Propofol attenuates myocardial lipid peroxidation during coronary artery bypass grafting surgery†

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Background. Propofol can scavenge free radicals because it has a chemical structure similar to antioxidants.

Methods. We examined if free radical scavenging occurs with propofol during CABG operations. We studied 24 patients undergoing CABG surgery for triple vessel disease, randomized into two groups. After induction of anaesthesia with fentanyl 10 μg kg^{-1} and midazolam 0.1 mg kg^{-1}, patients in the fentanyl group (n=14) received fentanyl infusion 10–30 μg kg^{-1} h^{-1} and patients in the propofol group (n=10) received propofol infusion 3–6 mg kg^{-1} h^{-1} for maintenance of anaesthesia. Atrial tissue biopsies were taken during cannulation for bypass, 45 min after cross-clamp insertion, 5 min after unclamping, and in the decannulation period. Lipid peroxidation was assessed by measurement of thiobarbituric acid reactive substances (TBARS) in the atrial tissue samples.

Results. Lipid peroxidation in the propofol group was less than in the fentanyl group (P<0.05) in all sampling periods. Lipid peroxidation in the fentanyl group increased significantly during cardiopulmonary bypass (CPB) (P<0.05), but no increase was found in the propofol group (P>0.05).

Conclusion. In clinical doses, propofol strongly attenuates lipid peroxidation during CABG surgery.

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Reintroduction of molecular oxygen into previously ischaemic tissue can further damage partially injured cells, an event known as 'reperfusion injury'. Oxygen supply leads to the formation of free oxygen radicals which react with polyunsaturated fatty acids of cell membranes forming lipid peroxides and hydroperoxides through a chain of reactions resulting in decreased membrane fluidity, increased membrane permeability, and finally disruption of the membranes.[1] Cross-clamping of the aorta and removal of the cross-clamp leads to ischaemic injury and then to reperfusion injury in the myocardial tissue. Myocardial cell injury can cause post-ischaemic dysfunction, myocardial stunning, reperfusion arrhythmias, and necrosis.[2–6]

As well as factors such as myocardial protection and the quality of anastomosis, prevention of reperfusion injury could allow safer weaning from cardiopulmonary bypass (CPB) and preserve stable haemodynamic conditions after surgery. The anaesthetic agents used may protect the myocardium against reperfusion injury.

Propofol resembles phenol-based anti-oxidants in chemical structure. It is a scavenger of free oxygen radicals *in vitro*,[7] *in vivo* in animals,[8 9] in man,[10] and on human cellular models during CPB.[11] The *in vivo* effects of propofol on human myocardial muscle have not been studied during CABG, so we investigated the effect of propofol infusion during the CABG operations on the concentration of end products of lipid peroxidation (malondialdehyde (MDA)) levels in human myocardial muscle.

Methods

After approval of the ethics committee and with informed consent, we studied 24 patients with triple vessel coronary artery disease about to have CABG surgery. We excluded patients with atrial fibrillation, an ejection fraction less than

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0.45, history of allergy to propofol or its preservatives, and other chronic diseases such as diabetes or chronic obstructive lung disease.

In all patients, anaesthesia was induced with fentanyl 10 μg kg^{-1} and midazolam 0.1 mg kg^{-1}, and muscle relaxation for tracheal intubation was facilitated with pancuronium 0.08 mg kg^{-1}. After cannulation of the right internal jugular vein and left radial artery, a pulmonary catheter was placed. Thereafter, patients were randomly allocated into two using computer generated random numbers: fentanyl group (n=14) and propofol group (n=10). Anaesthesia was maintained with fentanyl 10–30 μg kg^{-1} h^{-1} in the fentanyl group and propofol 3–6 mg kg^{-1} h^{-1} in the propofol group. All patients received midazolam 0.1 mg kg^{-1} hourly and pancuronium 0.04 mg kg^{-1} every 45 min. The lungs were mechanically ventilated with 8 ml kg^{-1} of 50% oxygen in air, except during total bypass when they were insufflated with the same gas mixture. Patients received anaesthetic drugs until they were transferred to the ICU. If indicated, dopamine 2–5 μg kg^{-1} h^{-1} was used.

A standard pump priming solution was used in each patient. The pump flow was kept constant at 2.4 litre $min^{-1}m^{-2}$ during the partial bypass and during the hypothermic total bypass (rectal temperature was 29–30°C) the pump flow was 2 litre min^{-1} m^{-2}. After application of the cross-clamp, St Thomas Cardioplegic Solution (Plegisol®) at +4°C was infused into the coronary arteries from a retrograde cannula, initially a volume of 1 litre and then 500 ml every 30 min.

Right atrial myocardial tissue samples were taken at the following times: during cannulation, 45 min after insertion of cross-clamp when the rectal temperature was 29°C, 5 min after unclamping, and at the end of decannulation procedure. At least 0.2 g of atrial myocardial tissue was taken from the right atrial tissue below the superior vena cava (SVC) cannulation suture where the circulation was intact, and then the suture of the venous canula was renewed below the biopsy area. For each sample, the same procedure was used to avoid sampling the possible partially ischaemic tissue created by the sutures of SVC canula. The atrial tissue samples were then immediately frozen at –70°C and kept frozen until analysis. At each sampling time, arterial pressure, heart rate, arterial blood gases, haemoglobin concentration, and cardiac output were recorded, and arterial oxygen content and cardiac index were calculated.

End products of lipid peroxidation in MDA levels in the samples were detected by the method described by Michara and Uchiyama.[12] Frozen tissues were immediately weighed and homogenized in 10 volumes of ice-cold phosphate buffer (50 mmol $litre^{-1}$, pH 7.4) using a glass–glass homogenizer (B. Brown, Germany). The homogenate (0.5 ml) was mixed with 3 ml of 1% H_3PO_4. After addition of 1 ml of TBA reagent (0.67%) the tubes were heated in boiling water for 45 min. The colour formed was extracted into 4 ml of n-butanol. After centrifugation, the colour intensity of the butanol layer was measured at 532 nm using a Shimadzu UV-120-02 model spectrophotometer. Tetramethoxypropane was used as the standard and concentrations of thiobarbituric acid reactive substances (TBARS) were calculated as nanomoles of MDA per gram of wet tissue.

Table 1 Characteristics of the study groups (mean (SD or range))

	Fentanyl group (n=14)	**Propofol group (n=10)**
Age (yr)	56 (45–68)	60 (50–70)
Basal surface area (m^2)	1.85 (0.05)	1.84 (0.06)
Extracorporeal circulation duration (min)	113 (30)	116 (35)
Cross-clamp duration (min)	57 (19)	55 (20)
Reperfusion duration (min)	49 (14)	53 (16)
Cross-clamp duration/reperfusion duration	1.2 (0.4)	1.04 (0.2)

The results were analysed by ANOVA for repeated measures test with Bonferroni t-procedure. The groups were also compared with the Mann–Whitney U test and the comparisons within the groups were analysed with Wilcoxon matched pairs test. In all comparisons P<0.05 was considered statistically significant.

Results

The characteristics of the two groups were similar in age, surface area, extracorporeal circulation time, total bypass time, and the duration of reperfusion (Table 1). Mean arterial pressure, arterial oxygen content, and cardiac index for the groups were not significantly different (Fig. 1A–C). The MDA concentrations (mean (SD)) during cannulation, cross-clamp, unclamping, and decannulation periods in the fentanyl group were 45.6 (7.9), 63.6 (15.9), 74.5 (14.9), and 72.8 (16) nmol (g wet tissue)$^{-1}$ and in the propofol group were 34.4 (7.2), 38.9 (18.2), 43.9 (16.8), and 43.8 (13.6) nmol (g wet tissue)$^{-1}$, respectively. The differences between the two study groups were significant at each time of sampling (P<0.05) (Fig. 2).

Comparisons in each group revealed a progressive statistically significant increase in lipid peroxidation products compared with the cannulation period in the fentanyl group but no difference in the propofol group for each sampling period (Fig. 2). Lipid peroxidation levels during the unclamping and decannulation periods were greater than in the cross-clamp period in the fentanyl group (P<0.05).

Discussion

Propofol stucturally resembles phenol-based anti-oxidants such as butyrated hydroxytoulene and α-tocopherol and it can act as a scavenger of free radicals. Murphy and coworkers[7] showed that each molecule of propofol inhibited two molecules of oxygen radicals *in vitro*. Some investigators claim that this effect is evident only at supramaximal blood levels of propofol, and has no clinical significance.[13]

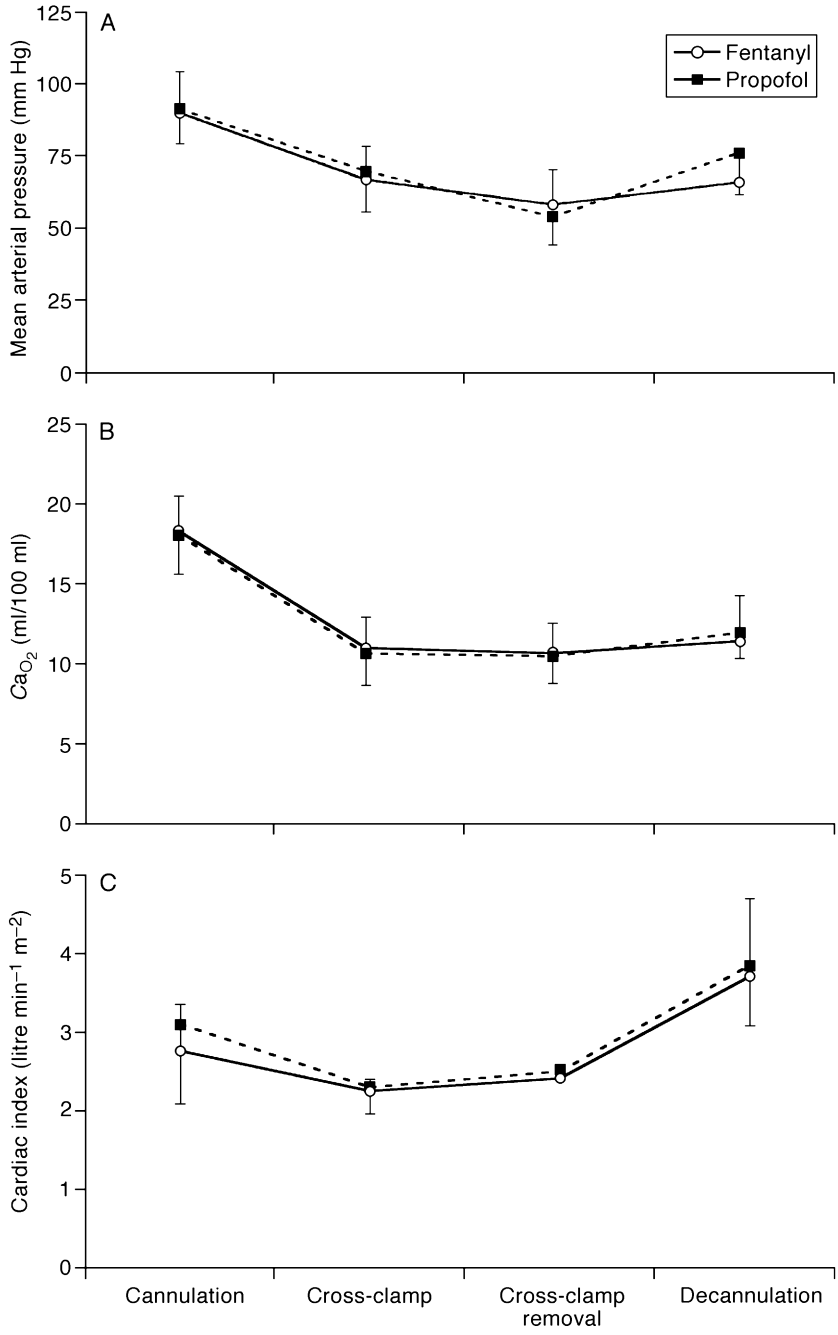

Fig 1 Mean arterial pressure. Arterial oxygen content, and cardiac index in both groups during sampling periods.

However, Murphy and colleagues[14 15] found that even at the anaesthetic blood concentrations, propofol inhibited the peroxidation of lipids, in proportion to the blood concentration of propofol. Propofol can attenuate lipid peroxidation on human skeletal muscle[10] and can increase the antioxidant capacity of erythrocytes (RBC) during CPB[11] but human myocardial muscle has not been studied during CPB.

MDA is one of several low-molecular-weight end products formed by decomposition of primary and secondary lipid peroxidation products. Many sophisticated assays are available for measurement of lipid peroxides.[16] However, the TBARS assay remains a test that provides a global measure of lipoperoxidation.[17] Coghlan and coworkers used the TBARS method to measure myocardial free radical generation in coronary sinus blood.[18] It has been used in myocardial tissue of the cats by Maulik and coworkers,[19] rats,[20] and other animals. This method of determination of MDA is a widely accepted and an easy way of assessing free radical insult to tissues.[10 21 22]

We investigated the effect of maintenance of anaesthesia with propofol on atrial myocardial tissue lipid peroxidation, compared with a fentanyl infusion, during CABG oper-

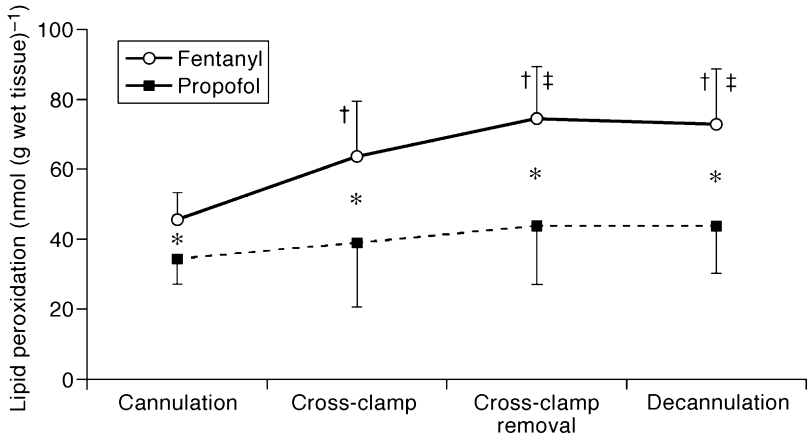

Fig 2 Changes in the lipid peroxidation during sampling periods. *$P<0.05$ (statistical difference between the groups); †$P<0.05$ (statistical difference within the group, when compared with cannulation); ‡$P<0.05$ (statistical difference within the group, when compared with cross-clamp).

ations. We chose fentanyl as a control substance rather than other i.v. anaesthetics such as thiopentone or barbiturates because the minimal antioxidant action of these anaesthetic agents might be a source of confusion.[23 24] Fentanyl, as far as we know, does not have antioxidant effects.[25 26] Opioids are also preferred by many anaesthesiologists for cardiac anaesthesia because of their non-cardiac depressant properties. Infusion of propofol 3–6 mg kg^{-1} h^{-1} caused a significant reduction in the atrial tissue lipid peroxidation when compared with the fentanyl group.

Ytreus and co-workers[1] suggested that lipid peroxidation normally occurs in the myocardial tissue in low levels even without ischaemia and reperfusion injury. We also found some degree of lipid peroxidation in the fentanyl group during the cannulation period, but the propofol infusion, started after induction of anaesthesia, significantly attenuated the lipid peroxidation levels in the propofol group. This difference is unlikely to be caused by haemodynamic, technical, and methodological differences between the groups, but can only be attributed to the propofol infusion. We could not obtain a pre-operative control value in our study as other authors have. Our results resemble those of Ytrehus and co-workers,[1] where propofol infusion significantly attenuated pre-exising lipid peroxidation.

During ischaemia, the cross-clamp period, lipid peroxidation levels in the myocardium tended to increase. Ferrari and co-workers[27] proposed that, during ischaemia, free radicals are still formed because of the residual molecular oxygen. Free radical generation was found in other studies,[28] and an experimental study by Hegstad and colleagues[29] found that lipid peroxides could accumulate during ischaemia. Our results support these findings, during the period of ischaemia during CPB in the fentanyl group, and propofol infusion prevented this increase. This suggests that free radical injury to the myocardial tissue could relatively be controlled by propofol infusion.

At the cross-clamp removal sampling time, when reperfusion injury was added to the injury of ischaemia, we observed a further statistically significant increase in lipid peroxidation. According to the electron spin resonance spectroscopy studies of Garlick and Baker and colleagues,[30 31] a large amount of free radicals are produced during the first few minutes of post-ischaemic reperfusion. These reactive oxygen species cause lipid peroxidation of the cellular and intracellular membranes. We observed an increase in lipid peroxidation products in the fentanyl group of our study, but in the propofol group no statistically significant increases in the lipid peroxidation, relative to the cannulation or the cross-clamp periods, were observed.

The sample at decannulation was the last time that a biopsy could be obtained without interfering with the integrity of the heart. In the fentanyl group lipid peroxidation was still greater than the propofol group. A burst of oxygen radical generation occurs during the early phase of reperfusion,[27 32 33] but reperfusion injury continues for some hours after unclamping.[28] Others have chosen different times after reperfusion for sampling.[34 35] We sampled both in the early stage after unclamping and in the late reperfusion stage, which was equal to the cross-clamp duration. Our findings support those of the previous studies, that reperfusion injury continued for some period after ischaemia,[28] and propofol relatively decreased this injury.

Kahraman and co-workers[10] used propofol in order to inhibit tourniquet-induced ischaemia and reperfusion injury, which reduced lipid peroxidation in muscle tissue. Hans and co-workers[36] examined the effect of TIVA with propofol in 18 neurosurgical patients assigned for cerebrospinal fluid shunting and found out that propofol increased the capacity of plasma to inhibit lipid peroxidation. Ansley and co-workers[11] showed that propofol at 3–6 mg kg^{-1} h^{-1} enhanced the antioxidant capacity of red blood cells. This is the first clinical study examining the antioxidant effects of propofol on myocardial muscle tissue during CABG operations. Our results support the results of Kahraman, Hans, and Ansley.[10 11 36]

Although the changes in lipid peroxidation did not relate to haemodynamic changes, it is inappropriate to link peroxidation to the outcome of the groups, because post-

operative measurements were not made. Our study was designed to investigate the biochemical *in vivo* effects of propofol on free radical injury during CPB. The effects of reduced free radical injury by propofol on clinical outcome should be the subject of a further clinical study.

We conclude that propofol effectively attenuates lipid peroxidation and could be used in an anaesthetic drug regimen during CABG if ischaemia and reperfusion injury is of concern.

References

1 Ytrehus K, Hegstad AC. Lipid peroxidation and membrane damage of the heart. *Acta Physiol Scand Suppl* 1991; **S599**: 81–91

2 Bolli R. Oxygen-derived free radicals and myocardial reperfusion injury: an overview. *Cardiovasc Drugs Ther* 1991; **5** (Suppl 2): 249–68

3 Nejima J, Knight DR, Fallon JT, *et al*. Superoxide dismutase reduces reperfusion arrhythmias but fails to salvage regional function or myocardium at risk in conscious dogs. *Circulation* 1989; **79**: 143–53

4 McCord JM. Oxygen-derived free radicals in postischemic tissue injury. *N Engl J Med* 1985; **312**: 159–63

5 Simpson PJ, Lucchesi BR. Free radicals and myocardial ischemia and reperfusion injury. *J Lab Clin Med* 1987; **110**: 13–30

6 Hammond B, Hess ML. The oxygen free radical system: potential mediator of myocardial injury. *J Am Coll Cardiol* 1985; **6**: 215–20

7 Murphy PG, Myers DS, Davies MJ, Webster NR, Jones JG. The antioxidant potential of propofol (2,6-diisopropylphenol). *Br J Anaesth* 1992; **68**: 613–8

8 Musacchio E, Rizzoli V, Bianchi M, Bindoli A, Galzigna L. Antioxidant action of propofol on liver microsomes mitochondria and brain synoptosomes in rat. *Pharmacol Toxicol* 1991; **69**: 75–7

9 Eriksson O, Pollesello P, Saris NEL. Inhibition of lipid peroxidation in isolated rat liver mitochondria by the general anesthetic propofol. *Biochem Pharmacol* 1992; **44**: **2**: 391–3

10 Kahraman S, Kilinç K, Dal D, Erdem K. Propofol attenuates formation of lipid peroxides in tourniquet-induced ischaemia-reperfusion injury. *Br J Anaesth* 1997; **78**: 279–81

11 Ansley DM, Sun J, Visser WA, *et al*. High dose propofol enhances red cell antioxidant capacity during CPB in humans. *Can J Anaesth* 1999; **46**: 641–8

12 Mihara M, Uchiyama M. Determination of malonaldehyde precursor in tissues by thiobarbituric acid test. *Ann Biochem* 1977; **86**: 271–78

13 Green TR, Bennet SR, Nelson VM. Specificity and properties of propofol as an antioxidant free radical scavenger. *Toxicol Appl Pharmacol* 1994; **129**: 163–9

14 Murphy PG, Davies MJ, Columb MO, Stratford N. Effect of propofol and thiopentone on free radical mediated oxidative stress of the erythrocyte. *Br J Anaesth* 1996; **76**: 536–43

15 Murphy PG, Bennett JR, Myers DS, Davies MJ, Jones JG. The effect of propofol anaesthesia on free radical-induced lipid peroxidation in rat liver microsomes. *Eur J Anaesth* 1993; **10**: 261–6

16 Esterbauer H. Estimation of peroxidative damage. A critical review. *Pathol Biol (Paris)* 1996; **44**: 25–8

17 Lefevre G, Beljean-Leymarie M, Beyerle F, *et al*. Evaluation of lipid peroxidation by measuring thiobarbituric acid reactive substances. *Ann Biol Clin (Paris)* 1998; **56**: 305–19

18 Coghlan JG, Fitter WD, Clutton SM, *et al*. Lipid peroxidation and changes in vitamine E levels during coronary artery bypass grafting. *J Thorac Cardiovasc Surg* 1993; **106**: 268–74

19 Maulik SK, Kumari R, Maulik M, Manchanda SC, Gupta SK. Captopril and its time of administration in myocardial ischaemic-reperfusion injury. *Pharmacol Res* 2001; **44**: 123–8

20 Kowluru RA, Engerman RL, Kern TS. Diabetes-induced metabolic abnormalities in myocardium: effect of antioxidant therapy. *Free Radic Res* 2000; **32**: 67–74

21 Inan C, Kilic I, Kilinc K, Kalayci O, Kotiloglu E. The effect of high dose antenatal vitamin E on hypoxia-induced changes in newborn rats. *Ped Res* 1995; **38**: 685–9

22 Akgur FM, Kilinç K, Aktug T, Olguner M. The effect of allopurinol pretreatment before detorting testicular torsion. *J Urol* 1994; **151**: 1715–7

23 Almaas R, Saugstad OD, Pleasure D, Rootwelt T. Effect of barbiturates on hydroxyl radicals, lipid peroxidation, and hypoxic cell death in human NT2-N neurons. *Anesthesiology* 2000; **92**: 764–74

24 Smith DS, Rehncrona S, Siesjo BK. Barbiturates as protective agents in brain ischemia and as free radical scavengers *in vitro*. *Acta Physiol Scand Suppl* 1980; **492**: 129–34

25 Chinev S, Bakalova S, Kovacheva S, Ribarov SR. Lipid peroxidation in rat lung induced by neuroleptanalgesia and its components. *Eur J Anaesthesiol* 1998; **15**: 686–94

26 Chinev S, Bakalova S, Peneva V, *et al*. Nitrous oxide with fentanyl and droperidol minimizes lipid peroxidation in the liver. *Eur J Anaesthesiol* 1995; **12**: 155–62

27 Ferrari R, Ceconi C, Curello S, *et al*. Oxygen free radicals and myocardial damage: protective role of thiol-containing agents. *Am J Med* 1991; **91** (Suppl 3C): 95S–105S

28 Bolli R, Patel BS, Jeroudi MO, Lai EK, McCay PB. Demonstration of free radical generation in 'stunned' myocardium of intact dogs with the use of the spin trap alpha-phenyl *N*-tert-butyl nitrone. *J Clin Invest* 1988; **82**: 476–85

29 Hegstad AC, Strand H, Ytrehus K. Selective measurments of phospholipid peroxidation in ischemic, reperfused and oxygen radical perfused isolated rat hearts. *J Mol Cell Cardiol* 1990; **22** (Suppl III): 118

30 Garlick PB, Davies MJ, Hearse DJ, Slater TF. Direct detection of free radicals in the reperfused heart using electron spin resonance spectroscopy. *Circ Res* 1987; **61**: 757–60

31 Baker JE, Felix CC, Olinger GN, Kalyanaraman B. Myocardial ischemia and reperfusion: direct evidence for free radical generation by electron spin resonance spectroscopy. *Proc Natl Acad Sci USA* 1988; **85**: 2786–9

32 Bolli R, Jeroudi MO, Patel BS, *et al*. Marked reduction of free radical generation and contractile dysfunction by antioxidant therapy begun at the time of reperfusion. Evidence that myocardial 'stunning' is a manifestation of reperfusion injury. *Circ Res* 1989; **65**: 607–22

33 Zweier JL, Flaherty JT, Weisfeldt ML. Direct measurment of free radical generation following reperfusion of ischemic myocardium. *Proc Natl Acad Sci USA* 1987; **84**: 1404–7

34 Jolly SR, Lucchesi BR. Effect of BW755C in an occlusion-reperfusion model of ischemic myocardial injury. *Am Heart J* 1983; **106**: 8–13

35 Kokita N, Hara A, Abiko Y, *et al*. Propofol improves functional and metabolic recovery in ischemic reperfused isolated rat hearts. *Anesth Analg* 1998; **86**: 252–8

36 Hans P, Deby-Dupont G, Deby C, *et al*. Increase in antioxidant capacity of plasma during propofol anesthesia. *J Neurosurg Anesthesiol* 1997; **9**: 234–6