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Propofol attenuates formation of lipid peroxides in tourniquet-induced ischaemia–reperfusion injury

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Summary

We studied 20 adult ASA I patients undergoing elective peripheral surgery allocated randomly to one of two groups. In the propofol group ($n=9$) anaesthesia was induced with propofol and fentanyl followed by continuous infusion of propofol. In the control group ($n=11$), after induction of anaesthesia with thiopentone and fentanyl, anaesthesia was maintained with isoflurane. Concentrations of lipid peroxides in both plasma and muscle tissue samples were measured as thiobarbituric acid-reacting substances (TBARS). Plasma TBARS concentrations increased significantly in the control group at 1,5,15,30 and 45 min after release of the tourniquet to mean 1.83 (SD 0.13), 2.00 (0.12), 2.25 (0.14), 2.30 (0.12) and 2.41 (0.14) μmol litre^{-1}, respectively, compared with pre-reperfusion values (1.64 (0.14) μmol litre^{-1}). In the propofol group this was significant only at 30 min (1.85 (0.03) *vs* 1.74 (0.04) μmol litre^{-1}). TBARS concentrations of reperfused muscle tissue were significantly higher than pre-reperfusion concentrations in the control group (70.30 (10.06) *vs* 52.13 (5.73) nmol/g wet tissue). We conclude that propofol attenuated ischaemia–reperfusion-induced lipid peroxidation in the therapeutic doses used in anaesthesia. (*Br. J. Anaesth.* 1997; **78**: 279–281).

Key words
Anaesthetics i.v., propofol. Muscle skeletal, lipid peroxides. Complications, ischaemia–reperfusion injury.

Re-introduction of oxygen to an ischaemic or hypoxic tissue has been shown to cause additional insult to the tissue (reperfusion injury). Reactive oxygen species which damage cellular components and initiate the lipid peroxidation process are known to be responsible for the ischaemia–reperfusion injury. Lipid peroxidation is a chain reaction leading to oxidation of polyunsaturated fatty acids which, in turn, disrupts the structure of biological membranes and produces toxic metabolites such as malondialdehyde (MDA). The thiobarbituric acid assay, which measures MDA, has served as the most commonly used method for measuring lipid peroxidation.

Propofol (2,6-diisopropylphenol) is chemically similar to phenol-based free radical scavengers such as butylated hydroxytoluene and the endogenous antioxidant α-tocopherol (vitamin E).[1–3] Propofol has been shown to exhibit significant antioxidant activity *in vitro*, reacting with free radicals to form a phenoxyl radical.[3 4] Although Green, Bennett and Nelson[1] suggested that the antioxidant activity of propofol may not be clinically relevant, this effect has also been demonstrated at anaesthetic concentrations.[2] However, no clinical study has been conducted in humans to investigate the antioxidant effect of anaesthetic doses of propofol in ischaemia–reperfusion injury of skeletal muscle.

Therefore, we have determined if infusion of propofol would prevent the formation of lipid peroxides as a result of tourniquet-induced ischaemia–reperfusion injury in surgery of the limbs.

Patients and methods

After obtaining Ethics Committee approval and informed patient consent, we studied 20 adult, ASA I patients undergoing peripheral surgery using a tourniquet. Patients were allocated randomly by closed envelope method to one of two groups: propofol ($n=9$) and isoflurane (control, $n=11$). No patient was receiving vitamin supplements. All patients were premedicated with diazepam 10 mg orally, 45 minutes before surgery. In the propofol group anaesthesia was induced with propofol 2–2.5 mg kg^{-1} and fentanyl 3 μg kg^{-1} followed by continuous infusion of propofol at a rate of 10 mg kg^{-1} h^{-1}, reducing to 8 and 6 mg kg^{-1} h^{-1}, respectively, at 10-min intervals. The maintenance dose was adjusted to clinical signs and anticipated demand. In the control group anaesthesia was induced with thiopentone 5 mg kg^{-1} and fentanyl 3 μg kg^{-1}, and maintained with 1% isoflurane. Vecuronium 0.1 mg kg^{-1} was given to facilitate tracheal intubation in both groups and the lungs were ventilated with 60% nitrogen in oxygen. The tourniquet was applied at a pressure approximately twice the systolic arterial pressure.

Using heparin-locked i.v. catheters, sequential

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venous blood samples were obtained from the antecubital vein of the contralateral arm. The dorsal vein of the foot was used for i.v. fluid and propofol infusions. During lower limb operations one arm was used for blood sampling and the other for i.v. fluid infusions.

Blood samples for lipid peroxidation analysis were obtained immediately before and 1, 5, 15, 30, 45 min after tourniquet release. Blood samples for measurement of blood urea nitrogen, creatinine, creatinine phosphokinase, lactate, sodium, potassium and calcium concentrations and muscle tissue biopsy samples were obtained immediately before (control) and 20 min after release of the tourniquet (reperfusion).

Plasma samples were centrifuged immediately. The supernatant and muscle tissue samples were stored at -70°C until analysis. Concentrations of lipid peroxides in both muscle tissue and plasma samples were measured as thiobarbituric acid-reacting substances (TBARS). 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.[5] Tetramethoxypropane was used as the standard and concentrations of TBARS were calculated as nanomoles of malondialdehyde (MDA) per gram of wet tissue. Lipid peroxide concentrations in plasma were measured as described by Wade and van Rij.[6] Concentrations of TBARS in serum were calculated as micromoles per litre.

Significant differences between groups were analysed using the Mann–Whitney *U* test. Intragroup comparisons were performed by Wilcoxon matched pairs test and $P<0.05$ was considered statistically significant.

Results

There were no significant differences between groups in age, weight, sex distribution or tourniquet time (table 1). Plasma concentrations of TBARS increased significantly in the control group at 1, 5, 15, 30 and 45 min after release of the tourniquet by mean 1.83 (SD 0.13), 2.00 (0.12), 2.25 (0.14), 2.30 (0.12) and 2.41 (0.14) μmol litre^{-1}, respectively, compared with pre-reperfusion values (1.64 (0.14) (μmol litre^{-1}) (fig. 1). In contrast, there were only slight increases at 15 , 30 and 45 min in the propofol group, and, this was significant only at 30 min (1.80 (0.03), 1.85 (0.03) and 1.83 (0.06) μmol litre^{-1}, respectively, *vs* 1.74 (0.04) μmol litre^{-1}).

TBARS concentrations in muscle tissue after 20 min of reperfusion were significantly higher than pre-reperfusion concentrations in the control group (70.30 (10.06) *vs* 52.13 (5.73) nmol/g wet tissue) while there were no significant differences in the propofol group (38.13 (5.26) *vs* 40.16 (5.68) nmol/g wet tissue). TBARS concentrations in reperfused muscle tissue were significantly different between groups ($P=0.0069$).

In two patients (22%) in the propofol group and four patients in the control group the anaesthetic was discontinued before 45 min of reperfusion. Plasma electrolyte, creatinine, blood urea nitrogen, lactate and creatinine phosphokinase concentrations were not significantly different after reperfusion in either group and differences between groups were not significant.

Table 1 Patient characteristics (mean (SD or range) or number)

	Group I (isoflurane)	Group II (propofol)
n	11	9
Age (yr)	36.55 (29–59)	36.77 (18–58)
Weight (kg)	73.34 (15.5)	80.55 (13.7)
Sex (M/F)	7/4	6/3
Operation (*n*)		
Median nerve exploration	3	3
Ulnar nerve exploration	1	—
Intramuscular haemangioma	2	1
Intramuscular hamartoma	1	1
Achilloplasty	2	1
Contracture release	2	3
Tourniquet time (min)	82.55 (26.1)	89.20 (23.7)
Anaesthesia time after tourniquet release (min)	46.36 (14.5)	47.78 (12.5)

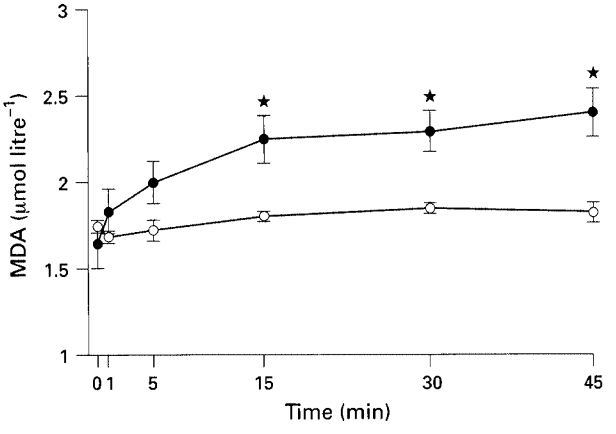

Figure 1 Plasma MDA concentrations before (0) and 1, 5, 15, 30 and 45 min after release of the tourniquet in the isoflurane (●) and propofol (○) groups. *$P<0.05$ between groups.

Discussion

Although skeletal muscle is fairly resistant to ischaemic injury, ischaemia–reperfusion injury has been documented in previous studies. Concannon and colleagues[7] showed a significant increase in MDA production within 2 h in a rabbit model of tourniquet-induced skeletal muscle ischaemia–reperfusion injury. The results of our study (35% increase in MDA concentrations in muscle tissue after 20 min of reperfusion and 47% increase in systemic blood within 45 min after reperfusion) were comparable with the results of Concannon and colleagues.

Studies using isolated organelles have shown that

propofol inhibits lipid peroxidation induced by oxidative stress in rat liver mitochondria, microsomes and brain synaptosomes.[2,4] However, its effects on skeletal muscle ischaemia–reperfusion injury have not been examined. Intentional ischaemia (tourniquet)–reperfusion for bloodless upper limb surgery has been accepted as a good human model for ischaemia–reperfusion injury. In this human model of ischaemia–reperfusion injury, we examined the effect of the i.v. anaesthetic propofol on lipid peroxide formation. As it was shown that maintenance of general anaesthesia with clinical relevant concentrations of nitrous oxide or isoflurane were not associated with an increase in toxic oxygen metabolites[8] we preferred to use isoflurane in the control group. While there was a significant increase in MDA concentrations in both plasma and muscle tissue in the isoflurane group, there was only a slight increase in plasma concentrations in the propofol group. The absence of a significant difference in biochemical variables at 20 min of reperfusion suggests that our measurements were probably performed after the initial rapid washout of accumulated tissue lactate and other substances.

We conclude that propofol attenuated ischaemia–reperfusion-induced lipid peroxidation when given in the therapeutic doses used in anaesthesia; this suggests that its oxygen free radical scavenging activity during anaesthesia is likely to be clinically significant.

References

1. Green TR, Bennett SR, Nelson VM. Specificity and properties of propofol as an antioxidant free radical scavenger. *Toxicology and Applied Pharmacology* 1994; **129**: 163–169.
2. Murphy PG, Bennett JR, Myers DS, Davies MJ, Jones JG. The effect of radical-induced in rat liver microsomes. *European Journal of Anaesthesiology* 1993; **10**: 261–266.
3. Aarts L, Van Der Hee R, Dekker I, Langemeijer H, Bast A. The widely used anesthetic agent propofol can replace alpha-tocopherol as an antioxidant. *FEBS Letters* 1995; **357**: 83–85.
4. Musacchio E, Rizzoli V, Bianchi M, Bindoli A, Galzigna L. Antioxidant action of propofol on liver microsomes, mitochondria and brain synaptosomes in the rat. *Pharmacology and Toxicology* 1991; **69**: 75–77.
5. Uchiyama M, Mihara S. Determination of malonaldehyde precursor in tissues by thiobarbituric acid test. *Analytical Biochemistry* 1978; **86**: 271–275.
6. Wade CR, van Rij AM. Plasma thiobarbituric acid reactivity: Reactions and the role of iron, antioxidants and lipid peroxy radicals on the quantitation of plasma lipid peroxides. *Life Sciences* 1988; **43**: 1085–1093.
7. Concannon MJ, Kester CG, Welsh CF, Puckett CL. Patterns of free-radical production after tourniquet ischemia: implications for the hand surgeon. *Plastic and Reconstructive Surgery* 1992; **89**: 846–852.
8. Wilson WC, Swetland JF, Benomof JL, Laborde P, Taylor R. General anesthesia and exhaled breath hydrogen peroxide. *Anesthesiology* 1992; **76**: 703–710.