COMPARISON OF CARDIAC PRECONDITIONING EFFECTS OF ISOFLURANE AND SEVOFLURANE IN CORONARY ARTERY BYPASS GRAFT SURGERIES DONE ON CARDIO PULMONARY BYPASS

Comparison of cardiac preconditioning effects of Isoflurane and Sevoflurane in Coronary Artery Bypass Graft surgeries done on Cardio Pulmonary Bypass

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CERTIFICATE

This is to certify that the dissertation entitled **"Comparison of cardiac preconditioning effects of Isoflurane and Sevoflurane in Coronary Artery Bypass Graft surgeries done on Cardio Pulmonary Bypass"** is the bonafide work by **Dr. D. SATHISH KUMAR**, in the Dept of Anaesthesiology, Christian Medical College ,Vellore during his two years (2005-2007) **M.D in Anaesthesiology Branch-X**, in the partial fulfillment of requirements for the award of Master of Anaesthesiology by The Tamilnadu Dr.M.G.R Medical University, Chennai.

Guide	**Head of Department**
Dr. Mary Korula, MD Professor	**Dr. Manickam Ponnaiah, MD** Prof. & Head,

Department of Anaesthesiology
Christian Medical College Hospital
Vellore 632004,
Tamil Nadu, India.

CONTENTS

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INTRODUCTION

Ischemic heart disease is one of the major causes of morbidity and mortality all over the world. Various medical and surgical strategies have been evolved to reduce the mortality from acute MI which includes the use of thrombolytic agents, β blockers, ACE inhibitors, antioxidants, PTCA and Coronary artery bypass surgery (CABG). CABG is still considered the gold standard. Many reports have shown that upto 90% of patients undergoing CABG surgery experience a fall in ejection fraction and cardiac index during the immediate post-operative period. This has been attributed to various factors of which ischemia associated with aortic cross clamping and inadequate myocardial protection during surgery seem to be the main causes. One intervention for myocardial preservation which has received universal acceptance and generated a lot of excitement is the phenomenon of "Myocardial Ischemic preconditioning". Myocardial preconditioning can be achieved by various methods. Recently several studies have reported cardioprotective effects of volatile agents in coronary surgery patients. This has been achieved with different volatile agents like halothane, enflurane, isoflurane and sevoflurane. Isoflurane and recently sevoflurane have been shown to be better preconditioning agents in some studies. My study is an attempt to determine if administration of either isoflurane or sevoflurane in

patients undergoing CABG surgery will improve postoperative myocardial contractility and function and also to determine which of these volatile agents would offer more cardiac protection when used as preconditioning and post conditioning agents throughout surgery including the cardio pulmonary bypass period.

AIM

To compare the cardioprotective properties of Isoflurane and Sevoflurane when used before, during and after Cardiopulmonary bypass (CPB) to improve postoperative outcome in coronary artery bypass graft surgeries by analyzing:

A) Hemodynamic parameters such as Invasive arterial blood pressure, Electrocardiogram, Cardiac index, Systemic vascular resistance and Central Venous pressure.

B) Postoperative measurements of Creatinine kinase-MB to compare degree of ischemia

C) Markers of Oxidative and nitrosative stress such as protein carbonyl content, malondialdehyde, protein thiols, total thiols and nitrates.

REVIEW OF LITERATURE

Purpose:

Our literature search is mainly stressed on the mechanism of myocardial ischemia, myocardial dysfunction both short and long term after bypass and ischemic reperfusion injury (IRI). We reviewed the main products utilized in the oxidative reaction and the byproducts of oxidative stress conditions. We also reviewed the methods by which IRI can be minimised or prevented, which involved mainly preconditioning the myocardium before the actual ischemic episode. The various techniques and agents that have been used to achieve this have been extensively reviewed especially the role of volatile anesthetic agents. Also the importance of timing the preconditioning agent, whether early or late preconditioning would help in minimising the detrimental effects and prevent neutrophil activation associated with IRI. Also the various biochemical and cardiac markers that would be most suitable and useful in designing our study were also reviewed.

Myocardial ischemia

Acute occlusion or progressive constriction of a coronary artery causes reduction or abolition of systolic shortening and thickening of the ischemic wall (1). Ischemic segments also demonstrate paradoxical wall motion (termed

post systolic shortening or post systolic thickening). These functional changes relate directly to the reduction in coronary blood flow.

Mechanism of Myocardial ischemia

The time course of the effects of ischemia on cardiac tissue is well known. There is a marked reduction of contractile function resulting from decreased ATP production a few seconds after the onset of ischemia. Leakage of potassium ions is responsible for the alteration of ST segments. Within minutes an intracellular acidosis develops associated with an increase in myoplasmic calcium and the beginning of cell swelling (2). Later, cellular lesions become irreversible. The ultra structure of the cells becomes altered and macromolecules (CK-MB, Troponins) are released. An increased concentration of cytosolic and mitochondrial calcium plays a central role in the damage to the cells and their membranes.

Myocardial ischemia occurs in the presence of fixed or dynamic coronary artery stenosis. The main cause of ischemia in fixed coronary stenosis includes tachycardia, excessive left ventricular filling and hypoxemia. In dynamic stenosis, in addition to the above mentioned factors, activation of sympathetic and parasympathetic systems are also involved. Moreover several endothelium derived mediators may enhance vasoconstriction (3).

Myocardial stunning (flow-contractility mismatch)

The term myocardial stunning was coined by Brownwald and Kloner (4) in 1982 to describe a reduction in function after a brief period of ischemia followed by reperfusion. The impairment of action could last for several hours to days at a time when coronary blood flow was normal and there was no obvious cellular damage. During the perioperative period a high proportion of adult patients suffer from episodes of myocardial ischemia, most of which are silent but can be prolonged. Importantly, silent myocardial ischemia is supposed to be a common cause of myocardial stunning and is a predictor of adverse cardiac outcome.

Mechanism of myocardial stunning

Myocardial ischemia followed by reperfusion causes reversible or irreversible damage depending on its duration. In stunning ischemic damage is, in principle reversible (5).

Three main mechanisms are involved in the establishment of stunned myocardium:

a) formation of free oxygen radicals

b) accumulation of intracellular calcium

c) Degradation of contractile proteins.

Free radicals do not have a single target, but adversely affect many components of the cell including sarcolemmal and sub cellular membranes of organelles. The role of free radicals in stunning is confirmed by the improved post-ischemic functional recovery in the presence of super oxide dismutase(6).

During ischemia and the early phase of reperfusion, there is an increase in the concentration of intracellular Ca^{2+}. Calcium overload can decrease the sensitivity of contractile proteins to Ca^{2+}, thus diminishing the developed force. Calcium overload may result from altered Na^{+}/Ca^{2+} antiport and from altered Ca^{2+} fluxes at the level of sarcoplasmic reticulum. Such alteration in calcium handling may be attributable to ischemia induced intracellular acidosis.

Myocardial Hibernation

The concept of myocardial hibernation was put forward by Rahimtoola in 1985(7, 8). In the hibernating myocardium, myocardial function is diminished as a consequence of insufficient coronary blood flow. However this reduction is not necessarily permanent: an improved balance of supply and demand may augment myocardial function (9, 10). The issue of myocardial hibernation is clinically important because the risk of adverse cardiac outcome in cardiac and non cardiac surgery increases with the reduction of the ejection fraction.

Mechanism of Myocardial hibernation

In hibernating myocardium, cardiac metabolism is down regulated. It has been proposed that abolition of contractility of hibernating cardiac tissue is attributable to chronic stunning caused by multiple episodes of severe ischemia followed by repetitive reperfusion. Other experimental models suggest that hibernation occurs as a result of chronic low flow state. In either case, hibernating myocardium should be salvageable by restitution of an adequate coronary blood flow.

Myocardial Preconditioning

In 1986 Reimer (11) and colleagues reported that a brief period of ischemia decreased the rate of ATP depletion during the further period of ischemia. Murry and colleagues (12) reported that brief periods of ischemia made the heart more resistant to infarction during subsequent periods of acute coronary occlusions, reducing the infarct size by 70-80%. This phenomenon, termed myocardial preconditioning is the most powerful means of achieving cardiac protection. It can be defined as

"An adaptive mechanism by which a brief period of reversible ischemia increases the heart's tolerance to a subsequent longer period of ischemia".

Two different time frames have been defined for pre-conditioning – early or the "classical pre-conditioning" which involves the activation of various membrane receptors and lasts only between 1 & 3hour and a late phenomenon – which is termed as the "second window" of protection begins 12-24 hours later and lasts for up to 72 hours and is called "late or delayed preconditioning"(13).

Preconditioning can result from successive episodes of angina or silent myocardial ischemia. Preconditioning also reduces the risk of ischemia induced ventricular tachycardia and ventricular fibrillation (14). During coronary angioplasty, sequential occlusions cause fewer anginas, smaller ST-segment changes and lesser lactate production (15). The damage occurs not

during the period of tissue ischemia but during the period of reperfusion. This injury is termed as

Ischemic Reperfusion Injury (IRI) and is responsible for paradoxical organ death and dysfunction after termination of the reperfusion period. The mechanism involved in IRI include

a) Reduction in high energy phosphate (ATP) levels for many hours after tissue ischemia.

b) Pro-inflammatory cell (neutrophilis and mast cells) mediated cellular and micro vascular injuries, through direct cellular toxicity of super oxide free radicals that are generated by these cells during ischemia and subsequent reperfusion.

c) Micro vascular dysfunction with platelet plugging and endothelial damage resulting in a no-reflow phenomenon with inadequate tissue perfusion during the reperfusion period.

d) Calcium overload mediated reperfusion

Ischemic stimuli cause the release of stress mediators such as adenosine, bradykinin, nor epinephrine and opioids. The mechanisms of preconditioning involve several types of triggers and mediators. Among them adenosine, bradykinin/opioid and adrenoceptors play an important role. Via G-proteins, phospholipase C and proteinkinase C, these receptors act on mitochondrial and sarcolemmal potassium ATP channels and calcium

channels. In order for ischemic or pharmacologic preconditioning to occur, it is necessary to reach an activation threshold. This threshold represents the sum of the activity of several mediators.

Role of Adenosine (ADO)

The role of adenosine in preconditioning has been well documented. Adenosine A1-receptor activation plays an important role. These receptors are coupled with K^+_{ATP} channels (16) via Gi-proteins. Activation of adenosine receptors decreases the production of reactive oxygen species and attenuates myocardial stunning (17). Adenosine does not protect against Ischemic reperfusion injury when administered at the time of reperfusion, which indicates that Adenosine receptor activation must precede prolonged ischemia (18, 19). The protective mechanisms of adenosine in Ischemic preconditioning have been thought to be by

a) better preservation of tissue ATP

b) inhibition of neutrophil and mast cell activity

c) antioxidant(22) and anti-free radical activity(21)

d) anti-platelet activity

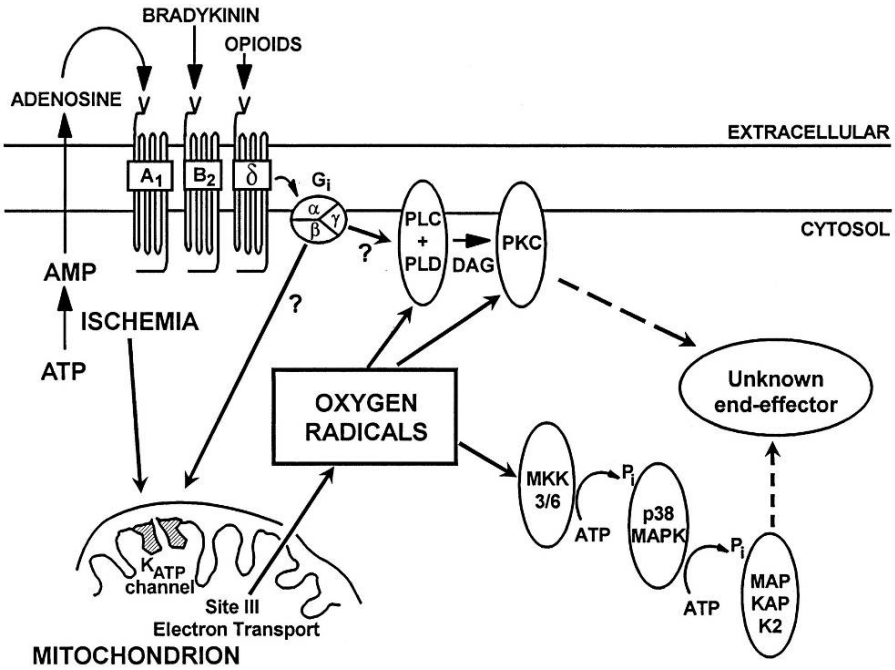
e) inhibition of no reflow phenomenon.(20)

f) Activation of K^+_{ATP} channels and reduction of calcium intracellularly.

g) Activation of protein kinase C and

h) Premature degradation of mast cells.(23,24)

Fig 1 . Role of Adenosine in Ischemic preconditioning



PLC➔Phospho lipase C DAG➔Diaceyl glycerol

PLD➔Phospho lipase D MAPK➔Mitogen activated Proteinkinase.

PKC➔Protein kinase C

Adenosine is known to inhibit neutrophil mediated free radical production through ADO2 receptor activation (23, 26) and may be responsible for limiting the degree of micro vascular injury during ischemia and reperfusion by inhibiting free radical mediated damage to endothelial cells and through its antiplatelet activity. Adenosine is a potent vasodilator of the vasculature ranging from major coronary arteries to microvasculature (18, 27). Adenosine in addition opposes the effects of a number of focally released vasoconstrictor substances like leucotrines, thrombaxane A2, endothelin and platelet activating factor (28). It also inhibits release of nor-adrenaline from the sympathetic mechanisms which in turn facilitates regional blood flow in a previously ischemic bed (19, 25, 28).

A3 receptors have been found to be present in mast cell membranes and they function to degranulate mast cells. Increased interstitial (29) ADO may be responsible for prematurely degranulating these mast cells during the preconditioning period. A brief IPC before a more prolonged ischemic period may wash out these mast cell products and prevent subsequent mast cell mediated tissue damage. It has been documented by Sandhu et al in rabbit myocardium that IPC prevented an increase in cAMP levels, which invariably occurred during sustained ischemia (30). There is usually an increase in intracellular cAMP during myocardial ischemia due to increased interstitial nor

epinephrine release and stimulation of α-1 receptors. The blunting of cAMP by the IPC may be caused by neither attenuation of nor epinephrine release during ischemia.

Role of Volatile agents

Ischemic preconditioning can also be mimicked by certain volatile agents in the myocardium (31-36). Studies on canine models of myocardial stunning by Warltier et al (32) have shown that Isoflurane, Halothane or Sevoflurane administered during an ischemic period but not during reperfusion period correlated with faster recovery of cardiac contractility. This study triggered a dramatic proliferation of investigations regarding proliferation protection by anesthetics. Volatile agents which are known to mimic IPC include isoflurane, sevoflurane, halothane and enflurane.(32,35,36)

Preconditioning by volatile agents is a promising therapeutic strategy to render myocardial tissue resistant to perioperative ischemia. It was hypothesized that Sevoflurane preconditioning would decrease postoperative release of brain natriuretic peptide, a biochemical marker for myocardial dysfunction (37).

Volatile anesthetics exert significant protection against myocardial ischemia (38) and excitotoxic cardiomyocyte death (39). One of the mechanisms by which volatile anesthetics induce protection in myocytes is

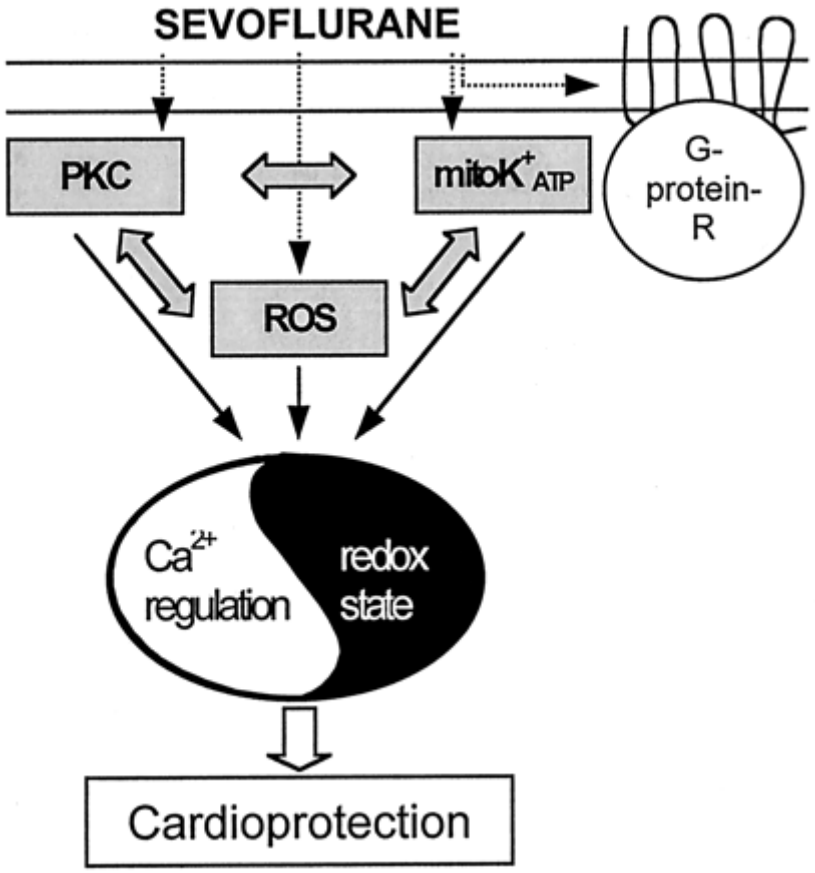
pharmacological preconditioning (40,41), the activation of potent endogenous protective mechanism in cardiac tissue against a variety of important stressors.

In contrast only a few studies have demonstrated the preconditioning effects of volatile agents in human myocardium (42-45). Cardioplegic arrest in patients undergoing coronary artery bypass graft (CABG) surgery is one of the few controlled models of human myocardial ischemia.

Sevoflurane and Desflurane

Further evidence for the protective properties of halogenated anesthetics was collected for halothane and isoflurane after 1997 (46-62). A new agent whose protection was demonstrated recently is sevoflurane. This anesthetic improves post-ischemic mechanical and coronary function and reduces infarct size (62-67). A beneficial effect of desflurane was suggested by a few groups (51, 66, 67). Further investigations are needed to determine the effect of desflurane in other experimental settings.

Fig 2. Proposed mechanism(s) underlying sevoflurane (sevo)-induced cardioprotection



Protein kinase C (PKC), mitochondrial (Mito) K^+_{ATP}, and reactive oxygen species (ROS) all contribute to the signal transduction of sevo-induced cardioprotection, suggesting that the three signaling elements are interactively arranged within a common signaling pathway. In addition, it cannot be excluded that sevo directly modulates G-protein coupled receptors (G-protein-R), PKC, Mito K^+_{ATP}, or ROS. Finally, it was suggested that the cardio protective effect results in modulation of the Ca^{2+} regulation and redox state of the cardiomyocyte.

Different approaches to assess myocardial injury

Several indices other than post-ischemic ventricular mechanical function have been examined to asses the degree of myocardial injury in the past few years. Reduction in infarct size has been well demonstrated with volatile anesthetics (46-48, 51, 55, 56, 58, 67). Results of infarction studies are more consistent than those of post-ischemic contractility. Kersten and coworkers administered isoflurane to dogs for 1 hour before 60-min LAD occlusion. The extent of infarct was reduced from 25% to 12%. According to Cope et al., preconditioning with halothane, enflurane or isoflurane diminishes infarct size to less than one third in rabbits (47).

Another approach to evaluate post-ischemic injury of the heart is to examine the coronary vasculature. Halothane, Isoflurane and Sevoflurane reduced the number of neutrophils sequestered in the coronary vasculature after ischemia (49, 54). A similar effect was also shown for platelets. Reduced neutrophil/platelet entrapment by anesthetics was accompanied by enhancement of post-ischemic mechanical function (49, 54, 62, 64).

Novalija and coworkers measured coronary flow changes in response to endothelial-dependent independent vasodilators. Sevoflurane preserved the reaction provoked by both types of vasodilators during the reperfusion period and was better than no treatment (65).

Mechanisms of protection by halogenated anesthetics

Before 1997, mechanisms underlying the cardiac protection were suggested to be preservation of ATP, reduction in calcium influx to the cell, inhibition of free radical formation and activation of K^+_{ATP} channels (68). Recently, progress in elucidating the mechanisms responsible for protection was achieved when investigators related ischemic preconditioning and anesthetic induced protection and also examined the coronary system

Preconditioning

Halogenated anesthetics dilate the coronary arteries via K^+_{ATP} channels, known to be key constituent of the ischemic preconditioning pathways. Cason et al hypothesized that halogenated anesthetics induce an ischemic preconditioning-like effect. They studied rabbit hearts in situ treated with 5-min coronary occlusion followed by reperfusion. Administration of 15 min isoflurane was followed by washout before 30-min coronary occlusion. In the ischemic preconditioning group infarction was reduced by 74% compared to the non pretreated group and reduced by 30% in the isoflurane group. Although less effective than ischemic preconditioning, isoflurane limited ischemic injury even though isoflurane was washed-out, i.e. isoflurane had an ischemic preconditioning effect (46). These findings indicate that the signals for halogenated anesthetics, like for brief periods of ischemia in ischemic

preconditioning, are preserved in intracellular components that can mediate protection against forthcoming ischemia. The protection by halogenated anesthetics can be reversed by a selective adenosine A1 receptor antagonist(61), a Gi protein inhibitor (58), PKC inhibitors (47, 59), and K^+_{ATP} channel blockers (48, 56, 57, 60, 61, 67). Contribution of mitochondrial K^+_{ATP} channel appears certain (46, 47), but not the sarcolemmal K^+_{ATP} channel. These observations strongly suggest that halogenated anesthetic agents provide protection via a mechanism similar to that of ischemic preconditioning. As the protection by halogenated anesthetics is not accompanied by augmented release of adenosine (47), it could be assumed that they stimulate adenosine receptors via a non adenosine mechanism, or up regulate the adenosine receptor G protein complex to promote the signal transduction downstream.

Coronary vasculature

Halothane, isoflurane and sevoflurane was shown to have reduced number of trapped neutrophils in the heart during reperfusion (49,54). The post ischemic expression of CD11 which forms an integrin with CD18 was also suppressed by volatile anesthetics (54). Kowalski et al showed that neutrophil adhesion was attenuated even when sevoflurane was administered only during reperfusion (49). These studies imply that halogenated anesthetics can act

directly on the neutrophils at the time of reperfusion. Volatile anesthetics also reduced platelet adhesion on the vascular wall after ischemia (62, 64) but Sevoflurane failed to reduce the expression of glycoprotein 11b/111a, a platelet adhesion molecule involved in the platelet-endothelium interaction (64). When ischemia is regional, one way to slow the progression of ischemic injury is to increase coronary collateral flow. Kersten et al showed that sevoflurane selectively increased collateral flow to the ischemic areas in the dogs with chronic LAD stenosis. This effect was not reversed by glibenclamide, a non-selective K^+_{ATP} channel blocker (69).

Cardiac surgery and some noncardiac procedures are associated with a significant risk of perioperative cardiac morbid events. Experimental data indicate that clinical concentrations of volatile general anesthetics protect the myocardium from ischemia and reperfusion injury, as shown by decreased infarct size and a more rapid recovery of contractile function on reperfusion. These anesthetics may also mediate protective effects in other organs, such as the brain and kidney. Recently, a number of reports have indicated that these experimentally observed protective effects may also have clinical implications in cardiac surgery. However, the impact of the use of volatile anesthetics on outcome measures, such as postoperative mortality and recovery in cardiac and noncardiac surgery, is yet to be determined (70).

Some of the studies also demonstrated that if the anesthetic was stopped more than 30min before the coronary artery was occluded the preconditioning effect was lost(70).

De Hert and colleagues (71) conducted a randomized study of 320 elective CABG patients, in which 80 patients received propofol, 80 midazolam , 80 sevoflurane and 80 desflurane. All patients received a remifentanil - based anesthetic regimen. They found a significant decrease in ICU and hospital length of stay in the volatile groups when compared with the non-volatile groups. Postoperative troponin I and inotrophic support was significantly lower in the volatile group.

Neutrophil mediated secretion and activation of matrix MMP-9.

Cardiopulmonary bypass (CPB) induces neutrophil activation, degranulation and a systemic inflammatory response. Matrix metalloproteinase (MMP-9) exists in neutrophils and is released on neutrophil activation. Increased levels of MMP-9 have been observed in patients undergoing CPB. Plasma levels and activity of MMP-9 increased significantly 2-6hr after beginning CPB. CPB caused an increase in the concentration and activity of plasma MMP-9 expression and production which suggests that MMP-9 is derived primarily from neutrophils and may contribute to the inflammatory response associated with CPB (72).

Previous studies have investigated the role of volatile anaesthetic agents in myocardial protection during coronary artery bypass graft (CABG) surgery, and some have identified beneficial effects. However, these studies have been too small to identify a significant effect on myocardial infarction (MI) or mortality. A systematic overview and meta-analysis of all randomized trials comparing volatile with non-volatile anaesthesia in CABG surgery identified 27 trials that included 2979 patients. There was no significant difference in myocardial ischemia, MI, intensive care unit length of stay or hospital mortality between the groups (all $P>0.05$). Post-bypass, patients randomized to receive volatile anaesthetics had 20% higher cardiac indices ($P=0.006$), significantly lower troponin I serum concentrations ($P=0.002$) and lesser requirement for inotropic support ($P=0.004$) compared with those randomized to receive i.v. anaesthetics. Duration of mechanical ventilation was reduced by 2.7 h ($P=0.04$), and there was a 1 day decrease in hospital length of stay ($P<0.001$). Some of these outcomes were based on a smaller number of trials because of incomplete data, largely because the individual trials focused on one or more surrogate endpoints. We found some evidence that volatile anaesthetic agents provide myocardial protection in CABG surgery, but larger adequately powered trials with agreed, defined outcomes need to be done to fully assess a possible beneficial effect of volatile anaesthetic agents on the risk of MI and mortality (73).

Ecto-5'-nucleotidase activity significantly increased in patients receiving isoflurane according to a preconditioning protocol. There is a large body of evidence implicating PKC as a critical mediator of the cardioprotective response to this preconditioning phenomenon.[75]

In the human heart, however, such an involvement has, until now, been exclusively based on in vitro studies that used cultured cardiomyocytes[76] or right atrial trabeculae[77] subjected to "simulated" ischemia/reperfusion (in fact, anoxia/reoxygenation) and showed that PKC agonists and antagonists trigger or blunt the preconditioning response, respectively. More direct evidence for a role of PKC has been the demonstration of the cytosol-to-membrane translocation of its α-isoform induced through the exposure of human cardiomyocytes to adenosine.[76] However, until the assay of the PKC isoforms most relevant to cardioprotection can be accurately performed in human biopsy samples taken during cardiac surgery, one has to rely on surrogate markers like ecto-5'-nucleotidase. This enzyme, which releases adenosine from 5'-cAMP, is 1 of the substrates that is phosphorylated by PKC, [78, 79] and elevation of its levels therefore stands as a reporter of PKC activation (80).

Platelets enhanced the ability of neutrophils to cause coronary endothelial dysfunction. This effect was prevented by isoflurane. This may be attributable to an inhibitory action on super oxide production by the neutrophils leading to reduced expression of endothelial adhesion molecules and in turn reduced neutrophil adherence (81).

Oxidative stress, nitric oxide and volatile anesthetic preconditioning

Ischaemia has been shown to compromise defence mechanisms against oxygen free radicals, mainly by decreasing levels of anti-oxidant enzymes such as mitochondrial superoxide dismutase and a decreasing tissue content of reduced glutathione (GSH). Reperfusion and the concomitant increase in oxygen then results in an increased production of free radicals, which result in oxidation of thiol groups (GSH and protein thiols) and lipid peroxidation [82]. Thus, reactive oxygen species (ROS) are central to cardiac ischemic and reperfusion injury. They have been implicated in a variety of phenomena in this context such as myocardial stunning, infarction and apoptosis, and possibly to the genesis of arrhythmias [83]. A recent study from India also showed that levels of malondialdehyde (a marker of lipid peroxidation) were significantly increased in patients with myocardial infarction, accompanied by a decrease in activities of anti-oxidant enzymes such as superoxide dismutase, catalase and glutathione reductase [84].

Nitric oxide is also now emerging as an important signaling molecule, and in an animal model of off-pump CABG, it has been shown that circulating levels of nitric oxide decrease by 10 minutes after reperfusion, but subsequently increase significantly by 24 hours after reperfusion [85]. High levels of nitric oxide can result in production of nitrosative species such as peroxynitrite, which can also produce lipid peroxidation [86]. Nitrate was measured as a marker for nitric oxide (87). Again, however, the beneficial effect of pre-conditioning by volatile anaesthetics has also been shown to require nitric oxide [88]. Here again the explanation could be differences in concentration. High concentrations of nitric oxide have been shown to be damaging in a number of contexts, while low levels are generally protective.

Female gender-induced reductions in infarct size are mediated by eNOS, but remote isoflurane exposure (1.0 MAC) before ischemia and reperfusion does not produce additional cardio protection *in vivo* (89).

MATERIALS AND METHODS

This randomized, blinded, clinical controlled trial was approved by the Research and ethics committee of our institution. Twenty patients scheduled to undergo elective CABG surgery on cardio pulmonary bypass pump for coronary artery disease were included in this study. The surgeon, the pump technician and biochemist was blinded to the drug, the anaesthetist involved with the surgery could not be blinded to the anaesthetic agent.

EXCLUSION CRITERIA:

a) Off pump CABG

b) Previous coronary (or) valvular heart surgery

c) Preoperative hemodynamic instability requiring medical or mechanical support

d) Severe hepatic disease (ALT or AST >150U/L)

e) Renal insufficiency (creatinine>1.5mg/dl.)\

f) COPD (FEV1<50% of predicted or <0.8L)

g) H/o neurological disturbances.

Randomization

Patients were randomly allocated into group A (isoflurane group) and group B (sevoflurane group) using computer generated random numbers. This randomization was done before the study began to assure equal allocation to the Group A and Group B. The randomization envelope was opened by an anesthetist, and accordingly that particular anesthetic agent either isoflurane or sevoflurane was used throughout the procedure.

Pre-op evaluation

Following a thorough preoperative check up the procedure was explained to the patient. After obtaining informed consent, patients are randomly allocated to one of the two groups.

Antiplatelet therapies were stopped 1wk before the operation and replaced by Heparin. ACE inhibitors are stopped one day prior to the surgery as a routine in our institution. Oral anti diabetic drugs were continued upto the night prior to surgery. Beta blockers and nitrates were continued upto the morning of surgery.

Patients were premedicated with benzodiazepines and opioids one hour prior to the procedure.

Monitors: Equipment used for monitoring the patient during the surgery

MONITORING

In the operating room patients received routine monitoring, including leads II and V on the electrocardiogram, O2 saturation by pulse oximetry, capnography, continuous radial arterial and central venous pressure and temperature monitoring which included nasopharyngeal ,rectal and blood. The hemodynamic measurements performed for intermittent cardiac output monitoring by thermo dilution method includes mean pulmonary artery wedge pressure (PCWP), systolic diastolic and mean systemic arterial pressure (SAP), systolic, diastolic and mean pulmonary arterial pressure (MPAP), mean right atrial pressure (RAP) and heart rate. Derived hemodynamic values of cardiac output, cardiac index (CI) and systemic vascular resistance (SVR) at induction were recorded. The Bi spectral index (BIS) monitor was attached to the patient and maintained at 40 – 60 throughout the surgery and during cardiopulmonary bypass.

Induction

After securing I.V. access, radial arterial blood pressure and other monitors like pulse oximetry (Spo2), electro cardiogram (ECG) were established. Before inducing the patient, a blood sample was taken for biochemical tests (Sample A). Patients were induced with midazolam (5mg), fentanyl (5-10mcs\kg), air oxygen and isoflurane (group A) or sevoflurane

(group B). When they were asleep, muscle relaxants (vecuronium or pancuronium or combination) was given. After intubation internal jugular vein on the right side of the neck was cannulated.

Pulmonary arterial catheter insertion

In all patients, a 7.5Fr Swan Ganz catheter was introduced through an 8.5Fr introducer sheath, sited in the right internal jugular vein. The distal lumen of the Swan Ganz catheter is connected to a pressure transducer to record the pressure waves continuously. Once the tip of the catheter reached the right atrium, as indicated by the pressure waveform, the balloon was inflated with 1.5ml of air and the catheter was gently floated through the right ventricle(RV) and finally into the pulmonary artery (PA). The catheter was guided till the tip was wedged in one of its branches as indicated by a PA wedge pressure tracing. At this position, if the balloon is deflated, the pulmonary artery pressure waveform should be seen. All the pressures were measured with the transducer kept at the level of the mid-axillary line. The cardiac output catheter has a thermistor at the tip which measures blood temperature continuously. Cardiac output is measured intermittently by injecting 10 ml cold saline in the CVP lumen (right atrial), the change in temperature was measured at the tip of the catheter from which the cardiac output was calculated.

Maintenance

Stress responses were tackled with incremental doses of fentanyl and isoflurane or sevoflurane according to the groups they were allocated. After going on pump, Isoflurane or Sevoflurane was administered via a special vaporizer attached to the pump maintaining the BIS around 40-60. During rewarming, muscle relaxants and opioids were given.

Two blood samples, one 60 minutes after aortic cross clamp release(Sample B) and the second (Sample C) 60 minutes after separation of pump are taken for biochemical analysis. After separation from the Cardiopulmonary bypass (CPB) pump, anesthesia is maintained with opioids, air, oxygen and isoflurane or sevoflurane according to the allotted group.

Surgical aspect

Median sternotomy and pericardiotomy were performed. After administration of 3mg/kg unfractionated heparin, the aortic cannula was secured in place. Activated coagulation time (ACT) was kept at 450secs or three times above the baseline ACT value throughout the cardiopulmonary bypass (CPB). Routine surgical techniques and cardio protective strategies were used in both groups. Cardiac protection was obtained with cold cardioplegic solution. Body temperature was cooled to 30°C-32°C on CPB. After the surgical procedure, the heart was reperfused and rewarmed to a

rectal temperature of 35°C-36°C. Inotropes mainly used was adrenaline in a dose of less than 0.1µg/kg/hr. This was started in both groups before separation from cardiopulmonary bypass and continued throughout post pump period and in the intensive care unit (ICU) depending upon the hemodynamic parameters of the patient. After neutralizing the heparin activity with protamine, the aortic cannula was removed. Protamine administration was further guided by activated coagulation time measurements aiming for a value of below baseline ACT. At the end of the procedure, patients were transferred to ICU, where they were kept sedated with morphine and midazolam. The patients were then weaned from the ventilator and tracheally extubated.

All patients included in this study were operated by a single surgeon in order to standardize the techniques, the duration of CPB and surgery. This is because duration of ischemia can influence Ischemic reperfusion injury (IRI).

Measurement of Cardiac output during surgery

Three pre-bypass measurements of cardiac output and cardiac indices were measured and the average was taken. Post-bypass, three measurements were taken and the averages of those were also taken.

Measurement of O2 free radicals and CK-MB

Blood samples were sent to biochemical lab for measurement of biochemical markers of oxidative stress during CABG. We estimated malondialdehyde as a marker of lipid peroxidation, protein carbonyl content as an indicator of protein oxidation, protein thiols and total thiols to evaluate thiol status and nitrate (the stable end product of nitric oxide) to evaluate nitrosative stress. We took 3 blood samples at different time points; sample A at pre induction, sample B one hour after cross clamp release and sample C one hour after separation from pump. Oxidative stress was expected to increase after reperfusion phase but this could not be measured as the presence of some anesthetic agent was required on pump due to ethical considerations. It was hoped that the samples collected before induction could be used as baseline and compared against the other time points where volatile anesthetic agent would be present. Measurement of protein carbonyls, malondialdehyde, nitrates, protein thiols and total thiols were done for each of these samples. The actual method of determining these are given in the appendix.

Initially a pilot study was done on five patients to decide which cardiac biochemical marker would be the best indicator of post CABG myocardial ischemia. Creatinine Kinase – MB (CK-MB) was seen to rise within 4 to 8 hrs and peak at 12hrs, while Troponin-I levels remained elevated for 72 hours.

From our pilot study we concluded that plasma CK-MB levels would be a better indicator for postoperative MI because of its rapid rise and fall than troponin I values. Blood samples were taken from all patients for determination of creatinine kinase-MB (CK-MB). These samples were obtained at arrival in the ICU (T0) and at 12hrs (T12) after arrival in the ICU.

Sample size

The sample size was calculated with the following formula based on a previous study by Cason, Brian A et al (46).

$$n = \frac{(Z_{\alpha/2} + Z_{1-\beta})^2 2S^2}{d^2}$$

$$S^2 = \frac{n_1 s_1^2 + n_2 s_2^2}{n_1 + n_2}$$

d = precision = 10.4

$$n = \frac{2(1.96+1.282)^2 (5.25)^2}{(10.4)^2} = 6 \text{ in each arm}$$

For a power of 90% and α = 5%, a sample size of 6 patients in each group was calculated to be appropriate.

Statistical analysis

Patient characteristics, hemodynamics parameters and biochemical markers were compared using non parametric Mann-Whitney, Wilcoxon signed rank test, paired t test and Fisher's exact test wherever appropriate**.**

RESULTS

20 patients scheduled for coronary artery bypass grafting on CPB were enrolled in the study. Two patients were not included in the analysis; one surgery was done off pump, hence excluded from the analysis. In the other patient, due to damage of coronary sinus during surgical manipulation there were problems of separation from CPB pump, the patient required an Intra aortic balloon pump (IABP) as well as a pacemaker for weaning from bypass.

Table 1 shows the demographic data of patients in both groups. There were no significant differences in age, weight, height and gender of the patients between the two groups. As can be seen from this table, there was only one female included in the study.

Table 2 describes the premorbidity status of all patients. There was no statistically significant differences between the two groups .One patient in Group 2 had aortic stenosis and another had four vessel disease.

Table 1-Demographic data

	Group 1(n=9)	**Group 2(n=9)**
Age(mean+/_SD)	55.67 ± 9.42	52.56 ± 7.98
Height(cms)	166.78 ± 6.8	163.11 ± 7.47
Weight(kg)	57.67 ± 7.890	65.89 ± 12.150
Sex(male: female)	8 : 1	9 : 0

Table 2 – Pre-operative morbidity

Morbidity factors		**Group1(n=9)**	**Group2(n=9)**
NYHA CLASS	I	0	0
	II	6	7
	III	3	2
	IV	0	0
LV dysfunction (angio)		2	4
Hypertension		5	5
Diabetes mellitus		2	4
Diseased vessel	Double	2	2
	Triple	7	6
	Four	0	1
Associated MR(functional)		1	2
Hypothyroidism		1	1
AS		0	1

Cardiac output (CO) (L/min)

The mean pre pump CO was 5.18 and 5.1L/min in Group 1 and Group 2 respectively. Likewise the mean post pump CO was 8.9 and 8.11L/min respectively.

There was no significant statistical difference between the 2 groups

Cardiac index (L/min/m²)

The mean pre pump CI was 3.12 and 2.84L/min/m² in Group 1 and Group 2 respectively. Likewise the mean post CPB CI was 5.32 and 5.01L/min/m² respectively. Here also we found no statistical difference between both groups.

Systemic vascular resistance (SVR) (dynes. sec. cm-5)

The SVR value in Group 1 was not statistically different when compared with Group 2 during pre and post CPB period. It was observed that in the post CPB period, there was fall in systemic vascular resistance in both Groups.

Other hemodynamic parameters were similar in both groups.

Table 4 gives the duration of CPB and ICU stay in both the groups. The CPB duration was more than 2hrs in 4 patients, 2 in isoflurane group and 2 in sevoflurane group. There was no difference in CPB duration between both the groups.

ICU stay exceeded 24 hrs in three patients, 2 in the isoflurane and 1 in sevoflurane group. But none of them stayed beyond 36 hrs in ICU.

Table 3

Haemodynamics with Isoflurane and Sevoflurane - Mean ±- SD

	Basal	Pre-CPB	Post-CPB
HR (bpm) Isoflurane Sevoflurane	 68.66 ± 8.12 80.8 ± 22.6	 75.66 ± 8.12 79.66 ± 12.3	 87.33 ± 8.29 89.33 ± 9.96
MAP(mmhg) Isoflurane Sevoflurane	 87.77 ± 7.57 90.33 ± 7.85	 74.11 ± 8.90 74.77 ± 8.01	 75.77 ± 8.12 73.33 ± 8.07
CVP(mmhg) Isoflurane Sevoflurane	 6.55 ± 2.78 6.22 ± 2.68	 7 ± 2.06 7.8 ± 2.36	 8.11 ± 1.16 6.88 ± 1.76
CO(L/min) Isoflurane Sevoflurane	 ____ ____	 5.18 ± 1.13 5.10 ± 0.98	 8.90 ± 1.70 8.11 ± 1.26
CI(L/min/m²) Isoflurane Sevoflurane	 _____ _____	 3.12 ± 0.61 2.84 ± 0.68	 5.32 ± 0.89 5.01 ± 0.73
SVR(dynes.sec/cm5) Isoflurane Sevoflurane	 _____ _____	 1087 ± 251 980 ± 178	 602 ± 155 677 ± 134

Table -4 CPB duration and ICU stay

	Group 1	Group 2
CPB DN (hrs)	1.5 ± 0.02	2 ± 0.02
ICU stay (hrs)	21.7 ± 6.86	19 ± 2.52

OXIDATIVE STRESS BIOCHEMICAL MARKERS

Protein Thiols

Figs 3.shows in the isoflurane group, protein thiol levels were not reduced at time B but at time period C, levels were reduced. In the sevo group, thiol levels were reduced both at time period B and C. But there was no statistically significant difference in the fall of thiol levels between the 2 groups ($P>0.05$)

Fig 4, 5, 6 and 7 shows the distribution pattern of protein thiol levels in B and C samples for the iso and sevo groups.

Fig 3. Levels of Protein thiols

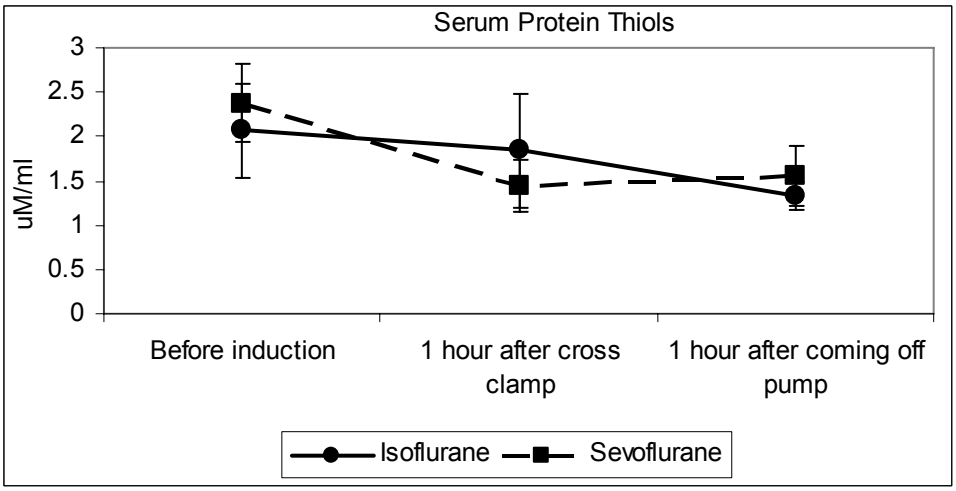


Fig 4. Pn thiol - Iso group

Sample B

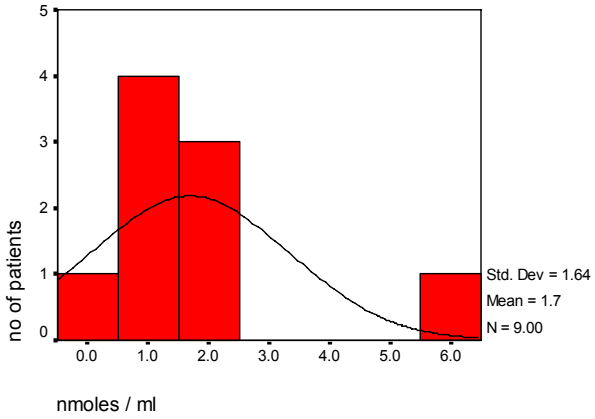


Fig 5.Pn thiol - sevo group

Sample B

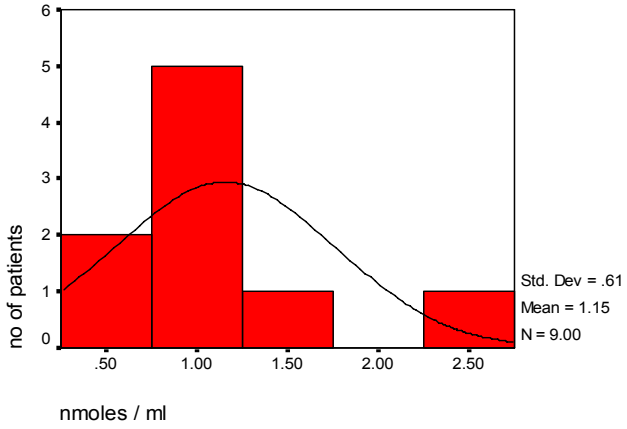


Fig 6. Pn thiol - Iso group

Sample C

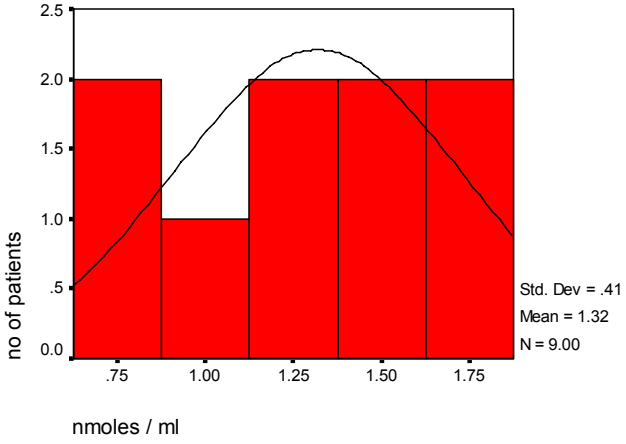
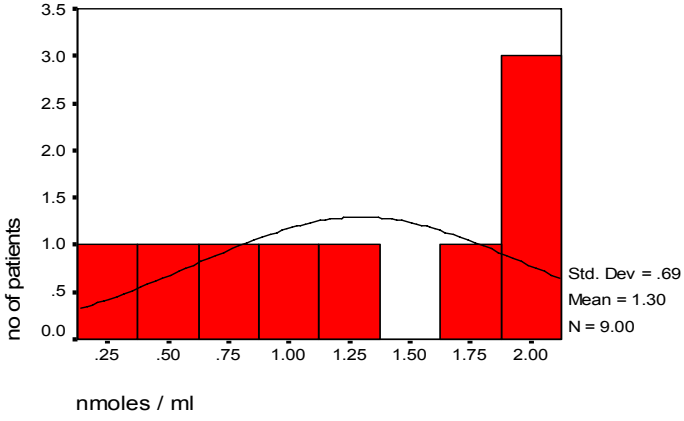


Fig 7.Pn thiol - Sevo group

Sample C



Total Thiols

In the isoflurane group the total thiol level were increased from the baseline value at time B, but was reduced below the baseline value at time C (fig 8.). In the sevoflurane group, total thiols were reduced below the baseline at time B, but increased and reached the baseline values at time C. There was no statistically significant difference between these two groups (P> 0.05).

Fig 9, 10 ,11 and 12 shows the distribution pattern of total thiols in B and C samples for the iso and sevo groups.

Fig 8. Levels of Total thiols

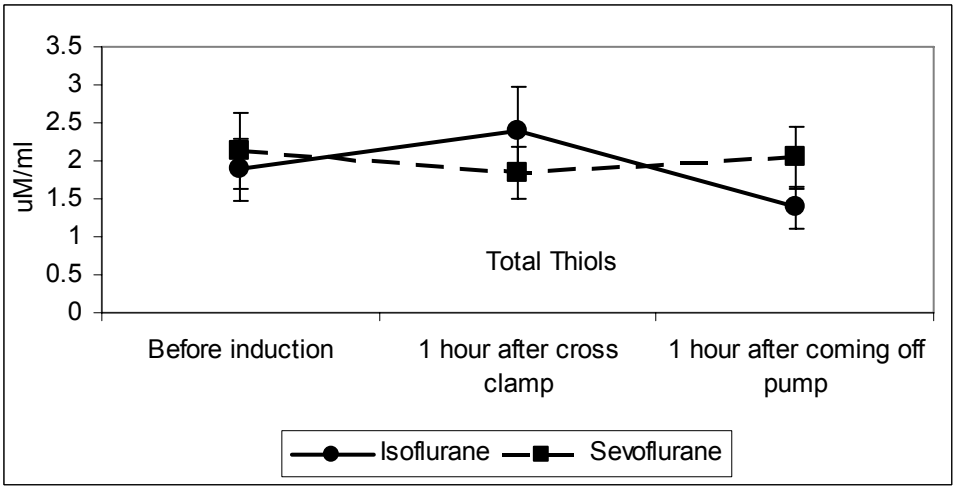


Fig 9.Total thiol - Iso group

Sample B

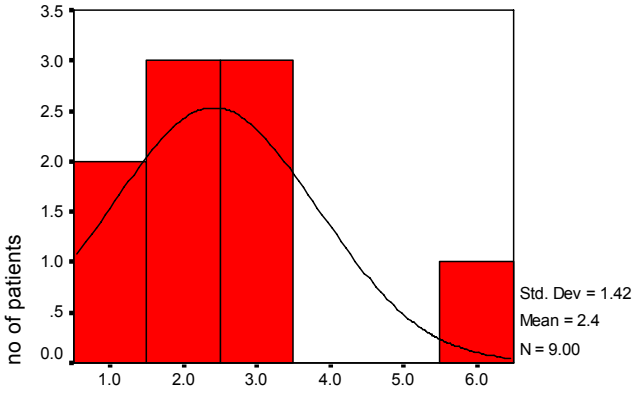


Fig 10. To thiol - Sevo group

Sample B

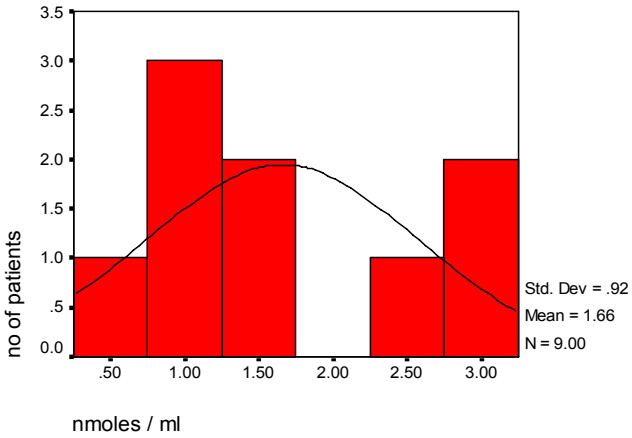


Fig 11. To thiol - Iso group

Sample C

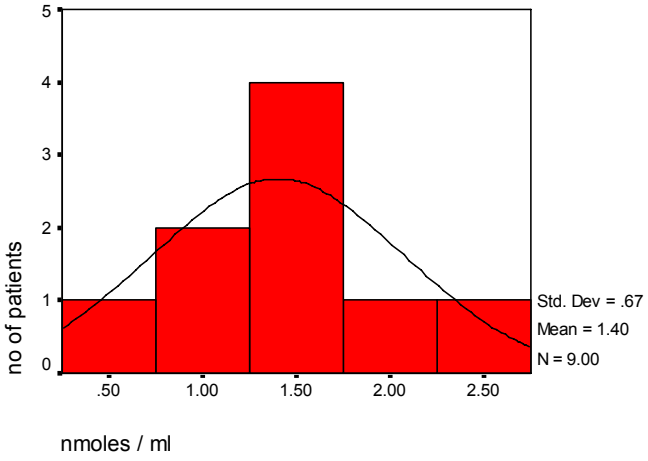
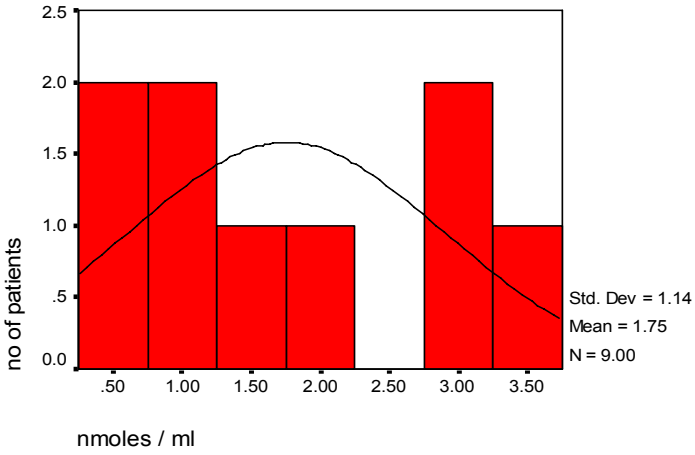


Fig 12. To thiol - Sevo group

Sample C



Malondialdehyde

Fig 13 shows malondialdehyde levels in the isoflurane group were decreased from baseline at time C, but there was no fall at time B. In the sevoflurane group though malondialdehyde levels reduced at time B and also at time C. This change in levels had statistical significance at time point C (P= 0.02).

We can see the distribution pattern for malondialdehyde levels in fig 14, 15, 16 and 17 for both iso and sevo groups.

Fig 13.Levels of Malondialdehyde

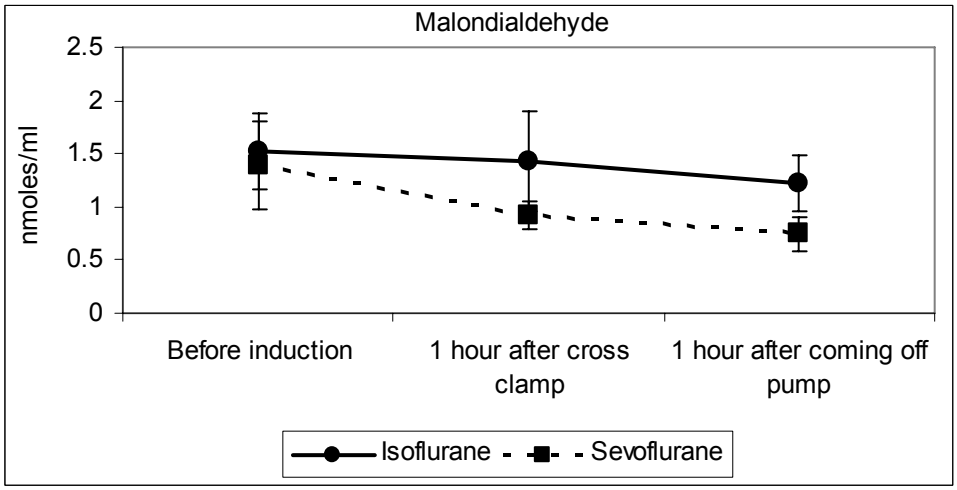


Fig 14.Mal level - Iso group

Sample B

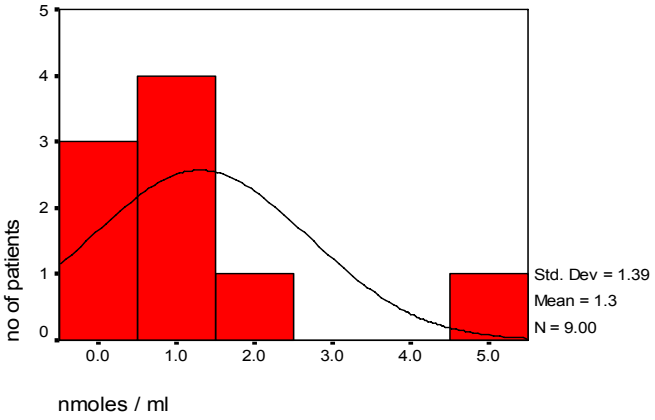


Fig 15. Mal Level - Sevo group

Sample B

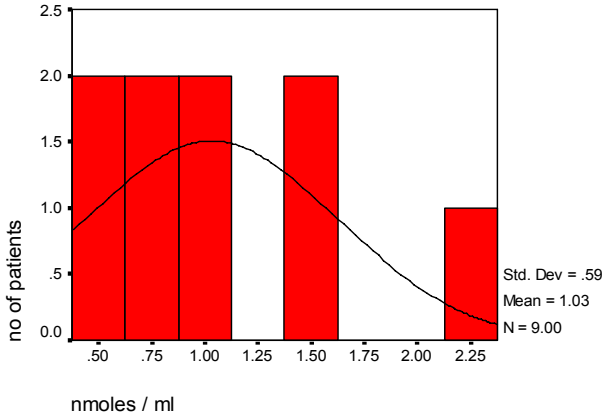


Fig 16.Mal Level - Iso group

Sample C

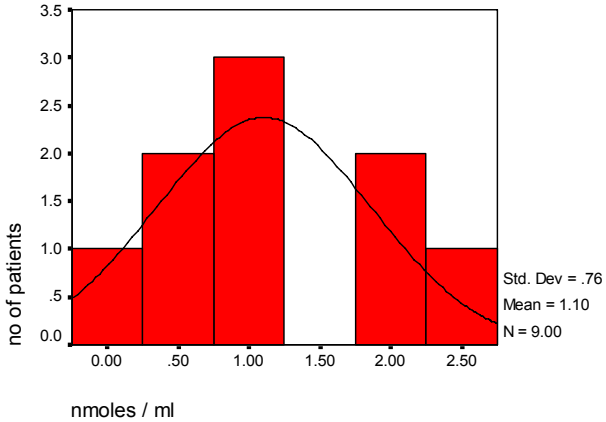
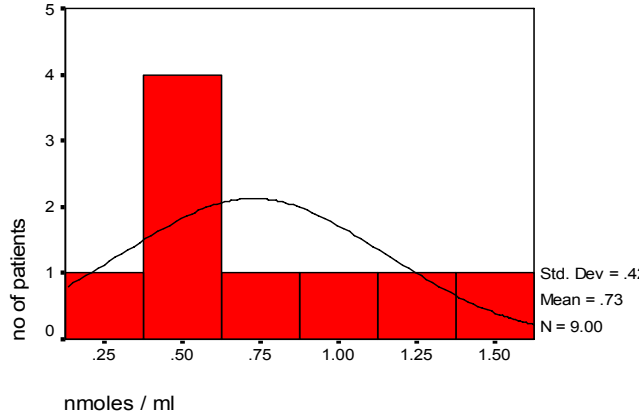


Fig 17.Mal Level - Sevo group

Sample C



Protein Carbonyls

In the isoflurane group, protein carbonyl levels were reduced from the baseline value at time B, and the same level maintained at time C. In the sevoflurane group, though there was a decline in protein carbonyl level at time B, there was a mild increase at time C but did not reach the baseline values. This difference was also not statistically significant between the 2 groups ($P>0.05$).

Fig 19 to 22 displays the distribution pattern of protein carbonyl levels for both iso and sevo groups at time period B and C.

Fig 18.Levels of Protein Carbonyls

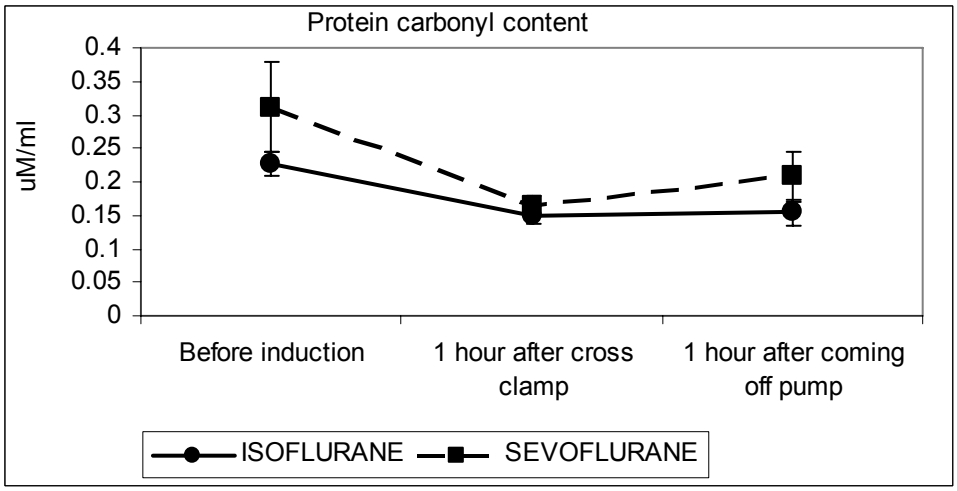


Fig 19.Pn carb - Iso group

Sample B

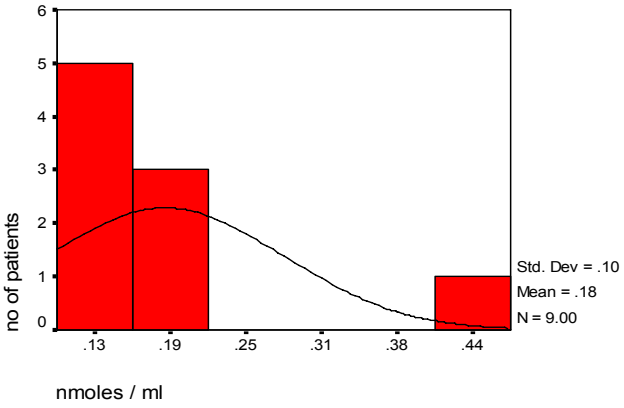


Fig 20.Pn carb - Sevo group

Sample B

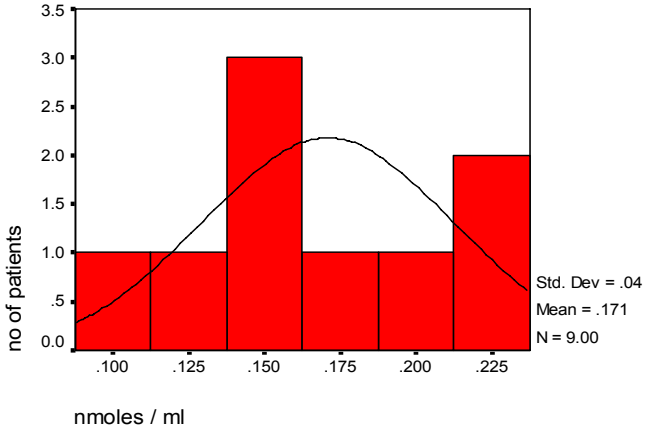


Fig 21.Pn carb - Iso group

Sample C

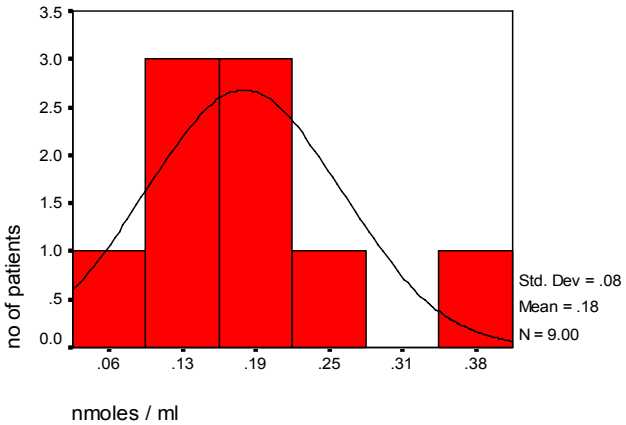
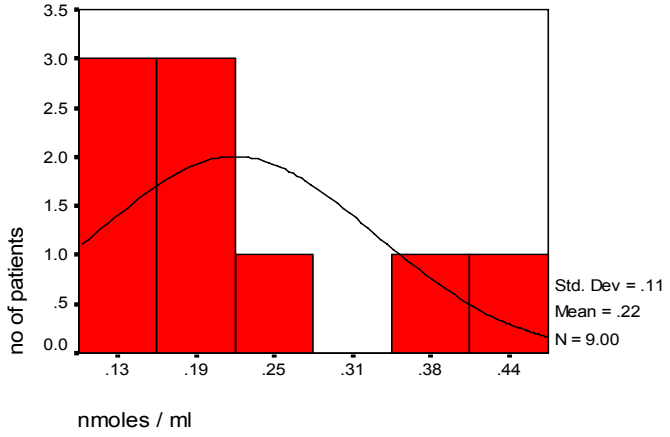


Fig 22.Pn carb - Sevo group

Sample C



Nitrosative Stress Bio chemical marker

Nitrates

From fig 23 it can be seen that in the iso group there was no significant decrease in nitrate levels at time B and C. But there was significant decline in nitrate level at time B and C in the sevo group. This was shown to be statistically significant **(P= 0.03).**

Fig 24 to 27 shows the distribution pattern of nitrate levels in both the groups at time period B and C.

Fig.23.Levels of Nitrates

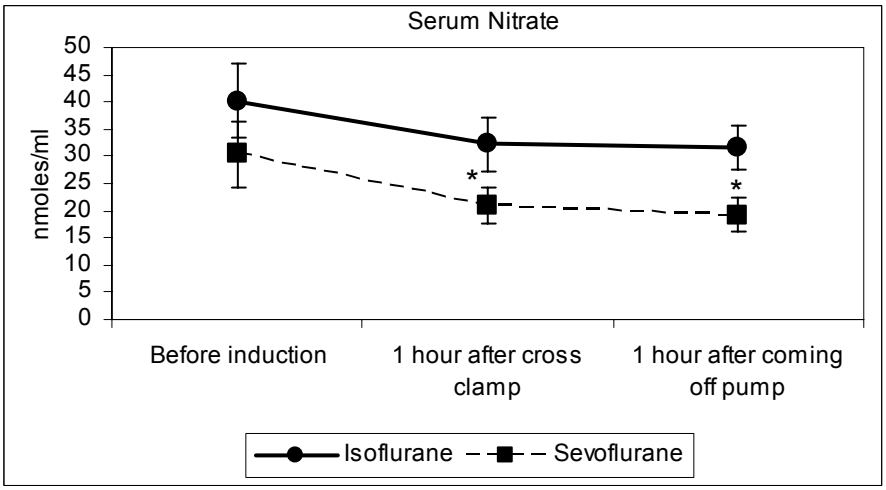


Fig 24.Nitrate levels in Iso group

Sample B

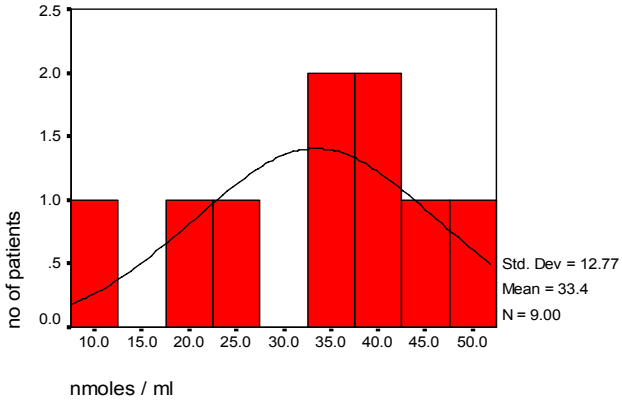


Fig 25.Nitrate levels in Sevogroup

Sample B

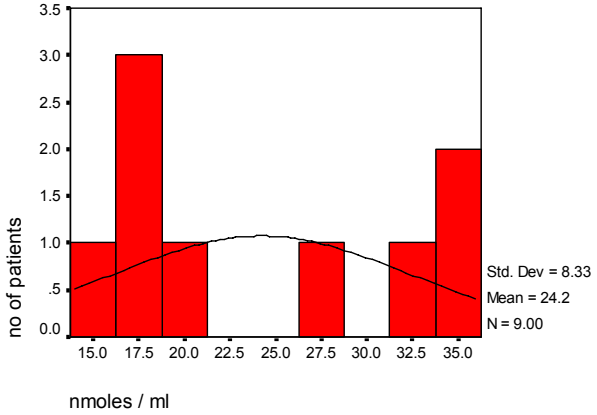


Fig 26.Nitate level in Iso group

Sample C

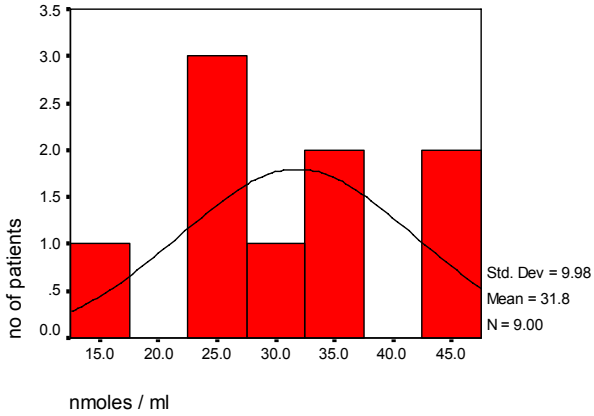
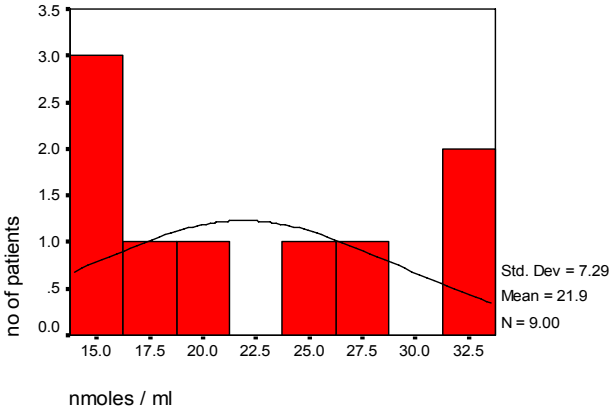


Fig 27.Nitate level in Sevogroup

Sample C



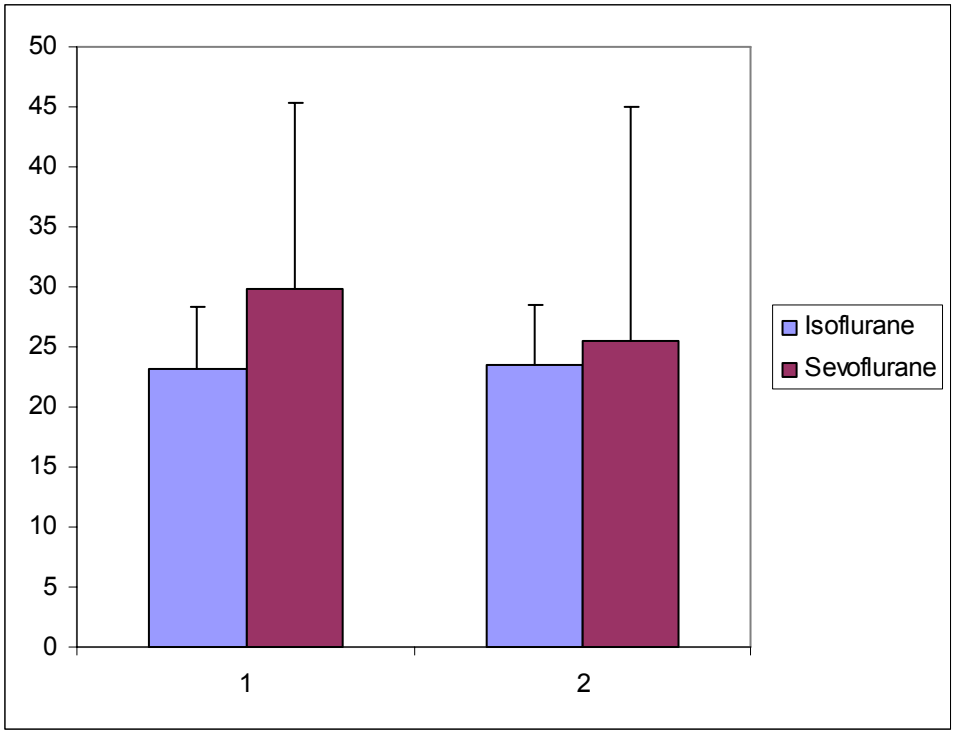
CARDIAC BIOCHEMICAL MARKERS

Table 5 shows the Creatinine Kinase MB (CK-MB) for both isoflurane and sevoflurane at 0 hr and 12 hrs after admitting into ICU. From fig 28 it is evident that CK-MB values were decreasing at 12 hrs in sevoflurane group. But this difference did not attain any statistical significance.

Table 5. Post operative CK-MB values

	Group 1 (n=9)	Group 2 (n=9)
CK-MB (T0) [U/L]	23.11 ± 5.2	29.89 ± 15.46
CK-MB (T12) [U/L]	23.44 ± 5.02	25.44 ± 19.6

Fig 28. Representation of postoperative CK-MB values



DISCUSSION

Coronary Artery Bypass Graft (CABG) surgery is still considered the best treatment for Myocardial Ischaemia (MI), but it has been reported that 90% of patients undergoing CABG surgery have a fall in ejection fraction and cardiac index during the immediate post-operative period even with cardioplegic protection and hypothermia widely practiced all over the world. This has been attributed to various factors including ischemia associated with aortic cross clamping, inadequate myocardial protection and the consequent ischaemic reperfusion injury (IRI) during surgery. In recent years it has been shown that myocardial ischemic preconditioning has been accepted as one of the interventions for myocardial preservation. More recently, studies have shown that myocardial preconditioning can be achieved with propofol, opiates and various volatile agents like halothane, enflurane, isoflurane, sevoflurane and desflurane.

Volatile agents have definitely been proven to be more effective than propofol or opiates (69). Only few studies have evaluated differences in preconditioning effects between the different volatile agents in human myocardium. Many animal studies have shown that isoflurane is better than halothane, enflurane and propofol in achieving cardiac protection and causing lesser ischaemic episodes in the postoperative period. Recent animal studies have suggested that sevoflurane may confer better cardiac protection. But

there are hardly any studies comparing isoflurane with sevoflurane. Hence we decided to compare these two agents to determine whether their continuous administration in patients undergoing CABG surgery will provide adequate cardiac protection. Earlier studies have shown that discontinuation of anesthetic agent more than 30min before occlusion of the coronary artery resulted in loss of the preconditioning effect (70). Hence we decided to use the volatile agents pre-pump, throughout CPB and also post pump to achieve both early (lasting between 1 to 3 hours) and late (lasting for upto 72 hours) pre-conditioning effects.

Generation of reactive oxygen species (ROS) is central to reperfusion injury and this is responsible for cellular toxicity and activation of proinflammatory cells such as neutrophils and mast cells. Oxygen free radical production can result in damage to a variety of cellular components resulting in peroxidation of lipids, oxidation of proteins and loss of cellular thiols. To evaluate the effect of sevoflurane and isoflurane on oxidative stress during CABG, we estimated malondialdehyde as a marker of lipid peroxidation, protein carbonyl content as an indicator of protein oxidation, protein thiols and total thiols to evaluate thiol status and nitrate (the stable end product of nitric oxide) to evaluate nitrosative stress.

Plasma levels of matrix metalloproteinase (MMP-9), a biochemical marker of neutrophil activation and brain natriuretic peptide (BNP), a marker of

myocardial dysfunction are increased during oxidative stress reactions. Volatile agents especially sevoflurane have shown to reduce the activation of neutrophils and reduce the BNP levels. Hence determination of these levels would probably give us the exact degree of oxidative stress. Unfortunately the facilities required to measure these parameters were unavailable in our institution and so we did not measure those values.

Initially a pilot study was done on five patients to decide which cardiac biochemical marker would be the best indicator of post CABG myocardial ischemia. In this study CK-MB values had declined by 12 hours post surgery while but troponin I values remained high. So we concluded that plasma CK-MB levels would be a better indicator for postoperative MI because of its rapid rise and fall than troponin I values. Normal CK-MB values in our institution are less than 24u/l and three times this value is taken as significant for ischemia along with ECG changes for ischemia.

All the CK-MB values decreased after 12 hours**.** (29.89 in iso vs 25.44 in the sevo) .The values were found to be lower in the sevoflurane group after 12 hrs though this did not reach statistical significance. There was no significant difference in morbidity in both the groups. There were no new ischemic changes or hemodynamic instability noticed in any of the patients in the immediate postoperative period or during their ICU stay except one patient who to be re explored for a surgical bleed.

According to our data, the hemodynamic parameters obtained with use of isoflurane and sevoflurane groups were comparable, with hardly any difference in cardiac index (CI) and systemic vascular resistance (SVR).

A sharp decline in SVR and large increase in CI was seen in all patients immediate postop, probably due to use of GTN and the volatile agent, both being vasodilators. (CI of 5.32 ± 0.89, SVR of 602 ± 155in isoflurane group during immediate post bypass compared with CI of 5.01 ± 0.73, SVR of 677 ± 134 in sevoflurane group).

This indicates that isoflurane and sevoflurane have similar cardiac effects in the immediate postoperative period. Kersten et al have demonstrated that sevoflurane selectively increased collateral flow to ischemic areas in the dogs with chronic LAD stenosis (69) and can maintain the vasodilating effect of independent vasodilators**.**

Intensive care unit (ICU) stay and discharge was also similar in both groups and no advantage of one group over the other was evident. This was also shown by De Hert and colleagues (71) who have shown that there is significant decrease in ICU and hospital length of stay in the volatile groups (sevoflurane and desflurane) when compared with the non-volatile agents (propofol and midazolam).

For evaluation of oxidative and nitrosative stress, 3 blood samples were taken at different time points; sample A at pre induction, sample B one hour after cross clamp release and sample C one hour after coming off bypass. Parameters of oxidative and nitrosative stress such as protein thiols, total thiols, protein carbonyls, malondialdehyde and nitrates were measured in each sample. Based on the literature, it was expected that oxidative stress would increase during the reperfusion phase, but this could not be measured in this study since an anaesthetic agent had to be present during CPB due to ethical considerations. It was hoped that the sample collected before induction could be used as a baseline, and compared against the other time points, where anaesthetic would be present.

Thiols are necessary for normal cellular function and also constitute important anti-oxidant defenses in cells. Thiol groups on proteins also have critical roles in protein function and are also susceptible to damage by ROS. Oxidative stress would result in a decrease in total thiol content, which may be accompanied by decreases in protein thiols if thiol groups on proteins are damaged.

As per our study change in serum total thiols during CABG surgery shows that patients in the isoflurane group had an increase in total thiols after initiation of ischemia by cross clamping. This could be due to a decrease in constitutive levels of free radicals because of a lack of oxygen, resulting in

sparing of thiol anti-oxidants. However after subsequent reperfusion (point C) thiol levels show a sharp decrease, which is expected since reperfusion would result in a significant increase in ROS production. It is interesting to note that the response with sevoflurane is different from that for isoflurane. Here serum total thiol shows a slight decrease during ischemia and then does not change during reperfusion, in fact showing a slight increase. This indicates that presence of sevoflurane is able to spare thiols during reperfusion, and has a beneficial effect during later stages. It should be noted however, that if neither isoflurane nor sevoflurane were used in pump, the changes in thiols probably would have been more dramatic.

Looking at protein thiols, our data shows that serum levels of protein thiols are decreased in both pre and post CPB period with both agents when compared to the initial baseline values. This indicates that oxidative stress during CABG did affect thiol groups on proteins. According to the data, fall in thiol levels were more with sevoflurane when compared with isoflurane, but this difference did not attain statistically significant value probably due to the small sample size.

Lipid peroxidation is another important indicator of damage during oxidative stress, and malondialdehyde is one of the main products of lipid peroxidation. An increase in levels of malondialdehyde in serum would thus be indicative of oxidative stress. According to our study, the levels of

malondialdehyde in serum from patients at different time points during CABG shows presence of sevoflurane results in a decrease in malondialdehyde levels suggestive of protection against oxidative stress. This was not evident in the isoflurane group, which did not change. After bypass, during the reperfusion phase, presence of isoflurane prevented any increase in malondialdehyde, suggesting protection against lipid peroxidation. The effect of sevoflurane during this phase is more dramatic, since the decrease in malondialdehyde levels initiated in the ischemic phase is sustained. This indicates that presence of sevoflurane provides better protection against lipid peroxidation both during pre as well as post bypass periods when compared to isoflurane. This is statistically significant ($P = 0.02$). Again it should be noted that presence of isoflurane prevented the expected increase in malondialdehyde during reperfusion, an effect which could not be demonstrated in this study due to absence of the control group due to ethical reasons.

Proteins are important targets of oxidative stress, and protein carbonyl content is a marker of protein oxidation. Oxidative stress in serum would thus result in an increase in protein carbonyl content. Our study shows that the levels of serum protein carbonyls is decreased in both isoflurane and sevoflurane treated groups. As can be seen, during the ischemic pre bypass

period patients in both groups show a decrease in protein carbonyl content. In the post bypass period during reperfusion, levels do not change very much, though the sevoflurane group does show a slight increase. All the above data indicates that protein carbonyl content is similar between the groups at the later time points B & C. This implies that presence of either anesthetic in pump during CABG prevents protein oxidation, and has a beneficial effect.

Nitric oxide (NO) is now recognized as an important signaling molecule and high levels of NO can have damaging effects. Interaction of NO and ROS such as superoxide can result in production of nitrosative species such as peroxynitrite, which can damage cellular components. Nitric oxide is rapidly metabolized to stable end products such as nitrate, and this was measured in serum from patients undergoing CABG as an indicator of nitrosative stress. High levels of nitrate reflect increased NO levels and are generally damaging. Our data shows that there was no increase in nitrate levels after ischemia, or after reperfusion, suggesting that anaesthetic preconditioning had a protective effect. The levels were lower in patients treated with sevoflurane when compared to the isoflurane group at both early reperfusion and immediate post bypass period when the oxidative stress is greatest. This suggests that sevoflurane provides better protection than isoflurane during these phases of CABG. This difference in levels between the two groups attained statistical significance (P=0.03) for the two later time points. However the use of Glyceryl

trinitrate (GTN) throughout the surgery would have been affected the levels of nitric oxide in plasma levels and hence it is difficult to come to a conclusion.

Paradoxically however, reactive oxygen species have also been implicated in the mechanism of anaesthetic preconditioning, which is cardio protective. It has been suggested that anaesthetic pre-conditioning increases generation of reactive oxygen species, mediated by a partial inhibition of mitochondrial respiration. One explanation for these opposing effects could be the relative concentrations of free radicals generated in ischemia reperfusion, versus pre-conditioning. Even though all the above data imply that there is oxidative stress involved during cardiopulmonary bypass, thiol levels probably might have been much lower and the levels of malondialdehyde, protein carbonyls and nitrates might have been much higher if we had not used either of these agents during CPB period. Since similar doses of narcotics, benzodiazepines, cardioplegia and hypothermia were used in all the patients in both the groups, potentiation of cardio protective effects with these should also be similar. Concentrations of volatile agents used in all the previous studies for cardioprotection have been in the ranges between 0.5 – 2 MAC which is relevant to clinical practice. In our study also we were able to achieve these cardiac effects within these concentrations of isoflurane and sevoflurane, though we actually used Bispectral Index (BIS) monitoring to maintain a value around 40-60 for adequate depth of anaesthesia.

In conclusion, our data in this study indicate that preconditioning with volatile anaesthetics such as isoflurane or sevoflurane definitely has beneficial effects in preventing oxidative stress during CABG. Among the two agents, isoflurane seems to provide better protection during the pre-bypass and early reperfusion period, while sevoflurane provides protection not only during early reperfusion but also in the immediate post operative period.

Limitations of our study

1) Current practice in most of the centres in India is not to use volatile agents during CPB, instead depending on high dose narcotics for analgesia, awareness and cardioprotection. Though we wanted to include this protocol for our control group, it was not approved by the Institutional Ethics Committee and hence we compared only these 2 groups (isoflurane and sevoflurane) for the study.

2) All the CABG patients are routinely on Glyceryl trinitrate (GTN) throughout the surgery, which leads to a loss of systemic vascular resistance (SVR) and increased cardiac index (CI) at the end of surgery and immediate post-operative period. We were not able to measure CI and SVR in the ICU post operatively. A normal SVR would have accurately reflected the true CI. Also the nitrate values obtained might have been influenced by GTN.

3) As a rule, in our hospital post CABG patients are ventilated in ICU overnight and weaned the next day morning. They are discharged from ICU after 24 hours, one day ICU stay was mandatory for all patients, so we were unable to compare ICU stay and early discharge between the 2 groups. Also long term morbidity between the two groups was not assessed.

4) Reactive oxygen species (ROS) have been implicated to have **a** significant role in preconditioning. Initial low concentrations of ROS have been shown to be protective, an effect which could not be demonstrated in this study due to absence of the control group due to ethical reasons. This could be the reason for some of the opposing effects seen before and after bypass.

5) We had only one female patient in the whole study, so we were not able to come to any gender based conclusions.

6) The cost of the study included the cost of a PA catheter, sophisticated monitoring equipment for pulmonary artery pressure, cardiac index measurements and estimation of the biochemical markers. The cost involved for all of the above is quite high for a developing country like India. To do a study with a larger sample size would require a lot of funds, hence this can only be taken as a pilot study.

CONCLUSIONS

1) Our study strongly indicates definite cardiac preconditioning effects with both the volatile agents isoflurane and sevoflurane in clinically relevant concentrations when administered throughout CABG surgery.

2) The evidence of oxidative stress with CABG surgery was shown by decreasing levels of biochemical markers. The preconditioning effects with these agents were evident from the ease of separation from CPB bypass and outcome of surgery in the immediate post-operative period as shown by the clinical and biochemical markers at different time intervals.

3) Among the two agents, isoflurane seems to provide better protection during the pre-bypass period and early reperfusion, while sevoflurane provides protection during early reperfusion as well as immediate post-bypass period when the oxidative stress is much greater.

Taking into account patient variability and the different mechanisms involved with different anaesthetic agents, larger adequately powered trials with defined outcomes need to be done to fully assess a possible beneficial effect of one volatile anaesthetic agent over the other and arrive at definite conclusions.

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PROFORMA

Comparison of cardiac preconditioning effects of isoflurane and sevoflurane in coronary artery bypass graft surgeries done on Cardio Pulmonary Bypass

Hosp.no: Serial no:

Name: Age: Sex:

Weight: Height: Date:

APPENDIX

Pre-operative Data

- Hypertension (Duration and Treatment)
- Diabetes mellitus (duration and treatment)
- Renal dysfunction (Serum creatinine value)
- COPD\Asthma (specify PFT report)
- Any other medical illness –Specify
- Number of diseased vessels & Any cardiac dysfunction
- Any other cardiac illness(valvular,congenital)
- Preoperative medications (antiplatelet & others)

Intra-operative data –

Baseline Prepump

Post pump

Heart rate

Arterial Bp

Saturation

CVP

BIS (Average) Prepump

Intrapump

Postpump

Prepump

Postpump

Cardiac output

Cardiac index

SVR

Biochemical parameters (samples to be taken from radial arterial line)

Sample A-Before induction

Sample B-One hour after Aortic cross clamp release

Sample C-One hour after coming off bypass

Duration of CPB :

Post-operative data

Biochemical marker

Creatinine kinase MB (0hr and 12 hrs later)

Duration of ICU stay

Any other problems in (ICU specify in detail)

INFORMATION TO THE PATIENT AND CONSENT FORM

Introduction

Hello, my name is Dr. Sathish Kumar D. and I am working in the department of Anesthesiology CMC Hospital Vellore. We are conducting a study that can help to influence cardiac function and postoperative benefits like reduced ICU stay. First, the procedure is done only in the presence of senior supervision inside the operation theatre with all the usual monitoring required for the surgery done in our centre.

Study procedure

As you know, CABG is done in the operation theatre. After taking you inside the operation theatre an intravenous line will be started. After establishing monitors, you will be asked to take a good breath through mask with oxygen. Depending on the randomization, you will be administered general anaesthesia using a gaseous agent (either Isoflurane or Sevoflurane). Your vitals will be monitored intraoperatively using standard monitoring. Biochemical tests for cardiac function will be sent during the procedure and also during the postoperative period in the ICU. At the end of the procedure you will be taken to the ICU without waking up, where you will be ventilated for a day with all the usual monitoring. Then you will regain consciousness and the endotracheal tube will be removed.

Benefits

By this study, we hope your cardiac function after surgery will become better. Further ICU stay and postoperative hospital stay will be shorter. You will be comfortable and asleep during the intra op and post op period. You will have adequate pain relief during the procedure.

Discomfort and risks

It will be the same like any other general anaesthesia for cardiac surgery. Additional blood tests and monitoring which are done to look for improvement in cardiac function will not cause any discomfort as you will be asleep then.

Confidentiality

Your name will not appear on the study records. Information related to you will be marked in a code sheet and only the study doctor will be able to link your name with the code number.

Participation in the study

Your participation in this study is entirely voluntary and you have the right to refuse to volunteer for this study. Your care will not be affected by this decision. However if you volunteer, you are required to sign or put your thumb print on the following consent form.

CONSENT

I have read this consent form and have discussed the procedure with Dr. D. Sathish Kumar. The details of this study have been explained to me. I have been given the opportunity to ask questions, which have been answered to my satisfaction. I understand that this study is voluntary. I understand that I can refuse to participate in the study and my care will not be affected by this study. I give my consent to be enrolled in this study.

Signature of the participant Signature of the Anaesthetist

Name of the patient:

Hospital No:

Date:

Measurement of protein carbonyls, protein thiols and total thiols

Blood samples are treated with 10ml 2-4 dinitrophenyl hydrazine dissolved in 2N hydro chloric acid(HCl) followed by incubation at room temperature for 1 hour. Trichloroacetic acid (10% final concentration) will then be added, followed by centrifugation to pellet the precipitated protein. To this pellet an equal volume of 1:1 (v/v) ethyl acetate: ethanol are added. Following centrifugation, the pellet is collected and dissolved in 1ml of 6M guanidine HCl. Absorbance at 366nm is then measured, and expressed as nmoles/mg protein. Total thiol content was measured using DTNB [dithio-bis-2-nitrobenzoic acid] and expressed as nmoles/mg protein.

Measurement of malondialdehyde

For measurement of malondialdehyde, serum samples was treated with 200microlitre of 8.1%SDS, 1.5ml of 20% glacial acetic acid pH 3.5 and 1.5ml of 0.8% thiobarbituric acid. The samples are incubated at 100°C for 1 hour. The absorbance is then read at 532nm and amount of malondialdehyde formed is calculated from a standard curve prepared using 1, 19,3,39 tetramethoxypropane and expressed as nmol/mg protein.

Estimation of serum nitrate levels

Estimation of nitrate and nitrite, the stable end products of nitric oxide oxidation, is a common indirect method to monitor NO levels. Nitrate in the samples must first be reduced to nitrite, which is then measured by the Griess reaction. Reduction of nitrate to nitrite will be carried out using a copper-cadmium alloy. Copper cadmium alloy is prepared by mixing molten copper with cadmium in the ratio 1:9. Filings of the alloy are then prepared, followed by activation as follows- The copper-cadmium alloy filings are washed twice with 100ml of de-ionized water, followed by two washed with 0.5N HCl. The activated filings are then washed with 0.1N HCl and stored in 0.1N HCl at 2-8^{o}C until use. For analysis of nitrate/nitrite, samples were incubated with the alloy filings in carbonate buffer for 1 hour at room temperature with shaking. The reaction is stopped by addition of 0.35M NaOH and 120mM $ZnSO_4$ solution, followed by vortexing. After standing for 10 minutes, the samples are centrifuged at 4000g for 10 minutes. Aliquots of the clear supernatant are treated with

1% sulfanilamide and 0.1% N-naphthylethylenediamine. After 10 minutes, the optical density is read at 545nm in a spectrophotometer.

1	S NO	Serial number	
2	HOS NO	Hospital number	
3	AGE	Age	
4	WT	Weight	
5	HT	Height	
6	WT	Weight	
7	NYHA class	New york Heart association Classification	
8	PREOP DATA	Preoperative morbidity	
	Bp	Hypertension	0-no hypertension 1 - hypertension
	DM	Diabetes mellitus	0 – non diabetic 1 - Diabetic
	rendys	Renal Dysfnction	0 – No dys function 1 – dysfunction present
	copd	Chronic obstructive pulmonary disease	0 – no copd 1 - copd
	ves_blk	Number of coronary vessels blocked	
	sys dysfn	Systolic dysfunction-Angiogram finding	0 – sys dys absent 1 – sys dys present
	Ef	Ejection fraction	
	Others	Other preoperative problems	0 – No problems
	MR-fnal	Functional Mitral regurgitation	1 – Functional mitral regurgitation
	hypothy	Hypothyroidism	2 – Hypothyroidism
	AS-cal	Calcific aortic stenosis	3 – calcific aortic stenosis
9	hr-base	Baseline heart rate	
	hr- pre	Heart rate prior to cardiopulmonary bypass (CPB)	
	hr-post	Heart rate after CPB	
	map-pre	Baseline mean arterial pressure	
	map- pre	Mean arterial pressure prior to CPB	
	map-post	Mean arterial pressure after CPB	
	SPO2	Baseline oxygen saturation	
	cvp-base	Baseline central venous pressure (CVP)	
	cvp-pre	CVP prior to CPB	
	cvp-post	CVP after CPB	

10	BIS - prepump - intrapump - postpump	Bi spectral index Prior to CPB During CPB After CPB	
11	CO -prepump -postpump	Cardiac output Prior to CPB After CPB	
12	CI prepu postpu	Cardiac index Prior to CPB After CPB	
13	SVR prepu pstpu	Systemic vascular resistance Prior to CPB After CPB	
14	prn thiols	Protein thiol levels	
15	total thiols	Total thiol levels	
16	mal-ald	Malondialdehyde levels	
17	prn corbns	Protein carbonyl levels	
18	nitrates	Nitrate levels	
19	CPB DN	Cardiopulmonry bypass duration	
20	CKMB0	Creatinine kinase-MB at 0hr in Intensive care stay(ICU)	
21	CKMB12	Creatinine kinase-mb at 12hrs in ICU	
22	ICUDN	Duration of stay in ICU	0 – no problems during ICU stay
23	OTHER - postop bld	Other problems in ICU Post operative bleeding	1 – Post operative bleeding
24	DRUG	Volatile anaesthetic agents used	1 – Isoflurane 2 - Sevoflurane

Name of the investigator **: *Dr. Sathish Kumar. D.***

Name of the Guide ***: Dr. Mary Korula M.D., D.A.***

Topic title: Comparison of cardiac preconditioning effects of Isoflurane and Sevoflurane in coronary artery bypass surgeries done on cardiopulmonary bypass

Course: Master in Anaesthesiology (Branch X)

ABSTRACT

BACKGROUND:

Preconditioning by volatile agents is a promising therapeutic strategy to render myocardial tissue resistant to perioperative ischemia in cardiac surgery. It was hypothesized that preconditioning with volatile anaesthetic agents isoflurane and sevoflurane would decrease oxidative stress during ischemic reperfusion injury and also prevent postoperative increase in creatinine kinase-MB, a marker of myocardial ischemia. But there are only few studies comparing these 2 volatile agents.

AIM:

To compare the cardioprotective properties of Isoflurane and Sevoflurane when used before, during and after Cardiopulmonary bypass (CPB) to improve postoperative outcome in coronary artery bypass graft surgeries by analyzing: haemodynamic parameters such as invasive arterial BP, ECG, cardiac index, systemic vascular resistance and CVP; postoperative measurements of CK-MB to compare degree of ischemia; biochemical markers of Oxidative and Nitrosative stress at different time intervals.

METHODS:

Twenty patients scheduled for CABG surgery on CPB were randomly assigned to preconditioning with isoflurane or sevoflurane throughout the surgical procedure. The agent used was blinded to surgeons, perfusionist and the biochemist. Biochemical markers of oxidative stress (thiols, malondialdehyde, protein carbonyls and nitrates) and myocardial ischemia (CK-MB) were determined. Cardiac index (CI) and systemic vascular resistance (SVR) were recorded perioperatively.

RESULTS:

Both isoflurane and sevoflurane preconditioning showed similar release of oxidative stress marker the thiols. Sevoflurane however showed significant reduction of malondialdehyde- a marker of lipid peroxidation (P=0.02) and nitrates – a marker of nitrosative stress(P=0.03) when compared to isoflurane. No difference in cardiac

indices were found between the two groups. Though CK-MB values for patients in sevoflurane group were lesser than the iso group, this did not attain statistical significance.

CONCLUSION:

Preconditioning with sevoflurane as well as isoflurane preserves myocardial function as assessed by biochemical markers in patients undergoing CABG on CPB pump.This study demonstrates that among these two agents, isoflurane provides more protection during the early reperfusion phase,while sevoflurane was effective in early reperfusion as well as in the immediate post-bypass period, when the oxidative stress is much greater.

Master Chart

S.NO.	HOS NO	AGE	WT	HT	NYHA	MI	PREOP DATA													
					class	prior	Bp			DM			rendys		COPD		ves_ blk	sys dysfn		Ef
1	749544c	53	53	168	2	1	nil	0	0	nil	0	0	nil	0	nil	0	4	nil	0	54
2	757681c	55	69	171	2	0	yes-2yrs	1	2	nil	0	0	nil	0	nil	0	2	nil	0	57
3	705336c	50	57	165	2	1	nil	0	0	yes-12yrs	1	12	nil	0	nil	0	2	sys dysfn	1	45
4	741096c	48	48	169	3	1	nil	0	0	nil	0	0	nil	0	nil	0	3	nil	0	44
5	790101c	58	58	172	2	0	yes-5yrs	1	5	nil	0	0	nil	0	nil	0	3	nil	0	56
6	786710c	53	74	169	2	0	yes-10yrs	1	10	yes-1yr	1	1	nil	0	nil	0	3	nil	0	45
7	785206c	53	61	166	2	0	nil	0	0	nil	0	0	nil	0	nil	0	3	nil	0	56
8	801796c	56	54	162	3	0	yes-2yrs	1	2	nil	0	0	nil	0	nil	0	2	nil	0	48
9	814094c	36	54	173	2	0	nil	0	0	nil	0	0	nil	0	nil	0	3	nil	0	59
10	820418c	61	62	165	2	0	yes-10yrs	1	10	yes-9yrs	1	9	nil	0	nil	0	2	nil	0	57
11	817253c	60																		
12	827061c	51	70	163	2	1	yes-4yrs	1	4	1year	1	1	nil	0	nil	0	3	sysdysfn	1	59
13	819500c	59	46	155	2	1	nil	0	0	nil	0	0	nil	0	nil	0	3	sys dysfn	1	42
14	821391c	34	93	170	3	1	1year	1	1	nil	0	0	nil	0	nil	0	3	sysdysfn	1	41
15	831929c	69	65	175	2	0	nil	0	0	nil	0	0	nil	0	nil	0	3	nil	0	58
16	837665c	56	60	160	2	0	26yrs	1	26	5yrs	1	5	nil	0	nil	0	3	nil	0	59
17	792635c	58	66	155	2	0	nil	0	0	nil	0	0	nil	0	nil	0	3	nil	0	57
18	840192c	64																		
19	908401B	55	62	166	3	1	yes-1yr	1	1	yes-3yrs	1	3	nil	0	nil	0	3	sys dysfn	1	44
20	819686c	54	56	165	3	1	yes-5yrs	1	5	nil	0	0	nil	0	nil	0	3	sys dysfn	1	41

Master Chart

												BIS		
others		hr-base	hr-pre	hr-post	map-base	map-pre	map-post	SPO2	cvp-base	cvp-pre	cvp-post	prepump	intrapump	postpump
nil	0	80	75	95	88	65	85	100	8	7	9	48	45	51
nil	0	68	75	95	87	65	80	99	11	6	8	51	46	55
nil	0	66	78	89	92	66	70	100	5	7	8	44	46	48
nil	0	60	65	75	86	78	70	100	4	5	8	45	50	43
nil	0	76	88	74	86	78	73	98	10	11	9	44	43	51
MR-fnal	1	56	68	88	75	82	88	100	5	5	8	44	41	48
nil	0	68	75	92	80	65	62	99	8	9	10	42	48	42
hypothy	2	62	69	83	99	80	75	100	3	5	7	42	48	40
nil	0	82	88	95	97	88	79	100	5	8	6	45	48	47
AS-cal	3	68	66	75	88	72	85	98	7	9	6	44	48	48
nil	0	60	85	95	100	72	68	100	7	8	7	44	41	47
MR-fnal	1	132	89	103	90	85	75	98	7	7	6	40	45	50
nil	0	90	98	86	84	85	70	100	6	8	5	44	48	47
nil	0	78	86	95	79	64	72	100	3	5	6	45	48	47
nil	0	65	68	78	88	70	64	100	3	5	5	44	48	50
hypothy	2	66	75	85	88	65	70	99	5	8	9	47	45	41
nil	0	73	85	95	96	85	80	100	6	8	10	48	42	47
MR-fnal	1	68	82	96	56	65	60	98	16	15	12	48	46	40

Master Chart

CO		CI		SVR		SAMPLEA					SAMPLEB			
prepump	postpump	prepu	postpu	prepu	pstpu	prn thiols	totalthiols	mal-alde	prn corbns	nitrates	prnthiols	totalthiols	mal-alde	prn corbns
4.5	8.6	2.8	4.9	1205	536	1.03	1.18	0.86	0.32	45.32	0.94	1.24	2.31	0.15
3.47	9.18	2.2	5.1	1220	605	2.12	2.59	1.98	0.17	62.77	1.61	2.18	1.36	0.10
4.11	8.11	2.51	6.1	1105	802	1.41	1.00	2.30	0.20	56.00	1.09	0.85	0.94	0.13
6.25	9.9	3.79	5.59	1234	625	0.41	0.74	2.00	0.23	32.30	0.80	0.56	4.50	0.14
5.8	9.4	2.95	5.3	895	559	2.68	1.71	0.41	0.27	56.44	1.41	2.50	0.21	0.21
5.1	6.75	2.07	4.45	1063	678	3.26	1.18	0.31	0.29	32.22	1.29	0.65	0.52	0.21
4.16	7.42	2.55	4.55	956	610	3.00	2.35	1.67	0.19	38.64	1.09	2.50	0.98	0.16
4.25	7.31	2.71	4.66	1279	416	1.94	1.12	1.96	0.19	32.22	1.67	1.65	1.08	0.11
5.45	12.4	3.32	7.45	980	930	5.06	3.94	3.21	0.31	42.23	1.58	2.56	2.46	0.15
6.31	9.8	3.76	5.84	912	785	4.79	4.97	0.47	0.86	19.55	1.18	1.68	0.75	0.21
4.66	7.17	2.65	4.17	1218	750	0.74	0.24	0.19	0.28	23.56	0.68	1.26	0.38	0.15
5.43	6.48	3.82	4.8	859	512	1.94	1.44	0.86	0.23	23.33	0.94	2.68	0.65	0.17
5.61	10.4	2.75	5.13	811	436	1.35	1.24	0.74	0.34	21.11	0.53	0.82	0.63	0.19
6.86	8.14	3.8	4.55	635	753	1.56	2.53	0.91	0.18	12.45	5.91	5.71	0.91	0.14
3.82	9.25	2.36	5.71	1333	475	0.88	1.00	0.84	0.29	60.00	0.80	1.41	0.32	0.21
4.58	7.12	3.07	4.23	709	649	1.47	2.76	0.92	0.18	19.00	0.94	2.88	1.38	0.11
2.86	7.25	1.65	2.75	1102	689	1.56	2.12	1.12	0.16	14.22	1.12	1.38	1.34	0.13
2.56	6.55	1.6	4.25	1124	589	1.35	1.59	1.23	0.22	17.11	1.41	1.50	1.13	0.12

Master Chart

	SAMPLEC					CPBDN							
nitrates	prnthiols	totalthiols	mal-alde	prn corbns	nitrates		CKMB_0	CKMB12	ICUDN	OTHER		DRUG	
33.25	0.94	0.62	1.52	0.16	27.26	2:20	27	11	28	po op bld	1	Sevo	2
49.23	0.82	1.82	2.30	0.12	36.61	1:15	28	20	20	nil	0	Iso	1
16.30	0.53	1.00	0.52	0.13	19.07	1:30	15	9	16	nil	0	Sevo	2
22.76	1.24	0.94	1.88	0.17	25.23	1:10	30	17	36	nil	0	Iso	1
34.48	1.59	0.94	0.52	0.18	45.33	1:40	28	27	25	nil	0	Iso	1
34.66	2.03	1.15	0.52	0.22	33.33	1:57	23	16	14	nil	0	Sevo	2
34.66	2.09	3.21	0.69	0.11	31.33	1:40	17	7	20	nil	0	Sevo	2
34.66	1.76	0.32	0.49	0.06	33.33	1:35	21	23	17	nil	0	Iso	1
39.00	1.62	1.35	1.93	0.25	26.88	1:15	18	15	20	nil	0	Iso	1
16.00	2.00	3.35	0.38	0.21	15.12	2:40	57	38	24	nil	0	Sevo	2
18.44	0.29	0.32	0.19	0.43	15.55	1:50	55	69	16	nil	0	Sevo	2
20.00	1.06	2.74	0.75	0.15	26.22	1:50	21	25	22	nil	0	Iso	1
17.35	0.85	1.82	0.58	0.18	16.55	2:00	21	24	18	nil	0	Sevo	2
11.56	1.82	1.59	1.08	0.11	14.66	1:40	14	28	19	nil	0	Iso	1
46.61	0.71	1.35	0.86	0.21	45.78	2:10	23	28	18	nil	0	Iso	1
19.00	1.15	1.44	0.97	0.14	14.86	1:20	29	20	21	nil	0	Sevo	2
16.88	1.85	2.06	1.96	0.15	8.88	2:50	31	39	23	nil	0	Iso	1
27.77	1.12	1.94	1.37	0.17	17.11	3:10	84	60	20	nil	0	Sevo	2