ANTIOXIDANT PROPERTIES OF PROPOFOL

IN CORONARY ARTERY BYPASS SURGERY

by

JIANHANG SUN

M.B., The Second Military Medical University of PLA, 1986

A THESIS SUBMITTED IN PARTIAL FULFULMENT OF

THE REQUIREMENTS FOR THE DEGREE OF

MASTER OF SCIENCE

in

THE FACULTY OF GRADUATE STUDIES

(Department of Surgery)

We accept this thesis as conforming

to the required standard

THE UNIVERSITY OF BRITISH COLUMBIA

September 1998

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Department of _Surgery_

The University of British Columbia
Vancouver, Canada

Date _September 22, 1998_

ABSTRACT

Ischaemia reperfusion injury (IRI) is considered one of the major causes of cardiopulmonary dysfunction in cardiopulmonary bypass (CPB) surgery. Reactive oxygen intermediates have been linked to IRI. Measures to increase antioxidant capacity, with vitamin C, vitamin E, and allopurinol, have markedly reduced tissue lipid peroxidation and resulted in improved cardiopulmonary function. An intravenous anaesthetic, propofol has been found to have antioxidant properties in cell culture and in animal studies. The antioxidant potential of propofol during CPB surgery has not been reported.

Following institutional approval and informed patient consent, 26 patients scheduled for CPB surgery were enrolled. Patients were anaesthetized with sufentanil-isoflurane (control, n=11), sufentanil-low dose propofol (1.5-2.5 mg/kg bolus then 100 µg/kg/min preCPB, 50 ug/kg/min intra-CPB; n=7), or high dose propofol (1.5-2.5 mg/kg then 200 µg/kg/min continous infusion; n=8). Venous blood was sampled for determination of red cell antioxidant capacity as MDA (malondialdehyde) production against in vitro oxidative challenge and plasma concentration of propofol. Clinical parameters of interest included perioperative inotropic requirement, haemodynamic changes, and lung oxygenation.

Red cell antioxidant capacity increased significantly only with high dose propofol. This effect continued 2 hours after separation from bypass. High dose and low dose propofol were associated with significantly less inotropic

(dopamine 3-5 µg/kg/min) requirement. Low dose propofol significantly increased the percentage of patients with normal cardiac index post-operatively. Improvement of cardiac function did not parallel the reduction of lipid peroxidation. Propofol's effects were limited to lipid membranes. The cardiodepresant effect of high dose propofol was manifested as relatively low cardiac index within the first three hours after operation. Lung oxygenation was superior with low dose propofol and isoflurane after 6 to 12 hours after operation, comparing to that within one hour after operation ($P < 0.05$).

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ACKNOWLEDGEMENTS

To my wife Jennifer and son Sean

who continually provide the inspiration,

and to my sister Liping Sun

for her unlimited support

Chapter 1

INTRODUCTION

1.1 Overview

Ischaemia reperfusion injury (IRI) has been considered as one of the major causes of cardiopulmonary dysfunction in coronary artery bypass (CAB) surgery [1-5]. Reactive oxygen intermediates have been linked to IRI [2,6-8]. Measures to increase antioxidant capacity, such as preoperative conditioning with vitamin C, vitamin E or allopurinol, have demonstrated markedly reduced tissue lipid peroxidation in both animal and human studies, in association with improved cardiopulmonary function [1,9-13]. An intravenous anaesthetic, 2,6-diisopropylphenol, or propofol, has been shown to have antioxidant activity in animal studies [14-18]. Although propofol anaesthesia has been used for CAB surgery since 1987, its protective effects as an antioxidant during surgery have not been reported.

1.2 Free Radicals

1.2.1 Free Radicals and Reactive Oxygen Intermediates (ROIs) [19]

Free radicals are molecules or fragments of molecules containing unpaired electrons in their outer orbitals. Unpaired electrons tend to acquire a pair; therefore, most free radicals are chemically highly reactive and, as a result, short-lived.

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Free radical reactions are chain reactions started by initiators causing hydrogen removal, which is followed by several steps of propagation in which the free radicals produced interact with one another and with surrounding molecules, and the reaction terminates when the free radicals are converted to nonradical products.

Reactive oxygen intermediates (ROIs) is a collective term for oxygen free radicals and reactive products of oxygen [20]. Free radicals of oxygen play an important role in biology and medicine. They are generated from oxygen by excitation (singlet oxygen) or reduction (superoxide anion, hydrogen peroxide, and hydroxyl radical). By definition, not all reactive products of the reduction or excitation of oxygen are free radicals (e.g., singlet oxygen, and hydrogen peroxide). However, their reactivity and reactions resemble those of free radicals. These products, together with the true free radicals, are called ROIs.

Well-recognized ROIs include superoxide radical (O_2^-), singlet oxygen($^1O_2$), hydrogen peroxide (H_2O_2), hydroxyl radical($\cdot OH$), peroxyl radical ($ROO\cdot$), perhydroxyl radical($HOO\cdot$), alkoxyl radical ($RO\cdot$), ferryl haem protein radical, and nitric oxide($NO\cdot$) [21].

The superoxide anion is comparatively unreactive and under physiological conditions is converted, by dismutation, to hydrogen peroxide. This is less reactive, longer lived and more lipophilic than the superoxide anion and it can diffuse considerable distances from its site of generation.

The major danger of increased tissue concentrations of hydrogen peroxide is the production of a hydroxyl radical by the Haber Weiss or Fenton reaction:

$$O_2^- + H_2O_2 \rightarrow O_2 + OH^- + \cdot OH$$
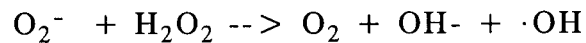
The hydroxyl radical has a short half-life, and is extremely reactive. For example, it will rapidly react with unsaturated fatty acid side chains, resulting in lipid peroxidation. Fortunately, the Haber-Weiss reaction is very slow under physiological conditions. It is, however, accelerated by metal catalysts. This process is called the Fenton reaction.

Exogenous H_2O_2 has been used as a source of ROIs for experimental study because (1) H_2O_2 and its metabolite $\cdot OH$ are known to be important in the pathogenesis of IRI; (2) H_2O_2 can penetrate the cell membrane, thereby reaching intracellular sites [15,22].

1.2.2 Free Radical Effects

Although oxygen is essential for aerobic life forms, too much oxygen or inappropriate metabolism of oxygen can be toxic to the organism [23].

Physiologically, free radicals are essential to many normal biological process [21]. (1) Superoxide can be generated by a variety of cells to perform useful functions in the body: they are part of the cascade of events in tissue response to invading microorganisms and foreign material through the antimicrobial action of phagocytic cells (neutrophils, monocytes, and eosinophils) [24,25]; (2) They are intermediates in and/or products of enzyme-catalyzed reactions, for example the action of xanthine oxidase;

(3) They are regulatory molecules in biochemical processes. For example, lymphocytes and fibroblasts constantly generate small amounts of superoxide radical as growth regulators [26]. (4) They are involved in cyclooxygenase and lipoxygenase action in eicosanoid metabolism. (5) They are involved in the synthesis of adrenocortical hormones and in the functional division of the cortex [25].

Pathologically, abnormal free radical reactions may be elicited by physiological free radicals if the control mechanisms are defective, or if there is a marked increase in the production of free radicals under normal conditions of self protection. Free radical reactions may damage virtually any biomolecule: enzymes, proteins, carbohydrates, lipids or nucleic acids. The genetic material, membranes composed predominantly of lipids and proteins, and subcellular structures (plasma membrane, mitochondria, microsomes and lysosomes) are all potential sites of lipid peroxidation damage [23,26]. No organ is spared from such oxidative damage. Aging and many disorders, such as cancer, atherosclerosis, and neural disorders, have all been related to such oxidative injury [26-28].

1.2.3 Mechanisms of the endogenous release of Free Radicals during Tissue Injury. [9,21,25,29]

1. Phagocyte recruitment and activation at the site of injury (Figure 1).

2. Xanthine oxidase and other superoxide-producing enzymes (Figure 2). Studies of xanthine oxidase distribution in cardiac tissue have shown that the

enzyme is localized in oxidation-sensitive vascular endothelial cells rather than in myocytes [30].

3. Disrupted mitochondrial electron transport, e.g. during ischemia, allowing leakage of electrons onto oxygen during reperfusion.

4. A number of cell types, including endothelial cells, macrophages, and smooth muscle cells, have been shown from studies in cell culture to be capable of producing superoxide radicals.

$$\text{NADPH} + 2\,O_2 \xrightarrow{\text{Oxidase}} \text{NADP}^+ + \text{H}^+ + 2\,O_2^{-\cdot}$$

$$\downarrow$$

$$H_2O_2$$

$$Cl^- \Big\downarrow \text{MPO}$$
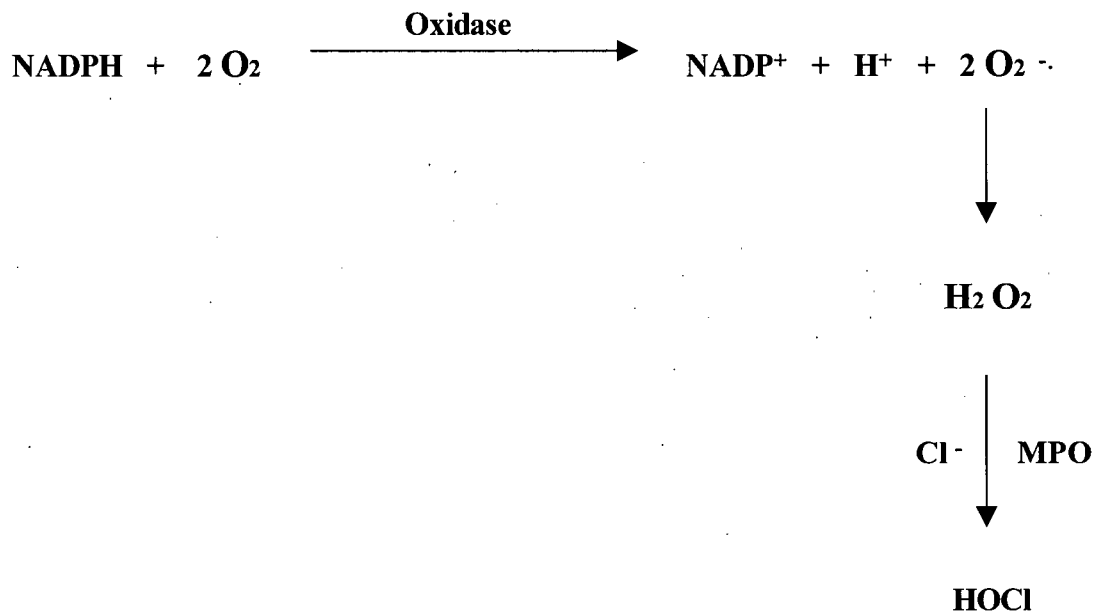
$$HOCl$$

Figure 1. Reactions involved in the generation of superoxide by NADPH oxidase and the respiratory burst of phagocytes. NADPH, nicotinamide adenine dinucleotide phosphate; MPO, myeloperoxidase; HOCl, hypochlorous acid.

Allopurinol

$$\Big\downarrow (-)$$

Xanthine oxidase

$$\begin{array}{l} \text{Xanthine} \\ \text{or} \\ \text{Hypoxanthine} \end{array} \quad \begin{array}{c} \text{NADPH} \longrightarrow \text{NADP}^+ + \text{H}^+ \\ \longrightarrow \text{Uric Acid} \\ O_2 \longrightarrow O_2^{-\cdot} \end{array}$$
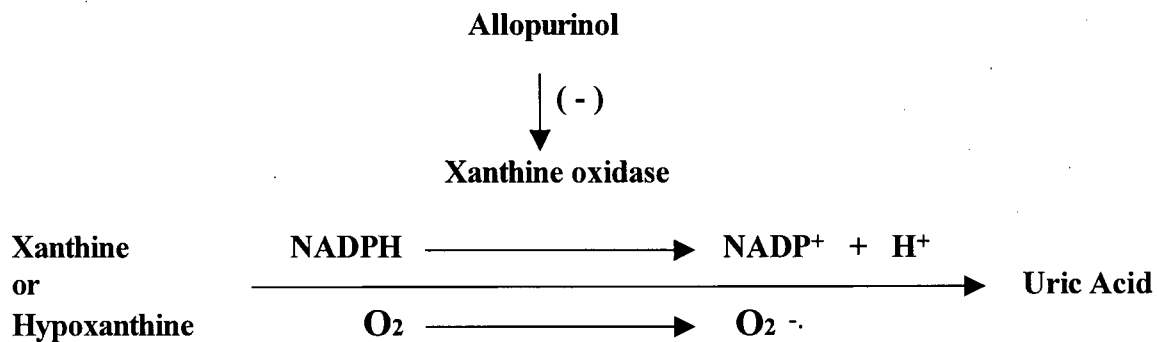
Figure 2. Xanthine oxidase and by-product superoxide radical formation.

1.3 Lipid Peroxidation and Detection

1.3.1 Lipid peroxidation.

The most common target of ROIs is membrane lipids because of their chemical nature (cholesterol unsaturation, conjugated unsaturated linkages of polyunsaturated fatty acids) and of their regular structural arrangement (monolayers in lipoproteins, bilayers in cell membranes) [31]. However, auto-oxidation of the lipids of living organisms is usually a slow, compartmentalized process because molecular oxygen is a weak oxidant [19].

Lipid peroxidation is a process in which a lipid free radical R· is formed by the effect of a free radical initiator. The free radical then reacts with molecular oxygen, producing a peroxy free radical ROO·. A chain reaction is started, the effect of which is not restricted to lipids, but may, under pathological conditions, damage neighboring biomolecules (proteins, carbohydrates, nucleic acids) [19]. Moreover, by mediation of stable products (comparing to unstable products) of the process, such as alkanes [19,32], remote molecules may also be damaged through local diffusion or circulation. Marked reduction of energy metabolism by aldehyde products of oxidation (malondialdehyde, hydroxypentenal, and hydroxynonenal), and by various hydroperoxides has been described [32], including inhibition of glycolytic enzymes, and reduction of mitochondrial respiration. Other membrane-bound enzymes are also affected [33].

1.3.2 Arachidonic Acid Metabolism and MDA Formation [25,34]

MDA (malondialdehyde) is formed from peroxidation of polyunsaturated fatty acids with conjugated double bounds, mainly via the arachidonic acid metabolism pathway in vivo. Malondialdehyde is formed not only as a result of cyclooxygenase activity as a by-product of thromboxane A2 (TxA2) synthesis, but also by lipoxygenase activity as a by-product of hydroperoxy fatty acids (Figure 3).

Phospholipid

$\downarrow$ Phospholipase A_2

Arachidonic acid

Cyclooxygenase   Lipoxygenase

PGG$_2$ Hydroperoxy fatty acids

$\downarrow$

PGH$_2$

PGD$_2$ PGE$_2$ PGF$_{2\alpha}$ PGI$_2$ TxA$_2$ HHT   MDA   Hydroxy fatty acids

+

6 $-$ keto $-$ PGF$_{2\alpha}$ TxB$_2$ MDA
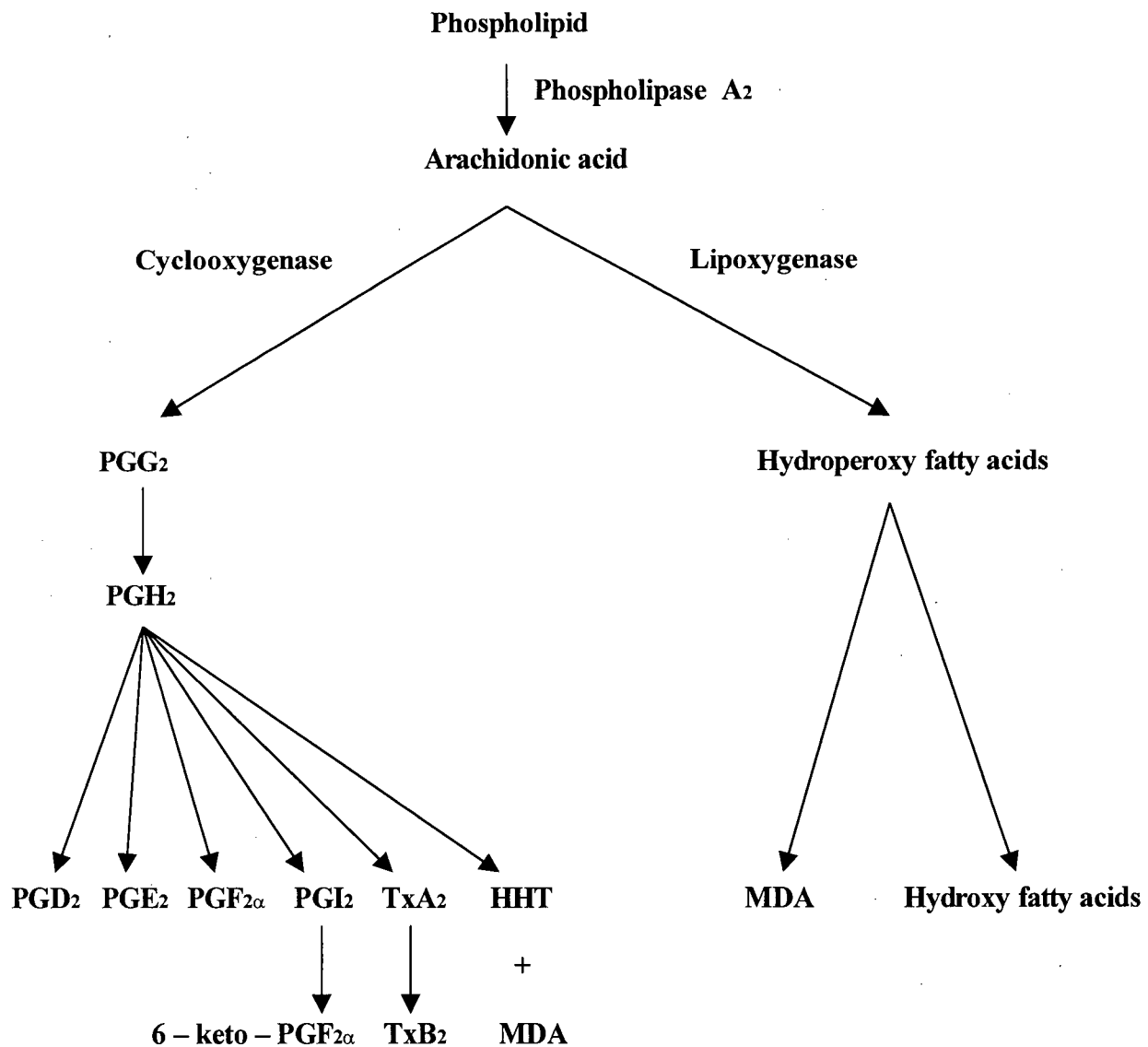
Figure 3. Pathway by which MDA is formed as a by-product in arachidonic acid metabolism. Tx A$_2$,

thromboxane A$_2$; Tx B$_2$, thromboxane B$_2$; HHT, (12S-12-hydroxy-5Z,8E,10E)-heptadecatrienic

acid; MDA, malondialdehyde.

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1.3.3 MDA: A Marker for Lipid Peroxidation

Malondialdehyde is metastable, and detectable in plasma [1,35-37], cells[38-40], tissues [15,41-43], and subcellular structures such as mitochondria [16,44] and microsomes [45]. The concentration of MDA provides a measure of the extent of lipid peroxidation and antioxidant status of the body [1,15,39,45,46]. Therefore, MDA has been widely used as a marker for lipid peroxidation.

In healthy persons, the average plasma MDA concentration is about 0.7 μmol /L for age less than 30 years, and about 1.2 μmol/L for individuals of more than 40 years [35]. These levels are undetectable with the classic thiobarbituric acid (TBA) assay, but can be detected by more sensitive and specific high-performance liquid chromatography (HPLC) with fluorescence detection [47-49].

1.3.4 Conjugated Dienes and Phospholipid Molar Ratio: Another Chemical Marker for Oxidative Injury

In vitro experiments showed that free radicals can cause isomerization of polyunsaturated fatty acyl chains, generating products with diene conjugation but without peroxidation [50,51]. The isomerization most studied has been the conversion of linoleic acid (18:2(9,12)) to 9,11-octadecadienoic acid (18:2(9,11)). This accounts for over 90% of the diene conjugation in human plasma, tissue, and tissue fluids [52]. HPLC can measure both the isomer 18:2(9,11) and the parent compound 18:2(9,12) in plasma. Molar ratio is the

ratio of 18:(9,11) to 18:2(9,12) [4,53]. The molar ratio in the free fatty acid fraction is affected by heparin, but the molar ratio in the phospholipid-esterified fraction is not [54]. Therefore the phospholipid molar ratio is measured.

1.4 Free Radical Generation and Myocardial Damage during CAB Surgery

1.4.1 Pattern of Systemic Free Radical Generation during CAB Surgery

HEART. Reperfusion of myocardium after the period of aortic cross-clamping or coronary occlusion can lead to the release of ROIs from a variety of pathways in phagocytes, endothelial and myocardial cells [8,55-58]. Serial blood sampling from systemic arterial, mixed venous and coronary sinus catheters reveals, however, that the increase in MDA and molar ratio in coronary venous blood is similar to, or less than, the increases in mixed venous and arterial blood [4]. Even though neutrophils have been shown to accumulate in the myocardium during early reperfusion, their activation during oxidative stress does not occur [2]. Thus, it seems unlikely that the myocardium is a major source of systemic ROIs during CPB.

LUNG. Activated neutrophils within the lungs may be important in the production of ROIs. The lungs are not ventilated, and they are partially collapsed during bypass. When pulmonary perfusion is restored at the cessation of bypass, there is white cell sequestration within the lungs [59-61]. Activated complement components [60-62], markers of white cell

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degranulation [61-63] and markers of free radical activity are all detectable in the pulmonary effluent blood at this time.

Bypass Apparatus. Neutrophils are activated in the circulation during bypass [64-66]. They stick to the surfaces of the tubes and to the oxygenator of the extracorporeal circulation apparatus. This is followed by rapid activation and degranulation [92]. Complement is also activated, probably via the alternative pathway, due to the contact of the blood with foreign surfaces, including the pump, tubing, oxygenator, and blood gas interface [67-71]. The activated complement in turn activates neutrophils to produce ROIs [72]. The effects of increased oxidative stress related to the bypass apparatus should occur mainly during, rather than following, bypass.

1.4.2 Myocardial Ischaemia Reperfusion Injury Caused By ROIs

Ischaemia reperfusion injury (IRI) is a very complex pathophysiological process. Factors associated with IRI include formation of ROIs, production of platelet activating factor (PAF), complement activation, platelet and leukocyte activation, production of arachidonic acid metabolites, formation of inflammatory mediators such as histamine, serotonin, kinin and bradykinin as well as immunologically-provoked types of acute reactions [72].

It is considered that in the complex mutifactorial pathogenesis of IRI, ROI formation, PAF production, and complement activation may play the most important roles [72-75]. However, this section of the thesis will ONLY focus on the pathophysiology of IRI caused by ROIs.

In the myocardium, the reduction of oxygen to water proceeds by two pathways. The mitochondrial cytochrome oxidase system reduces 95% of oxygen to water by tetravalent reduction without the production of ROIs. The remaining 5% of oxygen enters the univalent reductive pathway and several ROIs, such as superoxide anions, hydrogen peroxide and the hydroxyl radical, are produced [8].

The myocardial damage during CAB surgery likely starts during ischaemia. At this time, oxygen is no longer available, but a certain amount of ROIs might be still formed from residual molecular oxygen. (1) During this period, the components of the mitochondrial electron transport chain become reduced [76,77], allowing leakage of electrons onto residual molecular oxygen, leading to the formation of superoxide radicals [21]. Most likely, in the early phase of ischaemia, ROI production from mitochondria is neutralized by superoxide dismutase (SOD). Increasing the duration of ischaemia leads to a progressive decline in SOD activity, leaving the mitochondria less capable of dealing with the increased radical flux [78-80]. (2) ROIs may be generated by xanthine oxidase, because cardiac tissue produces uric acid and superoxide radicals during ischaemia and reperfusion [81]. (3) ROIs may be generated within membranes, in association with the arachidonic acid cascade.

During reperfusion, oxygen is available, and ROIs can be produced through the following mechanisms: (1) Reperfusion re-energizes the mitochondria, but electron egress through cytochrome oxidase is reduced

because of the lack of adenosine diphosphate (ADP) causing formation of ROIs [8]. (2) ROIs again may be produced through the xanthine oxidase pathway [81]. (3) Neutrophils could also be activated, generating ROIs. Even though neutrophils have not been demonstrated to contribute to ROI generation in the myocardium during reperfusion [2,6], there is evidence that neutrophils do accumulate in the myocardium during reperfusion [2,7]. Furthermore, the depletion of circulating neutrophils has been associated with decreased myocardial damage in models of ischaemia reperfusion injury [7,82].

Thus, it seems that ischaemia induces alterations capable of reducing endogenous defense mechanisms against ROI challenge. The prime alteration appears to lie at the level of mitochondrial antioxidant enzymes with SOD activity being reduced by 50% after severe ischaemia [8]. Furthermore, reperfusion is likely to stimulate the production of ROIs to an extent greater than the neutralizing capacity of mitochondrial antioxidant enzyme defenses. If ischaemia is brief (< 30 to 60 min), reperfusion may not result in irreversible oxidative damage [2,8], probably because the defense mechanisms are able to protect myocardial cells against the burst of ROIs generated by readmission of oxygen with coronary flow. Reperfusion after a longer period of ischaemia, when the defense mechanisms are impaired, causes myocardial damage, which may lead to irreversible loss of contractile function.

1.5   Endogenous Antioxidant Defense System

Cells and tissues usually have adequate intracellular and extracellular antioxidant defenses. Physiologically, those located intracellularly are appropriate for dealing with aberrant generation of ROIs; those located extracellularly are appropriate for binding metal ions, delocalised haem proteins and for inhibiting peroxidation processes [83]. The major antioxidants in human plasma and their effectiveness against ROIs generated in the aqueous phase are: ascorbate = protein bilirubin > urate > tocopherol. Plasma lipid peroxidation only occurs when ascorbate is completely consumed. In ascorbate-replete plasma, the lipids are protected [84]. The range of antioxidants and radical scavengers is summarized as follows:

1.5.1 Nonenzymic: Vitamin C and Vitamin E

These are chain breaking and hydroxyl radical scavenger antioxidants. Vitamin E is highly lipophilic. It contains shielding methyl groups in the vicinity of the phenolic hydroxyl group of the chromophoric moiety, and it is optimally positioned in the membrane by its phytyl side chain [85]. In myocardium, it has been identified in both myocardial cytosolic and mitochondrial membranes and presumably protects them from lipid peroxidation [86,87]. Vitamin C is water soluble. It is active in cytosol and in the extracellular fluid. It functions synergistically with vitamin E and it must be present to regenerate vitamin E.

1.5.2 Major Extracellular Protection Mechanisms [21]

1. Uric acid: scavenges hydroxyl radical and singlet oxygen;

   it is also a chelator of iron and copper.

2. Caeruloplasmin: acts as an antioxidant by virtue of its ferroxidase activity.

3. Transferrin: sequesters iron (III), rendering it unavailable for catalyzing the Haber-Weiss reaction which initiates lipid peroxidation or catalyzes the decomposition of lipid hydroperoxides.

4. Albumin: binds metals, especially copper but also iron weakly.

5. Beta carotene: transported primarily within low-density lipoproteins [88], scavenges peroxyl radicals [89].

1.5.3 Enzymic Antioxidant Defenses

1. Superoxide dismutase (SOD): is present in the manganese form (Mn-SOD) in mitochondria and in the copper or zinc form (Cu-SOD, Zn-SOD) in cytoplasm. It disposes of superoxide radicals by dismutation to hydrogen peroxide and oxygen, which is 10 times faster than by spontaneous decay [90].

2. Glutathione peroxidase (GPX): present in the cytosol, detoxifies hydrogen peroxide and lipid peroxides in the presence of reduced glutathione (GSH).

3. Catalase: detoxifies hydrogen peroxide

   In the myocardium, SOD and GPX are present at significant concentrations, while catalase, at very low concentration. SOD was shown as the first line of defense against ROI challenge, and GPX as the second line [8].

Red cells, in contrast to myocardial cells, have large amounts of catalase [91] as well as SOD and GPX. In animal studies, it was shown that human and murine red cells, probably by virtue of catalase activity, completely protected nucleated cells (L1210 murine leukemia) against exogenous oxidative challenge in vitro [91]. Reperfusion with human red cells increased ventricular function and decreased myocardial hydrogen peroxide levels of ischemic, isolated rat hearts. Reperfusion with red cells that lacked catalase (aminotriazole-treated) and/or glutathione (N-ethylmaleimide-treated) did not increase ventricular function or decrease myocardial hydrogen peroxide concentration as much as reperfusion with untreated red cells [92].

1.6 Preoperative Vitamin C, Vitamin E, and Allopurinol Therapy in Patients Undergoing CAB Surgery

According to a public survey, only 9% of the American population follow diets that meet the recommended guidelines for the antioxidants vitamin E and vitamin C. Furthermore, the low cholesterol diets prescribed for some patients may exacerbate any prior insufficiency of lipid-phase antioxidants [93].

Vitamin E probably is the most important antioxidant in the lipid phase [94]. Its concentration in myocardium was shown to decrease` during CAB surgery [95].

High dose vitamin C (250 mg/kg), given intravenously 30 min before CPB and at the time of aortic declamping (125 mg/kg each time), has shown

protective effects on myocardium, manifested as significantly lower plasma levels of MDA, CPK, CPK-MB, and LDH as well as significantly higher cardiac index after operation [36].

A popular regimen is vitamin C and vitamin E (1000 mg and 100 to 800 IU daily, up to one week before surgery) combined to give patients preoperatively [96-99]. Unfortunately, this regimen for CAB surgery has not resulted any measurable reduction in myocardial IRI although it prevented post-surgical vitamin E depletion in plasma [96]. In the treatment of atherosclerosis, this supplementation regimen has significantly reduced progression of atherosclerosis confirmed morphologically by high resolution B-mode ultrasonography [100] or by angiography [101].

Allopurinol is a purine analogue. It has been used effectively in the treatment of hyperuricemia [102,103]. Its antioxidant effects likely include selective inhibition of xanthine oxidase. It may also attenuate the increase of myeloperoxidase activity in IRI [9,104,105], presumably by decreasing influx of neutrophils during reperfusion. Other mechanisms of allopurinol in reducing IRI independent of xanthine oxidase inhibition may mainly include reduction in lysosomal enzyme release from neutrophils [106] and facilitation of mitochondrial electron transport [107].

In animal models of IRI, allopurinol has effectively increased cell and tissue antioxidant capacity, reduced lipid peroxidation, and significantly improved cardiopulmonary function [9,10,108-110].

In CAB surgery, preoperative conditioning with allopurinol alone or together with vitamin C and vitamin E has shown [1,11-13,111-116] improved myocardial contractility, increased incidence of spontaneous reversion to sinus rhythm, reduced metabolic changes (e.g. plasma MDA, CPK-MB)[1], and decreased hospital mortality rate.

1.7 EGb 761 and Carvedilol: Uncommonly Used Antioxidants for CAB Surgery

EGb 761 and Carvedilol are found to have antioxidant activity. They have rarely been tried as antioxidants in CBP surgery out of North America. Here is only a brief description of the two drugs.

1.7.1 EGb 761

EGb is a titrated and standardized extract of green leaves of Ginkgo Biloba. It is a complex mixture composed of flavonoid substances, terpenes, proanthocyanidins, organic acids, and other constituents [102]. The extract is standardized at 6 and 24% (w/w) of terpenes and flavonoid heterosides. As a free radical scavenger, its protective effects have been demonstrated on the mitochondrial [103,117,118], cellular [119,120], and tissue [119] levels by decreased MDA production [119,121], inhibited neutrophil function and adhesion to endothelium [122], decreased retinal [123], neural [117,120,124] and myocardial damage caused by ROIs.

In patients undergoing CAB surgery, pretreatment with EGb 761 was associated with decreased TBA-reactive species in myocardium, and myocardial improvement (not significantly) in postoperative recovery [121].

1.7.2 Carvedilol

Carvedilol is a vasodilating, beta-adrenoceptor antagonist currently used for the treatment of mild to moderate hypertension [125-127]. Carvedilol and some of its hydroxylated metabolites are potent antioxidants. In physiochemical, biochemical and cellular assays, carvedilol and several of its metabolites directly scavenged ROIs [128], prevented lipid peroxidation in cardiac and brain membranes, both in vitro and in vivo [129] and prevented the depletion of endogenous antioxidants, such as vitamin E and glutathione. Moreover, carvedilol and its metabolites prevented the oxidation of LDL (low density lipoprotein) to oxidized LDL, the latter being directly cytotoxic and known to activate monocytes/macrophages and to stimulate foam cell formation [130].

Carvedilol has been shown to produce significant cardioprotection in experimental animal models (using rat, dog, and pig) of acute myocardial infarction [131-133], with the most dramatic effect being observed in the pig model of myocardial IRI, where the reduction in infarct size reached 91%.

These results suggest its clinical use in patients undergoing CAB surgery [134].

1.8 Propofol: An Anesthetic and Antioxidant

Propofol is a short-acting intravenous anesthetic, which is highly lipid-soluble. It is widely used in both ambulatory and hospitalized patients. It permits both efficient control of anesthetic depth and rapid, controllable recovery [135]. As an antioxidant, propofol has been shown to have protective effects in attenuating myocardial IRI in animal models [14,15].

1.8.1 Structure

Propofol, 2,6-diisopropylphenol (molecular weight 178.27), is chemically similar to phenol-based free radical scavengers such as butylated hydroxytoluene and the endogenous antioxidant vitamin E (Figure 4) [17,136,137]. All these three compounds carry a hydroxyl substituent on a phenyl ring, which is known to confer free radical scavenging properties [136].

OH

(CH3)2 CH   CH(CH3)2

Propofol

OH

(CH3)3C   C(CH3)3

Butylated hydroxytoluene

CH3

CH3

HO

CH2 – (CH2 - CH2 - CH – CH2) - H

CH3   CH3   CH3

O   CH3

Vitamin E

CH3
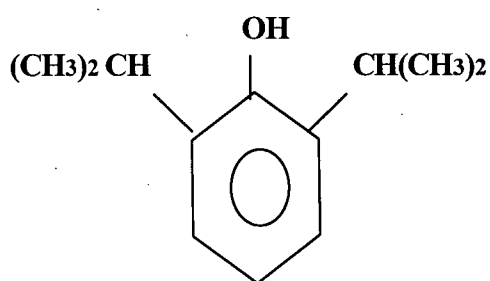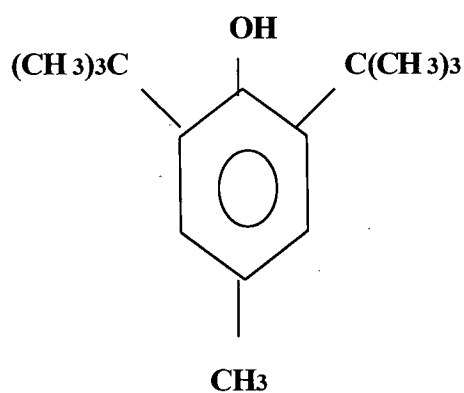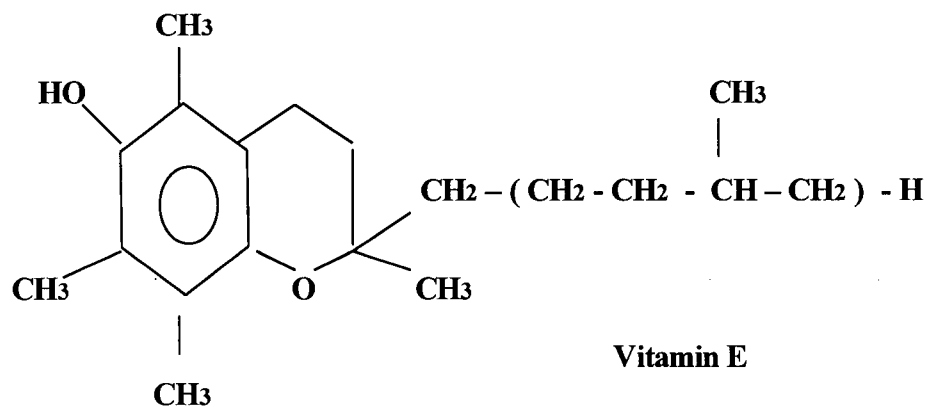
Figure 4. Structural formulae of propofol (2,6-diisopropylphenol), butylated hydroxytoluene, and vitamin E (alpha tocopherol). All three compounds carry a hydroxyl substituent on their phenol rings, which is considered to confer free radical scavenging properties.

1.8.2 Basic Pharmacokinetics [137]

Administration of propofol, 2-2.5mg/kg given iv over 15 seconds or less, produces unconsciousness within about 30 seconds. Awakening is very rapid and complete with minimal residual central nervous system effects, which seems to be the most important advantage over other drugs used to produce induction of anesthesia.

Clearance of propofol from the plasma exceeds hepatic blood flow, emphasizing that tissue uptake as well as metabolism is important in removal of this drug from the plasma. Less than 0.3% of a dose is excreted unchanged in the urine. The elimination half-life is 0.5 to 1.5 hours.

Despite the rapid clearance of propofol by metabolism, there is no evidence of impaired elimination in patients with cirrhosis or renal dysfunction. Patients older than 60 years of age exhibit a reduced rate of plasma clearance of propofol. There may be a modest cumulative effect, especially in elderly patients receiving continuous intravenous infusions.

Plasma Concentration of Propofol during CAB Surgery: Propofol is a weak organic acid that is bound extensively to plasma albumin, with a free fraction of only 2-3% [138]. Haemodilution during cardiopulmonary bypass is associated with decreases in the concentrations of plasma proteins, a decrease in total propofol concentration disproportional to the decreases in the concentrations of plasma proteins [139,140], and a 1.5 to 3 fold increase in the fraction of unbound propofol [141,142].

Disproportional decreases in total plasma concentration of propofol during bypass may be attributed not only to haemodilution but also to propofol sequestration within the extracorporeal circuit, because the decrease of propofol concentration was up to 50% more than that predicted by haemodilution alone [139,140].

Reasons for the increase in the fraction of unbound propofol: The use of heparin during bypass causes an increase in non-esterified fatty acids because of activation of lipoprotein lipase [143]. Non-esterified fatty acids are thought to be responsible for the decrease in drug binding to plasma proteins after heparin [144]. An increased free fraction of propofol may contribute to the prolonged effect of propofol when the plasma concentration of propofol was low [145].

1.8.3 Mechanisms of Antioxidant Activity

Phenol-based antioxidants (R-OH) scavenge ROIs (X·) by a process of hydrogen abstraction, and thereby themselves become a (less reactive) phenoxyl radical (R-·)

R-OH + X· ---> R-O· + XH
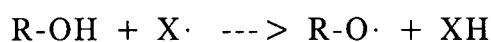
Spin resonance spectroscopy, one of the most specific methods for characterizing radical reactions [16,136,146,147], was used to investigate this mechanism.

An in vitro study was carried out in a liquid phase to examine the way in which propofol reacts with ROIs and, in particular, to identify if a propofol-

derived phenoxyl radical is generated [136]. Propofol was demonstrated to act as an antioxidant by reacting with free radicals to form a phenoxyl radical - a property common to all phenol-based free radical scavengers, such as vitamin E.

Another in vitro study was carried out using isolated rat liver mitochondria [16]. ^{1}H-NMR spectra of mitochondrial lipid extracts indicated that 95% of the added propofol remained intact after a 30 min incubation under conditions of low oxidative stress. The electron spin resonance spectrum of propofol, incubated in the presence of EDTA-Fe^{2+} and H_2O_2 as initiators of radical production, showed a radical that was most likely a decomposition product of the phenoxyl radical of propofol. It was concluded from the study that propofol acted as a chain reaction-breaking antioxidant by forming a stable radical [16,148].

1.8.4 Antioxidant Activities of Propofol in Animal Studies

1. Propofol Inhibits Lipid Peroxidation in Isolated Liver Mitochondria of the Rat.

1.1 μM propofol produced about 50% inhibition of MDA (or TBA-reactive substances) production [16,17], while 8 μM propofol completely suppressed the production of MDA [16].

2. Propofol Attenuates IRI in the Isolated Rat Heart. [14]

During reperfusion after global ischemia, 100 μM propofol increased coronary flow and reduced LDH release and the hearts showed lower left

ventricular end-diastolic pressure. Histologically, the injury was less severe in propofol-treated hearts in terms of interstitial edema, myofiber degeneration, and formation of myocardial hypercontraction bands.

3. Propofol Attenuates Mechanical and Metabolic Damage Induced by Exogenous Hydrogen Peroxide in the Isolated Rat Heart [15].

25 µM or 50 µM propofol completely suppressed the production of tissue MDA (Figure 5), significantly attenuated mechanical dysfunction (increased left ventricular end-diastolic pressure, decreased left ventricular pressure) and metabolic changes (decreased tissue concentrations of ATP and creatine phosphate) induced by exogenous hydrogen peroxide (600 µM).

4. In a previous study from our laboratory using a swine model of heart-lung transplantation, propofol at plasma concentrations of 10 to 20 µM significantly increased red cell antioxidant capacity against in vitro oxidative challenge (1.5 uM t-butyl hydrogen peroxide). This effect may be indicative of a generalized enhancement of tissue antioxidant status, and might confer protection against cardiopulmonary IRI [18,19].

5. In a study carried out in aqueous suspension, 50 µg/ml propofol decreased MDA production by 38%. [45]

6. Propofol did NOT:

(1) Propofol did not have any detectable effect on plasma or red cell antioxidant enzyme activity in a swine model of heart and lung transplantation. It did not prevent red cell glutathione depletion against in vitro peroxidative challenge [18], probably because propofol mainly acts at

the level of the plasma membrane as compared to the cytosolic or extracellular compartment.

(2) Propofol (25 µM or 50 µM) did not completely attenuate mechanical and metabolic changes induced by exogenous hydrogen peroxide in the isolated rat [15] or rabbit [149] heart, in spite of the complete inhibition of lipid peroxidation. Therefore, factors other than lipid peroxidation may play an important role in myocardial damage induced by ROIs. In fact, oxidation of protein or nonprotein sulfhydryl groups has been demonstrated to contribute to myocardial damage induced by ROIs [150]. These biochemical alterations, which are not attributed to lipid peroxidation of the cell membrane, may account for the incomplete protection by propofol of mechanical and metabolic derangements induced by ROIs [15].

(3) Propofol did not show any radical scavenging activity at concentrations < 10 µg/ml in an experiment carried out in aqueous suspension in vitro [45].
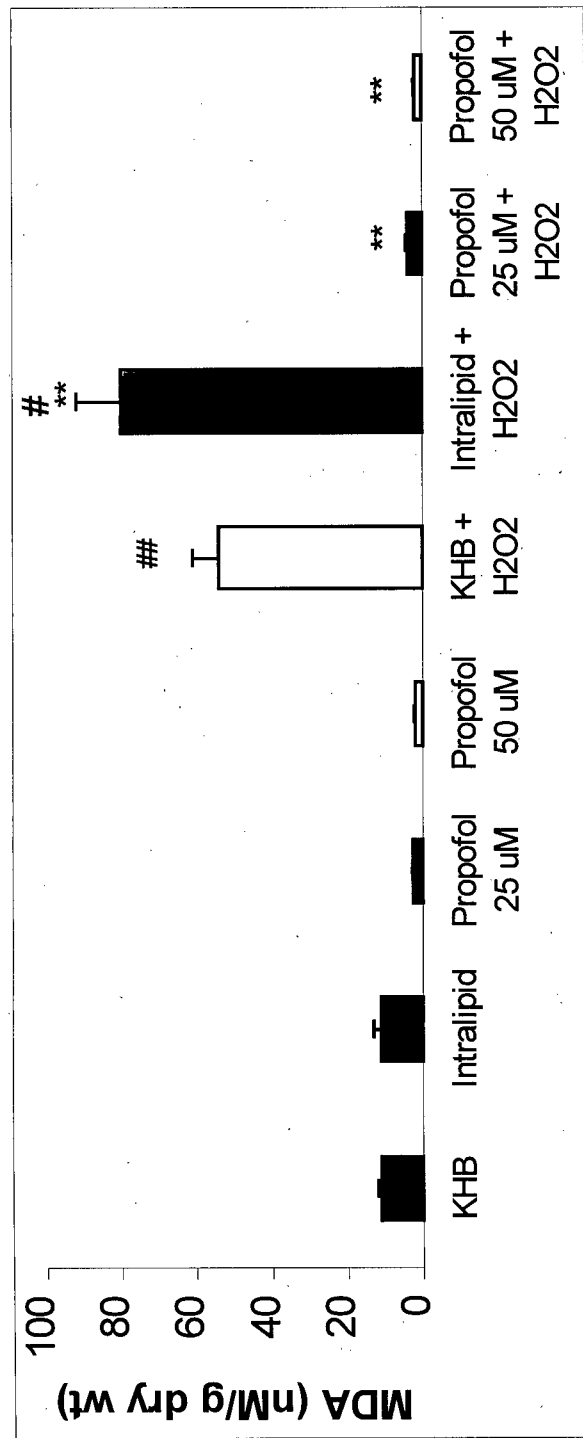
Figure 5. Effect of propofol in intralipid or intralipid alone on hydrogen peroxide-induced changes of the rat heart tissue concentration of MDA, which was measured 25 min after start of Krebs-Henseleit bicarbonate (KHB) buffer, intralipid, or propofol infusion (11 min after hydrogen peroxide infusion). ** $P < 0.01$ when compared with value in the hydrogen peroxide-treated KHB group. ## $P < 0.01$ when compared with the respective value in hydrogen peroxide-untreated (normal) heart. Data are expressed as mean ± SEM ($n = 8$ in each group). Adapted from Kokita and Hara, 1996 [67].

1.8.5 Propofol Anaesthesia in CAB Surgery

Propofol has the advantage of conveying very rapid and complete awakening, which results early extubation after surgery and early intensive care unit discharge [151,152]. It has been used since 1987 in CAB surgery as total intravenous anaesthesia [151, 153-172] or post-operative sedation [173-178].

Hypotension is a major side effect of propofol anaesthesia [137, 145,179]. This is due to a decrease in peripheral vascular resistance caused by propofol. [145,180,181].

Propofol as compared to isoflurane or enflurane did not show any significant changes [152,157,166,180] in terms of myocardial contractility [157,180], cardiac output [180], inotropic requirement [152], coronary perfusion pressure [157] or perioperative myocardial infarction [152]. In earlier studies, propofol was associated with decreased myocardial blood flow (by 26%), myocardial oxygen consumption (by 31%) [182], and cardiac index [113,182,183] and was suggested to lead to myocardial ischaemia in patients with coronary artery disease [182].

As an antioxidant the protective effects of propofol on myocardium in experimental studies were achieved at higher than recommended clinical doses for CAB surgery. Because of this, it was suggested that the scavenging activity of propofol during clinical anaesthesia is likely to be very limited [45]. Its potential as an antioxidant in CAB surgery has not been investigated. In our previous study in the swine model of ischaemia reperfusion injury

[18], propofol was shown to have antioxidant properties at clinically relevant plasma concentrations, which encouraged us to proceed with this clinical study.

Chapter 2

OBJECTIVES

The objectives of the thesis project were:

(1) to determine if propofol in recommended clinical dosage enhances red cell antioxidant capacity during coronary artery bypass (CAB) surgery;

(2) to investigate the effect of propofol on patient's cardiopulmonary functions perioperatively;

(3) to determine the maximally effective dose or maximal infusion rate of propofol as both an antioxidant and anesthetic for CAB surgery.

Because of the small sample size of the study (see chapter 4, section 4.1.2), this project was designed to show statistical differences only in laboratory data (red cell MDA production against in vitro oxidative challenge) rather than that in clinical data (e.g., cardiopulmonary functions).

Chapter 3

HYPOTHESES

1. Isoflurane (less than 2% end tidal) will not modify the red cell antioxidant status (experimental control).

Isoflurane has been reported to inhibit the production of superoxide radicals by neutrophils at concentrations in excess of 2% end tidal, and enhance the production of superoxide radicals by neutrophils at concentrations up to 2% [184]. The concentration of isoflurane for our study is less than 2% end tidal. This might slightly increase the production of superoxide radicals, but the magnitude of this effect should not be large enough to influence the red cell antioxidant status given the marked protection afforded by plasma antioxidants.

2. High dose propofol (200 µg/kg/min) will enhance antioxidant capacity of red cells against in vitro oxidative challenge, while low dose propofol (50 µg/kg/min) may not.

The antioxidant properties of propofol are dose-dependent. In an experiment carried out in aqueous suspension, propofol at concentrations < 10 µg/ml did not show any radical scavenging activity, whereas 50 µg/ml propofol decreased MDA production by 38%[45]. In our previous study in a swine model of heart-lung transplantation, propofol at plasma concentrations of 7-12 µg/ml and 12-25 µg/ml resulted in decreased red cell MDA production more than 25% and 50%[18]. Thus, by continous infusion with

high dose propofol, we may reach 7-12 μg/ml or more for the plasma concentration of propofol during bypass. However, with low dose propofol, the plasma concentration of propofol may be too low to protect the red cells against in vitro oxidative challenge.

3. 200 μg/kg/min is the maximal infusion rate of propofol for CAB surgery.

The antioxidant effects of propofol appear to be dose-dependent. In our previous study in swine, limited enhancement of antioxidant status was achieved with plasma propofol concentration greater than 25 μg/ml [18]. The maximum recommended clinical infusion rate of propofol for CAB surgery is 200 μg/kg/min [166]. At this rate, the plasma concentration of propofol is greater than 7 μg/ml.

4. The antioxidant effect of propofol on red cell MDA production may continue after bypass.

Propofol is highly lipophilic. It mainly distributes to membranous structures such as cytosolic and mitochondrial membranes. Using isolated rat liver mitochondria, it was shown, by spin resonance spectroscopy, that 95% of the propofol remained intact even after 30 min incubation under conditions of low oxidative stress, and propofol did not seem to be metabolized by mitochondria in vitro [16]. This implies that propofol may remain in the red cell membrane even after propofol anaesthesia is discontinued.

5. Propofol will improve the performance of the heart after bypass and will decrease inotropic support required perioperatively.

Allopurinol, an antioxidant when used preoperatively, effectively improved myocardial function, and reduced the inotropic support required perioperatively [1,11-13,111,112,114,116,185].

Given that propofol has protective effects on the myocardium against oxidative challenge, like allopurinol, it might lead to improved myocardial function, and decreased inotropic requirement perioperatively.

6. Propofol anesthesia will have the benefit of early extubation after CAB surgery.

Very rapid and complete awakening with minimal residual central nervous system effects is the most important advantage of propofol over other drugs used to produce induction of anesthesia [137]. The use of propofol anesthesia for CAB surgery has been reported to be responsible for early extubation [151,152].

Chapter 4

MATERIAL AND METHODS

4.1 Coronary Artery Bypass Surgery: A Model of Ischaemia Reperfusion Injury

Following institutional approval and informed patient consent, 26 patients scheduled for CAB surgery were randomly selected.

4.1.1 Selection Criteria

Inclusion criteria:

Male or female 35 years of age or older

Haemodynamically stable with documented coronary artery disease

History of recent (less than six months) myocardial infarction

Unstable angina

Left ventricle dysfunction

Exclusion criteria:

Haemodynamically unstable,

History of evolving myocardial infarction,

Acutely failed percutaneous transluminal coronary angioplasty or

previous CAB surgery,

ASA or steroid therapy within 7 days of surgery.

4.1.2 Study Design

Sample Size Determination. Because MDA has been widely used as a marker for lipid peroxidation [1,15,36,39,41,43,45,55] and the suppressed red cell MDA production may be indicative of generalized enhancement of tissue antioxidant status, the calculation of sample size for the study was based on the differences of red cell MDA production between isoflurane and propofol groups. At present, there are no data available on the relationship of red cell MDA production and propofol concentrations in patients undergoing CAB surgery. Therefore, data from a swine model of heart-lung transplantation [18] were used to calculate the sample size for this project. In the isoflurane group of the swine model, the average red cell MDA production ($\mu 0$) is about 80 nM/gm rbc with a standard deviation ($\sigma 0$) of about 30 nM/gm rbc. In the propofol group, the MDA production ($\mu 1$) is about 60 nM/gm rbc with a standard deviation ($\sigma 1$) of about 20 nM/gm rbc when the plasma propofol concentration is about 7 μg/ml (25% inhibition of MDA production). We define Type I error as 0.05 and type II error as 0.1 (i.e., test power 90%). Then for this one-tail test, $Z\alpha = 1.645$, and $Z\beta = 1.282$. According to Lachin's method of sample size determination and power analysis for clinical trials [186], the total sample size is

$$N = [\ (Z\alpha * \sigma 0 + Z\beta * \sigma 1)\ /\ (\mu 1 - \mu 0)\ ]^2\ = 14$$

Therefore, the total sample size is 14. That means for the high dose propofol group, in which plasma propofol concentration was expected to reach close to 7 μg/ml to significantly suppress red cell MDA production, at least 7 patients should be recruited for this group and for the isoflurane group.

Patients were randomly selected, and purposely assigned to one of the three study groups. The anesthetist involved in the care of the patient was aware of the experimental maneuver, which was blinded to the researchers responsible for the laboratory and clinical outcome analyses.

Control group (isoflurane-sufentanil):

    Sample size: 11 (7 have data on MDA production.)

    Anesthesia: Sufentanil 0.5-10 µg/kg

            Isoflurane (0-2%) in air/oxygen $<$ FiO2=0.5.

Low dose propofol group (propofol-sufentanil)

    Sample size: 7

    Anesthesia:

Propofol:	Induction	1.5-2.5 mg/kg, bolus
	Pre-CPB	100 µg/kg/min, continuous infusion
	Intra-CPB	50 µg/kg/min continuous infusion
	Post-CPB	as above
	In ICU	as above

    Sufentanil: 0.3-1 µg/kg initially,

           and then 1 µg/kg every 5 minutes if required to maintain

           haemodynamics $>$ 20% above ward control levels

High dose propofol group (propofol-sufentanil)

    Sample size: 8 (7 have data on MDA production.)

    Anesthesia:

Propofol: Induction   1.5-2.5 mg/kg, bolus

          Pre-CPB   200 µg/kg/min, continuous infusion

          Intra-CPB   as above

          Post-CPB   50 µg/kg/min, continuous infusion

          In ICU   as above

Sufentanil: the same as in the low dose propofol group

4.1.3 Blood Sample Collection

Venous blood sampling (7 ml each time) for the measurement of red cell MDA production was performed over seven time intervals at baseline, 30 min post-induction, 30 min cardiopulmonary bypass (CPB), 5, 10 and 30 min post aortic cross clamping (ACC), and 120 min post-CPB (Figure 6). These samples were immediately heparinized and stored in the cold room (4° C) until the next morning to be analyzed for peroxide-induced TBA formation. Venous blood for determining plasma concentration of propofol was collected over 4 time intervals at 30 min post-induction, 30 min CPB, 30 min post-ACC, and 120 min post-CPB (Figure 6). These samples were immediately heparinized and centrifuged (x1500, for 10 min). The plasma was placed in 1.5 ml cryovials and immediately frozen and stored at -70° C until they could be analyzed by HPLC.

R e d C e l l M D A

Preind   30'Postind   30'CPB   5'PostACC   10'PostACC 30'PostACC 120'PostCPB

Propofol Concentration
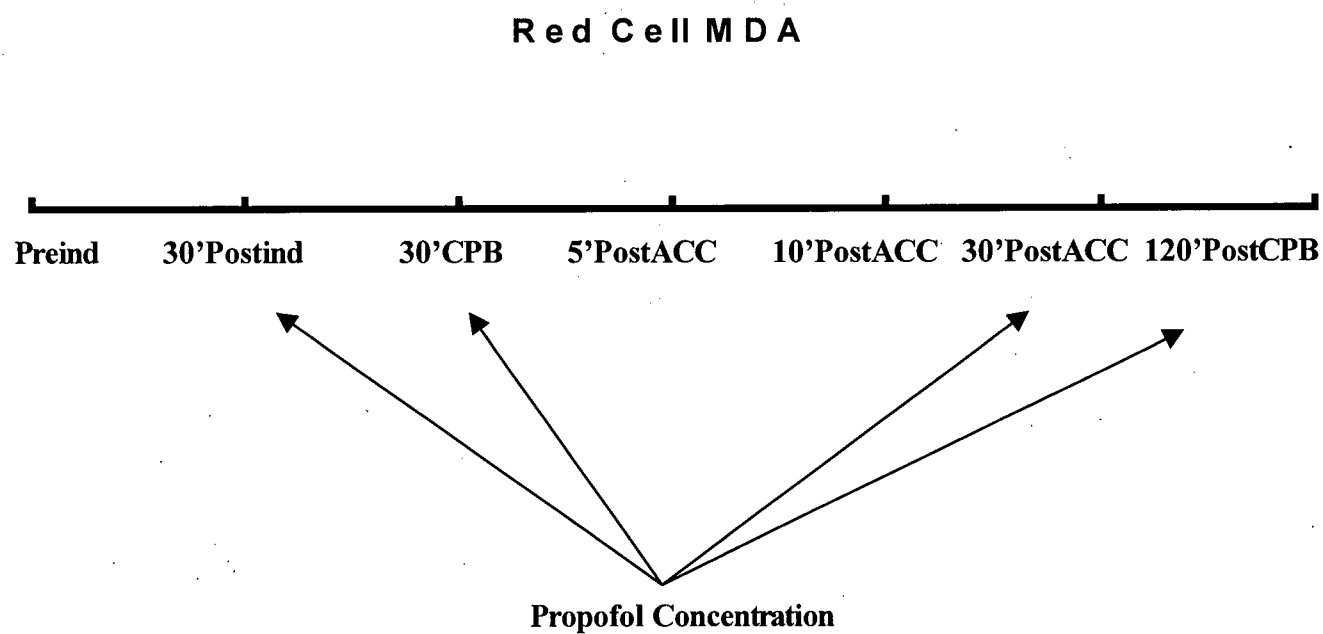
Figure 6. Schematic time-line representation of blood sampling for red cell MDA production and

plasma concentration of propofol.

4.1.4 Clinical Data Collection

Clinical data, notably perioperative inotropic requirement, post-operative cardiac index and post-operative extubation time, were taken from the medical records.

4.1.5 Cardiopulmonary Bypass

The patients were perfused during cardiopulmonary bypass with nonpulsatile flow at 2.0 to 2.8 $L/m^2/min$ with the use of standard Cobe pumps (Cobelaboratories, Lakewood, Colorado), maintaining mean arterial pressure at 55 to 70 mmHg. The oxygenator was a polypropylene hollow fiber Cobe Optima (Cobe Laboratories, Lakewood, Colorado) with a 1450 ml crystalloid primer. During bypass, the lungs were not ventilated and the endotracheal tube was open to the air, so that the lungs were flaccid and partially collapsed. Following cross-clamping of the aorta, the surgical technique consisted of sequential myocardial revascularization by vein and internal mammary artery grafts. Patients were cooled to 32° to 34° C. Systemic heparinization (300 U/kg) was instituted shortly before aortic cannulation and was reversed immediately before aortic decannulation with protamine (180-300 mg/m^2). The lungs were reventilated immediately before the cessation of bypass.

4.1.6 Data Analysis

The pooled t test is used to evaluate the significance of the differences

between two group means. The ANOVA test is used to evaluate the significance of the differences among the means of more than two groups. The Chi square test is used to evaluate the significance of the differences between percentages. When the smallest expected value is less than 5, Fisher's exact test is used instead of the Chi square test. A P value < 0.05 is considered to be significant.

4.2 Measurement of in vitro Red Cell MDA Production: TBA assay.

MDA is formed during ischemia-reperfusion injury through the arachidonic acid metabolism pathway (Figure 3). However, the concentration of MDA during CAB surgery is too low to be detected by our TBA assay. Therefore, we challenged the red cells in vitro with increasing concentrations (0.5, 1.0, 1.5, 2.0, 2.5 mM) of t-butylhydroperoxide, thereby achieving MDA levels high enough to be detected by this assay. This approach measures red cell antioxidant capacity in the presence of an in vitro oxidative challenge.

More specifically, the procedure used in our TBA assays was as follows (mainly adapted from a published report by Dr. Godin) [39]:

Reagents and solutions (for 35 test tubes):

Double-distilled water was used.

0.9% NaCl - NaN3 (saline/azide). NaCl (4.5 g) and azide (65 mg, from Sigma, Canada) was dissolved in 500 ml of distilled water.

28% TCA (trichloroacetic acid) - 0.1 M Na arsenite, from Sigma, Canada.

0.5% TBA (thiobarbituric acid) in 0.025 M NaOH. 105 mg TBA (Sigma, Canada) was dissolved in 10.5 ml 0.05 M NaOH and 10.5 ml distilled water.

tBHP (t-butylhydroperoxide). 100 µl of 70% tBHP (Sigma) was diluted with 6.9 ml saline/azide to get 100 mM TBH, which was further diluted with saline/azide to different concentrations of TBH (1.0, 2.0, 3.0, 4.0, 5.0). During peroxidative challenge, 0.5 ml tBHP in different concentrations is reacted with 0.5 ml of test sample to achieve the effective tBHP concentrations, which are 0.5, 1.0, 1.5, 2.0, 2.5 mM.

Procedure:

1) Centrifuge blood at 1000 rpm for 5 min at 4^0 C.

2) Remove plasma and white cells by aspiration.

3) Wash red cells twice with saline/azide by centrifugation.

4) Weigh aliquots of 50 µl packed red cells in 1.5 ml microcentrifuge tubes.

5) Add 0.45 ml cold saline/azide to the microcentrifuge tubes.

6) Preincubate for 5 min at 37^0 C.

7) Start peroxidative challenge with 0.5 ml tBHP (Sigma, Canada) in different concentrations and incubate 30 min at 37^0 C.

8) Stop reaction with 0.5 ml cold 28% TCA-arsenite.

9) Centrifuge 5 min at 12000 x g and transfer 1.0 ml aliquot of supernatant to 100 mm glass tubes.

10) Add 0.5 ml 0.5% TBA in 0.025 M NaOH to each glass tube.

11) Boil 15 min to develop the color for spectrophotometery.

12) Read absorbance at 532 nm and 453 nm with a Perkin Elmer Lambda 6 spectrophotometer.

13) Calculate the concentration of MDA.

delta = (abs@532 - blank@532) - 20% (abs@453 - blank@453)

MDA (nmoles/gm RBC) = (delta-0.0053)/1.931 * 50/RBC weight in gram

4.3 Measurement of Plasma Propofol Concentration by HPLC

High-performance liquid chromatography (HPLC) or gas chromatography (GC) [48,187-189] can determine propofol concentration in plasma or in whole blood. Both HPLC and GC methods had given equivalent results [188]. Fluorescence detection has been used for HPLC determination of propofol concentration, because it is more sensitive than classical ultraviolet detection.

Significant differences in propofol concentration between plasma and whole blood specimens were observed [188]. This discrepancy in concentrations resulted from the infusion or clearance of propofol, and the lag in redistribution across blood cell membranes. Whole blood sample collection is easier and more convenient for the clinician, but plasma concentrations are more directly related to the activity of a drug in the target organ than are whole blood concentrations. Therefore, plasma samples are preferred, but immediate centrifugation is needed.

The details of the determination of propofol concentrations in plasma by HPLC with fluorescence detection were as follows (mainly adapted from a published report by Dr. Plummer) [48]:

Reagents and solutions:

Double-distilled water was used. Cyclohexane (spectroscopic grade), 2-propanol, trifluoroacetic acid and sodium dihydrogen orthophosphate were obtained from Sigma (Canada). Acetonitrile (HPLC grade) was obtained from Caledon (Canada). Tetramethylammonium hydroxide (TMAH) (25% in methanol) and thymol were obtained from Fluka (Aldrich, Canada).

Internal standard solution. A solution of thymol was prepared in methanol (1 mg/ml) and further diluted with methanol to an appropriate working concentration.

Phosphate buffer solution (0.1 M). Sodium dihydrogen orthophosphate (13.6 g) was dissolved in 1 l of distilled water.

Dilute TMAH solution. TMAH (25% in methanol) (1.5 ml) was added to 2-propanol (18.5 ml).

HPLC mobile phase. The HPLC mobile phase consisted of 600 ml of acetonitrile, 400 ml of distilled water and 1 ml of trifluoroacetic acid. The mobile phase was degassed by the passage of helium prior to use.

Apparatus:

The high-performance liquid chromatograph used consisted of a Gold HPLC System (Beckman, Canada) set to deliver a solvent flow of 1.5 ml/min, and RF 5000 Flurometer (Shimaclzu, Canada) fluorescence detector. The excitation and emission wavelengths were 276 and 310 nm, respectively, and both monochromator slit widths were 10 nm. The signals were recorded using a Alltech Lichroshere RP 18 encpd reversed-phase column (5 μm particle size, 150 x 4.6 mm I.D.; Mandel Scientific, Canada was used at ambient temperature.

Procedure:

To a sample of plasma (0.5 ml), internal standard solution (20 μl), phosphate buffer (1 ml) and cyclohexane (5 ml) were added. The mixture was then placed on an inversion mixer for 15 min at 60 rpm. Following centrifugation (1150 x g for 5 min), an aliquot of the cyclohexane layer (4.5 ml) was transferred to a tube containing dilute TMAH solution (50 μl). The solvent was evaporated to dryness at ambient temperature under a stream of nitrogen. The dry residue was then redissolved in HPLC mobile phase (200 μl) and an aliquot (100 μl) of the solution was subjected to HPLC analysis. Typical chromatograms of extracts containing propofol are shown in Figure 7.

A calibration graph was prepared by the addition of known quantities of propofol to aliquots of control blood and extracted according to the above procedure. The peak-height ratio of propofol to thymol was plotted against the concentration of propofol added. The concentration of propofol in test

samples was calculated using the regression parameters obtained from the calibration graph.

Times (minutes)

A   B   C
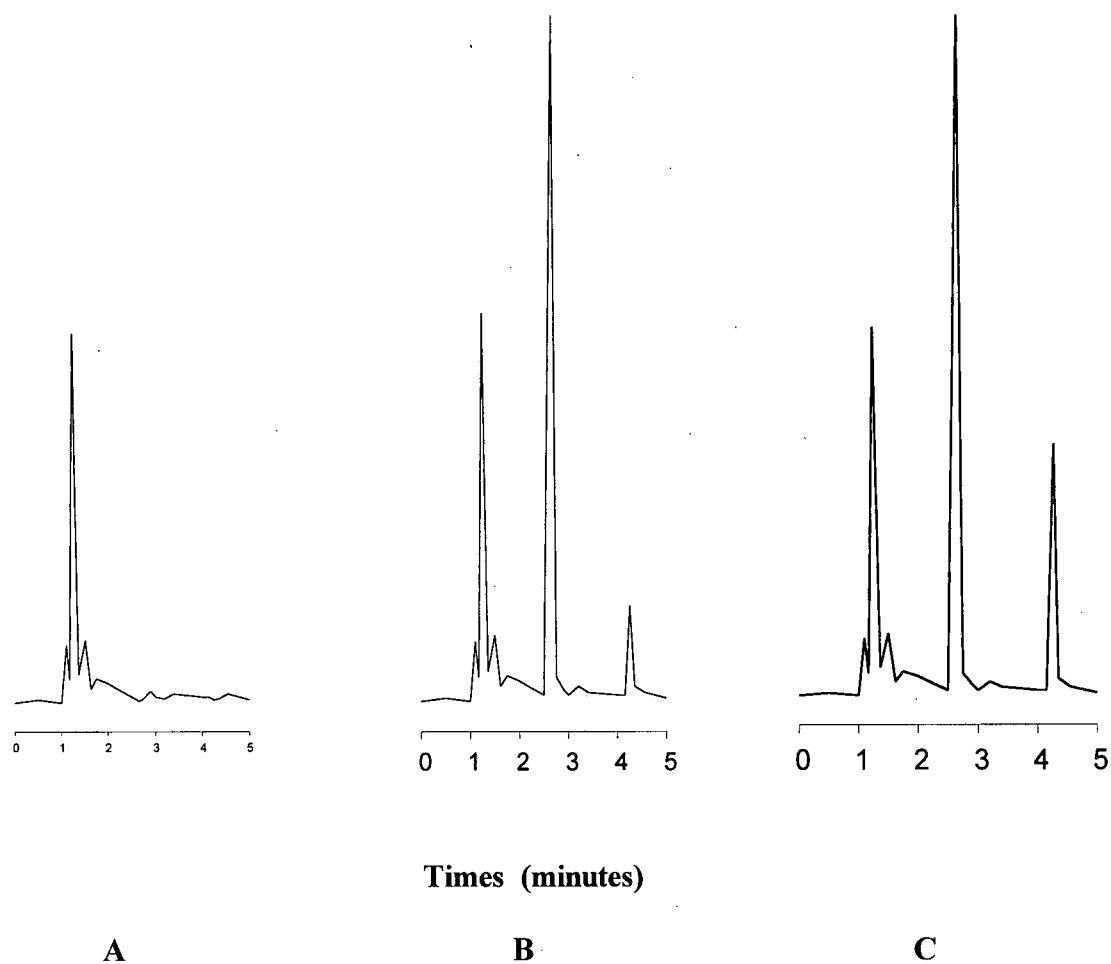
Figure 7. Schematic representation of chromatograms of extracts from (A) control

human blood, (B) blood containing propofol (1 μg/ml), and (C) blood obtained

from a patient having received propofol intravenously. Thymol was added as the

internal standard. Peaks 1 and 2 are thymol and propofol, respectively.

Chapter 5

RESULTS

5.1 Patient Profile

The patients' age, sex, body surface area and body weight are shown in Table 1. The hematocrit values pre-CPB and during CPB, the duration of CPB and aortic cross-clamping and extubation time, are also included in Table 1.

5.2 Red Cell MDA Production Following in vitro tBHP Challenge

5.2.1 tBHP Dose-Response Curve

The red cell MDA production following tBHP challenge is dose-dependent. For each sample, the MDA production begins to rise in response to 1.0 mM tBHP challenge and reaches a plateau when tBHP concentration is 2.0 mM. The steep portion of the curve is related to tBHP concentration between 1.0 and 2.0 mM. Therefore, from the various concentrations, 1.5 mM of tBHP was selected for the analysis of antioxidant capacity of red cells. Figure 8 exemplifies this sigmoid curve which is based on the average MDA production of the baseline (pre-induction) blood samples of the three groups (Table 2).

5.2.2 Red Cell MDA Production in Response to in vitro tBHP (1.5 mM) Challenge

Red cell MDA production is summarized in Table 3 and Figure 9.

The pattern of MDA production seems similar in the isoflurane and low dose propofol groups. A sustained significant decrease in MDA production is seen only with high dose propofol, and this effect appears to persist post-operatively.

5.3 Plasma Concentration of Propofol

Average plasma concentrations of propofol measured by HPLC are summarized in Table 3 and Figure 9. The average plasma concentration of propofol was decreased by 18% in low dose propofol and increased by 29% in high dose propofol at 30 min CPB compared to that before CPB (30 min post-induction). The propofol concentration was significantly higher in the high dose propofol group than in the low dose propofol group during CPB.

In the high dose propofol group, there was a correlation ($R = 0.78213$) between red cell MDA production (Y) and plasma concentration of propofol (X) (Figure 10): $Y = 35.945 - 16.75 \log X$

Such a correlation did not exist in the low dose propofol group.

5.4 Inotropic Requirement during CAB Surgery (Table 4, Figure 11)

5.4.1 Percentage of Patients Given Inotropic Drugs

There were no significant differences among the groups.

5.4.2 Percentage of Patients Given Dopamine (3-5 μ/kg/min)

The patients in isoflurane needed more dopamine than those in the low dose or high dose propofol groups (57% vs. 14%, P < 0.05).

5.4.3 Percentage of Patients Given Adrenaline

The patients in the isoflurane group needed more adrenaline than those in the low dose or high dose propofol groups (73% versus 43% or 57%). However, the differences did not attain statistical significance.

5.5　Haemodynamic Changes during 24 Hours Post-operation

5.5.1 Cardiac Index (Table 5, Figure 12)

The percentage of the cardiac index > 2.5 L/min/m2 in the low dose propofol group is significantly higher than that in the isoflurane group during the first 3 hours post-operation (Table 6, Figure 13). There are no significant differences among the groups otherwise.

5.5.2 Pulmonary Capillary Wedge Pressure (Table 5, Figure 12)

In the isoflurane group, the pulmonary capillary wedge pressure is significantly higher (P < 0.05) during the 19-24 hour period than it is at 1 or 2 hours post-operation. When it is plotted against cardiac index (Figure 14), one can see that the cardiac index is low even with high pulmonary capillary wedge pressure, and the percentage of cardiac index > 3 L/min/m2 is

significantly higher (P < 0.05) in the low dose propofol than that in isoflurane group.

5.5.3 Central Venous Pressure (Table 5, Figure 12)

The central venous pressure is relatively stable throughout the surgery. There are no significant differences among the groups.

5.6 Alveolar Arterial Oxygen Gradient during 24 Hours Post-operation (Table 7, Figure 15)

There is a significant improvement in oxygenation (P < 0.05) in the first 12 hours after surgery in the isoflurane or low dose propofol groups. There are no significant changes in the high dose propofol group.

Table 1. Patient Profile, by Group

		Sex					Hematocrit		Time		
	N	M	F	Age(yr)	BSA (m²)	Wt (kg)	Pre CPB	CPB	CPB(min)	ACC(min)	Extubation(hr)
Isoflurane	11	9	2	67 ± 3	1.97 ± .1	81 ± 6	.41 ± .01	.25 ± .01	150 ± 8	103 ± 7	14 ± 1
Propofol-Low	7	6	1	65 ± 5	1.94 ± .1	79 ± 7	.41 ± .01	.24 ± .02	185 ± 15	137 ± 14	13 ± 2
Propofol-High	8	8	0	65 ± 5	1.90 ± .06	77 ± 4	.42 ± .01	.25 ± .01	148 ± 17	107 ± 12	11 ± 2

Note: A total of 26 patients were recruited for the study. 7 out of each group were analyzed for red cell MDA production. BSA, body surface area; Wt,body weight; CPB,cardiopulmonary bypass; ACC, aortic cross clamping. There are no significant differences among the groups. Data are expressed as mean ± SEM.

Table 2.   tBHP-MDA Dose-Response Curve for Red Cells from the Three Experimental Groups. MDA Production (n moles/g rbc)

tBHP (mM)	0.5	1.0	1.5	2.0	2.5
Isoflurane (n = 7)	1.16 ± 0.47	45.6 ± 4.2	122 ± 5	190 ± 4	198 ± 7
Propofol-Low (n = 7)	2.73 ± 1.45	46.6 ± 7.5	122 ± 12	187 ± 10	193 ± 8
Propofol-High (n = 7)	1.91 ± 0.62	33.4 ± 3.6	106 ± 5	180 ± 6	196 ± 5

Note: Data are from the first baseline (pre-induction) samples only, used to show the tBHP-MDA dose-response curve. Data are expressed as mean $\pm$ SEM.

Table 3. Red Cell MDA Production and Plasma Concentration of Propofol at Various Time Intervals

	Preinductio	30' Induction	30' CPB	5' Post-AC	10' Post-ACC	30' Post-ACC	120' Post-ACC
MDA (n moles/g rbc)							
Isoflurane	122 ± 5	122 ± 5	139 ± 6	132 ± 8	131 ± 6	126 ± 6	127 ± 6 .
Propofol-Low	124 ± 12	92 ± 11	124 ± 13	122 ± 16	123 ± 12	123 ± 15	124 ± 13
Propofol-High	108 ± 5	51 ± 7 * #	43 ±2 * #	41 ± 5 * #	46 ± 7 * #	49 ± 5 * #	81 ± 9 * *
Propofol (μg/ml plasma)							
Propofol-Low(N=7)		5.1 ± .8	4.0 ± .4			1.8 ± .2	2.2 ± .3
Propofol-High(N=5)		10.9 ± 2.9	11.8±1.6 +			8.0 ± .8 #	3.4 ± .5 +

Note: Red cell MDA production is based on in vitro 1.5 mM tBHP challenge. * P < 0.0001 between the isoflurane and high dose propofol group, # P < 0.005 between the low dose and high dose propofol groups in red cell MDA production or plasma concentration of propofol, ** P < 0.05 between the high dose and low dose propofol or isoflurane groups, + P < 0.05 between the low dose and high dose propofol groups. Data are expressed as mean ± SEM.

Table 4. Inotropic Requirement during CAB Surgery, by Group

Inotrope Required	Any Inotropic Drug	Dopamine 3-5 µg/kg/min	Adrenaline
Isoflurane	10 / 11 (91)	4 / 7 (57) * +	8 / 11 (73)
Propofol-Low	7 / 7 (100)	1 / 7 (14)	3 / 7 (43)
Propofol-High	6 / 7 (86)	1 / 7 (14)	4 / 7 (57)

Note: Data are expressed as number (%). * P < 0.05 the isoflurane group versus low dose propofol group, + P < 0.05 the isoflurane group versus high dose propofol group for dopamine 3-5 µg/kg/min.

Table 5. Cardiac Index, Pulmonary Capillary Wedge Pressure, and Central Venous Pressure during 24 Hours Post-operation

Time (hr)	1	2	3	4	5	6	7-12	13-18	19-24
CI (L/min/m2)									
Isoflurane	2.4 ± 0.1	2.8 ± 0.3	2.8 ± 0.3	2.8 ± 0.2	2.9 ± 0.4	2.6 ± 0.2	2.9 ± 0.2	3.1 ± 0.2	2.9 ± 0.2
Propofol-Low	3.3 ± 0.6	3.6 ± 0.7	3.5 ± 0.7	2.5 ± 0.2	3.3 ± 0.3	2.7 ± 0.3	3.4 ± 0.3	3.2 ± 0.2	2.8 ± 0.2
Propofol-High	2.6 ± 0.4	3.0 ± 0.05	2.3 ± 0.2	3.3 ± 0.6	2.7 ± 0.3	2.9	3.1 ± 0.2	3.8 ± 0.8	3.5 ± 0.6
PCWP (mm Hg)									
Isoflurane	11 ± 1	12 ± 2	14 ± 1	15 ± 1	15 ± 2	15 ± 1	14 ± 2	13 ± 1	17 ± 1
Propofol-Low	16 ± 2	13 ± 1	13 ± 3	15 ± 2	12 ± 2	14 ± 3	14 ± 2	13 ± 2	15 ± 4
Propofol-High	13 ± 1	18 ± 2	14 ± 2	14 ± 3	13 ± 2	14 ± 2	12 ± 2	11 ± 1	16 ± 7
CVP (mm Hg)									
Isoflurane	10 ± 1	12 ± 2	12 ± 2	13 ± 2	12 ± 2	13 ± 1	12 ± 1	11 ± 1	13 ± 2
Propofol-Low	12 ± 2	12 ± 1	12 ± 1	13 ± 1	10 ± 0	11 ± 2	10 ± 1	10 ± 2	12 ± 1
Propofol-High	9 ± 0	14 ± 2	14 ± 4	12 ± 1	11 ± 1	10	10 ± 1	10 ± 2	10 ± 2

Note: CI, cardiac index; PCWP, pulmonary capillary wedge pressure; CVP, central venous pressure. P >0.05 among groups.

Table 6. Percentage of Patients with a Cardiac Index > 2.5 L/min/m^2 during 24 hours Post-operation.

Time (hr)	1 - 3	4 - 6	7 - 12	13 - 18	19 - 24
Isoflurane	9 / 22 (41) *	6 / 17 (35)	6 / 8 (75)	7 / 10 (70)	5 / 5 (100)
Propofol-Low	10 / 13 (77) *	3 / 11 (27)	6 / 7 (86)	5 / 5 (100)	2 / 3 (67)
Propofol-High	4 / 5 (80)	4 / 7 (57)	4 / 4 (100)	3 / 4 (75)	2 / 2 (100)

Note: * $P < 0.05$ between the isoflurane and low dose propofol groups. Data are expressed as number (%).

Table 7. Alveolar Arterial Oxygen Gradient (mmHg)

	Admission	1 – 6 Hrs Post-operation	6– 12 Hrs Post-operation
Isoflurane	195 ± 19 * #	148 ± 18 *	124 ± 21 #
Propofol-Low	145 ± 9 **	94 ± 6 **	97 ± 26
Propofol-High	162 ± 19	149 ± 27	127 ± 32

Note: In the isoflurane group, * P < 0.05 (# P < 0.001) between admission and 1-6 hours (6-12 hours) postoperation. In the low dose propofol group, ** P < 0.0005 between admission and 1-6 hours postoperation. P > 0.05 between each group at any time or duration. Data are expressed as mean ± SEM.
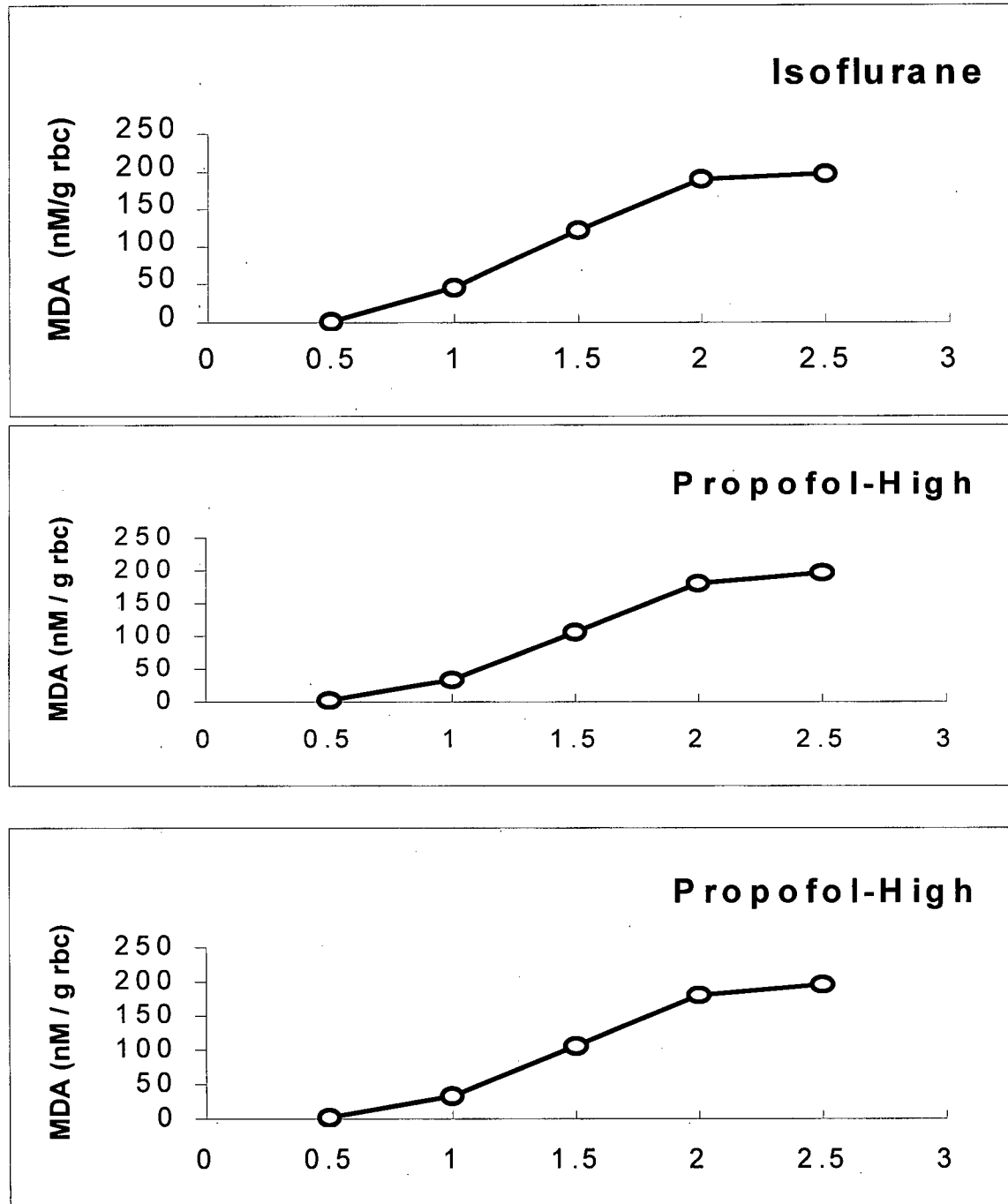
Figure 8. t BHP dose-response curve for red cell MDA production. Data is exemplified

only from the blood samples taken at preinduction. Data are expressed as mean ± SEM.
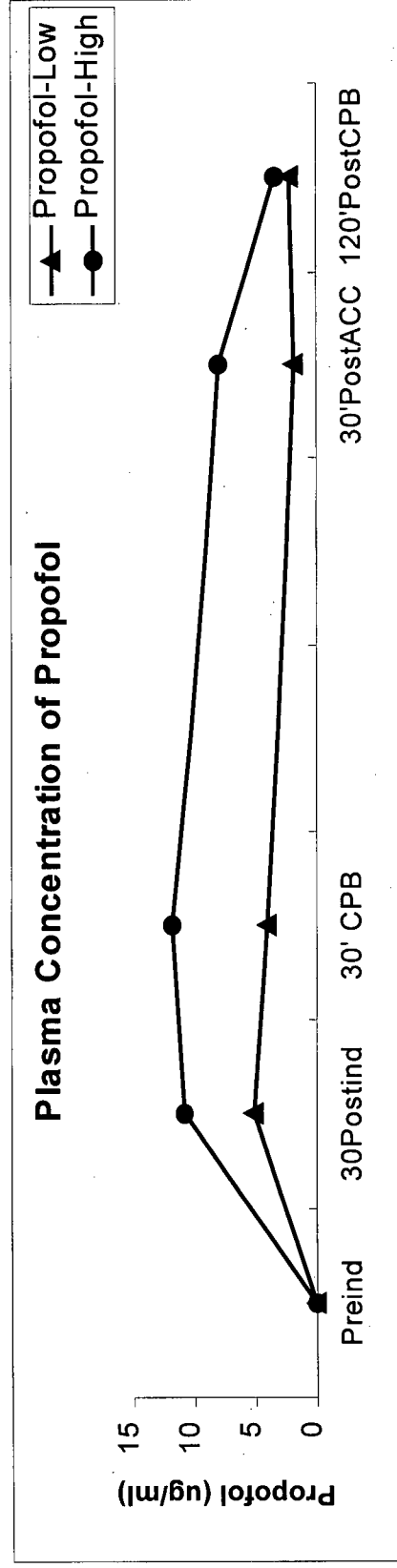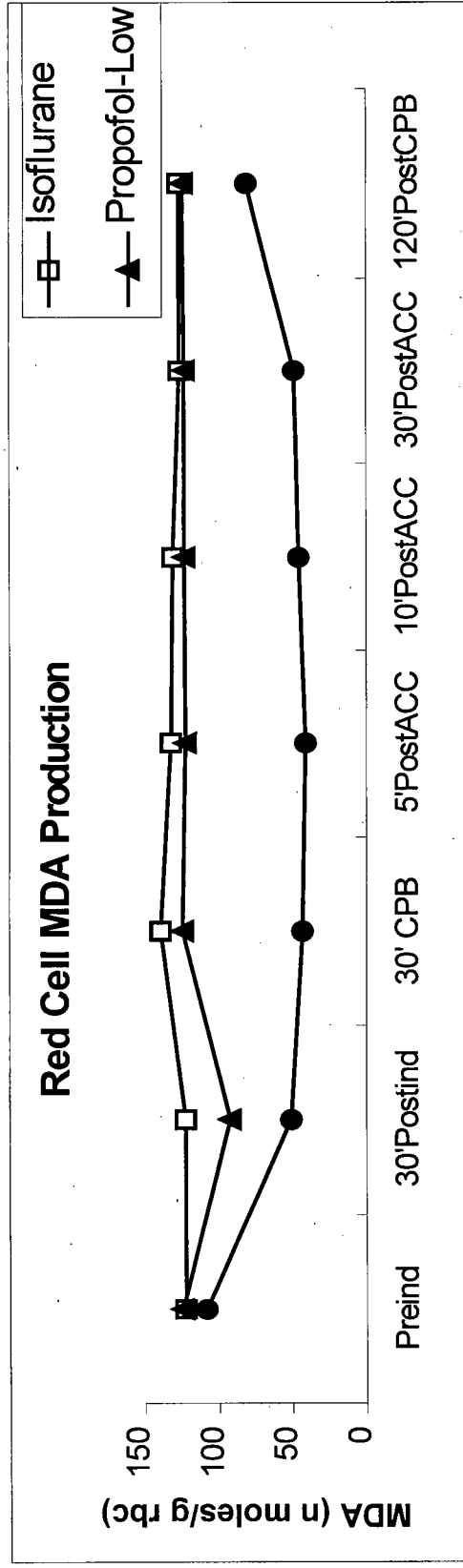
Figure 9. Red cell MDA production and plasma concentration of propofol. A sustained significant decrease in red cell MDA production is seen only in the high dose propofol group. The plasma concentration of propofol is significantly higher at 30 min post ACC and 120 min post CPB in high dose than that in the low dose propofol group, P < 0.05.
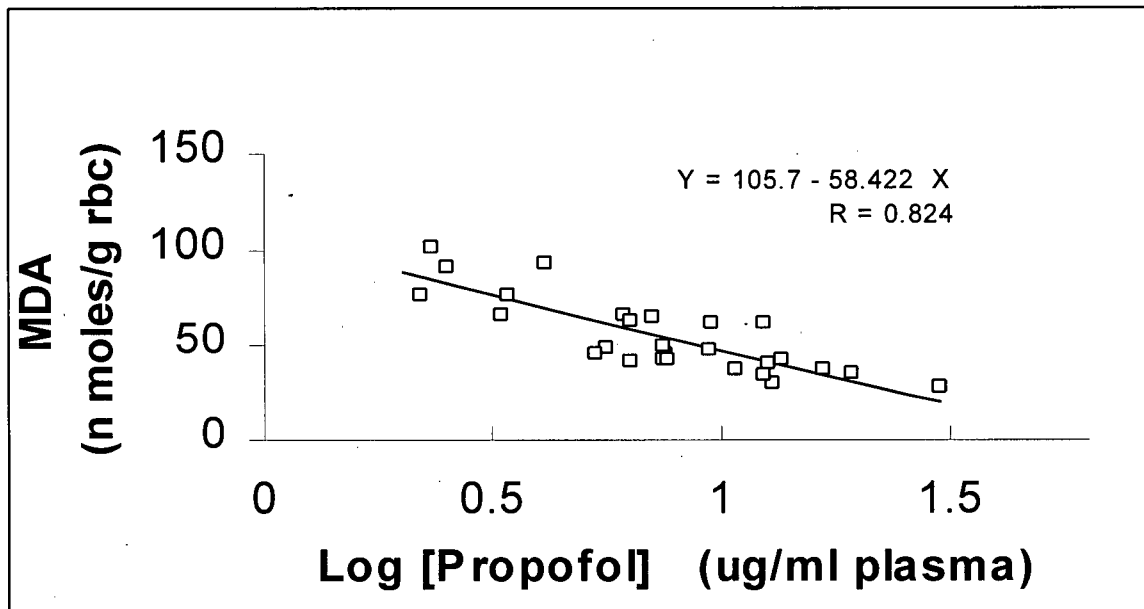
Figure 10. The relationship of red cell MDA production and plasma concentration of propofol in the high dose propofol group.
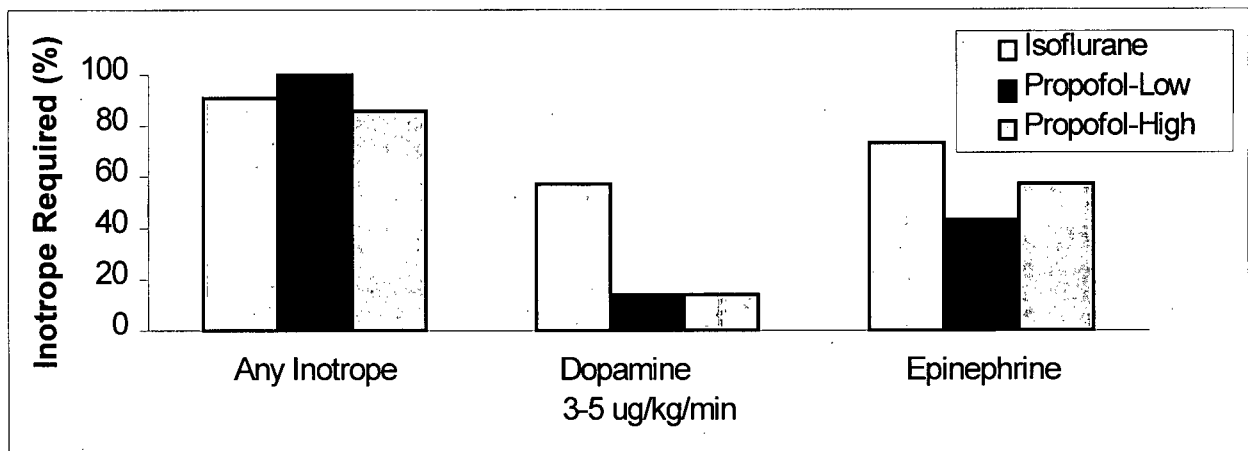
Figure 11. Inotropic requirement during CAB surgery. The requirement for dopamine 3-5 μg/kg/min is significantly less in the low dose or high dose propofol groups than that in the isoflurane group, P < 0.05.
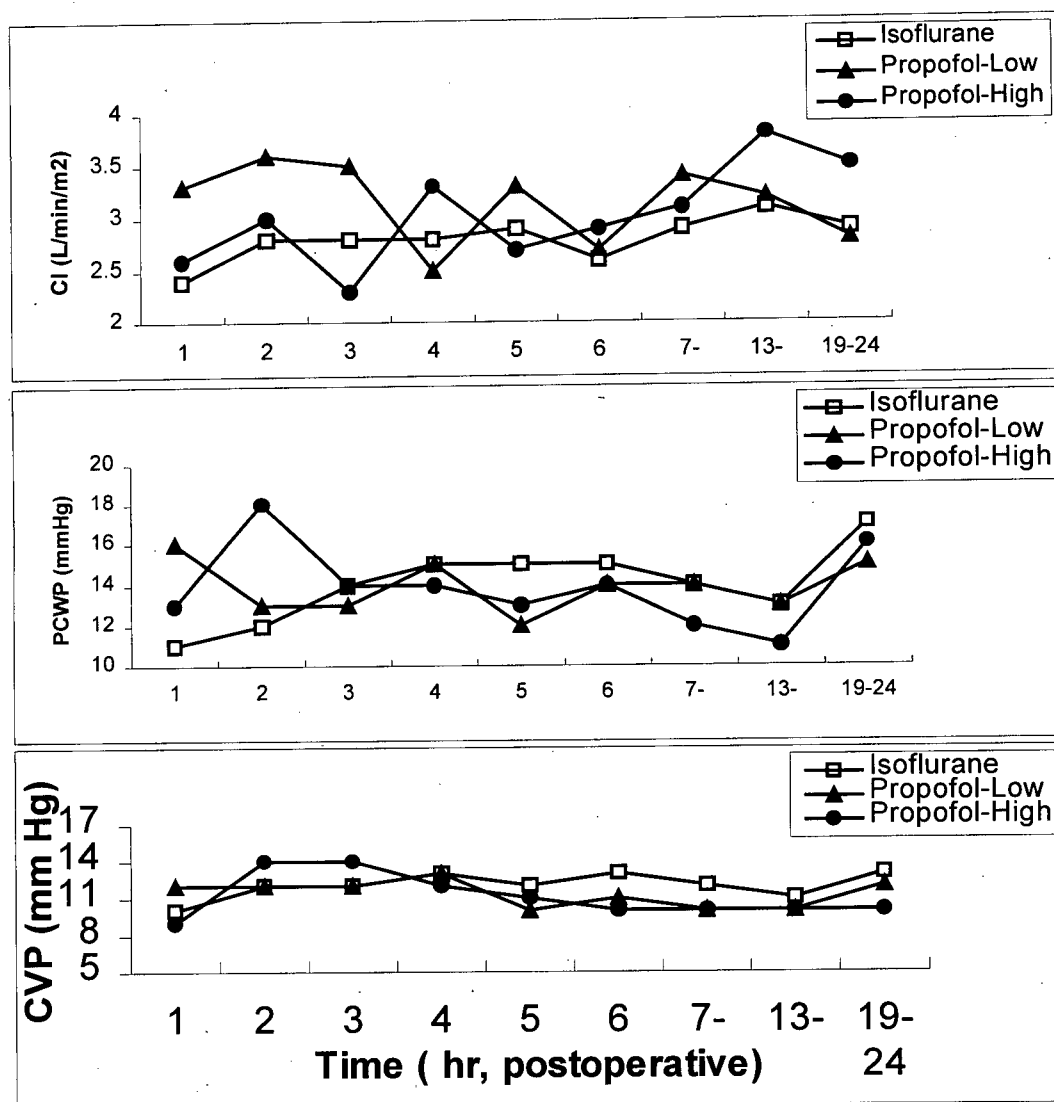
Figure 12. Hemodynamic changes during 24 hours post-operatively. For cardiac index and central venous pressure, there are no significant differences between the groups using the pooled t test. In the isoflurane group, the pulmonary capillary wedge pressure is significantly higher (P < 0.05) during 19-24 hour period than it is at 1 or 2 hours post-operatively.
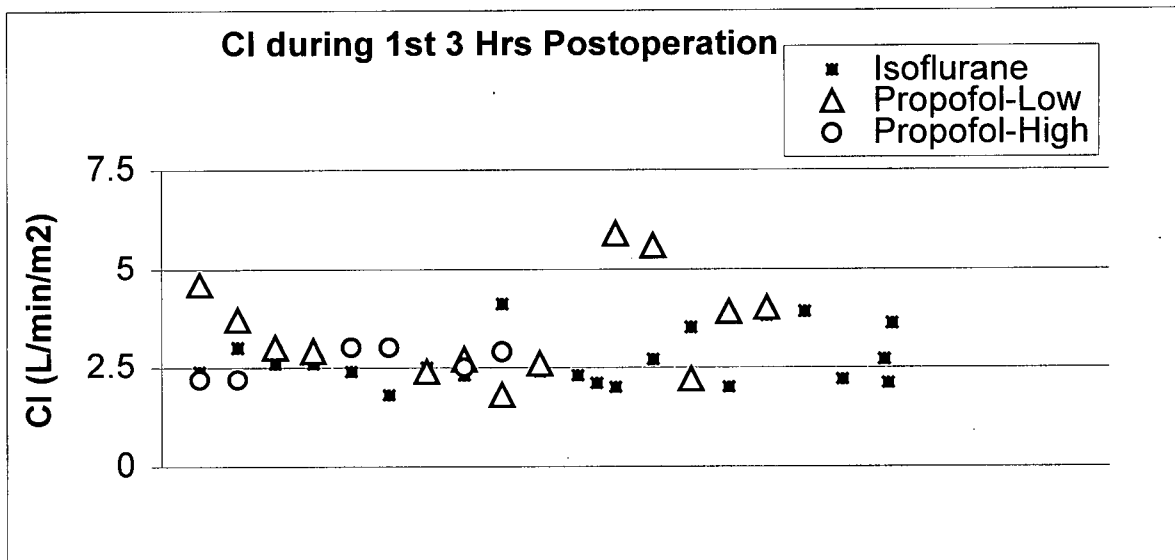
Figure 13. Cardiac index during the firsr 3 hours post-operatively. The percentage of patients with a CI > 2.5 L/min/m^2 in the low dose propofol group is significantly higher than that in the isoflurane group. There are no significant differences between the high dose and low dose propofol or isoflurane groups.
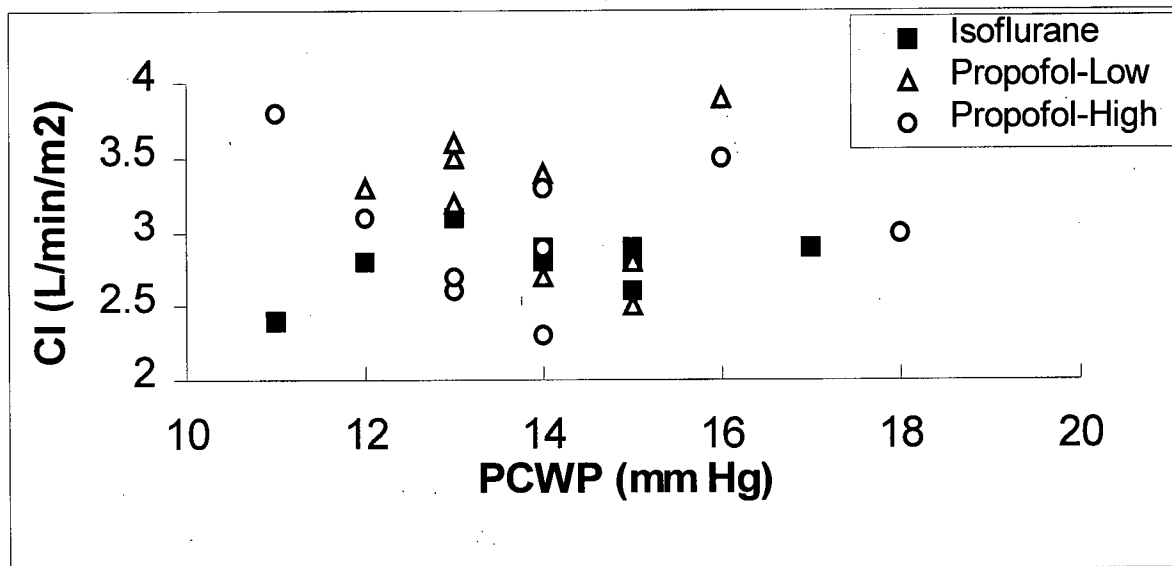
Figure 14. Cardiac index versus pulmonary capillary wedge pressure during 24 hours post-operatively. Data are expressed as mean of different time intervals as seen in Figure 11. The CI is low in the isoflurane group, even with high PCWP. The CI is high in the low dose propofol group, in which the aortic cross-clamping time is longest among three groups. There is no difference between the high dose and low dose propofol or the isoflurane groups. The percentage of patients with a CI > 3 L/min/m^2 is significantly higher in the low dose propofol group (6 put of 8) than that in the isoflurane group(1 out of 8), P < 0.05.
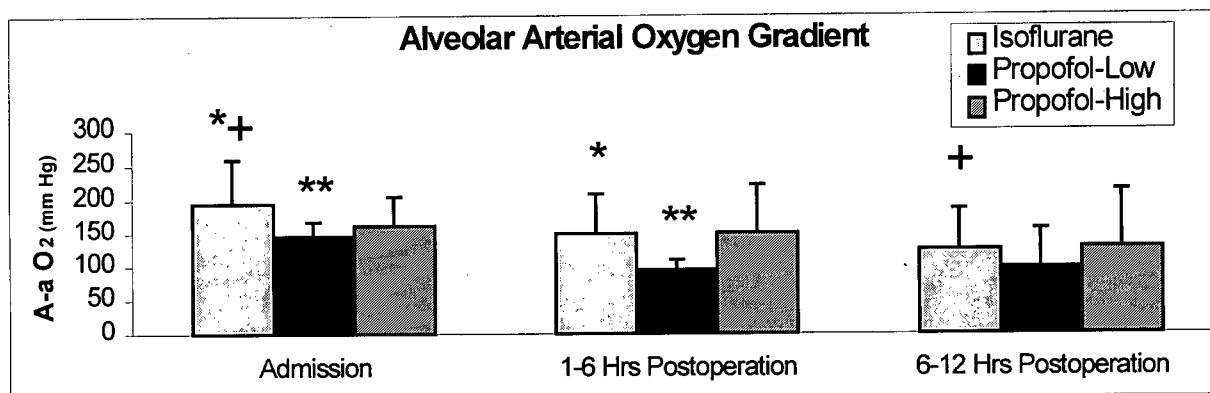
Figure 15. Alveolar arterial oxygen gradient during 24 hours post-operation. * P < 0.05
(+ P < 0.001) between 1-6 (6-12) hours post-operation and admission in the isoflurane group.
**, P < 0.001 between 1-6 hours post-operation and admission in the low dose propofol
group.

Chapter 6

DISCUSSION

6.1 Evaluation of Hypotheses

Isoflurane at a concentration less than 2% end tidal, used as control for this study, did not modify red cell antioxidant status. The red cell antioxidant capacity did not change significantly 30 min post-induction in comparison to pre-induction.

Reperfusion itself did not reduce the red cell antioxidant capacity in any of the groups. This may be because ROIs produced from heart and lungs were largely detoxified by plasma antioxidant systems before they reached the red cells to exert significant effects. This implicates the bypass machine as a major contributor to the systemic production of ROIs.

The antioxidant properties of propofol are dose-dependent. High dose propofol significantly increased the red cell antioxidant capacity throughout the surgery. This effect appears to continue post-surgically, even at two hours after bypass when the plasma propofol concentration was very low. Low dose propofol, on the other hand, increased the red cell antioxidant capacity before bypass, but this effect was not sustained during or after bypass.

By continuous infusion with high dose propofol, the average plasma concentration of propofol reached 7.2 μg/ml before bypass and 9.3 μg/ml while on bypass, with resultant suppression of red cell MDA production of

more than 50%. This effect on MDA production is greater than it was in our previous swine model [18], in which the swine red cell MDA production was suppressed by only 25% when the plasma propofol concentration was between 7 and 12 μg/ml. In the latter swine model, a decrease in red cell MDA production of 50% required a plasma propofol concentration of 12 to 25 μg/ml.

Low dose propofol had cardiopulmonary protective effects, manifested as significantly higher percentage of patients with cardiac index $>$ 2.5 L/min/m^2 during the 1st 3 hours post-operatively and with cardiac index $>$ 3 L/min/m^2 during 24 hours post-operatively compared to that with isoflurane (P $<$ 0.05), and a greater degree of recovery in lung oxygenation 1-12 hours after surgery as compared with isoflurane (P $<$ 0.001). High dose propofol did not increase cardiac index post-operatively. High dose, as well as low dose propofol, were associated with significantly less inotropic requirement, and prevented the increase in pulmonary capillary wedge pressure which was seen in the isoflurane group. Isoflurane did not show any detectable protective effect on the heart. Moreover, it was associated with a significantly increased pulmonary capillary wedge pressure without any increase in cardiac index. However, isoflurane is associated with greater recovery in lung oxygenation 1-12 hours post-operation compared with that of the same group at admission to ICU after operation, P $<$ 0.001.

The average extubation time in the high dose propofol group was shorter than that in the isoflurane controls, but the difference was not statistically significant [151,152].

6.2 What Does Increased Red Cell Antioxidant Capacity Mean?

6.2.1 Generalized Enhancement of Tissue Oxidant Status

The relationship of red cell antioxidant status and tissue antioxidative capacity has not been clarified, partially because human tissues, especially heart and lung, can not be obtained for analysis. Thus, the relationship can only be inferred indirectly from the organ functional performance or from animal experiments.

In a swine model of heart-lung transplantation [39], the susceptibility of swine red cells to in vitro oxidative challenge was significantly reduced, manifested as significantly decreased red cell MDA production. Red cell MDA levels correlated significantly with the functional viability of the transplanted lung, as assessed by lung water content – a convenient measure of lung functional integrity. In a comparative study on red cells of diabetic rats and human diabetics [192], the red cell production of MDA was increased in response to in vitro challenge with hydrogen peroxide. In the diabetic patients, the extent of this increase in susceptibility of red cell lipids to oxidation paralleled the severity of diabetic complications present.

Toth et al. [190] found that perfusion of isolated rat lungs with free radical generating systems caused vascular leakage and edema [190]. The inclusion of red cells in this model prevented such oxidant-induced damage. Van Asbeck et al. [191] reported that prior insufflation of red cells into rat lungs protected the animals against early death due to oxygen toxicity. Furthermore, it was found that catalase [91,92] and/or glutathione [92] present in intact red cells can decrease myocardial hydrogen peroxide levels and reperfusion injury [92], and prevent death of somatic cells resulting from in vitro oxidative challenge [91].

Therefore, it seems that red cell antioxidant capacity is associated with tissue oxidative status, and intact red cells can protect tissues from oxidative stress. This is more important for tissues such as human heart, which unlike red cells is vulnerable to oxidative injury due to their poor oxidative status relative to red cells - including very low concentrations of catalase [9,92].

6.2.2 Partially Improved Cardiopulmonary Function

ROIs are generated during CPB and have been implicated as a cause of myocardial injury [8,193,194]. As noted earlier, ROIs may damage any biomolecule including lipids, proteins, carbohydrates, and nucleic acids [26]

As mentioned, the heart and lungs may be protected by the antioxidant capacity of intact red cells or by a generalized antioxidant action of propofol manifest in oxidation-sensitive tissues as well as red cells. In our study, propofol improved cardiopulmonary function. This may be due to its free

radical scavenging activity against locally or systemically produced ROIs. The enhanced tissue antioxidative status would be expected to preserve cell membrane integrity, with resultant improved cardiopulmonary function. Similar results have been achieved in animal models of IRI [14,15,149].

It should be noted, however, that the improvement in cardiopulmonary function within 24 hours post-operatively did not parallel the inhibition of in vitro red cell lipid peroxidation in this study, where significant cardiopulmonary protection was also seen in the low dose propofol group in the absence of red cell protection. Other investigators have reported that even complete elimination of lipid peroxidation induced by exogenous hydrogen peroxide only mildly attenuated the cardiac dysfunction in the isolated rat [15] or rabbit [149] heart. Therefore, factors other than lipid peroxidation may play a more important role [195-197]. Oxidation of protein or nonprotein sulfhydryl groups [195] and nucleic acids [196] have been considered as potential causes of diminished myocardial contractile performance. In this multifactorial pathogenesis of IRI during CAB surgery, factors such as production of PAF or other mediators may also play an important role [72,73,75].

6.3   Propofol Concentration and Its Protective Effects

Propofol in plasma is bound extensively to plasma albumin, with a free fraction of only 2-3% [138]. It is this small free fraction, however, that may determine the protective effect of propofol on red cells and tissues. The

amount of propofol in blood cells and in plasma accounts for the total blood concentration of propofol. There is a lag in propofol distribution into red cells and tissues, which explains why the plasma concentration is more than the whole blood concentration during continuous infusion. Fan et al. reported that plasma concentrations were 30% higher than whole blood concentrations during propofol infusion if the blood was centrifuged immediately to determine the plasma concentrations; even following storage for 1 hour before centrifugation, the plasma concentration was still 10% higher than that in whole blood [188]. In our study, we were not able to establish a correlation between plasma propofol concentration and red cell antioxidant status in the low dose propofol group. It may be that the amount of propofol distributed into red cells from the plasma was too small to cause a significant change in susceptibility to oxidative challenge. On the other hand, in the high dose propofol group, a significant correlation was established, paralleling the result we obtained in the swine model [18]. Under the latter condition, the amount of propofol in red cells was sufficient to reduce peroxide-induced red cell MDA production.

With the initiation of CPB, hematocrit was decreased by 39%. Interestingly, the plasma concentration of propofol was decreased by only 18% in the low dose propofol group and was increased by 29% in the high dose propofol group after 30 min of CPB. It has been reported that plasma propofol concentration decreases more than predicted on the basis of acute haemodilution alone [140]. This suggests the binding of propofol to the

extracorporeal circuit. At this point, it is difficult to explain this discrepancy.

Unlike the plasma concentration of propofol, the free fraction of propofol in plasma behaves differently during CPB. The free fraction of propofol does not decrease during CPB. Indeed, a 1.5 to 3 fold increase in this free fraction was reported [141,142]. This may be because heparin activates lipoprotein lipase to hydrolyze plasma triglycerides into non-esterified fatty acids [143,198]. These compounds competitively inhibit the binding of various drugs including propofol to plasma proteins [144]. Unfortunately, we did not measure the free fraction of propofol in plasma. We observed the sustained suppression of red cell MDA production in the high dose propofol group even at 2 hours post CPB when the plasma propofol concentration was very low. This may possibly be because of an unchanged (or increased) free fraction of propofol in plasma perioperatively, and a cumulative effect of propofol with continuous infusion.

6.4   MDA Production, Red Cell Antioxidant Status, and TBA Assay

Malondialdehyde is formed during lipid peroxidation. It has been widely used as a marker for lipid peroxidation [1,15,36,39,41,43,45,55]. There are basically two assays available for the measurement of MDA in plasma, cells, and tissues: the TBA assay and HPLC. The TBA assay is based on the reactivity of the colorless malondialdehyde (MDA) with thiobarbituric acid (TBA) to produce a red adduct, which is measurable by spectrophotometry. In

contrast to HPLC, the TBA assay is a convenient, easy to perform, rapid, and cost-effective test. It is, however, not very sensitive and not very specific [76,199-202]. Normal levels of MDA in plasma or cells or tissues are too low to be detected by this test. Besides MDA, there is the generation of non-lipid-related, malondialdehyde-like, TBA-reactive substances that lead to overestimation of the MDA production [203]. The nature of this substance is not clear, but sialic acid, glycoconjugates, sugars and, in general, aldehydes have been demonstrated to be able to cross-react with TBA [202,204,205]. For example, auto-oxidation of linoleic acid generates at least nine breakdown products that give positive responses to the TBA reaction [206].

Fluorometric detection has been used to improve the specificity of the TBA assay [207]. With respect to the red cell TBA assay, Gilbert et al. recommended a method to correct for errors caused by generation of interfering compounds during red cell lipid peroxidation [208]. The interfering compounds are not considered as the products of lipid peroxidation but are derived from erythrocyte hemolysate and reduced glutathione. Interference results from carryover absorbance at 532 nm, equivalent to 20% of the intensity of the maximum absorption peak at 453 nm. The improved accuracy of the method was corroborated with a specific HPLC. This method formed the basis for our TBA assay.

6.5   Influence of Normothermia or Hypothermia for CPB on Red Cell Antioxidant Status and Cardiopulmonary Function

Hypothermia is commonly used for CPB with relatively long periods of perfusion, while normothermic CPB is employed for relatively short operative procedures and otherwise uncomplicated cases. In this study, we had one normothermic CPB patient in each group.

Induced hypothermia for CPB itself is beneficial for the patient [209]. It apparently does not have any adverse physiological consequences [210]. It does greatly depress body metabolism [210], which might reduce the production of ROIs because of decreased oxygen consumption, especially for the ischemic and reperfused heart and lungs, in which antioxidant systems may be impaired during IRI [78-80]. Red cells are somewhat different. They do not experience IRI during CPB as do the heart and lungs, and their antioxidant defense is relatively intact. Their antioxidant status might be enhanced to some degree, because of reduced body metabolism with possibly reduced total production of ROIs.

However, in this study we did not notice any significant differences between hypothermic and normothermic CPB in terms of red cell antioxidant status, plasma concentration of propofol, or cardiopulmonary function. This may be because the effect of hypothermia on ROI production might be negligible in comparison to that of ischemia-reperfusion and the bypass machine. In fact, even with deep hypothermia (15° to 20°C), tissue lipid peroxidation is still prevalent [211]. Unfortunately, no study has been done to compare hypothermic and normothermic CPB in terms of oxidative or

antioxidative status, and our small sample size makes it too early to make any conclusions on this important matter.

Chapter 7

CONCLUSION AND RECOMMENDATIONS FOR FURTHER WORK

The antioxidant effect of propofol on red cells is dose-dependent. High dose propofol significantly enhanced red cell antioxidant capacity. Plasma concentrations of propofol in the range of 7 to 9 μg/ml suppressed red cell MDA production by more than 50%. This effect was associated with improved cardiac function compared to isoflurane, as manifested by significantly less inotropic requirement (dopamine 3.5 μg/kg/min), and stable pulmonary capillary wedge pressure (In the isoflurane group, it was significantly increased at about 24 hours compared to 1 or 2 hours after surgery without an increase in cardiac index.) and/or significantly higher percentage of patients with normal cardiac index post-surgically. However, the suppression of lipid peroxidation did not parallel the improvement in cardiopulmonary function. Because of its high lipid solubility, propofol distributes to membranous structures such as plasma and mitochondrial membranes, reducing the damaging effects by ROIs on them. However, it probably could not prevent the oxidation of structures such as protein or nonprotein sulfhydryl groups and nucleic acids, which have been considered important in myocardial contractility. Meanwhile, high dose propofol, like other anesthetics, also has negative inotropic effects on the heart. Compared to low dose propofol, it was associated with a low (not statistically significant in our study probably because of small sample size) cardiac index during early (1 to 3 hours post-

operative) period of time after the operation, and not significant recovery in lung oxygenation within 12 hours post-operatively. However, the long-term effect of high dose propofol on cardiopulmonary function could not be determined by the study.

An expanded clinical trial to determine the effects of propofol on cardiopulmonary mobility and mortality following CAB surgery is required. Intermediate doses of propofol may be tried to optimize the balance of increasing cardiopulmonary function with the suppression of lipid peroxidation and avoiding the potential detrimental effects of high dose propofol on the heart and lungs in the immediate post-operative period.

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