiography are useful in the diagnosis of left ventricular hypertrophy, seen as an increase in wall thickness (Nishimura, Gibbons and Tajik 2003).

2.4.1 Doppler Echocardiography

Doppler echocardiography measures the velocity of moving red blood cells and has become a non invasive alternative to cardiac catheterization for assessment of hemodynamics. Pulsed-wave Doppler measures the blood flow velocity in a specific location on the two-dimensional echocardiographic image and displays the velocity in a spectral pattern using time as the x-axis. Continuous-wave Doppler echocardiography can measure high velocities of blood flow directed along the line of the Doppler beam such as occur in the presence of valve stenosis, valve regurgitation, or intracardiac shunts. These high velocities can be used to determine intracardiac pressure gradients by a modified Bernoulli equation.

\[
\text{Pressure change} = 4 \times (\text{Velocity})^2
\]

2.4.2 Assessment of Ventricular Function

2.4.2.1 Systolic Function

2.4.2.1.1 Global Function

Two parameters are most frequently used to express left ventricular (LV) global systolic function

1. Fractional Shortening or ejection fraction and
2. Cardiac output

Fractional shortening is a percent change in LV cavity dimension with systolic contraction and can be calculated from the following equation.
**Fractional Shortening**

\[
\text{Fractional Shortening} = \frac{\text{LVEDD} - \text{LVESD}}{\text{LVEDD}} \times 100\%
\]

Where \( \text{LVEDD} = \text{LV end diastolic dimension} \)

\( \text{LVESD} = \text{LV end systolic dimension} \)

Ejection fraction represents stroke volume as percent of end diastolic LV volume; hence its determination requires LV volume measurement.

\[
\text{Ejection Fraction} = \frac{\text{EDV} - \text{ESV}}{\text{EDV}} \times 100\%
\]

Where \( \text{EDV} = \text{end diastolic volume} \)

\( \text{ESV} = \text{end systolic volume} \)

**Cardiac output** is the product of stroke volume and heart rate. Stroke volume is determined by two-dimensional volumetric measurement as in calculation of ejection fraction or by Doppler method using LV outflow tract diameter and time velocity integral.

Flow across an orifice is equal to the product of the cross sectional area (CSA) of the orifice and flow velocity. Since flow velocity varies during a flow ejection period, individual velocities of Doppler spectrum need to be summed (integrated) to measure total flow during a given ejection period. The sum of velocities is called time velocity integral (TVI) and is equal to the area enclosed by the baseline and Doppler spectrum. TVI can be readily measured by the built-in calculation package in the ultrasound unit. Once TVI is determined, stroke volume (SV) is calculated by multiplying TVI by CSA. Cardiac output is obtained by multiplying stroke volume by heart rate. The most frequently utilized location for stroke volume determination is the left ventricular outflow tract (LVOT).
2.4.2.1.2 Regional Function

LV regional wall motion analysis is usually based on grading of contractility of individual segments. Based on the contractility of the individual segments a numerical scoring system has been adopted in which higher scores indicate more severe wall motion abnormality.

1 = normal, 2 = hypokinesis, 3 = akinesis, 4 = dyskinesis, 5 = aneurysmal

2.4.2.2 Diastolic Function

Diastole begins with myocardial relaxation at the end of systolic contraction (aortic valve closure) and ventricular pressure starts to decline (isovolumic relaxation). When the left atrial pressure exceeds the LV pressure, the mitral valve opens to allow an early (E) rapid filling phase of the left ventricle. The left ventricular pressure continues to decline after the onset of E, but soon increases again with continuous ventricular filling equalizing with left atrial pressure, resulting in a period of diastasis.

Another bolus of ventricular filling occurs with left atrial contraction (A). Therefore, diastole is divided into four phases.

1. Isovolumetric relaxation time (IVRT) (Aortic valve closure to mitral valve opening)
2. Rapid filling phase
3. Diastasis
4. Atrial filling phase

The transmitral pressure changes during diastole are reflected by pulsed-wave Doppler mitral flow velocities. Mitral flow velocities are measured by pulsed-wave Doppler with the sample volume placed between the leaflet tips, and the following diastolic filling parameters are derived; IVRT, early filling velocity (E), late filling velocity (A), and deceleration time (DT) of E (Oh, Seward and Tajik 1994).
Based on the Doppler velocity patterns the diastolic filling abnormalities can be classified into:

1. Relaxation abnormality
2. Restrictive Physiology
3. Pseudonormalization

**Abnormal Relaxation**

When myocardial relaxation is the predominant diastolic abnormality, IVRT is prolonged and the initial decline in LV pressure is slow. Hence, early filling is reduced and there is a large compensatory filling with atrial contraction. The ventricular muscle continues to relax even after the opening of the mitral valve, and it takes longer to equalize ventricular pressure with atrial pressure, resulting in a longer DT. Therefore abnormal myocardial relaxation is characterized by a constellation of abnormalities consisting of:

1. Prolong IVRT (≥ 110 sec.)
2. Low E velocity and high A velocity
3. Reversed E/A ratio (<1.0)
4. Prolonged DT (≥ 240 Sec)

**Restrictive Physiology**

When ventricular compliance is decreased the rise in ventricular diastolic pressure is very rapid during early filling phase (short DT) and the elevated LV end diastolic pressure minimizes ventricular filling due to atrial contraction (decreased A). With the resultant high left atrial pressure, the IVRT becomes shortened and the E velocity is high. This particular diastolic filling pattern is indicative of restrictive physiology characterized by the following diastolic parameters:

1. Shortened IVRT (<60 msec)
2. High E velocity and low A velocity
3. Increased E/A ratio (≥ 2.0)
4. Shortened DT (≤ 150 msec)

Restrictive physiology pattern is seen whenever LV diastolic pressure rises rapidly and end diastolic pressure is high as in LV failure, restrictive cardiomyopathy, volume overload and severe acute aortic regurgitation.

**Pseudonormalization**

When relaxation abnormality and restrictive hemodynamics coexist, Doppler features of the latter predominate. However there is a transitional period when they become 'pseudonormalized'. The pseudonormalization pattern is present when the left atrial pressure rises moderately in the setting of abnormal myocardial relaxation, producing a diastolic filling pattern similar to normal pattern.
AIMS AND OBJECTIVES
3.0 AIMS AND OBJECTIVES

1. To study the interaction of aspirin (a non selective NSAID) with angiotensin-converting enzyme (ACE) inhibitor on methylprednisolone-induced hypertension in rats.

2. To study the interaction of a selective COX-2 inhibitor with antihypertensive agent in spontaneously hypertensive rats.

3. To study the interaction of aspirin with angiotensin II receptor antagonist on L-NAME-induced hypertension in rats.

4. To assess by echocardiography, the acute effect of aspirin administration on left ventricular systolic and diastolic performance in patients of severe congestive heart failure who are on antifailure treatment including ACE inhibitors and/or angiotensin receptor blockers.
4.0 MATERIALS

4.1 Animals

Male albino Wistar rats : Central Animal House, Jamia Hamdard
Spontaneously Hypertensive rats : Ranbaxy Laboratories Ltd

4.2 Equipments

Electronic microbalance : Mettler Toledo
LE 5002 Storage Pressure Meter : Letica Scientific Instruments
Flame Photometer Mediflame 127 : Systronics
Binocular Research Microscope with photomicrographic attachment : Olympus (Vanox)
DMLP Microscope : Leica
Micropipette : J Microlab
Microcentrifuge RM 12C : Remi Equipments

4.3 Drugs and Chemicals

Methylprednisolone acetate
(Debo-Medrol™ Sterile aqueous suspension) : Pharmacia & Upjohn
Lisinopril : Ranbaxy Laboratories Ltd
Aspirin : Ranbaxy Laboratories Ltd
Drug interaction studies between NSAIDs and drugs used in cardiovascular disorders

Rofecoxib : Ranbaxy Laboratories Ltd.

Telmisartan : Unichem Laboratories

L-N-nitro-L Arginine Methyl Ester (L-NAME) : Sigma Aldrich

Ouabain : Hi Media Laboratories

Ammonium oxalate : Merck

Tris Buffer (Tris hydroxymethylaminomethane) : CDH

Disodium hydrogen phosphate (M.W. 142) : CDH

Sodium hydrogen orthophosphate (Dihydrate) : Loba Chemie

Sucrose : S.d. fine Chemicals

Potassium chloride : Merck

Magnesium chloride : Merck

Formaldehyde : CDH

Glutaraldehyde : AIIMS Laboratories

All the chemicals and reagents used were A.R. grade only.
LE 5002 Storage Pressure Meter
METHODS AND RESULTS
5.1. Protocol 1

To study the interaction of aspirin (a non selective NSAID) with angiotensin-converting enzyme (ACE) inhibitor on methylprednisolone-induced hypertension in rats
METHODS

The protocol was approved by the Institutional Animal Care and Ethical Committee of Jamia Hamdard. All animals received human care in compliance with the principles formulated by the committee for the purpose of control and supervision of experimental animals.

Fifty six male Wistar rats (150-250g) were housed in a temperature controlled room, 25 ± 2 °C and allowed standard laboratory chow and tap water ad libitum.

The animals were divided into seven groups and received the following treatment for two weeks:

**Group I**: Control, received 1% gum acacia

**Group II**: Methylprednisolone acetate suspension (MP) 20 mg/kg/week s.c.

**Group III**: Methylprednisolone acetate suspension (MP) 20 mg/kg/week s.c. + Lisinopril (LS) 15 mg/kg/d p.o.

**Group IV**: Methylprednisolone acetate suspension (MP) 20 mg/kg/week s.c. + Lisinopril (LS) 15 mg/kg/d p.o. + Aspirin (ASA) 100 mg/kg/d p.o.

**Group V**: Methylprednisolone acetate suspension (MP) 20 mg/kg/week s.c. + Lisinopril (LS) 15 mg/kg/d p.o. + Aspirin (ASA) 25 mg/kg/d p.o.

**Group VI**: Lisinopril (LS) 15 mg/kg/d p.o. + Aspirin (ASA) 100 mg/kg/d p.o.

**Group VII**: Lisinopril (LS) 15 mg/kg/d p.o.

LS and ASA were administered to the animals by dissolving in glass-distilled water and suspending in 1% gum acacia dispersion, respectively. The drug doses were adjusted based on the daily recordings of animal body weight.

**Induction of Hypertension**

Hypertension was induced by s.c. administration of a long acting MP preparation (Depo-Medrol, Upjohn) given at a dose of 20mg/kg/week for two weeks (Ribeiro et al. 1981; Eljovich and Krakoff 1980).
Measurement of Arterial Pressure and Heart Rate in Conscious Rats

Control measurements were made of body weights daily. The systolic blood pressure (SBP) and heart rates were recorded in unanaesthetised rats by tail cuff plethysymography using LE 5002 storage pressure meter (Stoelting, USA). The animals were observed daily for any mortality.

Histopathology

At the end of two weeks the animals were sacrificed and tissue pieces from kidney and heart were collected in 10% formal saline for proper fixation. These tissues were processed and embedded in paraffin wax. Sections 5-6 µ in thickness were cut and stained with haematoxylin and eosin staining procedure for examination under microscope.

Statistical Analysis

Data are expressed as mean ± SEM. Kruskal Wallis test was applied, followed by post-hoc analysis for evaluation of SBP and heart rates at two weeks interval. The mortality and body weights were calculated using Chi-square and paired 't' test respectively.

RESULTS

Effect of Drug Treatments on Arterial Pressure and Heart Rate in Conscious Rats

Table 1.1 shows the SBP values and heart rates with different drug treatments. As may be seen a significant rise of 36 ± 4mm Hg (p = 0.001) was obtained in SBP during the two week treatment with MP. LS treatment reduced the pressure significantly in both MP treated group and control. Addition of ASA 25 or 100 mg/kg/d p.o. did not reduce the hypotensive response of LS. Treatment with methylprednisolone did not change heart rate. Similarly different groups did not show statistical difference between heart rates except that LS treatment alone increased heart rate significantly compared to control.

Effect on Body Weights
Table 1.2 summarizes initial and final body weights of different drug treatments. Animals in control group showed an increase in body weights. MP treatment was associated with loss of body weights. LS treatment alone (Group VII) prevented the growth of the animals. Similarly addition of ASA to LS (Group VI) retarded the body weights of animals.

**Effect on Mortality**

During the two weeks study, the group receiving a combination of ASA 100 mg/kg/d with LS and MP (Group IV) had a significant mortality of 70% as against animals receiving a combination of LS and MP (Group III) (p = 0.0198) (Table 1.1):

ASA when given at 25 mg/kg/d with LS and MP (Group V) had lower mortality of 16% compared to animals receiving LS and MP (Group III) and was not different statistically. There was no mortality in control and MP treated animals. All the mortalities occurred during the second week of drug treatment.
TABLE – 1.1

Effect of Lisinopril (LS) and combination of Lisinopril (LS) and Aspirin (ASA) on Heart Rate, Systolic Blood Pressure (SBP), and Mortality on Methylprednisolone (MP) -Induced Hypertension in Rats.

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Second Week</th>
<th>Mortality</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Heart Rate (beats/ minute)</td>
<td>SBP (mmHg)</td>
</tr>
<tr>
<td>I</td>
<td>Control</td>
<td>319.50 ± 6.29</td>
<td>101.38 ± 4.44</td>
</tr>
<tr>
<td>II</td>
<td>MP (20 mg/kg/week), s.c.</td>
<td>352.10 ± 15.17</td>
<td>137 ± 4.23 †</td>
</tr>
<tr>
<td>III</td>
<td>MP (20 mg/kg/week), s.c. + LS (15 mg/kg/d), p.o.</td>
<td>337.25 ± 11.58</td>
<td>90.38 ± 4.04 ‡</td>
</tr>
<tr>
<td>IV</td>
<td>MP (20 mg/kg/week), s.c. + LS (15 mg/kg/d), p.o. + ASA (100 mg/kg/d), p.o.</td>
<td>358.30 ± 14.51</td>
<td>91.60 ± 6.76</td>
</tr>
<tr>
<td>V</td>
<td>MP (20 mg/kg/week), s.c. + LS (15 mg/kg/d), p.o. + ASA (25 mg/kg/d), p.o.</td>
<td>434 ± 20.76</td>
<td>84 ± 11.09</td>
</tr>
<tr>
<td>VI</td>
<td>LS (15 mg/kg/d), p.o. + ASA (100 mg/kg/d), p.o.</td>
<td>340.40 ± 5.32</td>
<td>60.46 ± 6.36</td>
</tr>
<tr>
<td>VII</td>
<td>LS (15 mg/kg/d), p.o.</td>
<td>404.40 ±18.07**</td>
<td>69.66 ± 5.30 §</td>
</tr>
</tbody>
</table>

* IV Vs III (P<0.05)
†, §, **, II Vs I, VII Vs I, VII Vs I respectively (P < 0.01)
‡ III Vs II (P<0.001)
**TABLE 1.2**

Effect of Lisinopril (LS) and combination of Lisinopril and Aspirin (ASA) on Body Weights of Rats treated with Methylprednisolone (MP)

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Bodyweight (g)</th>
<th>Δ W (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Initial</td>
<td>Final</td>
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<tr>
<td>I</td>
<td>Control</td>
<td>171.88 ± 7.55</td>
<td>198 ± 11.00</td>
</tr>
<tr>
<td>II</td>
<td>MP (20 mg/kg/week)</td>
<td>207.50 ± 4.29</td>
<td>159.40 ± 3.59</td>
</tr>
<tr>
<td>III</td>
<td>MP (20 mg/kg/week) + LS (15 mg/kg/d)</td>
<td>182.20 ± 5.31</td>
<td>125.11 ± 4.77</td>
</tr>
<tr>
<td>IV</td>
<td>(MP (20 mg/kg/week) + LS (15 mg/kg/d) + ASA (100 mg/kg/d)</td>
<td>177.10 ± 6.33</td>
<td>125.20 ± 11.27</td>
</tr>
<tr>
<td>V</td>
<td>(MP 20 mg/kg/week) + LS (15 mg/kg/d) + ASA (25 mg/kg/d)</td>
<td>157.66 ± 4.05</td>
<td>96.80 ± 3.77</td>
</tr>
<tr>
<td>VI</td>
<td>LS (15 mg/kg/d) + ASA (100 mg/kg/d)</td>
<td>177.66 ± 4.01</td>
<td>147.60 ± 3.91</td>
</tr>
<tr>
<td>VII</td>
<td>LS (15 mg/kg/d)</td>
<td>145 ± 5.43</td>
<td>149.33 ± 6.60</td>
</tr>
</tbody>
</table>

**Initial Vs Final (P<0.01)**
Histopathology

Effect on Cardiac Muscle

The cross section (C.S.) and longitudinal section (L.S.) of cardiac muscle of normal rat are shown in Fig. 1.1a and 1.1b respectively. The cardiac muscle fibers of this group (I) revealed presence of nucleus with distinctive nucleoli, well preserved cytoplasm and fibroblast connective tissue nucleus.

The sections of cardiac muscle of rat treated with MP (Group II) revealed marked degenerative changes characterized by coagulative necrosis and pyknotic nuclei devoid of nucleoli (Fig. 1.2a and 1.2b). Treatment with LS (Group III) for a period of two weeks reversed these changes and protected the myocardium against infarction induced by MP (Figs. 1.3a and 1.3b). However, LS when combined with ASA 100 mg/kg/d (Group IV) revealed marked degenerative changes of muscle fiber characterized by coagulative necrosis, pink eosinophilic cytoplasm and marked interstitial edema in connective tissues spaces of hypertensive rats (Fig. 1.4a). The L.S. also showed loss of striations of muscle fibers with pyknotic nuclei and interstitial edema (Fig. 1.4b).

Treatment of ASA 25 mg/kg/d with LS (Group V) showed variability of degenerative changes in the muscle fibers, cytoplasm and nuclei indicating moderate changes in the cardiac muscle fiber of hypertensive rats (Figs. 1.5a and 1.5b). The interstitial edema of cardiac muscle was less compared to Group IV. The sections treated with LS + ASA (100 mg/kg/d) (Group VI) and LS only (Group VII) showed normal structure.

Effect on Kidney

Figs. 1.1c and 1.1d show cortical and medullary sections of kidney from normal rat. Histopathological examination of sections of kidney treated with MP (Group II) revealed early degenerative changes of glomerular endothelium simulating acute progressive glomerular nephritis (Fig. 1.2c and 1.2d). Treatment with LS at 15 mg/kg/d (Group III) for two weeks revealed well protective effect over changes induced by MP treatment (Figs. 1.3c and 1.3d). The combination of ASA 100 mg/kg/d with LS and MP (Group IV)
revealed degenerative changes of proximal and medullary regions showing presence of hyaline casts simulating to acute progressive glomerular nephritis (Figs 1.4c and 1.4d). Similarly kidney sections treated with ASA, 25mg/kg/d with MP and LS (Group V) showed atrophied glomerulus, desquamation of distal convoluted tubules and hyaline casts, at places (Figs. 1.5c and 1.5d). The sections treated with ASA 100 mg/kg/d + LS 15 mg kg/d (Group VI) and LS only (Group VI), were found to be normal.
Photomicrographs – Cardiac Tissue

Group I (Control Normal Rat Heart)

Figure 1.1a Cross section (C.S.) of cardiac muscle fiber of normal rat reveals presence of (a) oval nucleus with distinctive nucleoli (b) well preserved cytoplasm (c) distinctive fibroblast connective tissue nucleus. H. & E. x400

Figure 1.1b Longitudinal section (L.S.) of normal rat heart revealing myocardial fibers running in parallel direction with presence of (a) elliptical nucleus with nucleoli (b) well preserved cytoplasm having cross striations. H. & E. x400

Group II (Methylprednisolone (MP), 20 mg/kg/week)

Figure 1.2a C.S. of group II revealing marked degenerative changes simulating myocardial infarction (a) coagulative necrosis devoid of nucleus and nucleoli. At few places coagulative necrosis of cytoplasm of muscle fibres with (b) pyknotic nuclei is also seen (c) widening of interstitial spaces. H. & E. x400

Figure 1.2b L.S. revealing degenerative changes of muscle fibers with (a) loss of cross striations of cytoplasm (b) pyknotic nuclei devoid of nucleoli. However the interstitial spaces of muscle fiber (c) do not show any widening of spaces. H. & E. x400
Photomicrographs – Cardiac Tissue

Group III (MP, 20 mg/kg/week + LS, 15 mg/kg/d)

Figure 1.3a C.S. of cardiac muscle of group III reveals (a) well preserved nucleus and nucleoli (b) cytoplasm is well preserved (c) interstitial spaces do not show any widening or edema. H. & E. x400

Figure 1.3b L.S. revealing myocardial fibers running in parallel direction with presence of (a) elliptical nucleus with nucleoli (b) well preserved cytoplasm having cross striations (c) presence of distinct fibroblast connective tissue. H. & E. x400

Group IV (MP, 20 mg/kg/week+ LS, 15 mg/kg/d + ASA, 100 mg/kg/d)

Figure 1.4a C.S. of cardiac muscle of rat in group IV revealed marked degenerative changes of muscle fiber (a) coagulative necrosis of cytoplasm devoid of nucleus and nucleoli (c) marked edema in the interstitial connective tissue spaces. H. & E. x400

Figure 1.4b L.S. revealing (a) loss of striations of muscle fiber with (b) pyknotic nucleus & (c) marked interstitial edema in the connective tissue spaces. H. & E. x400
Photomicrographs – Cardiac Tissue

Group V (MP, 20 mg/kg/week + LS, 15 mg/kg/d + ASA, 25 mg/kg/d)

Figure 1.5a C.S. of cardiac muscle of group V shows variability of degenerative changes in the muscle fiber, cytoplasm and nucleus. At places: (a) condensed nucleus and moderate degenerative changes in the cytoplasm are seen. Another area of the same section revealed (b) coagulative necrosis which is devoid of nucleus and nucleoli thereby revealing moderate changes in the cardiac muscle fiber. H. & E. x400

Figure 1.5b L.S. of cardiac muscle of group V revealing, at places; (a) the muscle fiber with moderate changes in the cytoplasm and well protected nucleus (b) pyknotic nucleus and cytoplasm with necrotic changes (c) the interstitial edema is also quite less. H. & E. x 400
Photomicrographs – Renal Tissue

Group I (Control Normal Rat Kidney)

Figure 1.1c Section of kidney of control rat reveals (a) well preserved glomerulus and bowman's capsule (b) Proximal convoluted tubule showing epithelium attached to basement membrane of tubules having distinct nucleus, cytoplasm and (c) lumen of the tubule. H. & E. x250

Group II (Methylprednisolone (MP), 20 mg/ kg /week)

Figure 1.2c Kidney section of Group II revealing (a) early degenerative changes of glomerular endothelium, due to decreased glomerular filtration rate (b) early degenerative changes of proximal tubule with degeneration of epithelial cells projecting towards (c) lumen of tubule; thereby leading to changes in kidney simulating acute progressive glomerular nephritis. H.&E. x250

Figure 1.2d Medullary area of methylprednisolone treated group showing (a) desquamation of epithelium but no casts. H. & E. x250
Photomicrographs – Renal Tissue

Group III (MP, 20 mg/kg/week + LS, 15 mg/kg/d)

Figure 1.3c The section of kidney in group III revealed well preserved (a) glomerulus with endothelial lining and basement membrane. (b) Proximal tubules reveal well brought out epithelial cells having well preserved nucleus and cytoplasm including (c) lumen which are devoid of any casts. H. & E. x250

Figure 1.3d Distal convoluted tubules showing structure towards normalcy. H. & E. x250

Group IV (MP, 20 mg/kg/week + LS, 15 mg/kg/d + ASA, 100 mg/kg/day)

Figure 1.4c Section of kidney reveals (a) degenerative changes of glomerular endothelial cells with decreased glomerular filtration rate. The renal tubules reveal (b) degenerative changes and desquamation of convoluted tubule epithelium. H. & E. x250

Figure 1.4d Medullary region shows distal convoluted tubules characterized by (a) desquamation and necrosis of epithelium (b) presence of hyaline casts in the lumen of tubules thereby revealing changes simulating acute progressive glomerular nephritis. H. & E. x250
Photomicrographs – Renal Tissue

Group V (MP, 20 mg/kg/week + LS, 15 mg/kg/d + ASA, 25 mg/kg/d)

**Figure 1.5c** Kidney section of group V revealing atrophied glomerulus but basement lining of bowman’s capsule is well intact. H. & E. x250.

**Figure 1.5d** Medullary section of group V revealing (a) desquamation of epithelium of distal convoluted tubule and at places, (b) hyaline casts. H. & E. x250.
5.2 PROTOCOL-2

To study the interaction of a selective COX-2 inhibitor with antihypertensive agent in spontaneously hypertensive rats
METHODS

Ten weeks old, twenty five male spontaneously hypertensive rats (SHRs) were obtained from Ranbaxy Laboratories Limited and were acclimatized to a climate controlled facility with 12 hours alternating light and dark cycles for three weeks and allowed laboratory chow and water ad libitum. The animals were trained for three weeks in restrainers for measurement of blood pressure.

The baseline measurements of blood pressure were taken on 16-week-old SHRs which then received following treatment for two weeks.

Group I : SHR (positive control) received no treatment
Group II : Lisinopril (LS) 15 mg/kg/d
Group III : Lisinopril (LS) 15 mg/kg/d + Rofecoxib (RF) 20 mg/kg/d
Group IV : Rofecoxib (RF) 20 mg/kg/d

LS and RF were administered to the animals orally by dissolving in glass distilled water and 1% gum acacia dispersion respectively. The drug doses were adjusted based on the daily recordings of the animal body weight. The doses of both lisinopril and rofecoxib were selected based on previous experiments carried out in rats (Brilla et al. 1991; Gretzer et al. 2001; Laudanno et al. 2001)

Determination of Arterial Pressure and Heart Rate in Conscious Rats

The systolic blood pressure (SBP) and heart rates were measured in each rat by use of tail-cuff plethysmography using LE 5002 storage pressure meter (Stoelting's, USA). Measurements were started one week before treatment and performed on conscious, restrained rats previously trained for the procedure. Pressure measurements were made at weekly intervals during the treatment period (deBlois et al. 1997; Black et al. 1997). Seven consistent readings for blood pressure and heart rates were recorded for each animal which were then averaged.
Determination of Sodium Hydrogen Exchange (NHE) in Erythrocytes

The sodium hydrogen exchange was determined after two weeks of treatment and compared with the age matched Wistar rats.

The sodium influx into the erythrocytes was determined by the method of (Scholz et al. 1992, 1993). Blood was drawn from retrobulbar plexus under light ether anesthesia and prevented from coagulation by addition of ammonium oxalate. The haematocrit of the sample was determined in duplicate by centrifugation. Aliquots of 100 micro liters were taken to measure the initial sodium content of the erythrocytes.

To determine the amiloride sensitive sodium influx into the erythrocytes 100 micro liters of each blood sample was added to 5 ml of buffer made hyperosmolar by sucrose (in mM/l: NaCl 140, KCl 3, Sucrose 150, Ouabain 0.1, tris - hydroxy methyl aminomethane 20, pH 7.4) and incubated for 60 minutes at 37° C. Subsequently the erythrocytes were washed three times in ice-cold MgCl₂- ouabain solution (in mM/l: MgCl₂ 112, Ouabain 0.1) with centrifugation. For determination of intracellular Na⁺ content, the cells were haemolyzed in distilled water in a final volume of 2.0 ml, the cell membrane were centrifuged and sodium concentration of the haemolysate was measured by flame photometry.

Net influx of sodium into the erythrocytes was calculated from the difference between the initial sodium content and the sodium content after the incubation.

Histopathology

At the end of two weeks the animals were sacrificed and tissue pieces from heart and kidneys were collected in 10% formal saline for proper fixation. The tissues were processed and embedded in paraffin wax. Sections 5 - 6 microns in thickness were cut and stained with haematoxylin and eosin staining procedure for examination under microscope.
**Statistical Analysis**

Descriptive statistics i.e. mean and standard deviation was calculated for each variable analyzed. Values are expressed as mean ± SEM. Non parametric one way analysis of variance (ANOVA) (Kruskal-Wallis test) was performed to see the difference among the groups for systolic blood pressure (SBP) and heart rates at each point of time whereas to see the trend within the groups, repeated measure ANOVA (Friedman test) was performed. The difference in mean Na⁺ influx into the erythrocytes was determined by one way ANOVA (Kruskal-Wallis test) followed by post hoc analysis (Newman-Keuls procedure) to compare the difference between the groups.

SAS (8.0) statistical package was used for the analysis and p<0.05 was considered to be statistically significant.

**RESULTS**

**Effect of Drug Treatments on Arterial Pressure and Heart Rate**

Lisinopril treatment was associated with decrease in systolic blood pressure of 21% (95% CI: 11%, 28%) at 1st week and 27% (95% CI: -153%, 208%) at 2nd week (Table-2.1). For the combination of rofecoxib with lisinopril, systolic blood pressure decreased 3% (95% CI: -18%, 26%) at 1st week and 37% (95% CI: 8%, 61%) at 2nd week of treatment. Rofecoxib treatment alone (Group IV) caused a decrease of 0.34% (95% CI: -14%, 15%) in SHRs during 1st week of treatment and the animals in this group died by the 2nd week of treatment.

Table-2.2 shows the effect of drug treatments on heart rates of SHRs. One way and two ways analysis of variance (ANOVA) showed no significant difference among the groups.

**Effect on Sodium Hydrogen Exchange (NHE) Activity**

Table 2.3 shows that 18 weeks old SHRs had significantly decreased Na⁺ influx than their age matched Wistar rats (p<0.05). Lisinopril treatment increased Na⁺ influx...
significantly to Wistar rats while combination of rofecoxib with lisinopril was devoid of any activity on Na$^+$ influx into the erythrocytes after two weeks of treatment.
<table>
<thead>
<tr>
<th>Group</th>
<th>Percent Change from Baseline and Baseline (CT)</th>
<th>SBP (mmHg)</th>
<th>Body Weight (E)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SHR + RP (20mg/kg), p.o.</td>
<td>206.6 ± 8.82</td>
<td>223.66 ± 6.33</td>
<td>229.46 ± 5.33</td>
</tr>
<tr>
<td>SHR + LS (15mg/kg), p.o.</td>
<td>210 ± 9.30</td>
<td>225.83 ± 13.9</td>
<td>247.16 ± 26.35</td>
</tr>
<tr>
<td>SHR + LS (15mg/kg), p.o.</td>
<td>210 ± 9.30</td>
<td>225.83 ± 13.9</td>
<td>247.16 ± 26.35</td>
</tr>
<tr>
<td>SHR Control</td>
<td>220.29 ± 7.59</td>
<td>250.29 ± 12.39</td>
<td>197 ± 15.39</td>
</tr>
<tr>
<td>Pre-Week</td>
<td>220.29 ± 7.59</td>
<td>250.29 ± 12.39</td>
<td>197 ± 15.39</td>
</tr>
<tr>
<td>1st Week</td>
<td>220.29 ± 7.59</td>
<td>250.29 ± 12.39</td>
<td>197 ± 15.39</td>
</tr>
<tr>
<td>2nd Week</td>
<td>220.29 ± 7.59</td>
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<td>220.29 ± 7.59</td>
<td>250.29 ± 12.39</td>
<td>197 ± 15.39</td>
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<tr>
<td>4th Week</td>
<td>220.29 ± 7.59</td>
<td>250.29 ± 12.39</td>
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</tr>
<tr>
<td>5th Week</td>
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<tr>
<td>7th Week</td>
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<tr>
<td>8th Week</td>
<td>220.29 ± 7.59</td>
<td>250.29 ± 12.39</td>
<td>197 ± 15.39</td>
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<tr>
<td>9th Week</td>
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<td>250.29 ± 12.39</td>
<td>197 ± 15.39</td>
</tr>
<tr>
<td>10th Week</td>
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</tr>
<tr>
<td>11th Week</td>
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<tr>
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<tr>
<td>14th Week</td>
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</tr>
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<td>15th Week</td>
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<td>250.29 ± 12.39</td>
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<td>17th Week</td>
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<tr>
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<td>250.29 ± 12.39</td>
<td>197 ± 15.39</td>
</tr>
<tr>
<td>22nd Week</td>
<td>220.29 ± 7.59</td>
<td>250.29 ± 12.39</td>
<td>197 ± 15.39</td>
</tr>
<tr>
<td>23rd Week</td>
<td>220.29 ± 7.59</td>
<td>250.29 ± 12.39</td>
<td>197 ± 15.39</td>
</tr>
<tr>
<td>24th Week</td>
<td>220.29 ± 7.59</td>
<td>250.29 ± 12.39</td>
<td>197 ± 15.39</td>
</tr>
<tr>
<td>25th Week</td>
<td>220.29 ± 7.59</td>
<td>250.29 ± 12.39</td>
<td>197 ± 15.39</td>
</tr>
<tr>
<td>26th Week</td>
<td>220.29 ± 7.59</td>
<td>250.29 ± 12.39</td>
<td>197 ± 15.39</td>
</tr>
<tr>
<td>27th Week</td>
<td>220.29 ± 7.59</td>
<td>250.29 ± 12.39</td>
<td>197 ± 15.39</td>
</tr>
<tr>
<td>28th Week</td>
<td>220.29 ± 7.59</td>
<td>250.29 ± 12.39</td>
<td>197 ± 15.39</td>
</tr>
<tr>
<td>29th Week</td>
<td>220.29 ± 7.59</td>
<td>250.29 ± 12.39</td>
<td>197 ± 15.39</td>
</tr>
<tr>
<td>30th Week</td>
<td>220.29 ± 7.59</td>
<td>250.29 ± 12.39</td>
<td>197 ± 15.39</td>
</tr>
</tbody>
</table>

Hypotensive Rats (SHR)

Effect of Lisinopril (L) and combination of Lisinopril (L) and Rofecoxib (R) on Systolic Blood Pressure (SBP) of Spontaneously Hypertensive Rats (SHR)
<table>
<thead>
<tr>
<th>SHR + RP (20 mg/kg/d)</th>
<th>SHR + LS (15 mg/kg/d)</th>
<th>SHR Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>II</td>
<td>I</td>
<td>III</td>
</tr>
<tr>
<td>II</td>
<td>I</td>
<td>I</td>
</tr>
<tr>
<td>I</td>
<td>I</td>
<td></td>
</tr>
<tr>
<td>365 ± 42.21</td>
<td>393 ± 9.71</td>
<td>330 ± 9.62</td>
</tr>
<tr>
<td>377.6 ± 26.99</td>
<td>408.6 ± 22.77</td>
<td>348.8 ± 6.99</td>
</tr>
<tr>
<td>16.5 ± 2.5</td>
<td>19.1 ± 1.5</td>
<td>15.8 ± 0.6</td>
</tr>
<tr>
<td>6% - 50% (39%)</td>
<td>12% - 24% (0.7%)</td>
<td>9% - 16% (5%)</td>
</tr>
</tbody>
</table>

**Effect of Lisinopril (LS) and combination of Lisinopril (LS) and Rotecoxib (RP) on Heart Rate of Spontaneously Hypertensive Rats**

**Table 2.2**
<table>
<thead>
<tr>
<th></th>
<th>SHR + LS (15 mg/kg/p) + RF (20 mg/kg/p)</th>
<th>(p&lt;0.05)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.1 \pm 0.6</td>
<td>SHR</td>
<td>(p&lt;0.05)</td>
</tr>
<tr>
<td>3.3 \pm 0.3</td>
<td>SHIR control</td>
<td>(p&lt;0.05)</td>
</tr>
<tr>
<td>1.4 \pm 0.7</td>
<td>SHR control</td>
<td>(p&lt;0.05)</td>
</tr>
<tr>
<td>3.3 \pm 0.7</td>
<td>Wistar control</td>
<td>(p&lt;0.05)</td>
</tr>
<tr>
<td>Net NE influx (mEq/L)</td>
<td>Treatment</td>
<td>(p&lt;0.05)</td>
</tr>
</tbody>
</table>

18-week-old spontaneously hypertensive rats (SHR)

Effect of Listeriol (LS) and combination of Listeriol (LS) and Potocobol (RF) on sodium hydrogen exchange of Erythrocytes of

TABLE 2.3
Histopathology

Effect on Cardiac Muscle

Majority of cardiac muscle fibers of 18 weeks old SHRs revealed pyknotic nuclei and degenerative changes characterized by loss of cross striations and eosinophilic cytoplasm (Figs 2.1a and 2.1b). Treatment with lisinopril for a period of two weeks reversed these changes by demonstrating the presence of vesicular nucleus and well preserved cytoplasm thus showing the protective effect of this drug on myocardium of SHRs (Figs. 2.2a and 2.2b). However, lisinopril when given in combination with rofecoxib revealed necrosis of myocardium characterized by loss of cross striations of all the muscle fibers, pyknotic nuclei without nucleoli and eosinophilic cytoplasm (Figs. 2.3a and 2.3b), thus revealing changes which did not appear reversible as were seen with lisinopril treatment alone. The sections of SHRs treated with RF alone also showed degenerative changes of myocardium (Figs. 2.4a and 2.4b).

Effect on Kidney

The sections of kidney of 18-week-old SHRs revealed reduction in size of glomeruli without any inflammatory changes thereby indicating nephrosis (Fig. 2.1c) and desquamation of tubular epithelium from medullary region (Fig. 2.1d). Treatment with lisinopril for two weeks revealed well brought out Bowman's capsule, proximal convoluted tubules and distal convoluted tubules having epithelial tubular lining attached to the basement membrane (Figs. 2.2c and 2.2d). Lisinopril when combined with rofecoxib revealed atrophied glomeruli (Fig. 2.3c), desquamation of epithelial cells and proteinous material in the lumen of distal convoluted tubule (Fig. 2.3d) but well preserved proximal convoluted tubule thereby, revealing mild changes in the kidneys. Treatment with rofecoxib alone also showed atrophied glomerulus and desquamation of distal convoluted tubules (Figs. 2.4c and 2.4d).
Drug interaction studies between NSAIDs and drugs used in cardiovascular disorders

Photomicrographs – Cardiac Tissue

Group I (Control SHR)

Figure 2.1a C.S. of cardiac muscle of SHR reveals (a) pyknotic nuclei in majority of the cardiac muscle fibers & cytoplasm reveals degenerative changes characterized by (b) eosinophilic cytoplasm. H. &E. x 400

Fig 2.1 b L.S. of cardiac muscle of SHR reveals degenerative changes characterized by (a) pyknotic nucleus with loss of cross striations of muscle fibers & (b) eosinophilic cytoplasm. H. &E. x 400

Group II (SHR + LS, 15 mg/kg/d)

Figure 2.2a C.S. of cardiac muscle reveals (a) vesicular nucleus (b) well preserved cytoplasm & (c) connective tissue fibers, indicating protective effect of LS on myocardium. H.&E. x 400

Figure 2.2b L.S. of cardiac muscle reveals (a) prominent vesicular nucleus (b) well preserved cytoplasm and cross striations. In between longitudinal muscle fibers (c) interstitial connective tissue fibers are seen. H.&E. x 400
Photomicrographs – Cardiac Tissue

Group III (SHR + LS, 15 mg/kg/d + RF, 20 mg/kg/d)

Figure 2.3a C.S. of cardiac muscle of LS+RF treated group reveals (a) majority of muscle fibers devoid of nucleus & nucleoli and cytoplasm reveals degenerative changes leading to necrosis with eosinophilic appearance. H.&E. x 400

Figure 2.3b L.S. of cardiac muscle of the same group reveals, at places; (a) pyknotic nucleus without any nucleoli and (b) loss of cross striations of all the muscle fibers. H.&E. x 400

Group IV (SHR + RF, 20 mg/kg/d)

Figure 2.4a C.S. of RF treated group reveals that (a) majority of cells are devoid of nucleus and nucleoli and cytoplasm reveals degenerative changes leading to necrosis representing eosinophilic appearance. H.&E. x 400

Figure 2.4b L.S. of RF treated group reveals muscle fibers running in longitudinal direction with (a) pyknotic nuclei & (b) cytoplasm with loss of cross striations. H.&E. x 400
Photomicrographs – Renal Tissue

Group I (Control SHR)

**Figure 2.1c** C.S. of kidney of 18 weeks old SHR reveals (a) reduction in size of glomerulus without any inflammatory changes thereby indicating nephrosis & (b) proximal convoluted tubules do not show much change. H.&E. x 250

**Figure 2.1d** Another section of the same group from medullary region reveals (a) desquamation of tubular epithelium. H.&E. x 250

Group II (SHR + LS, 15 mg/kg/d)

**Figure 2.2c** Kidney section from cortical area treated with LS reveals (a) well brought out glomerulus, (b) bowman’s capsule & (c) well preserved proximal convoluted tubules. H.&E. x 250

**Figure 2.2d** Another area of the same group from medullary region reveals well brought out (a) distal convoluted tubules having epithelial tubular lining attached to basement membrane. H.&E. x 250
Photomicrographs – Renal Tissue

**Group III (SHR + LS, 15mg/kg/d + RF, 20 mg/kg/d)**

**Figure 2.3c** The cortical area of kidney section treated with LS+ RF reveals (a) atrophied glomerulus but (b) the proximal convoluted tubules are well preserved thereby revealing mild changes in the kidneys. H.&E. x 250

**Figure 2.3d** The section from medullary region reveals (a) desquamation of epithelial cells in distal convoluted tubules & (b) eosinophilic proteinaceous material lying in the lumen of the tubules. H.&E. x 250

**Group IV (SHR + RF, 20 mg/kg/d)**

**Figure 2.4c** Cortical region of kidney treated with RF only reveals (a) atrophied glomerulus without any damage to proximal convoluted tubules. H.&E. x 250

**Figure 2.4d** Another section from medullary region reveals (a) desquamation of distal convoluted tubules thereby revealing nephrotic changes in the kidney. H.&E. x 250
5.3 Protocol 3

To study the interaction of aspirin with angiotensin II receptor antagonist on L-NAME-induced hypertension in rats
METHODOLOGY

Thirty-six male Wistar rats weighing (50-70g) were obtained from Central Animal House, Jamia Hamdard and maintained at standard laboratory chow and water ad libitum throughout the study. All experiments were performed according to the guidelines of the institutional ethical committee CPCSEA (Committee for the purpose of control and supervision of experimental animals). Rats were randomly divided into five study groups that received drug concentrations for four weeks as follows.

Group I: received no drug and served as control
Group II: \( N^\text{6} \) Nitro-L-arginine-methyl ester (L-NAME) hydrochloride; 75mg/kg/d
Group III: L-NAME; 75mg/kg/d + Telmisartan (TM) 10mg/kg/d
Group IV: L-NAME; 75mg/kg/d + Telmisartan (TM) 10mg/kg/d + Acetylsalicylic acid (ASA); 100mg/kg/d
Group V: L-NAME; 75mg/kg/d + ASA 100mg/kg/d

L-NAME hydrochloride was dissolved in distilled water. Telmisartan and ASA were suspended in 0.4% xanthan gum. All drugs were administered by oral route. Fresh solutions were prepared every second day.

The liquid intake of rats was recorded thrice weekly and solid food consumption recorded twice weekly. Body weights were taken every alternate day.

Measurement of Systolic Blood Pressure (SBP) and Heart Rate

Fifteen days before the beginning of the experiment, rats were trained daily for measurement of SBP by the tail cuff method. Baseline measurements were then taken and the arterial pressure and heart rates subsequently measured at weekly or fortnightly intervals in conscious rats during drug treatment.

Morphometric Analysis

At the end of the experiment, the animals were sacrificed by an overdose of pentobarbital sodium (100 mg/kg, i.p.). In five control rats and the same number of L-NAME treated rats, the chest was opened and the cardiovascular system perfused under
constant perfusion pressure of 120mm Hg with glutaraldehyd fixative for 10 minutes via a cannula placed in the left ventricle. After perfusion, the middle part of the common carotid artery was excised, divided into 1 mm long segments and fixed in the same fixative. The samples were exposed to osmium tetroxide (OsO₄), stained en block with uranyl acetate, dehydrated through a graded series of alcohols and embedded in a mixture of Durcupan ACM. Subsequently semithin sections were cut perpendicularly to the long axis of the vessels and stained with methylene blue. The arterial wall thickness (tunica intima and tunica media) of the respective vessel was measured in semithin sections at about 45° intervals around the vessel circumference (Kristek and Gerova 1996; Kristek et al. 1996).

Thus the average of four measurements of the inner diameter and thirty two measurements of arterial wall thickness from each animal were taken as representative of each parameter. Intima and media thickness was determined with the use of an overall microscope magnification of x400 whereas the inner diameter was determined at x100. Findings were averaged for each group and the wall thickness and diameter ratio was then calculated for each group (Rossi and Colomini-Netto 2001).

In the remaining animals the heart and kidneys were excised and the heart weights were taken for calculating the heart weight/body weight ratio and then the heart and kidney tissues were subjected to histological examination.

Statistical Analysis

All data are expressed as mean ± SEM. Comparisons among different groups were performed by ANOVA and multiple comparison analyses using Tukey test were also performed to explore the difference among groups.

Heart weight and body weight ratio was analyzed by Kruskal-Wallis one way ANOVA on ranks. A value of p<0.05 was considered significant.
RESULTS

Effect of Drug Treatments on Systolic Blood Pressure (SBP) and Heart Rate

Table 3.1 depicts the SBP values among different groups at baseline and after 11\textsuperscript{th} week and 14\textsuperscript{th} week of drug treatment. At baseline all the groups had similar values of SBP and there was no statistical difference among the groups.

Treatment with L-NAME for four weeks caused a progressive rise in blood pressure of Wistar rats (Fig. 1). Two weeks and four weeks of treatment with L-NAME caused an increase in SBP of 30 mmHg and 93 mmHg compared to baseline respectively and the rise was statistically significant after 11\textsuperscript{th} week (p=0.018) and after 14\textsuperscript{th} week (p<0.001) compared to control.

Telmisartan caused a decrease in SBP after 11\textsuperscript{th} week and after four weeks of treatment with L-NAME though the decrease was not statistically significant. The combination of ASA with telmisartan (group III) caused a significant decrease in SBP of L-NAME treated rats compared to group II, III and V after four weeks of treatment (p<0.001). The combination of ASA with L-NAME (group V) also caused a significant increase in systolic blood pressure after four weeks of treatment compared to control.

Table 3.2 depicts heart rate values of telmisartan and its combination with ASA after four weeks of treatment on L-NAME induced hypertension in rats. L-NAME caused a significant decrease in heart rates at 11\textsuperscript{th} week (p=0.005) and after four weeks (p=0.010) of treatment compared to baseline values. No significant difference was observed among different treatment groups when data was analyzed by two way ANOVA followed by Tukey test.
Morphometric Analysis

Effect on Inner Diameter and Wall Thickness

As shown in Table 3.3, L-NAME treatment caused a significant decrease in inner diameter of carotid arteries of rats compared to control (group II vs. I, *P*=0.033). Telmisartan treatment caused significant increase in the inner diameter (group III vs. II, *P*=0.010) and restored the value towards normal arteries. The combination of ASA with telmisartan had no significant effect on inner diameter.

Similarly L-NAME treatment significantly increased media and intimal thickening of carotid arteries compared to control (*P*<0.001). Treatment with telmisartan for four weeks caused a significant decrease in wall thickness compared to L-NAME treated group (III vs. II, *P*<0.001) and the thickness was even lesser compared to control. The combination of ASA with telmisartan (group IV) did not cause any significant change when compared to telmisartan alone (group III) on L-NAME induced hypertension in rats.

Effect on Heart Weight /Body Weight

Table 3.4 depicts the data with different drug treatments on heart weight/body weight. None of the groups were found to be statistically different from each others.

Effect on Body Weights, Food Intake and Water Intake

Fig. 2 shows the time course of body weights in different treatment groups. L-NAME treated group appeared generally healthy but gained weight at a slightly lower rate than untreated rats. Weekly increases in body weights were similar in all groups, although tendency towards lower body weights were observed in groups treated with L-NAME. Telmisartan caused further delay in body weights. Acetyl salicylic acid had negative effect on body weights and further delayed the increase when combined with telmisartan or L-NAME alone.

The average daily solid food consumption and water intake are shown in Fig. 3 and Fig. 4 respectively.
There was no difference in food consumption at different time intervals within the groups when analyzed by one way ANOVA. L-NAME treated group consumed less average food than control. Similarly telmisartan retarded average daily food intake. ASA when combined with telmisartan increased food intake during 11nd week and 111rd week and decreased intake during fourth week. However, ASA when combined with L-NAME alone decreased food intake during 11nd and 111rd week.

Fig. 4 shows the water intake in different groups. There was much disparity in water intake among different treatment groups. However little decrease in water intake than control was observed in L-NAME and L-NAME +ASA treated groups during 1st week to 111th week.
Drug interaction studies between NSAIDs and drugs used in cardiovascular disorders

**TABLE - 3.1**

Effect of Telmisartan (TM) and combination of Telmisartan (TM) and Aspirin (ASA) on Systolic Blood pressure (SBP) of L-NAME-Induced Hypertensive Rats

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Systolic Blood Pressure (SBP) (mmHg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Baseline</td>
</tr>
<tr>
<td>I</td>
<td>Control</td>
<td>80.88 ± 3.34</td>
</tr>
<tr>
<td>II</td>
<td>L-NAME (75mg/kg/d), p.o.</td>
<td>89.33 ± 0.80</td>
</tr>
<tr>
<td>III</td>
<td>L-NAME (75mg/kg/d), p.o. + TM (10mg/kg/d), p.o.</td>
<td>95.17 ± 2.47</td>
</tr>
<tr>
<td>IV</td>
<td>L-NAME (75mg/kg/d), p.o. + TM (10mg/kg/d), p.o. + ASA (100mg/kg/d), p.o.</td>
<td>89.80 ± 1.28</td>
</tr>
<tr>
<td>V</td>
<td>L-NAME (75mg/kg/d), p.o. + ASA (100mg/kg/d), p.o.</td>
<td>102.83 ± 3.34</td>
</tr>
</tbody>
</table>

* II vs I, V vs I (p<0.05)

*** II vs I, (p<0.001)

††† IV vs II, III, V (p<0.001)

§§§ V vs I, IV, (p<0.001)
TABLE - 3.2

Effect of Telmisartan (TM) and combination of Telmisartan (TM) and Aspirin (ASA) on Heart rate of L-NAME-Induced Hypertensive Rats

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Heart Rate (beats/ minute)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Baseline</td>
</tr>
<tr>
<td>I</td>
<td>Control</td>
<td>348.13 ± 12.13</td>
</tr>
<tr>
<td>(n=8)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>II</td>
<td>L-NAME (75mg/kg/d)</td>
<td>374 ± 10.33</td>
</tr>
<tr>
<td>(n=10)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>III</td>
<td>L-NAME (75mg/kg/d) + TM (10mg/kg/d)</td>
<td>346.17 ± 8.63</td>
</tr>
<tr>
<td>(n=6)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IV</td>
<td>L-NAME (75mg/kg/d) + TM (10mg/kg/d) + ASA (100mg/kg/d)</td>
<td>343.20 ± 14.06</td>
</tr>
<tr>
<td>(n=6)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>V</td>
<td>L-NAME (75mg/kg/d) + ASA (100mg/kg/d)</td>
<td>343.83 ± 10.21</td>
</tr>
<tr>
<td>(n=6)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\* II\textsuperscript{nd} week vs baseline (p<0.05)

\** IV\textsuperscript{th} week vs baseline (p<0.01)
**Drug interaction studies between NSAIDs and drugs used in cardiovascular disorders**

**TABLE 3.3**

Effect of Telmisartan (TM) and combination of Telmisartan (TM) and Aspirin (ASA) on Inner Diameter and Wall Thickness of L-NAME-Induced Hypertensive Rats

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Inner Diameter (µm)</th>
<th>Wall Thickness (µm)</th>
<th>Ratio (Wall thickness/diameter ± 10^6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Control</td>
<td>486.55 ± 33.41</td>
<td>35.72 ± 1.21</td>
<td>74 ± 0.005</td>
</tr>
<tr>
<td>II</td>
<td>L-NAME (75mg/kg/d)</td>
<td>396.42 ± 15.90*</td>
<td>57.01 ± 1.80**</td>
<td>144.70 ± 0.008*</td>
</tr>
<tr>
<td>III</td>
<td>L-NAME (75mg/kg/d) + TM (10mg/kg/d)</td>
<td>505.30 ± 19.80**</td>
<td>27.57 ± 0.48†††</td>
<td>54.60 ± 0.001†</td>
</tr>
<tr>
<td>IV</td>
<td>L-NAME (75mg/kg/d) + TM (10mg/kg/d) + ASA (100mg/kg/d)</td>
<td>464 ± 8.33</td>
<td>30.85 ± 3.06سلط</td>
<td>66.70 ± 0.007‡</td>
</tr>
<tr>
<td>V</td>
<td>L-NAME (75mg/kg/d) + ASA (100mg/kg/d)</td>
<td>448 ± 4.62</td>
<td>48.58 ± 0.81§</td>
<td>108.4 ± 0.001§</td>
</tr>
</tbody>
</table>

* II vs I, † III vs II, ‡ IV vs II (p<0.05)
** III vs. II (p<0.01)
*** II vs. I (p<0.001)
††† III vs. II (p<0.001)
سلط IV vs. II (p<0.001), IV vs. III, IV (P<0.001), V vs. I (p<0.003)
§ V vs. II (p<0.033), V vs. III, IV (P<0.001), V vs. I (p<0.003)
Drug interaction studies between NSAIDs and drugs used in cardiovascular disorders

**TABLE - 3.4**

Effect of Telmisartan (TM) and combination of Telmisartan (TM) and Aspirin (ASA) on Heart Weight/ Body Weight Ratio of L-NAME-Induced Hypertensive Rats

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Heart Weight /Body Weight mg/g</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Control</td>
<td>3.21 ± 0.22</td>
</tr>
<tr>
<td>II</td>
<td>L-NAME (75mg/kg/d)</td>
<td>3.27 ± 0.11</td>
</tr>
<tr>
<td>III</td>
<td>L-NAME (75mg/kg/d) + TM (10mg/kg/d)</td>
<td>2.86 ± 0.02</td>
</tr>
<tr>
<td>IV</td>
<td>L-NAME (75mg/kg/d) + TM (10mg/kg/d) + ASA (100mg/kg/d)</td>
<td>3.15 ± 0.08</td>
</tr>
<tr>
<td>V</td>
<td>L-NAME (75mg/kg/d) + ASA (100mg/kg/d)</td>
<td>3.47 ± 0.10</td>
</tr>
</tbody>
</table>
Effect of Telmisartan (TM) and combination of Telmisartan (TM) & Aspirin (ASA) on Systolic Blood Pressure (SBP) of L-NAME-induced hypertensive rats.

Fig 1

Effect of Telmisartan (TM) and combination of Telmisartan (TM) and Aspirin (ASA) on Body Weights of L-NAME-induced hypertensive rats

Fig 2
Effect of Telmisartan (TM) and combination of Telmisartan (TM) and Aspirin (ASA) on Average Daily Food Intake of L-NAME-induced hypertensive rats

![Food Intake Graph](chart1)

**Fig 3**

Effect of Telmisartan (TM) and combination of Telmisartan (TM) and Aspirin (ASA) on Average Daily Water Intake of L-NAME-induced hypertensive rats

![Water Intake Graph](chart2)

**Fig 4**
Histopathology

Effect on Cardiac Muscle

Fig. 3.1a and 3.1b show the cross section and longitudinal sections of normal rat heart. Treatment with L-NAME for four weeks caused loss of nucleus with nucleoli in majority of cells, coagulative necrosis and eosinophilic cytoplasm (Fig. 3.2a). However, few cells revealed pyknotic nuclei indicating early degenerative changes. The L.S. also showed early degenerative changes of nucleus, loss of cross striations and interstitial edema (Fig. 3.2b). Thus L-NAME caused moderate type of toxicity in cardiac tissue.

Telmisartan treatment with L-NAME showed very minimal degenerative changes in cross section (Fig. 3.3a) and the longitudinal section of this group showed well preserved nucleus with nucleoli and cross striations (Fig. 3.3b). However, at few places, interstitial edema revealed moderate protective effect with telmisartan.

The combination of ASA with telmisartan caused severe type of toxicity in cardiac muscle fibers which was characterized by loss of nucleus and nucleoli, loss of cross striations, necrosis of cytoplasm and interstitial edema (Figs 3.4a and 3.4b). L-NAME combination with ASA also revealed moderate to severe type of toxicity characterized by necrosis of cytoplasm, loss of cross striations, nucleus and nucleoli and widened interstitial spaces (Figs. 3.5a and 3.5b).

Effect on Kidney

Figs. 3.1c and 3.1d show cortical and medullary sections of normal rat kidney respectively. L-NAME treatment for four weeks caused atrophy of glomerulus and degenerative changes in proximal convoluted tubules (Fig. 3.2c); however medullary region showed well preserved distal convoluted tubules (Fig. 3.2d). Telmisartan treatment with L-NAME had protective effect on glomerulus and Bowman’s capsule (Fig. 3.3c). Distal convoluted tubules were normal (Fig. 3.3d) but combination of ASA again interfered with protective effect of telmisartan by revealing atrophied glomeruli (Fig. 3.4c). Distal convoluted tubules were normal (Fig. 3.4d). The combination of ASA with L-NAME showed hemorrhage, degenerative changes in proximal convoluted tubules (Fig. 3.5c) and congestion of inter tubular vessels with red blood corpuscles (Fig. 3.5d).
Photomicrographs – Cardiac Tissue

Group I (Control Normal Rat Heart)

Figure 3.1a  C.S. of cardiac muscle of normal rat revealing cytoplasm with (a) vesicular nucleus and nucleoli. H. & E. x400

Figure 3.1b  L.S. revealing muscle fibers running in parallel direction having clear cut cross striations and (a) elliptical nucleus with nucleoli. H. & E. x400

Group II (L-NAME, 75mg/kg/d)

Figure 3.2a  C.S. revealing (a) pyknotic nucleus but  b) majority of cells without nucleus and nucleoli and revealing eosinophilic cytoplasm and coagulative necrosis. H. & E. x400

Figure 3.2b  L.S. showing early degenerative changes of (a) nucleus (b) loss of cross striations and (c) widened interstitial spaces revealing edema. H. & E. x400
Photomicrographs – Cardiac Tissue

Group III (L-NAME, 75 mg/kg/d + Telmisartan (TM), 10 mg/kg/d)

Figure 3.3a  C.S. shows (a) very minimal degenerative changes of nucleus. At places a few cells reveal loss of nucleoli and (b) interstitial spaces are widened, thereby revealing moderate type of protective effect of telmisartan. H. & E. x400

Figure 3.3b  L.S. shows (a) well preserved nucleus with nucleoli and cross striations are also preserved. However, at few places (b) interstitial spaces are widened. H. & E. x400

Group IV (L-NAME, 75 mg/kg/d + TM, 10 mg/kg/d + ASA, 100mg/kg/d)

Figure 3.4a  C.S. shows majority of muscle fibers revealing (a) loss of nucleus and nucleoli and necrosis of cytoplasm (c) interstitial edema. H. & E. x400

Figure 3.4b  L.S. reveals (a) loss of cross striations leading to necrosis and eosinophilic cytoplasm (c) widened interstitial spaces. H. & E. x400
Photomicrographs – Cardiac Tissue

Group V (L-NAME, 75 mg/kg/d + ASA, 100 mg/kg/d)

Figure 3.5a  C.S. reveals; in majority of cells (a) necrosis of cytoplasm and loss of nucleus with nucleoli. H. & E. ×400

Figure 3.5b  L.S. reveals (a) loss of cross striations with necrosis of muscle fibers and (c) widened interstitial spaces. H. & E. ×400
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Photomicrographs – Renal Tissue

Group I (Control Normal Rat Heart)

Figure 3.1c  Cortical section showing (a) well protected glomerulus and bowman's capsule (b) well protected proximal convoluted tubules. H. & E. x250

Figure 3.1d  Distal convoluted tubules are normal with (b) well protected basement membrane. H. & E. x250

Group II (L-NAME, 75 mg/kg/d)

Figure 3.2c  Cortical section reveals (a) atrophied glomerulus (b) proximal convoluted tubules show desquamation of epithelial lining indicating degenerative changes. H. & E. x250

Figure 3.2d  Medullary region revealing well preserved (b) epithelial lining of distal convoluted tubules with basement membrane. H. & E. x250
Photomicrographs – Renal Tissue

Group III (L-NAME, 75 mg/kg/d + TM, 10 mg/kg/d)

Figure 3.3c Cortex of kidney revealing (a) well protected glomerulus with Bowman’s capsule. H. & E. x250

Figure 3.3d Distal convoluted tubules are well protected (b) without any desquamation of epithelial cells. H. & E. x250

Group IV (L-NAME, 75mg/kg/d + TM, 10mg/kg/d + ASA, 100mg/kg/d)

Figure 3.4c Various gradations of atrophy in glomeruli showing moderate type of damage. H. & E. x250

Figure 3.4d Distal convoluted tubules are well preserved. H. & E. x250
Photomicrographs – Renal Tissue

Group V (L-NAME, 75 mg/kg/d + ASA, 100mg/kg/d)

Figure 3.5c Cortex shows (a) atrophied glomerulus (b) hemorrhage and (c) degenerative changes in proximal convoluted tubules. H. & E. x250

Figure 3.5d Medullary region revealing (a) intertubular vessels congested with red blood corpuscles. H. & E. x250
5.4 Protocol 4

To assess by echocardiography, the acute effect of aspirin administration on left ventricular systolic and diastolic performance in patients of severe congestive heart failure who are on anti failure treatment including ACE inhibitors and/or angiotensin receptor blockers
PATIENTS AND METHODS

Patients of severe congestive heart failure (stage D) who were in sinus rhythm and were stable for at least 6 weeks on regular and optimal anti failure treatment including digoxin, betablockers, diuretics, spirinolactone and ACE inhibitors/ angiotensin receptor blockers were considered for inclusion. Patients who had recently required dose adjustments, or who had recent change in clinical status were excluded. Patients who had milder degrees of congestive failure with clinical remission of heart failure on treatment (stage C congestive heart failure) were also excluded.

Echocardiographic study: Patients were asked to come to hospital in the morning for echocardiography which was done by a single operator (Dr. Rajiv Bajaj) using HP 450 machine and 4S phased array transducer. The heart rate and blood pressure were measured at the start of the study using standard techniques and a mercury sphygmomanometer. The basal study was done during the morning hours, and 325 mg of soluble dispersible aspirin was administered at the end of the study. The study was repeated four hours after aspirin administration.

The Aortic valve ejection time and the isovolumic contraction time were measured from the M-mode cut of the aorta in left parasternal view using simultaneous ECG recordings. The left ventricular dimensions were measured using M-mode echocardiography. The velocity time integral of the aortic outflow tract was measured by pulsed Doppler signal with the sample volume kept just below the aortic valve. Diastolic function was assessed by the mitral inflow signal E and A velocities and the E deceleration time. The pulmonary artery pressure was estimated by the tricuspid regurgitation jet velocity using continuous wave Doppler. All echocardiographic measurements were averaged from three consecutive beats during quiet respiration.

Fractional shortening was calculated from the left ventricular dimensions using the formula:

\[
\text{Fractional Shortening} \% = \left( \frac{LVEDD - LVESD}{LVEDD} \right) \times 100
\]

Where LVEDD = Left ventricular end diastolic dimension
LVESD = Left ventricular end systolic dimension
Pre and post aspirin wall stress was compared by calculating the product of systolic blood pressure and left ventricular end systolic dimension.

Statistical Analysis

Descriptive statistics i.e. mean ± SEM were calculated for all paired observations at baseline and then after treatment. Wilcoxon signed rank was used to compare the means in the two groups using SPSS 10.0 as statistical software. The level of significance was set at P≤0.05.

RESULTS

We studied seven patients, 6 males and 1 female aged 29 to 57 years, Body Mass Index 24.47 ± 1.039 kg/m²; suffering from Dilated Cardiomyopathy (n=4) or coronary artery disease (n=3), stage D for one to five years.

All the patients were on multiple anti failure therapy (digoxin, 5; aldactone, 6; furosemide, 5; Betablocker, 5; ACE inhibitor alone, 3; Angiotensin II receptor blocker alone, 2; and combination of ACE inhibitor and Angiotensin II receptor blocker, 2).

The echocardiographic and hemodynamic measurements before and four hours after 325 mg p.o. aspirin appear in Table 4.1.

There was a significant increase in aortic ejection time from 262.14 ± 18.92 to 291.43±30.37 msec after administration of aspirin. This was associated with a fall in systolic blood pressure (SBP) from 102.74±3.77 to 92.83±4.15 mmHg, fall in diastolic blood pressure (DBP) from 74.29±3.17 to 70±5.63 mmHg; fall in isovolumetric contraction time (IVCT) from 119.29 ± 14 to 110 ± 11.19 msec; fall in left ventricular end diastolic dimension (LVEDD) from 6.98 ± 0.28 to 6.94 ± 0.25 cm and fall in left ventricular end systolic dimension (LVESD) from 6.27 ± 0.25 to 6.05 ± 0.31 cm and an increase in fractional shortening from 0.10 ± 0.01 to 0.13 ± 0.02.

There was no change in mitral E and A velocities and heart rate. However, there was a decrease in mitral E wave deceleration time, aortic peak velocity, calculated wall stress
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(SBP x LVESD) and aortic velocity heart rate product as an index of cardiac output which did not reach statistical significance.
**TABLE – 4.1**

**Effect of Single-dose Aspirin on Hemodynamics and Echocardiographic Parameters in Patients of Congestive Heart Failure**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Pretreatment</th>
<th>Post- treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>102.74 ± 3.77</td>
<td>92.83 ± 4.15</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>74.29 ± 3.17</td>
<td>70 ± 5.63</td>
</tr>
<tr>
<td>Heart Rate (beats/minute)</td>
<td>78.43 ± 5.53</td>
<td>75.50 ± 6.14</td>
</tr>
<tr>
<td>M-Mode Aortic ejection time (msec)</td>
<td>262.14 ± 18.92</td>
<td>291.43 ± 30.37*</td>
</tr>
<tr>
<td>Isovolumic contraction time (IVCT) (msec)</td>
<td>119.29 ± 14.00</td>
<td>110 ± 11.19</td>
</tr>
<tr>
<td>Left ventricular end diastolic dimension (LVEDD) (cm)</td>
<td>6.98 ± 0.28</td>
<td>6.94 ± 0.25</td>
</tr>
<tr>
<td>Left ventricular end systolic dimension (LVESD) (cm)</td>
<td>6.27 ± 0.25</td>
<td>6.05 ± 0.31</td>
</tr>
<tr>
<td>Mitral E peak velocity (cm/sec)</td>
<td>96.86 ± 7.39</td>
<td>98.2 ± 9.10</td>
</tr>
<tr>
<td>Mitral A peak velocity (cm/sec)</td>
<td>53 ± 11.16</td>
<td>54 ± 12.19</td>
</tr>
<tr>
<td>Mitral E wave deceleration time (DT) (msec)</td>
<td>636.29 ± 205.70</td>
<td>462.29 ± 159.10</td>
</tr>
<tr>
<td>Aortic peak Velocity (msec)</td>
<td>69.10 ± 11.18</td>
<td>65.86 ± 9.13</td>
</tr>
<tr>
<td>Fractional shortening</td>
<td>0.10 ± 0.01</td>
<td>0.13 ± 0.02</td>
</tr>
<tr>
<td>Wall Stress (SBP x LVESD)</td>
<td>646.24 ± 41.21</td>
<td>580.28 ± 52.57</td>
</tr>
<tr>
<td>Cardiac Output (Aortic velocity x Heart rate)</td>
<td>5344.91 ± 757.23</td>
<td>5134 ± 973.19</td>
</tr>
</tbody>
</table>

* p < 0.05
DISCUSSION
6.0 DISCUSSION

6.1 Interaction of aspirin with angiotensin-converting enzyme (ACE) inhibitor on methylprednisolone-induced hypertension in rats.

Aspirin is the cornerstone in the treatment of acute coronary syndromes and has been shown to reduce the risk of vascular events like non fatal myocardial infarction, non fatal stroke or vascular death, not just among patients with unstable angina, acute myocardial infarction, or a past history myocardial infarction, stroke, or transient ischemic attack but also in the large category of other patients at increased risk of occlusive vascular disease; that is, patients having coronary vascular procedures and patients with stable angina, valvular heart disease, atrial fibrillation and peripheral vascular disease (ISIS-2 collaborative group 1988; Antiplatelet Trialists' Collaboration 1994). ACE inhibitors have been shown to be beneficial not only in hypertension but also in patients with congestive heart failure, myocardial infarction and left ventricular dysfunction (Cohn et al. 1991; Kober et al. 1995).

Considering the expanding therapeutic roles of ASA and ACE inhibitors it is not uncommon to encounter patients with cardiovascular diseases taking both drugs. However, the beneficial effects of ACE inhibitors have been shown to be attenuated by concomitant aspirin therapy in patients; where low dosage ASA appeared to interact little & high dosage produced more significant interaction (Nawarskas and Spinler 1998; Guazzi et al. 1998).

In our study two doses of ASA i.e. 100 mg/kg/d and 25 mg/kg/d were chosen. Several investigators have studied the differential effects of aspirin on platelet aggregation & thrombus formation. Potent antithrombotic activity in arterioles was manifested at doses of 2.5 - 50 mg / kg in an in vivo model of thrombus formation; the effect being more pronounced at higher doses (Nagamatsu et al. 1999). Aspirin (10 and 30 mg / kg i.v.) had little effect in terms of preventing photo chemically induced arterial thrombosis in the rat femoral artery (Shimazawa et al. 1997).
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A synergistic effect of ticlopidine / aspirin association was demonstrated with regard to ADP and collagen induced platelet aggregation and also in experimental models including silk thread-induced thrombosis where a total inhibition of thrombosis formation was observed at highest dosage of ticlopidine & aspirin tested (100 + 100 mg/kg p.o.) (Herbert et al. 1996).

Kalkman et al. (1995) carefully selected and validated the dose of 25 mg/kg/d ASA which caused marked inhibition of thromboxane production in rats but did not interfere with prostacyclin production reflecting the aims of low dose aspirin treatment in man.

In the present study ASA 25 or 100 mg/kg/d did not attenuate the hypotensive response of lisinopril when given to hypertensive rats. However the latter dose of ASA produced significant mortality compared to lower dose, when administered concurrently with ACE inhibitor.

Although clinically the low dose of ASA i.e. 25 mg/kg/d showed less mortality compared to 100 mg/kg/d; moderate and variable toxic effects were observed with this dose on pathological examination of various organs like heart & kidneys. Thus even the low dose of ASA had detrimental effects on cardiac and renal tissues when given to hypertensive rats.

In the study conducted by Durak et al. (2001), the researchers found that antioxidant system was impaired in the heart tissue from guinea pigs and in erythrocytes from volunteer subjects receiving aspirin treatment. Their results suggest that high-dose aspirin but not the lower concentration exerted significant toxicity to guinea pig myocardium. However, lower dose aspirin caused peroxidation in the human erythrocytes due to its oxidation potential.

Histopathological examination of cardiac and renal tissues of normal rats treated with combination of LS and ASA and LS only showed normal structures. Therefore it appears that the combination of ASA and ACE inhibitor is detrimental only in degenerative condition like hypertension. Both 'Aspirin Myocardial
Infarction Study’ (AMIS) (Aspirin Myocardial Infarction Study Research Group 1980) and ‘Persantine-Aspirin Reinfarction Study’ (Klimt et al. 1986) showed that ASA may be helpful in patients with well preserved ventricular function but harmful in those with considerable damage.

The exact mechanism of interaction between aspirin and ACE inhibitor is unknown. One proposed theory relates to the counteractive pharmacodynamics of ACE inhibitors and aspirin. ACE inhibitors stimulate prostaglandin synthesis by blocking the enzyme kininase II which impedes the degradation of bradykinin. Bradykinin then augments the release of kinin- mediated prostaglandins (vasodilation) (Swartz et al. 1980; Moore et al. 1981). Aspirin on the other hand, blocks cyclooxygenase which inhibits prostaglandin synthesis (Vane 1971). Therefore the mechanism action of aspirin may blunt the beneficial effects of ACE inhibitors.

Boger et al. (1996) found that low dose ASA, although exerting a significant inhibiting effect on TXA2 formation, did not modulate the hypotensive or humoral effects of captopril in healthy human subjects. Low dose ASA is known to inhibit platelet cyclooxygenase activity and, thus thromboxane formation relatively selectively (Patrano et al. 1985), although a slight decrease in prostacyclin formation seems to be unavoidable even with very low doses of ASA (Boger et al. 1993). Therefore an interaction between ASA and ACE inhibitor might be possible even with low dose ASA.

In our study probably, the myocardial damage induced by ACE inhibitor and ASA combination in glucocorticoid induced hypertension is due to sequential blockade of the enzymes phospholipase A2 and cyclooxygenase.

A third isoform of COX, COX-3 has been proposed which unlike COX-1 and COX-2 does not produce pro-inflammatory prostanoids but produces anti-inflammatory members of that family ; this isoform was found to be induced during the resolution of an inflammatory response and identified by use of specific antibodies (Willoughby 2000). If this hypothesis is true, expression of this third
inducible isoform of COX could result in the typical periods of remission seen in many clinical cases of chronic inflammatory diseases and aspirin probably by acting on such an enzyme system may retard normal periods of remission.

In conclusion, even in absence of concluding evidence, regarding the mechanism of action, it is clear that aspirin should be given cautiously in patients receiving ACE inhibitors or avoided in view of availability of equally promising ways of inhibiting platelet aggregation with glycoprotein IIb / IIIa receptor antagonists.

6.2 Interaction of a selective COX-2 inhibitor with antihypertensive agent in spontaneously hypertensive rats

Cyclooxygenase-2 (COX-2) inhibitors have been shown to combine similar efficacy to classical NSAIDs with less risk of gastrointestinal complications (Abramson et al. 2001). However, from a cardiovascular stand point COX-2 inhibitors have been the topic of much debate and discussion. The concern about the use of these agents stemmed from the observations suggesting that under physiological conditions COX-2 is a major source of endothelium derived prostacyclin (PGI\(_2\)) and that selective blockade of COX-2 may result in a disproportionate and unopposed increase in COX-1 derived thromboxane (TXA\(_2\)); an endothelium derived contracting factor and prothrombotic molecule, relative to PGI\(_2\). Such an imbalance might favour endothelial dysfunction and uncover a prothrombotic phenotype (Verma and Szmitko 2003). Thus by inhibiting synthesis of prostacyclin in the vascular wall but not platelet thromboxane production, COX-2 inhibitors could theoretically, increase the risk of cardiovascular events (Cleland 2002).

In our study the concurrent administration of rofecoxib with lisinopril for two weeks attenuated the protective effect of lisinopril in myocardium of 18 weeks old SHRs with mild deterrent changes in kidneys. This was evident from necrotic changes in cardiac muscle fibers of the group receiving combination of rofecoxib and lisinopril which did not appear reversible at all as were seen in the group
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receiving lisinopril treatment alone. Similarly rofecoxib attenuated the protective effect of lisinopril in renal tissue of SHR to some extent.

The combination of rofecoxib with lisinopril also prevented the antihypertensive effect of lisinopril almost completely during the first week of treatment without any apparent effect on heart rate. However, the hypotensive effect of lisinopril was restored during the second week of drug treatment. Therefore in our study, we found that rofecoxib had only short term impact on the antihypertensive activity of lisinopril.

There is data suggesting transient decreases in creatinine clearance seen after initiating NSAIDs therapy in humans but renal function generally reverts to patients' baseline quickly. In a double blind study comparing rofecoxib 50mg/day with indomethacin 80mg three times daily for two weeks; blood pressure did not change in any group but transient decreases in sodium excretion of similar magnitude were observed in rofecoxib and indomethacin groups with sodium excretion returning to baseline by day three of therapy (Catella-Lawson et al. 1999). Similar results were obtained in other study comparing renal effects of celecoxib and naproxen (Whelton et al. 1999).

In an experiment conducted on normotensive Wistar Kyoto rats and young Spontaneously Hypertensive rats, chronic treatment with rofecoxib for four weeks increased systolic blood pressure in both rat strains in a dose dependent manner (Höcherl et al. 2002). In contrast to our experiment however, which used fully developed hypertensive rats, this work was carried out during the developmental phase of Spontaneously Hypertensive rats beginning at age three weeks of their lives.

A significantly higher incidence of blood pressure destabilization and clinically significant edema was observed with rofecoxib than with celecoxib among older, hypertensive patients with osteoarthritis in some head to head, randomized trials comparing rofecoxib with celecoxib (Whelton et al. 2001, 2002).
Long term administration of NSAIDs has resulted in renal papillary necrosis and other renal injury (Sandler et al 1991; Whelton and Hamilton 1991). Renal toxicity has also been seen in patients in whom renal prostaglandins have a compensatory role in the maintenance of renal perfusion. In these patients administration of a NSAID may cause a dose dependent reduction in prostaglandin formation and, secondarily in renal blood flow, which may precipitate overt renal decompensation. Patients at greatest risk include those with impaired renal function, heart failure or those taking diuretics or ACE inhibitors and the elderly (Kendell and Horton 1990). Since it is now known that COX-2 is also constitutively expressed in regions of kidney (Harris et al. 1994) and has a potential role in the regulation of vascular tone and renin release (Harris and Breyer 2001; Lorenz et al. 1993); inhibition of this isoenzyme would therefore account for the common renal adverse effects associated with non selective NSAID therapy.

COX-2 is also expressed in cardiomyocytes in patients with heart failure and has caused increased production of prostacyclin and PGE$_2$ during episodes of chest pain in patients with unstable angina (FitzGerald et al. 1986). In animal model of myocardial ischemia, COX-2 has been shown to afford cardioprotection during late phase of ischemic preconditioning (Shinmura et al. 2000). Therefore, if up regulation of endothelial COX-2 represents a protective mechanism against vascular injury, inhibition of COX-2 without concomitant inhibition of platelet TXA$_2$ production may influence the overall prothrombotic/antithrombotic equilibrium and exacerbate the potential for thrombotic complications in patients at risk.

However recent reports indicate adverse cardiovascular and cerebrovascular events even with traditional NSAIDs like naproxen on long term use (WHO Pharmaceuticals Newsletter, 2005). The US Food and Drug Administration (FDA 2005) has already issued an alert for practitioners regarding the possible risk of serious cardiovascular events in patients receiving celecoxib and has also requested that the package insert for all NSAIDs be revised to include a
contraindication for use in patients immediately post-operative from coronary artery bypass (CABG) surgery.

The interaction between ACE inhibitors and NSAIDs including aspirin is primarily because of compensatory hemodynamic mechanisms of heart failure. Since ACE inhibitors share and enhance the effects of these desirable compensatory mechanisms by enhancing production of vasodilatory prostaglandins, they are particularly susceptible to the interaction with NSAIDs and subsequently incur a loss of benefits (Hall 2000).

Therefore for patients requiring long term treatment for heart failure it is important to maintain the integrity of prostaglandin system by avoiding use of both traditional NSAIDs including aspirin and selective COX-2 inhibitors. Selective COX-2 inhibitors should be prescribed with caution, only in patients with conditions for which these drugs have proven efficacy and with careful monitoring of outcomes and events. This is particularly important in the elderly, in patients with cardiovascular/renal disease, and in patients with other risk factors that might predispose them to adverse events.

In the present study an attempt was also made to determine the NHE activity in erythrocytes of SHRs and the influence of concomitant administration of lisinopril and rofecoxib on it.

In spite of substantial research efforts the etiology and pathogenesis of human hypertension remains unclear. Because SHRs follow the same progression of hypertension as human hypertension with prehypertensive, developing and sustained hypertensive phases, they have the potential to be used in studies of the cause and development of hypertension Many investigators have described raised intracellular Na⁺ contents mainly in the peripheral blood cells in both experimental and clinical hypertension (Ambrosioni et al. 1981; Hilton 1986; Parker and Berkowitz 1983) although unchanged and reduced intracellular Na⁺ has been reported as well (Simon 1989). The Na⁺/H⁺ exchanger is activated in a substantial number of patients with essential hypertension and therefore has been seen as an
intermediate phenotype in this disorder. This transport protein more precisely the ubiquitously expressed NHE-1 isoform, plays a dominant role in cellular pH and volume homeostasis and may also contribute to the initiation of cell growth or proliferation (Siffert and Düssing 1995).

In our study a paradoxical decrease of Na\(^+\) influx was found in 18 weeks old SHRs compared with their age matched Wistar rats. Lisinopril treatment restored these values to Wistar control while concurrent rofecoxib administration did not interfere with such activity of lisinopril.

This is in contrast to the findings of Orlov et al. (1989) who reported an enhanced rate of Na\(^+\)/H\(^+\) in erythrocytes of Japanese Spontaneously Hypertensive rats but not in Milan hypertensive rats. Rosskopf et al. (1993) describe in their article that a broad overlap of Na\(^+\)/H\(^+\) exchange activity in normotensive and hypertensive individuals as well as its distinct elevations in some hypertensive patients could lead to the alternate view of two different subgroups in hypertension characterized by either "high" or "low" antiport activity. This hypothesis is further supported by findings of Canessa et al. (1991), who reported a bimodal distribution of NHE activity in red blood cells of forty two hypertensive patients of which only 45% displayed enhanced activity. The total number of patients characterized in terms of "low" (or "normal") versus "increased" NHE activity is yet limited and more studies are required regarding the epidemiology of NHE activity in normotension and hypertension and to clarify the role of NHE in the pathogenesis of hypertension. Pharmacological studies with NHE inhibitors have already shown promising results in animal models of ischemia and reperfusion injury (Karmazyn et al. 2001) and may become novel tool for pharmacological modulation in hypertension.
6.3 Interaction of aspirin with angiotensin II receptor antagonist on L-NAME-induced hypertension in rats

Long term treatment of rats with nitric oxide (NO) synthase inhibitor, N^6-Nitro-L-arginine methyl ester (L-NAME) results in the development of sustained hypertension in normotensive rats (Ribeiro et al. 1992; Morton et al. 1993), left ventricular hypertrophy (Bernatova et al. 1996; Johnson and Freeman 1992) and vascular remodeling (Babal et al. 1997; Bernatova et al. 1999). The mechanism of these alterations however, is not completely understood. Besides the attenuation of vascular relaxation, activation of both the renin angiotensin system (RAS) and the sympathetic nervous system are considered (Rees et al. 1989; Jover et al. 1993; Sander et al. 1995). Chronic inhibition of NO synthase by L-NAME also induces structural vascular changes with increased wall to lumen ratio and perivascular fibrosis (Numaguchi et al. 1995; Bartunek et al. 2000). Furthermore the increase in the number of angiotensin 1 (AT_1) receptors and plasma aldosterone concentration may contribute to the development of the L-NAME induced hypertension as well as to the development of left ventricular hypertrophy and structural remodeling of the heart in later phases of L-NAME treatment (Katoh et al. 1998; Linz et al. 1995 b).

ACE inhibitors are effective antihypertensive agents which can reduce left ventricular enlargement and the formation of collagen and have been found to preserve cardiac function in a variety of experimental models of hypertension as well as in essential hypertension in humans (Brilla et al. 1991; Linz et al. 1997; Simko 1996).

Non peptide Ang II receptor antagonists are drugs that, like ACE inhibitors reduce blood pressure in both renin dependent and renin independent models of hypertension (Smith et al. 1992). We had selected telmisartan as AT_1 receptor antagonist to see its effect on L-NAME induced hypertension and to find out whether ASA interferes with blood pressure lowering effect or cardiorenoprotective effect of telmisartan. In the present study chronic blockade of NO formation by administration of L-NAME caused a progressive rise in blood
pressure in normotensive Wistar rats. L-NAME also produced bradycardia in rats. Telmisartan caused a decrease of 14 mmHg by the end of treatment while ASA when combined with telmisartan restored blood pressure to almost normal when given along with L-NAME.

It has been suggested that intact NO and prostaglandin systems are necessary for the full antihypertensive efficacy of AT_1 receptor antagonists and that increased NO production by AT_2 receptors might mediate the hypotensive effect of losartan (Cachoimph et al. 1995). Consequently a non significant decrease in SBP observed during concomitant administration of telmisartan and L-NAME could be due to the inhibition of telmisartan stimulated NO production by L-NAME. The mechanism underlying the participation of prostaglandins in the vasodepressor effect of telmisartan could be an increase in the prostaglandin synthesis as there are reports suggesting losartan to act as potent stimulus for both prostaglandin E_2 and F_2 in cultured endothelial cells (Jaiswal et al. 1991). Therefore inhibition of TXA_2 but not PGI_2 by the antiplatelet dose of ASA might explain the vasodepressor effect observed with telmisartan administered concurrently with ASA and L-NAME.

The observed daily food consumption in different groups was in accord with the assumed adequate dietary intake 15g/d per rat for growing rats or adult rats at maintenance. The slightly higher average consumption of solid food in control rats, in comparison with the average consumption of L-NAME treated rats is in agreement with the findings of Rossi and Colombini-Netto (2001).

The total heart weight/body weight ratio in our study did not change in any group despite hypertension. Many studies have not found clear myocardial hypertrophy of the whole heart (Arnal et al. 1992, 1993) or the left ventricle (Bartunek et al. 2000; Suo et al. 2002) after oral L-NAME treatment for 4 to 8 weeks. L-NAME has been reported to produce negative metabolic effects that suppress the increase in left ventricular mass in response to sustained pressure overload (Bartunek et al. 2000). This same mechanism could also contribute to the weight gain retardation of animals in L-NAME treated group. In contrast, treatment with L-NAME for 6
and 8 weeks has been shown to result in significant increase in heart weight, body weight ratio (Delacretaz et al. 1994; Kristek and Gerova 1996; Kristek et al. 1996). Bernatová et al. (1996, 2000) focusing in particular on the left ventricle later found an increase in left ventricular weight in L-NAME treated rats.

In our experiment, structural remodeling of the carotid artery wall was observed as indicated by 60% increase in media and intima thickness and a decrease in inner diameter during long-term NO synthase inhibition. These findings are in agreement with the data of Kristek et al. (1996) and Delacretaz et al. (1994). Telmisartan treatment reversed these changes even below normal when given along L-NAME while concomitant ASA administration did not interfere with this effect of telmisartan. Our study indicates that ASA does not interfere with structural effects of AT₁ receptor antagonist on vessel walls.

The histological examination however, revealed moderate protective effect of telmisartan on myocardium and kidney which was abolished by concomitant treatment with ASA.

6.4. Acute effect of aspirin administration on left ventricular systolic and diastolic performance in patients of severe congestive heart failure

Several studies have suggested that aspirin may interfere with the hemodynamic effects of angiotensin-converting enzyme inhibitors in patients with severe heart failure. Two recent prospective randomized studies, viz. Warfarin/Aspirin Study in Heart Failure (WASH) and the Warfarin And Antiplatelet Therapy in Chronic Heart Failure (WATCH) have found that aspirin is associated with more frequent hospitalizations for worsening heart failure in patients of chronic heart failure (Cleland et al. 2004; Massie et al. 2004) and should not be routinely used in CHF patients (Massie 2005).

We found a significant increase in aortic ejection time associated with fall in systolic blood pressure, diastolic blood pressure, isovolumetric contraction time, left ventricular end systolic and end diastolic dimensions, together with an increase
in fractional shortening. This indicates a fall in peripheral vascular resistance on acute administration of aspirin to patients of advanced congestive heart failure on decongestive therapy. However, a fall in aortic peak velocity, mitral E deceleration time and aortic velocity-heart rate product suggest that there is additional myocardial depression.

Hall et al. (1992) found that a dose of 350 mg aspirin interfered with hemodynamics of enalapril. He reported that enalapril alone led to a significant decrease in systemic vascular resistance, left ventricular filling pressure and total pulmonary resistance together with an increase in cardiac output in patients of severe heart failure. When aspirin was added, enalapril did not have a significant effect on any of these values.

Similarly 325mg aspirin when given daily for eight weeks to twenty six patients with primary dilated cardiomyopathy, worsened pulmonary diffusion for carbon monoxide, peak exercise oxygen uptake and the ventilatory response to exercise in the presence of ACE inhibition (Guazzi et al. 1999). Such negative interactions on pulmonary hemodynamics were not observed when aspirin was combined with angiotensin receptor blocker (Guazzi et al. 1997 b) or when ACE inhibitor was combined with a potent antiplatelet agent, ticlopidine (Spaulding et al. 1998).

Non-steroidal anti-inflammatory drugs have little effect on renal function in normal human subjects, presumably because the production of vasodilatory prostaglandins has only a minor role in sodium-replete individuals. However, these drugs decrease renal blood flow and glomerular filtration rate in patients with congestive heart failure, hepatic cirrhosis with ascites and in those who are hypovolemic (Oates et al. 1988). In such individuals renal perfusion is more dependent upon vasodilatory prostaglandins which oppose the increased vasoconstrictive influence of norepinephrine and angiotensin II that occur in these individuals.

In chronic compensated moderate heart failure the renin-angiotensin-aldosterone system is not activated but it can be activated by a low sodium diet and/or the
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The renal vasoconstrictor action of angiotensin II, vasopressin, and renal sympathetic nerve activity, mechanisms that may be stimulated in heart failure are attenuated by renal synthesis of vasodilator prostaglandins such as PGE\(_2\) and PG\(_{12}\) in the cortical and medullary collecting ducts and glomeruli (Riegger et al. 1991). Administration of ASA in doses that reduce the synthesis of renal PGE\(_2\) significantly reduces the renal sodium excretion and may produce adverse hemodynamic effects, such as decreases in the cardiac index and increases in the pulmonary capillary wedge pressure, mean arterial pressure and systemic vascular resistance as has been reported with indomethacin in patients of congestive heart failure particularly associated with hyponatremia (Dzau et al. 1984).

It has been speculated that low doses of aspirin are antiaggregatory while sparing the effects or having much less effect on prostacyclin formation (Weksler et al. 1983; Hanley et al. 1981; Ellis et al. 1980). However, there is uncertainty over the optimum dose regimen for aspirin, since although it inhibits platelet thromboxane production for many days, the magnitude and duration of its effects on PG\(_{12}\) production by vascular endothelium \textit{in vivo} is unknown (Heavey et al. 1985). In addition different formulations of aspirin inhibit platelet thromboxane and prostacyclin synthesis differentially; soluble aspirin causing greater inhibition of both thromboxane and prostacyclin than enteric coated formulations (James et al. 1991; de la Cruz et al. 2002). On the other hand there have been reports indicating that both low and high single dose of aspirin (40, 75, 300 or 325mg) inhibit both TXA\(_2\) and prostacyclin production in humans suggesting that selective thromboxane inhibition is not possible (Klein et al. 1987; Hanley and Bevan 1985).

Limitations of Study

The number of patients studied was small. We have studied only the acute effects of aspirin using 325mg dose and have not studied the effects of chronic moderate dose aspirin therapy. Since Stage D patients seldom have a stable clinical state, we found that chronic effects of aspirin were difficult to study due to constant dose.
adjustment of decongestive drugs. We conclude that aspirin has detrimental effect on the clinical status in patients of advanced congestive heart failure, who are on multiple anti failure medication and should be used with caution in such patients.

Recent studies have suggested that some patients experience recurrent vascular events despite treatment with aspirin, a phenomenon that has been called 'aspirin resistance' (Sanderson et al. 2005). Higher doses of aspirin are becoming popular to overcome aspirin resistance (Roller et al. 2002; Maly et al. 2005). In such instances the effect of aspirin in patients of heart failure will be all the more deleterious and therefore alternate therapy with warfarin or other antiplatelet agents such as clopidogrel should be considered.