Cardiothoracic Anesthesia, Respiration and Airway

Laboratory investigation: Effects of propofol on the systemic inflammatory response during aortic surgery

[Essai en laboratoire : les effets du propofol sur la réaction inflammatoire généralisée pendant une opération de l'aorte]

José M. Rodríguez-López MD,* Pilar Sánchez-Conde MD,* Francisco S. Lozano MD,† Juan L. Nicolás MBBS,‡ Francisco J. García-Criado MD,† Carlos Cascajo MD,† Clemente Muriel MD*

Purpose: A laboratory investigation was undertaken to assess the effects of propofol on renal function, through modulation of the systemic inflammatory response, in an *in vivo* experimental model of aortic surgery in comparison with sevoflurane.

Methods: Twenty young male piglets were anesthetized with either propofol 4 $mg \cdot kg^{-1} \cdot hr^{-1}$ ($n = 10$) or sevoflurane 1.5% end-tidal concentration ($n = 10$). Animals were subjected to aorta-aortic bypass with suprarenal aortic clamping for 30 min. At specific intervals (basal -before the start of surgery; reperfusion 15 min after unclamping the aorta; at 24, 48 and 72 hr after surgery, and on the seventh day after surgery) the levels of the following were determined: plasma creatinine, renal myeloperoxidase, tumour necrosis factor-α, interleukin 1-ß, and interferon-γ; kidney superoxide anion and its detoxifying enzyme superoxidase dismutase, kidney malondialdehyde and the activity of inducible nitric oxide synthase. Seven days after surgery, the animals were anesthetized using the described techniques, and after blood withdrawal and kidney sampling they were sacrificed.

Results: In comparison with sevoflurane, propofol was associated with a lower concentration of plasma creatinine ($P < 0.05$) together with lower concentrations of myeloperoxidase, tumour necrosis factor-α, interleukin 1-ß, interferon-γ, superoxide anion and superoxidase dismutase, malondialdehyde and inducible nitric oxide synthase ($P < 0.05$).

Conclusion: In an experimental model of aortic reconstructive surgery, and compared with sevoflurane, propofol anesthesia is associated with less neutrophil infiltration, lower plasma proinflammatory cytokine levels, lower production of oxygen free radicals, less lipid peroxidation, and reduced inducible nitric oxide synthase activity. These observations suggest a possible renal protective effect of propofol in this surgical setting.

***Objectif** : Un essai en laboratoire a été entrepris pour évaluer les effets du propofol sur la fonction rénale, à travers la modulation de la réaction inflammatoire généralisée, chez un modèle expérimental in vivo de chirurgie aortique et en comparaison avec le sévoflurane.*

***Méthode** : Vingt jeunes porcelets ont été anesthésiés avec 4 $mg \cdot kg^{-1} \cdot h^{-1}$ de propofol ($n = 10$) ou de sévoflurane à une concentration télé-expiratoire de 1,5 % ($n = 10$). Ils ont subi un pontage aorto-aortique avec clampage aortique pendant 30 min. À des moments spécifiques (au départ - avant le début de l'opération ; pendant la reperfusion 15 min après le déclampage de l'aorte ; à 24, 48 et 72 h après l'opération et au septième jour postopératoire), les niveaux suivants ont été déterminés : la créatinine plasmatique, la myéloperoxydase rénale, le facteur-α nécrosant tumoral, l'interleukine 1-ß et l'interféron-γ ; l'anion de superoxyde rénal et son enzyme de détoxification superoxydase dismutase, la*

From the Departments of Anesthesiology,* and Surgery,† University Hospital of Salamanca, Salamanca; and the Department of Anesthesiology,‡ Rodríguez-Chamorro Hospital, Zamora, Spain.

Address correspondence to: Dr. José María Rodríguez-López, Servicio de Anestesiología, Hospital Universitario de Salamanca, Paseo de San Vicente, 58-182, 37007, Salamanca, Spain. Phone: 0034-923291353; E-mail: jmrodlop@terra.es

The present study has been supported by the Instituto de Salud Carlos III and grant FISS 00/1093 from the Ministerio de Sanidad, Spain.

Accepted for publication January 30, 2006.

Revision accepted March 13, 2006.

This article is accompanied by an editorial. Please see Can J Anesth 2006; 53: 643–5.

malondialdéhyde rénale et l'activité de l'oxyde nitrique synthase inductible. Sept jours après l'opération, les animaux ont été anesthésiés selon les techniques décrites et, après le retrait du sang et la prise d'un échantillon rénal, ont été sacrifiés.

***Résultats** : Comparé au sévoflurane, le propofol a été associé à une plus faible concentration plasmatique de créatinine, ($P < 0,05$) et à de plus faibles concentrations de myéloperoxydase, de facteur-α nécrosant tumoral, d'interleukine 1-ß, d'interféron-γ, d'anion de superoxyde et de superoxyde dismutase, de malondialdéhyde et d'oxyde nitrique synthase inductible ($P < 0,05$).*

***Conclusion** : Pour un modèle expérimental de reconstruction aortique, et comparé à l'anesthésie au sévoflurane, l'anesthésie au propofol est associée à moins d'infiltration de neutrophiles, à des niveaux plasmatiques inférieurs de cytokine pro-inflammatoire, à une plus faible production de radicaux libres d'oxygène, à moins de peroxydation lipidique et à une activité réduite de l'oxyde nitrique synthase inductible. Ces observations indiquent un effet rénal protecteur possible du propofol dans ce contexte chirurgical.*

ABDOMINAL aortic surgery induces a systemic inflammatory response (SIR) due to the effects of aortic clamping and secondary ischemia-reperfusion (IR). The organic dysfunction brought about by IR injury is due to cellular damage secondary to the production of oxidant agents, which leads to the destruction of cell membranes through peroxidation of their lipids. These disturbances are increased in conditions of hyperoxia and may lead to cell death.[1–3] Recently, it has been reported that anesthetic agents may offer some protection against such lesions induced by IR, and hence knowledge of the mechanisms involved may provide anesthesiologists with strategies to lower morbidity and improve patient survival.[4] Propofol, a highly lipid soluble hypnotic agent, has proven antioxidant activity in both *in vitro* and *in vivo* studies; this is based on the fact that its chemical structure is similar to that of a natural antioxidant: i.e., vitamin E.[5–7] In this sense, several studies have demonstrated that propofol acts as a scavenger of oxygen free radicals, decreasing lipid peroxidation and increasing the antioxidant capacity of erythrocytes and other tissues in organs such as liver, kidney, heart and lung.[8,9] Other studies that have shown that volatile anesthetics exert a protective role in different organs when they are administered before and after ischemia.[10–12]

In keeping with the above, the aims of the present study were to develop an experimental model comparable to human clinical practice – abdominal aortic surgery with renal IR, which causes important but reversible renal ischemia - with a view to assessing the effect of anesthetic technique on renal injury through a comparison of different mediators of the SIR. Specifically, we sought to compare the potential renal protective effects of propofol *vs* sevoflurane at clinically relevant anesthetic concentrations.

Methods

This study was approved by the Committee for Animal Research of the University of Salamanca. We studied 20 male pigs (3–4 months old; 20–25 kg body weight), who received care for at least one week prior to the experimental period at the Biohealth Centre of Animal Research at the University of Salamanca. The animals were subjected to light/dark cycles of 12 hr, a constant temperature (21°C), and controlled ingestion of food and water. Twelve hours before the start of the experiments the animals were fasted, providing only water ad libitum.

Based upon the type of maintenance hypnotic agent used, the animals were divided into two groups: in ten animals, hypnosis was maintained with propofol (Group P); the other ten animals received sevoflurane (Group S). The study was blinded by ensuring the investigators did not know to which group the analyzed samples belonged. Anesthesia proceeded as follows: all animals were premedicated with ketamine 20 $mg \cdot kg^{-1}$ *im*, diazepam 0.5 $mg \cdot kg^{-1}$ *im* and atropine 0.05 $mg \cdot kg^{-1}$ *im*. Following premedication, a dorsal ear vein was cannulated with a 20-G *iv* catheter, through which *iv* anesthetic drugs were administered. Both induction and maintenance of anesthesia proceeded under conditions identical to those employed in humans. For induction, animals in Group P received propofol 1.5 $mg \cdot kg^{-1}$ *iv*, and animals in Group S received thiopental sodium 3 $mg \cdot kg^{-1}$ *iv*. These drugs were titrated gradually until hypnosis had been achieved in each animal, without causing apnea. The animals were placed on a table under restraint, while monitoring their electrocardiograms and heart rates. The trachea of each animal was intubated under direct laryngoscopy with the aid of Magill forceps, using a #5 endotracheal tube. The animals were then connected to a ventilator (Boyle 2000 anesthesia station, Datex-Ohmeda, Essex, UK) with the following parameters: tidal volume, 10 $mL \cdot kg^{-1}$; respiratory rate, 15 min; inspiration/expiration rate 1:2, and an oxygen/air mixture of 50%. Mivacurium chloride 0.2 $mg \cdot kg^{-1}$ *iv* was administered for muscle relaxation, and all pigs received cefazolin 1 g *iv*. Maintenance of anesthesia was provided by propofol 4 $mg \cdot kg^{-1} \cdot hr^{-1}$ *iv* in Group P or by sevoflurane 1.5% inspired in Group S. Animals in both groups received fentanyl 2 $\mu g \cdot kg^{-1} \cdot hr^{-1}$ *iv* and mivacurium chloride 1 $mg \cdot kg^{-1} \cdot hr^{-1}$ *iv*.

Following sterile cervical dissection, the internal jugular vein was cannulated with a double-lumen polyethylene catheter through which fluids (normal saline at 10 $mL \cdot kg^{-1} \cdot hr^{-1}$) were administered, together with anesthetic medications. The carotid artery was cannulated with a 20-G arterial catheter to monitor invasive arterial pressure and to obtain blood samples. Hemodynamic monitoring consisted of continuous electrocardiogram, heart rate and invasive arterial pressure (Monitor Dash 3000, Marquette Medical Systems, Freiburg, Germany). Routine ventilatory parameters were recorded.

The animals were subjected to a median laparotomy under aseptic conditions. The retroperitoneum was dissected to expose the abdominal aorta from the renal arteries to the aortic bifurcation. Prior to aortic clamping, heparin was administered (1 $mg \cdot kg^{-1}$ *iv*). The aorta was clamped suprarenally (below the superior mesenteric artery) and distally, immediately proximal to the iliac arteries. Whenever clamping extended beyond 30 min, the suprarenal clamp was moved from the suprarenal to an infrarenal position. Two 1-cm aortotomies were established to perform aorto-aortic bypass, with a latero-terminal anastomosis using a 6-mm diameter collagen-lined Dacron tube graft (previously submerged for 15 min in a solution of rifampicin). Finally, the clamps were removed and sodium bicarbonate was infused (1 $mEq \cdot kg^{-1}$ *iv*). Hemostasis was restored and the laparotomy was closed. With the exception of anatomical differences, the surgical technique was designed to mirror the surgical approach used in humans. Anesthesia was reversed by discontinuing the infusion of propofol or sevoflurane, depending on the group. When animals had recovered spontaneous breathing, the endotracheal tube was removed, and they were returned to the designated animal care area. Metamizol 1.2 g *iv* was administered for postoperative analgesia.

During surgery, the following hemodynamic variables were recorded: heart rate, and systolic, diastolic and mean arterial pressure. Intraoperatively and over the next seven days, blood was sampled, and the kidneys were biopsied at the following times: basal (before the start of surgery); reperfusion (15 min after unclamping the aorta); at 24, 48 and 72 hr after surgery, and on the seventh day after surgery. From 24 until 72 hr postoperatively percutaneous renal biopsies were performed under an *im* injection of ketamine hydrochloride (20 $mg \cdot kg^{-1}$) together with diazepam (0.5 $mg \cdot kg^{-1}$) and atropine (0.05 $mg \cdot kg^{-1}$). A Bard Monopty Biopsy Instrument (Covington, GA, USA) was employed and the needle was guided by ultrasonography (Sigma AC Start; Kontron Instruments, Montigny le Bretoneux, France). The following variables were recorded: plasma creatinine levels as a measure of kidney function; renal myeloperoxidase (MPO) as a measure of the degree of neutrophil infiltration into tissues; plasma proinflammatory cytokine levels, tumour necrosis factor-α (TNF-α) interleukin 1-ß (IL-1ß), and interferon-gamma γ (INF-γ); renal superoxide anion (SOA) and its detoxifying enzyme superoxide-dismutase (SOD), and renal malondialdehyde (MDA) to assess lipid peroxidation. Finally, the activity of inducible nitric oxide synthase (iNOS) was assessed 72 hr after surgery. On the seventh day, the animals were anesthetized using the techniques described above, and after the withdrawal of blood and kidney samples, were sacrificed by administration of *iv* potassium chloride.

Determination of plasma creatinine levels

As a marker of renal function, serum creatinine concentrations were determined, using a Hitachi 747-200 automatic analyzer (Boerhinger Mannheim, Indianapolis, IN, USA).

Determination of MPOin kidney tissue

The presence of MPO, an enzyme specific for neutrophils and used as an index for the assessment of neutrophil accumulation, was determined in kidney tissue with the method of Bradley,[13] as modified by Mullane.[14] We have reported this technique previously.[15,16] After collection, the samples were immediately weighed on ice, homogenized in phosphate buffer, frozen in liquid nitrogen and stored at -80°C until assayed. A double-beam spectrophotometer was used. Kidney samples were obtained by direct or percutaneous biopsy.

Determination of proinflammatory cytokines

Commercial kits were used for the determination of TNF-α, IL-1ß and INF-γ (factor-XX ELISA kit, A. Menarini Diagnostics, Menarini Labs, Badalona, Spain) based on enzyme-linked immunosorbent assay (ELISA). Recordings were carried out on a plate reader (GEST, General Elisa System Technology, Menarini) for the automatic ELISA technique, with the possibility of reading three plates at the same time. Both the supernatants from cell cultures and the serum samples could be tested with the kit. In our case, we used blood samples, which must not be hemolyzed. Immediately after withdrawal, the blood samples were centrifuged and the serum separated, divided into aliquots and placed in Eppendorf tubes. These were then frozen and stored at -80°C in a manner to avoid freezing and thawing processes.

The assay is an ELISA kit that uses many different antibodies. First, we used a 96-well plate with an anti-cytokine monoclonal antibody adherant to each well, to capture the corresponding cytokine present in the samples and standards, which were added in duplicate to each of the wells together with the corresponding blanks. After the plates had been washed to remove the non-adhering material, polyclonal anticytokine-conjugated peroxidase was added. Then, the plates were washed again to remove the non-adhering material and the substrate solution was added, thus initiating the peroxidase catalysis. The colour change was achieved by acidification. Absorbance was measured on a plate reader (General Elisa System Technology, Menarini) at a wavelength of 450 nm, the results obtained being proportional to the amounts of cytokine in the samples, which were calculated by interpolation with the curve of the standards.

Determination of oxygen free radicals in kidney tissue
Once withdrawn, for washing, the samples were placed in the homogenized buffer at a temperature between 0° and 4°C to minimize oxidative processes. Samples were then weighed and homogenized. The homogenates were centrifuged at 100,000 x g for 60 min at 4°C. The soluble fraction obtained was divided into aliquots and placed in Eppendorf tubes and stored at -80°C until sample processing. Determination of the rate of SOA production was accomplished with a modification of the technique described by Forman and Boveris.[17] Protein concentrations were measured spectrophotometrically, using the Bradford method.[18] The enzyme activity of SOD was measured following the technique reported by Misra *et al.*[19] We have reported all these techniques previously.[15,16]

Determination of malondialdehyde in kidney tissue
Malondialdehyde and four-hydroxyalkanes are the most important products in the decomposition of unsaturated fatty acids. Quantification of these aldehydes is a suitable index of lipid peroxidation.[20]

Malondialdehyde was determined in kidney samples using the following reagents: 0.1 M phosphate buffer, pH 7.4, and a stock solution composed of 15% trichloroacetic acid, 0.37% thiobarbituric acid and 0.25 N hydrochloric acid. Water was added to a final volume of 50 mL. The samples were weighed and 10 mL of the buffer was added, after which the tissue was homogenized. Then, 1 mL of the homogenate was mixed with 2 mL of the stock solution, incubating the resulting mixture for 30 min at 100°C. It was then cooled on ice and centrifuged for 15 min at 3000 rpm and left to stand. The supernatant was read at a wavelength of 535 nm.

Expression of iNOS in kidney tissue
Kidney samples were obtained by percutaneous biopsy (72 hr after surgery). The samples were immediately frozen in liquid nitrogen and stored in sealed tubes (-80°C). The frozen material was sliced and 3 mL of lysis buffer (40 mM NaCl, 15 mM EDTA, 10% glycerol, 20 mM Tris base, pH 8) per gram of tissue was added at 4°C. Two protease inhibitors were added to this buffer: 2 mM phenylmethylsulfonylfluoride and 50 $\mu g \cdot mL^{-1}$ of trypsin inhibitor. The mixture was homogenized at 4°C and the residues of the lyzate were collected in an Eppendorf tube, passing them through a 21-G needle. The samples were kept on ice for 30-60 min and then centrifuged at 4°C and 15.000 x g for 20 min (Mikro 12–24 centrifuge, Hettich, Germany). The supernatant was stored in aliquots at -20°C for determination of protein content according to the method of Bradford[18] and for Western blot assays. Primary and secondary antibodies were used (Santa Cruz Biotechnology, Santa Cruz, CA, USA). After digitization of the radioautographs (Scanner® model VM 6552 Trust, Dordrecht, the Netherlands and the Adobe Photoshop™ 3.0 program, Uxbridge, UK), optical densities were read with the MacBAS V2.2 program (Fuji Medical Systems Inc., Stamford, CT, USA).

Statistical analyses
Data were analyzed using the Fisher exact test and analysis of variance (Student-Newman-Keuls or Scheffe test for normally distributed data and the Kruskall-Wallis Z test for data not normally distributed). Results are expressed as means ± standard error of the mean. Statistical significance was set at P value of < 0.05.

Results

Survival
All animals survived with a functioning aortic bypass, regardless of group allocation, and were healthy after seven days

Kidney function
Plasma creatinine concentrations after IR were significantly increased over time in both groups in comparison with the basal levels observed before the start of surgery ($P < 0.01$). After 24 hr, the magnitude of the change was significantly smaller in the animals anesthetized with propofol ($P < 0.05$). On the seventh day, creatinine values tended to decrease in both groups (Figure 1).

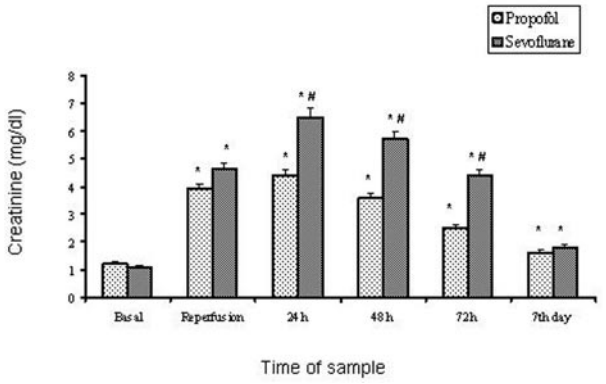

FIGURE 1 Serum concentrations of creatinine for each anesthetic group. Data are presented as mean ± standard error of the mean. * $P < 0.01$ *vs* basal; # $P < 0.05$ *vs* propofol.

TABLE I Myeloperoxidase activity in renal tissue

MPO (UI·g^{-1})	*Propofol (n = 10)*	*Sevoflurane (n = 10)*
Basal	3.6 ± 0.8	3.7 ± 0.5
Reperfusion	30.5 ± 6.3*	31.4 ± 7.4*
24 hr	39.5 ± 8.5*	68.4 ± 11.3*†
48 hr	101.6 ± 20.1*	228.1 ± 45.4*†
72 hr	118.7 ± 24.9*	249.6 ± 51.2*†
7th day	10.3 ± 2.2*	30.4 ± 6.8*

MPO = myeloperoxidase. Data are presented as mean ± standard error of the mean. * $P < 0.01$ *vs* basal; † $P < 0.05$ *vs* propofol.

TABLE II Serum concentrations of cytokines

Cytokine	*Propofol (n = 10)*	*Sevoflurane (n = 10)*
TNF-α (pg·mL^{-1})		
Basal	18.2 ± 4.1	18.7 ± 3.3
Reperfusion	114.1 ± 22.4*	114.4 ± 27.7*
24 hr	209.2 ± 38.9*	359.9 ± 50.2*†
48 hr	245.5 ± 52.7*	379.2 ± 67.8*†
72 hr	258.8 ± 52.6*	417.4 ± 68.5*†
7th day	125.5 ± 20.5*	132.2 ± 26.4*
IL-1ß (pg·mL^{-1})		
Basal	47.4 ± 9.5	50.8 ± 9.6
Reperfusion	111.8 ± 14.1*	112.2 ± 17.1*
24 hr	197.3 ± 12.8*	339.1 ± 29.6*†
48 hr	221.3 ± 30.5*	372.4 ± 36.4*†
72 hr	257.3 ± 34.4*	396.2 ± 39.9*†
7th day	106.5 ± 12*	109.8 ± 14.3*
INF-γ (pg·mL^{-1})		
Basal	38.4 ± 6.3	40.3 ± 4.3
Reperfusion	83.4 ± 11.9*	94.3 ± 17.4*
24 hr	196.7 ± 26.4*	330.3 ± 29.2*†
48 hr	197.2 ± 35.1*	363.9 ± 40.5*†
72 hr	199.4 ± 44.6*	345.6 ± 39.8*†
7th day	121.7 ± 23.2*	130.7 ± 21.7*

TNF-α = tumour necrosis factor-alpha; IL-1ß = interleukin-1beta; INF-γ = interferon-gamma. Data are presented as mean ± standard error of the mean. * $P < 0.01$ *vs* basal; † $P < 0.05$ *vs* propofol.

Myeloperoxidase activity in kidney tissue

A significant increase was observed over time with respect to activity of MPO in the kidney tissue of both groups of animals after IR ($P < 0.01$). In the sevoflurane group, from 24 hr and at 48 and 72 hr, a significant increase ($P < 0.05$) was seen in kidney MPO activity in comparison with the pigs anesthetized with propofol (68.65 ± 4 IU·g tissue, 228.1 ± 15.6 IU·g tissue, 249.6 ± 27.8 IU·g^{-1} tissue *vs* 39.5 ± 4 IU·g^{-1} tissue, 101.6 ± 20 IU·g^{-1} tissue, 118.7 ± 29.4 IU·g^{-1} of tissue, respectively, Table I).

Plasma proinflammatory cytokine levels

In the animals anesthetized with propofol, a smaller change in the cytokines TNF-α, IL-1ß and INF-γ was observed after IR as compared with the pigs anesthetized with sevoflurane ($P < 0.05$, Table II). The maximum amount of TNF-α was observed in the plasma of the animals anesthetized with sevoflurane at 72 hr in comparison with those receiving propofol during the same period (417.4 ± 68.5 pg·mL^{-1} *vs* 258.8 ± 52.6 pg·mL^{-1}). The same was the case with the IL-1ß plasma concentrations (396.2 ± 39.9 pg·mL^{-1} *vs* 257.3 ± 34.4 pg·mL^{-1}) and the concentrations of INF-γ (345.6 ± 39.8 pg·mL^{-1} *vs* 199.4 ± 44.6 pg·mL^{-1}).

Superoxide anion and superoxide-dismutase levels in kidney tissue

Superoxide anion and superoxide-dismutase levels in kidney tissue increased significantly in both groups after IR in comparison with the values observed following induction of anesthesia and before the start of surgery ($P < 0.01$). In the animals anesthetized with sevoflurane, from 24 hr, and up to 72 hr, increases in SOA and SOD concentrations were significantly greater than observed in the animals anesthetized with propofol ($P < 0.05$). In the determination carried out on the seventh day, a decrease in the concentrations of SOA and SOD was observed in both groups (Table III).

Malondialdehyde levels in kidney tissue

Ischemia-reperfusion was associated with a significant increase in the production of kidney MDA in both groups of animals ($P < 0.01$), but in animals anesthetized with sevoflurane, between 24 and 72 hr, a 1.5-fold increase in MDA levels was observed in com-

TABLE III Renal tissue oxygen free radicals

OFRs	*Propofol (n = 10)*	*Sevoflurane (n = 10)*
SOA (nmol·mg^{-1} protein·min^{-1})		
Basal	5.1 ± 0.8	5.4 ± 0.6
Reperfusion	95.1 ± 11.3*	95.9 ± 14.4*
24 hr	152 ± 22.5*	296.7 ± 29.3*†
48 hr	141.5 ± 24.1*	274.9 ± 31.4*†
72 hr	134.7 ± 20.9*	259.8 ± 30.2*†
7th day	29.6 ± 12.6*	30.4 ± 14.8*
SOD (U·mg^{-1} protein)		
Basal	55.5 ± 12.5	56 ± 11.1
Reperfusion	130.2 ± 31.4*	130.9 ± 38.4*
24 hr	241.7 ± 40.5*	369.5 ± 47.3*†
48 hr	200.9 ± 38.6*	327.9 ± 41.9*†
72 hr	166.4 ± 33.8*	285.1 ± 34.8*†
7th day	134.5 ± 35.7*	135.7 ± 39.5*

OFRs = oxygen free radicals; SOA = superoxide anion; SOD = superoxide dismutase. Data are presented as mean ± standard error of the mean. * $P < 0.01$ *vs* basal; † $P < 0.05$ *vs* propofol.

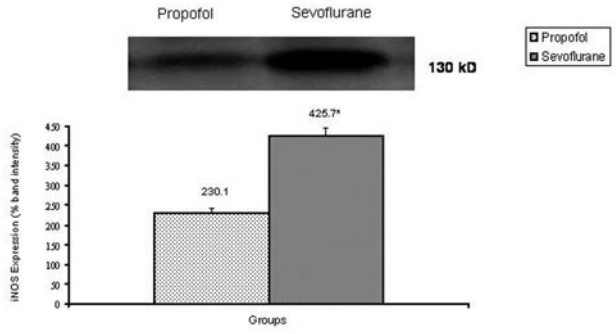

FIGURE 3 Inducible nitric oxide synthase (iNOS) expression in the kidney 72 hr after surgery. Top; a representative Western blot for iNOS. Bottom; mean ± standard error of the mean of two different blots. Animals anesthetized with sevoflurane (*$P < 0.01$) show increased iNOS levels with respect to animals anesthetized with propofol.

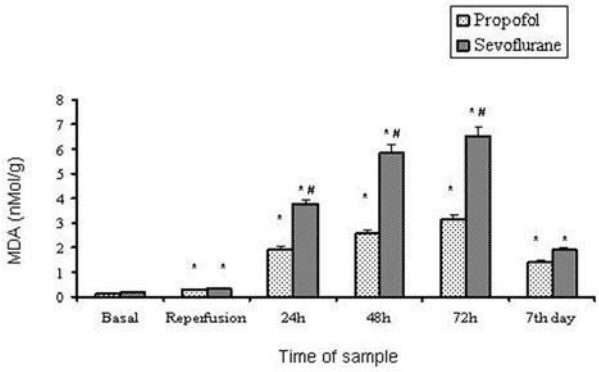

FIGURE 2 Malondialdehyde (MPO) activity in renal tissue for each anesthetic group. Data are presented as mean ± standard error of the mean. *$P < 0.01$ *vs* basal; #$P < 0.05$ *vs* propofol.

parison with measured concentrations in the animals anesthetized with propofol ($P < 0.05$, Figure 2).

Inducible nitric oxide synthase activity in kidney tissue

Ischemia-reperfusion was associated with greater activation of iNOS in kidney tissue at 72 hr in animals anesthetized with sevoflurane (iNOS protein band intensity 425.7%) as compared with those receiving propofol (iNOS protein band intensity 230.1%, $P < 0.05$, Figure 3).

Discussion

Aortic clamping elicits an IR syndrome that affects all organs and tissues (with different levels of tolerance to ischemia). Additionally, the existence of the SIR means that the sequelae are not only local, but may also affect several other distant organs, causing dysfunction and multiorgan failure (kidney, intestinal). Our findings show that renal ischemia after suprarenal aortic clamping induces severe kidney damage, characterized by an increase in plasma creatinine levels, neutrophil infiltration (as assessed by the increased MPO activity), an increase in oxygen free radicals (OFR) production (SOA and SOD) and an increase in plasma proinflammatory cytokine levels. The results reported here show that animals anesthetized with propofol have less neutrophil infiltration of renal tissue, lower plasma concentrations of proinflammatory cytokines, reduced OFR production, less lipid peroxidation, and less iNOS activity. These findings are important indicators of propofol's favourable moderation of altered renal function associated with aortic cross-clamping and Dacron tube grafting. It is surprising that the peak serum creatinine occurred at 24 hr and the other measures of mediators peaked at 72 hr, but we have not found a physiopathological explanation.

Usually under experimental conditions, renal function is completely recovered after seven days, even in situations of severe renal failure if there is no persistent hypotension or sepsis. Thus, we limited the duration of the study to seven days. Previous experimental work carried out by our group, with a clamping time of more than 30 min, induced situations of paraple-

gia, intra-abdominal infection, and/or postoperative mortality. It is notable that in this study, all animals survived with a functioning aortic bypass, regardless of group allocation, and were healthy after seven days

Several studies have demonstrated the antioxidant properties of propofol in both *in vivo* and *in vitro* models,[5–7] suggesting the potential benefit of the drug as an antioxidant in situations of IR that lead to SIR. Other investigators have also reported that, *in vivo*, propofol increases the antioxidant capacity of erythrocytes,[2,20] decreases lipid peroxidation and preserves myocardial contractile function,[21] while appearing to decrease OFR production.[22] Several *in vitro* studies demonstrate that propofol inhibits lipid peroxidation in human liver microsomes[23] and in different tissues of Wistar rats in which, additionally, increases in reduced glutathione levels have been reported.[24,25] Corcoran *et al.*,[26] showed that the administration of propofol before aortic cross-clamp release in patients undergoing elective coronary artery bypass graft surgery decreases myocardial lipid peroxidation, attenuates the inflammatory response to myocardial reperfusion, and limits the inflammatory cascade.

The antioxidant properties of propofol, observed both *in vivo* and *in vitro,* are evident at drug doses comparable to those used in clinical anesthesia to achieve plasma levels that will allow appropriate induction and maintenance of anesthesia.[5,20,22–24,27] Several studies suggest that it is the active principle of propofol and not its solvent (Intralipid®) which exerts the antioxidant action.[26] Certain immunomodulatory effects of propofol, such as suppression of respiratory bursts of neutrophils by propofol, may be caused by intralipid, while other actions, such as the ability to scavenge free radicals, appear to be a property of propofol itself.[6,8,23] Propofol's beneficial properties have been shown to be independent of intralipid.[26]

Attempts to link the propofol molecule and the observed antioxidant effect in SIR have been developed on the basis of different studies. Murphy *et al.*[6] reported that on the basis of its chemical structure, propofol must behave in a similar fashion to vitamin E, by binding to cell membranes or their phospholipids, reacting with the peroxylipid radicals that are formed when lipid peroxidation begins, and giving rise to a stable phenoxy- radical that would not propagate lipid peroxidation in the cell membrane. Kahraman *et al.*[8] suggest that propofol is in itself a scavenger of peroxynitrites, preventing or decreasing their noxious action. As in the case of others studies,[2,9,24,25] these authors propose that propofol would inhibit, or at least decrease lipid peroxidation. Studies on antioxidant defensive cell enzyme systems by De La Cruz *et al.*,[24,25] suggest that, at clinically relevant doses, apart from inhibiting lipid peroxidation, propofol may also act on enzyme systems (in particular on the glutathione system) which would lead to a decrease in the activity of glutathione peroxidase and an increase in the activities of glutathione reductase and glutathione transferase. This effect would yield an increase in cellular deposits of reduced glutathione, and hence in defensive cellular deposits of antioxidants, thereby protecting tissues from oxidative stress. Other studies have failed to demonstrate a protective effect of propofol on myocardial function during ischemia and reperfusion.[28,29] Javadov *et al.*,[30] suggest that the mechanism by which propofol may confer protection against myocardial reperfusion injury appears to be independent of adenosine triphosphate-sensitive potassium channels, and may be attributable to an action at the mitochondrial transition pore.

Our observation that propofol decreases lipid peroxidation, is consistent with other investigations.[5,20,21] We observed that MDA values were significantly lower in the animals receiving propofol from 24 up to 72 hr. The reduced neutrophil infiltration observed in the group of animals anesthetized with propofol, reflected in the lower activity of kidney MPO after reperfusion, is an important element in identifying the possible mechanism of propofol's moderation of the SIR. Neutrophils play a crucial role in the propagation of the damage caused by ischemia, and above all by reperfusion, since these white blood cells are able to release cytokines, oxygen free radicals and other proinflammatory substances. Kato *et al.*,[4] reported that in their *in vitro* experimental models, propofol decreased neutrophil activity, although they failed to offer any mechanism of action through which this might occur. Sevoflurane also appears to exhibit cardioprotective effects against reperfusion injury, and this effect has been attributed to its free radical scavenging properties and the reduction of postischemic adhesion of neutrophils.[31,32] Recently, a small study comparing sevoflurane with propofol showed that sevoflurane was superior to propofol in terms of reperfusion injury and contractile function in humans,[33] although the two groups were not homogenous with respect to preoperative ejection fraction and the number of surgical techniques. In our study, in animals anesthetized with propofol, we observed lower plasma inflammatory cytokine levels, these being able to stimulate and increase neutrophil infiltration. Neutrophils are responsible for the synthesis of these cytokines, and in turn are targets of the actions of the latter. Additionally, TNF-α is a potent inducer of SOA release by other neutrophils, which would increase

damage to other cells, amongst them, endothelial cells, favouring a greater migration of neutrophils.[34] The lower TNF-α levels in the animals anesthetized with propofol would decrease this process of autocrine neutrophil activation.

Tumour necrosis factor-α, IL-1ß and INF-γ are able to induce iNOS expression in different types of cells;[35–37] this response to the mediators of inflammation is associated with an excessive production of nitric oxide (NO) and may serve an additional role as part of the innate immune response-programmed cell death. The role of NO in IR is controversial, but its cytotoxic effect is believed to be due to the formation of free radicals derived from NO, such as the peroxynitrites generated when NO is combined with SOA. Furthermore, NO is a potent vasodilator, and it is known that the reestablishment of blood flow to ischemic tissues may exacerbate the tissue lesion, leading to a lesion due to reperfusion. Inhibition of iNOS activity would lead to a decrease in the tissue lesion due to reperfusion.[38] The decrease in cytokine levels in animals anesthetized with propofol is associated with reduced activation of iNOS in reperfusion, with a reduced release of NO, less vasodilatation, less migration and neutrophil infiltration, and reduced peroxynitrite formation, accompanied by less extensive tissue injury. In our case, the reduced formation of peroxynitrites could be explained on the basis of either a reduced activation of iNOS, or reduced production of SOA observed in the animals anesthetized with propofol.

Indeed, we observed less iNOS activity in animals anesthetized with propofol at 72 hr after surgery; this could be explained by the lower levels of proinflammatory cytokines and a reduced production of SOA. Inducible NO synthase activity is controlled mainly by gene expression and mainly at transcriptional levels through the activation of several transcription factors, such as nuclear transcription factor kappa B (NFκB).[39] In some *in vitro* studies carried out in experimental models with macrophages cultured in a medium with lipopolysaccharide (an inducer of iNOS) propofol was added at clinical therapeutic concentrations; the authors reported an inhibition of iNOS activity and of the mRNA for iNOS. All this suggests a possible direct action of propofol at transcription factor level that would govern iNOS activity,[40] or alternatively, an indirect action through the decreases in SOA, TNF-α and IL-1ß, each being an inducer of NFκB expression.[41,42] In our study, after the IR in animals anesthetized with propofol, we observed a decrease in SOA, TNF-α and Il-1ß levels, which could lead to a reduced expression of NFκB.

In conclusion, the results of this *in vivo* experimental model of reversible renal IR show that, in comparison with sevoflurane, clinically relevant doses of propofol decrease lipid peroxidation and increase the antioxidant capacity of the kidney. Propofol also plays a role in modulation of the mechanisms involved in SIR, with reduced accumulation of neutrophils in kidney tissue, and moderation of the release of proinflammatory cytokines. Our results also show that propofol has the ability to reduce the synthesis of NO through the inhibition of renal iNOS during reperfusion. The cumulative effects result in a moderation of altered renal function associated with aortic cross clamping and Dacron grafting. In clinical practice, whether this protective action of propofol exerts a beneficial outcome effect in patients presenting for major vascular surgery warrants further investigation.

References

1 *Ross S, Foex P.* Protective effects of anaesthetics in reversible and irreversible ischaemia-reperfusion injury. Br J Anaesth 1999; 82: 622–32.

2 *Runzer TD, Ansley DM, Godin DV, Chambers GK.* Tissue antioxidant capacity during anesthesia: propofol enhances in vivo red cell and tissue antioxidant capacity in a rat model. Anesth Analg 2002; 94: 89–93.

3 *Huang CJ, Slovin PN, Nielsen RB, Skimming JW.* Diprivan attenuates the cytotoxicity of nitric oxide in cultured human bronchial epithelial cells. Intensive Care Med 2002; 28: 1145–50.

4 *Kato R, Foex P.* Myocardial protection by anesthetic agents against ischemia-reperfusion injury: an updater for anesthesiologist. Can J Anesth 2002; 49: 777–91.

5 *Ansley DM, Lee JU, Godin DV, Garnett ME, Qayumi AK.* Propofol enhances red cell antioxidant capacity in swine and humans. Can J Anaesth 1998; 45: 233–9.

6 *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.

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

8 *Kahraman S, Demiryurek AT.* Propofol is a peroxynitrite scavenger. Anesth Analg 1997; 84: 1127–9.

9 *Mouithys-Mickalad A, Hans P, Deby-Dupont G, Hoebeke M, Deby C, Lamy M.* Propofol reacts with peroxynitrite to form a phenoxyl radical: demonstration by electron spin resonance. Biochem Biophys Res Commun 1998; 249: 833–7.

10 *Warltier DC, Al-Wathiqui MH, Kampine JP, Schmeling WT.* Recovery of contractile function of stunned myocardium in chronically instrumented dogs is enhanced by halothane or isoflurane. Anesthesiology 1988; 69:

552–65.

11 *Marijic J, Stowe DF, Turner LA, Kampine JP, Bosnjak ZJ.* Differential protective effects of halothane and isoflurane against hypoxic and reoxigenation injury in the isolated guinea pig heart. Anesthesiology 1990; 73: 976–83.

12 *Varadarajan SG, An J, Novalija E, Stowe DF.* Sevoflurane before or after ischemia improves contractile and metabolic function while reducing myoplasmic Ca^{2+} loading in intact hearts. Anesthesiology 2002; 96: 125–33.

13 *Bradley PP, Priebat DA, Christensen RD, Rothstein G.* Measurement of cutaneous inflammation: estimation of neutrophil content with an enzyme marker. J Invest Dermatol 1982; 78: 206–9.

14 *Mullane KM, Kraemer R, Smith B.* Myeloperoxidase activity as a quantitative assessment of neutrophil infiltration into ischemic myocardium. J Pharmacol Methods 1985; 14: 157–67.

15 *García-Criado FJ, Eleno N, Santos-Benito F, et al.* Protective effect of exogenous nitric oxide on the renal function and inflammatory response in a model of ischemia-reperfusion. Transplantation 1998; 66: 982–90.

16 *Lozano FS, García-Criado FJ, Fresnadillo MJ, García E, García JE, Gómez-Alonso A.* Systemic inflammatory response induced by dacron graft and modulation by antimicrobial agents: experimental study. J Surg Res 2002; 107: 7–13.

17 *Forman HJ, Boveris A.* Superoxide radical and hydrogen peroxide in mitochondria. *In*: Pryor WA (Ed.). Free Radicals in Biology, vol V. New York: Academic Press; 1982; 5: 65–90.

18 *Bradford MM.* A rapid and sensitive method for the quantitation of microgram quantites of protein utilizing the principle of protein-dye binding. Anal Biochem 1976; 72: 248–54.

19 *Misra HP, Fridovich Y.* The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase. J Biol Chem 1972; 247: 3170–5.

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

21 *Yoo KY, Yang SY, Lee J, et al.* Intracoronary propofol attenuates myocardial but not coronary endothelial dysfunction after brief ischaemia and reperfusion in dogs. Br J Anaesth 1999; 82: 90–6.

22 *Cheng YJ, Wang YP, Chien CT, Chen CF.* Small-dose propofol sedation attenuates the formation of reactive oxygen species in tourniquet-induced ischemia-reperfusion injury under spinal anesthesia. Anesth Analg 2002; 94: 1617–20.

23 *Bao YP, Williamson G, Tew D, et al.* Antioxidant effects of propofol in human hepatic microsomes: concentration effects and clinical relevance. Br J Anaesth 1998; 81: 584–9.

24 *De La Cruz JP, Villalobos MA, Sedeño G, Sánchez de la Cuesta F.* Effect of propofol on oxidative stress in an in vitro model of anoxia-reoxygenation in the rat brain. Brain Res 1998; 800: 136–44.

25 *De La Cruz JP, Sedeño G, Carmona JA, Sánchez de la Cuesta F.* The in vitro effects of propofol on tissular oxidative stress in the rat. Anesth Analg 1998; 87: 1141–6.

26 *Corcoran TB, Engel A, Sakamoto H, et al.* The effects of propofol on lipid peroxidation and inflammatory response in elective coronary artery bypass grafting. J Cardiothorac Vasc Anesth 2004; 18: 592–604.

27 *Grasshoff C, Gillessen T.* The effect of propofol on increased superoxide concentration in cultured rat cerebrocortical neurons after stimulation of N-methyl-D-aspartate receptors. Anesth Analg 2002; 95: 920–2.

28 *Ross S, Muñoz H, Piriou V, Ryder A, Foex P.* A comparison of the effects of fentanyl and propofol on left ventricular contractility during myocardial stunning. Acta Anaesthesiol Scand 1998; 42: 23–31.

29 *Ebel D, Schlack W, Comfere T, Preckel B, Thamer V.* Effect of propofol on reperfusion injury after regional ischaemia in the isolated rat heart. Br J Anaesth 1999; 83: 903–8.

30 *Javadov SA, Lim KH, Kerr PM, Suleiman MS, Angelini GD, Halestrap AP.* Protection of hearts from reperfusion injury by propofol is associated with inhibition of the mitochondrial permeability transition. Cardiovasc Res 2000; 45: 360–9.

31 *Kowalski C, Zahler S, Becker BF, et al.* Halothane, isoflurane, and sevoflurane reduce postischemic adhesion of neutrophils in the coronary system. Anesthesiology 1997; 86: 188–95.

32 *Heindl B, Reichle FM, Zahler S, Conzen PF, Becker BF.* Sevoflurane and isoflurane protect the reperfused guinea pig heart by reducing postischemic adhesion of polymorphonuclear neutrophils. Anesthesiology 1999; 91: 521–30.

33 *De Hert SG, ten Broecke PW, Mertens E, et al.* Sevoflurane but not propofol preserves myocardial function in coronary surgery patients. Anesthesiology 2002; 97: 42–9.

34 *García-Criado FJ, Palma-Vargas JM, Valdunciel JJ, et al.* Tacrolimus (FK506) down-regulates free radical tissue levels, serum cytokines, and neutrophil infiltration after severe liver ischemia. Transplantation 1997; 64: 594–8.

35 *MacNaul KL, Hutchinson NI.* Differential expression of iNOS and cNOS mRNA in human vascular

smooth muscle cells and endothelial cells under normal and inflammatory conditions. Biochem Biophys Res Commun 1993; 196: 1330–4.

36 *Beasley D, Schwartz JH, Brenner BM.* Interleukin-1 induces prolonged L-arginine-dependent cyclic guanosine monophosphate and nitrite production in rat vascular smooth muscle cells. J Clin Invest 1991; 87: 602–8.

37 *Balligand JL, Ungureanu-Longrois D, Simmons WW, et al.* Induction of NO synthase in rat cardiac microvascular endothelial cells by IL-1ß and INF-γ. Am J Physiol 1995; 268: H1293–303.

38 *Wang P, Zweier JL.* Measurement of nitric oxide and peroxynitrite generation in the postischemic heart. Evidence for peroxynitrite-mediated reperfusion injury. J Biol Chem 1996; 271: 29223–30.

39 *Xie QW, Kashiwabara Y, Nathan C.* Role of transcription factor NF- κB/Rel in induction of nitric oxide synthase. J Biol Chem 1994; 269: 4705–8.

40 *Chen RM, Wu GJ, Tai YT, et al.* Propofol reduces nitric oxide biosynthesis in lipopolysaccharide-activated macrophages by downregulating the expression of inducible nitric oxide synthase. Arch Toxicol 2003; 77: 418–23.

41 *Lowenthal JW, Ballard DW, Bohnlein E, Greene WC.* Tumor necrosis factor alpha induces proteins that bind specifically to kappa B-like enhancer elements and regulate interleukin 2 receptor alpha-chain gene expression in primary human T lymphocytes. Proc Natl Acad Sci USA 1989; 86: 2331–5.

42 *Osborn L, Kunkel S, Nabel GJ.* Tumor necrosis factor alpha and interleukin 1 stimulate the human immunodeficiency virus enhancer by activation of the nuclear factor kappa B. Proc Natl Acad Sci USA 1989; 86: 2336–40.