Intensive Care Med (2007) 33:845–850
DOI 10.1007/s00134-007-0585-3

BRIEF REPORT

Christian Maier
Angelika Scheuerle
Balázs Hauser
Hubert Schelzig
Csaba Szabó
Peter Radermacher
Jochen Kick

The selective poly(ADP)ribose-polymerase 1 inhibitor INO1001 reduces spinal cord injury during porcine aortic cross-clamping-induced ischemia/reperfusion injury

Received: 7 December 2006
Accepted: 12 February 2007
Published online: 15 March 2007

Electronic supplementary material
The online version of this article (doi:10.1007/s00134-007-0585-3) contains supplementary material, which is available to authorized users.

C. Maier and A. Scheuerle equally contributed to this manuscript.

C. Maier · B. Hauser · P. Radermacher (✉)
Universitätsklinikum, Sektion Anästhesiologische Pathophysiologie und Verfahrensentwicklung,
89073 Ulm, Germany
e-mail:
peter.radermacher@medizin.uni-ulm.de
Tel.: +49-731-50060160
Fax: +49-731-50060162

C. Maier · H. Schelzig · J. Kick
Universitätsklinikum, Abteilung Thorax- und Gefäßchirurgie,
89070 Ulm, Germany

A. Scheuerle
Universitätsklinikum, Sektion Neuropathologie,
89081 Ulm, Germany

B. Hauser
Semmelweis Egyetem, Aneszteziológiai és Intenzív Terápiás Klinika,
1125 Budapest, Hungary

C. Szabó
University of Medicine and Dentistry, Department of Surgery,
Newark N.J., USA

Abstract *Objective:* It is well-established that poly(ADP)ribose-polymerase (PARP) assumes major importance during ischemic brain damage, and the selective PARP-1 inhibitor PJ34 reduced spinal cord damage in murine aortic occlusion-induced ischemia/reperfusion injury. We investigated the effect of the PARP-1 inhibitor INO1001 on aortic-occlusion-related porcine spinal cord injury. *Design and setting:* Prospective, randomized, controlled experimental study in an animal laboratory. *Patients and participants:* Ten anesthetized, mechanically ventilated, and instrumented pigs. *Interventions:* Animals underwent 45 min of thoracic aortic cross-clamping after receiving vehicle ($n = 5$) or intravenous INO1001 ($n = 5$, total dose 4 mg/kg administered both before clamping and during reperfusion). During reperfusion continuous intravenous norepinephrine was incrementally adjusted to maintain blood pressure at or above 80% of the preclamping level. Plasma INO1001 levels were analyzed by HPLC. After 4 h of reperfusion spinal cord biopsy samples were analyzed for neuronal damage (hematoxyline-eosine and Nissl staining), expression of the cyclin-dependent kinase inhibitor genes p21 and p27 (immunohistochemistry), and apoptosis (terminal deoxynucleotidyl transferase mediated nick end labeling assay). *Measurements and results:* Plasma INO1001 levels were 0.8–2.3 and 0.30–0.76 mM before and after clamping, respectively. While 3–5% of the spinal cord neurons were irreversibly damaged in the INO1001 animals, the neuronal cell injury was three times higher in the control group. Neither p21 and p27 expression nor apoptosis showed any intergroup difference. *Conclusions:* The selective PARP-1 inhibitor INO1001 markedly reduced aortic occlusion-induced spinal cord injury. Given the close correlation reported in the literature between morphological damage and impaired spinal cord function, INO1001 may improve spinal cord recovery after thoracic aortic cross-clamping.

Keywords Poly(ADP)ribose-polymerase 1 · Aortic cross-clamping · Spinal cord · Nissl staining · Cyclin dependent kinase inhibitor gene · p21, p27

Introduction

It is well-established that poly(ADP)ribose-polymerase (PARP) assumes major importance during ischemic brain damage [1], and that PARP inhibitors attenuate tissue injury after transient cerebral ischemia [2–6]. Furthermore, the PARP inhibitor PJ34 reduced spinal cord injury in a murine model of thoracoabdominal aortic ischemia-reperfusion injury [7]. We have recently demonstrated in a clinically relevant porcine model of ischemia/reperfusion (I/R) injury induced by thoracic aortic cross-clamping that the novel, highly selective PARP-1 inhibitor INO1001 facilitated hemodynamic stabilization during the early reperfusion period without affecting DNA integrity or repair [8]. Therefore in the present study we tested the hypothesis that INO1001 would reduce spinal cord injury [8, 9]. The data presented were obtained in a subset of animals that had been studied in the experimental series investigating the hemodynamic and renal effects of INO1001 [8].

Material and methods

The experiments were performed in adherence to the National Institute of Health Guidelines on the Use of Laboratory Animals. The experimental protocol was approved by the University Animal Care Committee and the federal authorities for animal research (Tübingen, Germany). Ten domestic pigs of either sex with a mean body weight of 48 g (range 43–58) were used. The anesthesic procedure, surgical preparation, placement of catheters, physiological measurements [8, 9], and measurement of INO1001 [10] and tumor necrosis factor-α [11] plasma concentrations have been described in detail previously.

Postmortem spinal cord analysis

In addition to hematoxylin-eosin staining, postmortem spinal cord cross-sections were analyzed after nuclear cresyl violet staining (Nissl staining) [12]. Immunohistochemistry for p21 and p27 gene expression and the determination of the number of apoptotic nuclei were performed using specific antibodies and the terminal deoxynucleotidyl transferase mediated nick end labeling (TUNEL) assay as described previously [8, 9, 13, 14].

Experimental protocol

After 120 min of postsurgery recovery time animals were randomly assigned to receive either the vehicle (glucose 5%) or the INO1001 starting 90 min before clamping. Animals received a total of 4 mg kg^{-1} INO1001: after an initial bolus (2 mg kg^{-1} h^{-1} over 30 min) INO1001 (1 mg kg^{-1} h^{-1}) was infused for 60 min until immediately before clamping, stopped during the clamping period, and restarted again (0.5 mg kg^{-1} h^{-1}) after declamping during the remaining 4 h of the experiment. This approach was chosen so that plasma levels were higher immediately prior to clamping in order to provide a sufficient "loading up" of the tissues. Furthermore, total doses of 1 mg kg^{-1} and 4 mg kg^{-1} of INO1001 in dogs [15–17] and pigs [18] had virtually completely abolished poly(ADP-ribose)-staining in the heart, lung, and intestine after cardiopulmonary bypass. After baseline data collection the aorta was occluded for 45 min, which was verified by the disappearance of the blood pressure signal distal (MAPdist) to the clamping. This clamping period was chosen to avoid both the large spinal cord infarction over several segments reported in pigs after a clamping period of 60 min or longer [19] and the fairly mild histological damage observed after only 30 min of clamping [9, 12]. During the clamping period intravenous nitroglycerin (1.7 mg min^{-1}), esmolol (16.5 mg min^{-1}), and adenosine-5′-triphosphate (2–10 mg min^{-1}) were adjusted to maintain mean blood pressure proximal of the aortic tourniquet (MAPprox) at 80–120% of the baseline value. In addition to 10 ml kg^{-1} h^{-1} Ringer's solution infused throughout the experiment, 1500 ml hydroxyethylstarch was infused during the clamping period to optimize preload prior to the declamping and during the first 30 min of reperfusion to prevent declamping-associated hypotension. Continuous intravenous norepinephrine was incrementally adjusted as long as needed to maintain MAPprox higher than 80% of baseline. Further data sets were obtained 120 and 240 min after declamping. At the end of the experiment the spinal cord was taken for hematoxylin-eosin and Nissl staining as well as for immunohistochemistry.

Statistical analysis

All data are presented as median and range. After exclusion of normal distribution using the Kolmogorov–Smirnov test, Friedman's repeated measures analysis of variance on ranks with post-hoc multiple comparison procedure (Dunn's method) was used for data analysis within the experimental group. The Mann–Whitney rank sum test was performed to analyze intergroup differences. Differences with a p value less than 0.05 were regarded as statistically significant.

Results

Hemodynamic, gas exchange, and acid-base data are summarized in the accompanying Electronic Supplementary Material (ESM; S.T1). Except for a clinically negligible, albeit statistically significant lower MAPprox

and MAPdist in the INO1001-treated animals at the end of the experiment, none of hemodynamic, gas exchange, or acid-base variables or tumor necrosis factor-α levels showed any intergroup difference. The duration of the norepinephrine infusion and the total norepinephrine dose administered tended to be lower in the INO1001-treated animals, without, however, reaching statistical significance. S.F1 shows that the INO1001 plasma concentrations were always at least three times higher than 0.1 mM.

Immunohistochemistry showed extremely rare staining for apoptotic nuclei (data not shown), and staining for the cyclin-dependent kinase inhibitor (CDKI) genes p21 and p27 did not differ between the two groups (ESM, S.T2). Hematoxylin-eosin staining showed only minor inflammation as detected by the presence of lymphocytes, which also did not differ between the groups. By contrast, there was marked neuronal damage which presented as vacuolization and cytoplasmic swelling. Some vacuoles were surrounded by membranes, indicating that these vacuoles were due to lysosomal swelling as occurs during cellular edema. In the INO1001-treated animals this neuronal damage was markedly less pronounced, particularly in the central and anterior gray matter of the spinal cord. Typical examples are demonstrated in Fig. 1. This result was underscored by the Nissl staining (Fig. 2),

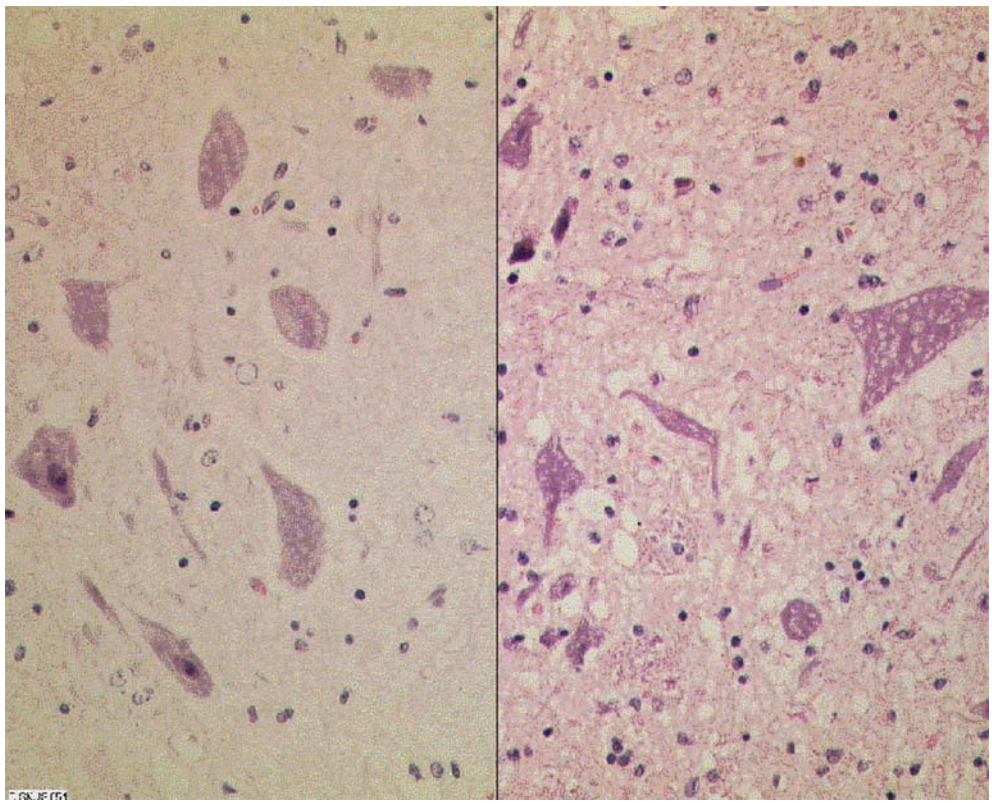

Fig. 1 Hematoxylin-eosin staining in an INO1001-treated animal (*left*) and an animal of the vehicle group (*right*). Note the well-maintained nerve cells in the central and anterior gray matter of the spinal cord after treatment with INO1001, while the control animal (*right*) showed strongly swollen nerve cell with cytoplasmic vacuolization and a disintegrating nerve cell, with greatly reduced staining

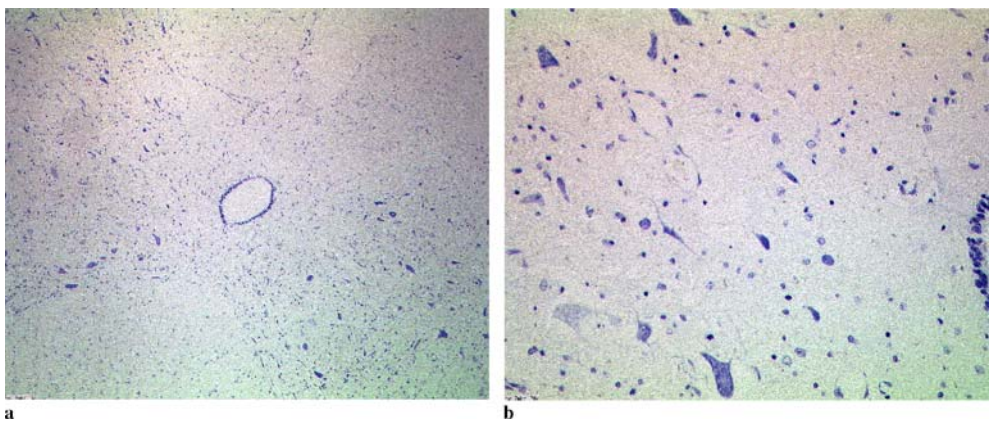

Fig. 2 Cresyl-violet (Nissl) staining in a control animal showing overview of the spinal cord near the central canal with basally contiguous sections of the anterior gray matter at × 5 (**a**) and a frontal section of the central and anterior gray matter of the spinal cord × 20 (**b**) magnification, the latter demonstrating disintegrating nerve cells, with greatly reduced staining

Table 1 Proportion of damaged neurons in the total number of neurons; data are medians of the mean of all fields of view in each animal (*parentheses* range)

	Control	INO1001	*p*
Neurons close to the spinal canal	11% (5–14)	3% (2–5)	0.008
All neurons (anterior horn and close to spinal canal)	14% (10–14)	4% (3–6)	0.008

the quantitative analysis showing a 70% reduction in the number of damaged neurons as a fraction of the total number of neurons evaluated (see Table 1).

Discussion

The present study tested the hypothesis that the protective effect of the selective PARP-1 inhibitor PJ34 reported in a murine model could be confirmed using another PARP-1 inhibitor, INO1001, in a clinically relevant porcine model of thoracic aortic cross-clamping-induced I/R injury. Swine were investigated because of their striking similarity with humans with respect to both the arterial vascularization of the spinal cord [20–22] and their susceptibility to oxidative stress and tissue antioxidant profiles [23, 24].

Our data that INO1001 reduces neuronal damage in the spinal cord confirm previous data using PJ34 in mice [7]. However, no difference was observed in the expression of the CDKI genes p21 and p27 or in the number of apoptotic nuclei. In fact, we found hardly any apoptotic cells, regardless of the treatment. This observation seems to be in contrast with literature findings reporting a reduced number of TUNEL-positive cells 24 h postischemia in rats after pretreatment with the PARP inhibitor 3-aminobenzamide prior to a 2-h middle cerebral artery occlusion [5]. Other authors, however, have not found different density of TUNEL labeling despite a decreased total number of TUNEL-positive cells reflecting the reduced infarct size in $PARP^{-/-}$ mice and animals pretreated with 3-aminobenzamide [2]. Equivocal reports are available on the occurrence of apoptotic cells after spinal cord I/R injury: Short-term aortic occlusion of 15–40 min was affiliated with neuronal apoptosis in rodents [25–28], but more recent studies have not confirmed these findings [29, 30]. Our observation of marked neuronal damage without TUNEL-positive neurons well agrees with recent data by Papakostas et al. [31] who also did not find TUNEL-positive neurons after 45 min of aortic occlusion in pigs, which was referred to motor neuron death from necrosis. Finally, our findings are further underscored by the similar p21 and p27 expression: albeit the enhanced expression of p21 in neurons surviving transient forebrain ischemia was referred to as an adaptive response to cerebral ischemia and reperfusion [32] and spinal cord demyelination [33, 34], p21 and p27 expression in other tissues subjected to I/R injury [35–37] was directly related to the amount of DNA damage and/or apoptosis. Since the increased expression of the cell cycle regulatory CDKI genes is referred to facilitate DNA repair [38] rather than being a marker of neuronal cell death [39], it is consequently unlikely that different CDKI gene expression is observed when only negligible apoptosis is present.

Limitations of the study

One limitation of our study certainly is that we do not have a direct confirmation of efficient PARP blockade since we performed neither tissue poly(ADP-ribose) staining nor direct measurements of PARP activity. The INO1001 blood levels, however, were always at least three times higher than those previously resulting in near-complete PARP inhibition [10, 40, 41]. Furthermore, the INO1001 dose chosen in our experiment had abolished tissue poly(ADP-ribose)-staining after cardiopulmonary bypass [15–18], hence suggesting efficient PARP inhibition. Unfortunately, we were unable to assess spinal cord function using neurological scoring or evoked motor potentials. Thus we can only speculate whether the reduced spinal cord morphological damage was associated with improved neurological outcome. It should be noted, however, that several previous reports from porcine models with aortic occlusion times of 30–60 min showed a close correlation between the degree of histomorphological damage in the spinal cord and the severity of neurological impairment using the Tarlov scale [12, 19, 31, 42–44] and/or evoked motor potentials [19]. Finally, recent experiments showed that after 45 min of aortic occlusion resulted in complete loss of evoked motor potentials [19] and paraplegia (unpublished data). Thus it is likely that the marked reduction in neuronal damage was affiliated with improved spinal cord function.

Conclusion

In a clinically relevant porcine model of aortic cross-clamping the selective PARP-1 inhibitor INO1001 markedly reduced the I/R-induced morphological spinal cord injury. Given the close correlation reported in the literature between spinal cord morphological damage and impaired function, PARP inhibition may be a promising approach to improve spinal cord recovery after aortic cross-clamping.

Acknowledgements. This study was supported by the Deutsche Forschungsgemeinschaft (DFG Sche 899/2-1). B.H. was the recipient of a Roman Herzog research fellowship of the Alexander von Humboldt Stiftung and the Gemeinnützige Hertie Stiftung. INO1001 was kindly provided by Drs. Garry Southan and Andrew Salzman (Inotek Pharmaceuticals Corp., Beverly, Mass., USA). Special thanks are dedicated to Wolfgang Siegler, Tanja Schulz, and Ingrid Eble for their skillful technical assistance.

References

1. Chiarugi A (2005) Poly(ADP-ribosyl)-ation and stroke. Pharmacol Res 52:15–24
2. Endres M, Wang ZQ, Namura S, Waeber C, Moskowitz MA (1997) Ischemic brain injury is mediated by the activation of poly(ADP-ribose)-polymerase. J Cereb Blood Flow Metab 11:1143–1151
3. Takahashi K, Pieper AA, Croul SE, Zhang J, Snyder SH, Greenberg JH (1999) Post-treatment with an inhibitor of poly(ADP-ribose)polymerase attenuates cerebral damage in focal ischemia. Brain Res 829:46–54
4. Takahashi K, Greenberg JH (1999) The effect of reperfusion on neuroprotection using an inhibitor of poly(ADP-ribose)polymerase. Neuroreport 10:2017–2022
5. Koh SH, Park Y, Song CW, Kim JG, Kim K, Kim J, Kim MH, Lee SR, Kim DW, Yu HJ, Chang DI, Hwang SJ, Kim SH (2004) The effect of PARP inhibitor on ischaemic cell death, its related inflammation and survival signals. Eur J Neurosci 20:1461–1472
6. Sharma SS, Munusamy S, Thiyagarajan M, Kaul CL (2004) Neuroprotective effect of peroxynitrite decomposition catalyst and poly(adenosine diphosphate-ribose)polymerase inhibitor alone and in combination in rats with focal cerebral ischemia. J Neurosurg 101:669–675
7. Casey PJ, Black JH, Szabó C, Frosch M, Albadawi H, Chen M, Cambria RP, Watkins MT (2005) Poly(adenosine diphosphate-ribose)polymerase inhibition modulates spinal cord dysfunction after thoracoabdominal aortic ischemia-reperfusion. J Vasc Surg 41:99–107
8. Hauser B, Gröger M, Ehrmann U, Albicini M, Brückner UB, Schelzig H, Venkatesh B, Li H, Szabó C, Speit G, Radermacher P, Kick J (2006) The PARP-1 inhibitor INO-1001 facilitates hemodynamic stabilization without affecting DNA repair in porcine aortic cross-clamping-induced ischemia/reperfusion. Shock 25:633–640
9. Kick J, Hauser B, Bracht H, Albicini M, Öter S, Simon F, Ehrmann U, Garrel C, Sträter J, Brückner UB, Leverve XM, Schelzig H, Speit G, Radermacher P, Muth CM (2006) Effects of a cantaloupe melon extract/wheat gliadin biopolymer during aortic cross-clamping. Intensive Care Med (in press)
10. Xiao CY, Chen M, Zsengeller Z, Li H, Kiss L, Kollai M, Szabó C (2005) Poly-(ADP-Ribose)polymerase promotes cardiac remodeling, contractile failure, and translocation of apoptosis-inducing factor in a murine experimental model of aortic banding and heart failure. J Pharmacol Exp Ther 312:891–898
11. Hauser B, Kick J, Iványi Z, Asfar P, Ehrmann U, Muth CM, Albicini M, Wachter U, Vogt J, Bauer M, Brückner UB, Bracht H (2006) Effects of 15-deoxy$^{\Delta\ 12:14}$-prostaglandin-J_2 during hyperdynamic porcine endotoxemia. Intensive Care Med 32:659–665
12. Maharajh GS, Pascoe EA, Halliday WC, Grocott HP, Thiessen DB, Girling LG, Cheang MS, Mutch WA (1996) Neurological outcome in a porcine model of descending thoracic aortic surgery. Left atrial-femoral artery bypass versus clamp/repair. Stroke 27:2095–2100
13. Schelzig H, Chkhotua AB, Wiegand P, Grosse S, Reis S, Art M, Abendroth D (2003) Effect of ischemia/reperfusion on telomere length and CDKI genes expression in a concordant ex-vivo hemoperfusion model of primate kidneys. Ann Transplant 8:17–21
14. Chkhotua AB, Schelzig H, Wiegand P, Grosse S, Reis S, Art M, Abendroth D (2005) Influence of ischaemia/reperfusion and LFA-1 inhibition on telomere lengths and CDKI genes in ex vivo haemoperfusion of primate kidneys. Transpl Int 17:692–698
15. Szabó G, Soos P, Mandera S, Heger U, Flechtenmacher C, Bahrle S, Seres L, Cziraki A, Gries A, Zsengeller Z, Vahl CF, Hagl S, Szabó C (2004) INO-1001 a novel poly(ADP-ribose)-polymerase (PARP) inhibitor improves cardiac and pulmonary function after crystalloid cardioplegia and extracorporal circulation. Shock 21:426–432
16. Szabó G, Soos P, Mandera S, Heger U, Flechtenmacher C, Seres L, Zsengeller Z, Sack FU, Szabó C, Hagl S (2004) Mesenteric injury after cardiopulmonary bypass: role of poly(adenosine 5'-diphosphate-ribose)polymerase. Crit Care Med 32:2392–2397
17. Szabó G, Soos P, Heger U, Flechtenmacher C, Bahrle S, Zsengeller Z, Szabó C, Hagl S (2005) Poly(ADP-ribose)polymerase inhibition attenuates biventricular reperfusion injury after orthotopic heart transplantation. Eur J Cardiothorac Surg 27:226–234
18. Khan TA, Ruel M, Bianchi C, Voisine P, Komjáti K, Szabó C, Sellke FW (2003) Poly(ADP-ribose)polymerase inhibition improves postischemic myocardial function after cardioplegia-cardiopulmonary bypass. J Am Coll Surg 197:270–277
19. Meylaerts SA, De Haan P, Kalkman CJ, Jaspers J, Vanicky I, Jacobs MJ (2000) Prevention of paraplegia in pigs by selective segmental artery perfusion during aortic cross-clamping. J Vasc Surg 32:160–170
20. Lazorthes G, Gouaze A, Zadeh JO, Santini JJ, Lazorthes Y, Burdin P (1971) Arterial vascularization of the spinal cord. J Neurosurg 35:253–262
21. Domisse GF (1974) The blood supply of the spinal cord. J Bone Joint Surg Br 56:225–235
22. Wadouh F, Lindemann EM, Arndt C, Hetzer R, Borst HG (1984) The arteria radicularis magna anterior as a decisive factor influencing spinal cord damage during aortic occlusion. J Thorac Cardiovasc Surg 88:1–10
23. Godin DV, Garnett ME (1992) Species-related variations in tissue antioxidant status. I. Differences in antioxidant enzyme profiles. Comp Biochem Physiol B Biochem Mol Biol 103:737–742
24. Godin DV, Garnett ME (1992) Species-related variations in tissue antioxidant status. II. Differences in susceptibility to oxidative challenge. Comp Biochem Physiol B Biochem Mol Biol 103:743–748

25. Kato H, Kanellopoulos GK, Matsuo S, Wu YJ, Jaquin MF, Hsu CY, Choi DW, Kouchoukos NT (1997) Protection of rat spinal cord from ischemia with dextorphan and cycloheximide: effects on necrosis and apoptosis. J Thorac Cardiovasc Surg 114:609–618
26. Kato H, Kanellopoulos GK, Matsuo S, Wu YJ, Jaquin MF, Hsu CY, Kouchoukos NT, Choi DW (1997) Neuronal apoptosis and necrosis following spinal cord ischemia in the rat. Exp Neurol 148:464–474
27. Kanellopoulos GF, Kato H, Wu Y, Dougenis D, Mackey M, Hsu CY, Kouchoukos NT (1997) Neuronal cell death in the ischemic spinal cord: the effect of methylprednisolone. Ann Thorac Surg 64:1279–1286
28. Mackey ME, Wu Y, Hu R, DeMaro JA, Jaquin MF, Kanellopoulos GK, Hsu CY, Kouchoukos NT (1997) Cell death suggestive of apoptosis after spinal cord ischemia in rabbits. Stroke 28:2012–2017
29. Kiyoshima T, Fukuda S, Mastumoto M, Iida Y, Oka S, Nakakimura K, Skabe T (2003) Lack of evidence for apoptosis as a cause of delayed onset paraplegia after spinal cord ischemia in rabbits. Anesth Analg 96:839–846
30. Lee JC, Hwang IK, Park SK, Yoo KY, Seo K, Kang TC, Oh YS, Won MH (2005) Histochemical and electron microscopic study in motor neuron degeneration following transient spinal cord ischemia at normothermic conditions in rabbits. Anat Histol Embryol 34:252–257
31. Papakostas JC, Matsagas MI, Toumpoulis IK, Malamou-Mitsi VD, Pappa LS, Gkrepi C, Anagnostopoulos CE, Kappas AM (2006) Evolution of spinal cord injury in a porcine model of prolonged aortic occlusion. J Surg Res 133:159–166
32. van Lookeren Campagne M, Gill R (1998) Increased expression of cyclin G1 and $p21^{WAF1/CIP1}$ in neurons following transient forebrain ischemia: comparison with early DNA damage. J Neurosci Res 53:279–296
33. Crockett DP, Burshteyn M, Garcia C, Muggironi M, Casaccia-Bonnefil P (2005) Number of oligodendrocyte progenitors recruited to the lesioned spinal cord is modulated by the levels of the cell cycle regulatory protein $p27^{Kip-1}$. Glia 49:301–308
34. Tanaka H, Yamashita T, Yachi K, Fujiwara T, Yoshikawa H, Tohyama M (2004) Cytoplasmatic $p21^{Cip1/WAF1}$ enhances axonal regeneration and functional recovery after spinal cord injury in rats. Neuroscience 127:155–164
35. Didenko VV, Wang X, Yang L, Horsnby PJ (1996) Expression of $p21^{WAF1/CIP1/SDI1}$ and p53 in apoptotic cells in the adrenal cortex and induction by ischemia/reperfusion injury. J Clin Invest 97:1723–1731
36. Megyesi J, Andrade L, Vieira JM, Safirstein RL, Price PM (2001) Positive effect of the induction of $p21^{WAF1/CIP1}$ on the course of ischemic acute renal failure. Kidney Int 60:2164–2172
37. Corbucci GG, Perrino C, Donato G, Ricchi A, Lettieri B, Troncone G, Indolfi C, Chiariello M, Avvedimento EV (2004) Transient and reversible deoxyribonucleic acid damage in human left ventricle under controlled ischemia and reperfusion. J Am Coll Cardiol 43:1992–1999
38. O'Reilly MA (2001) DNA damage and cell cycle checkpoints in hyperoxic lung injury: braking to facilitate repair. Am J Physiol 281:L291–L305
39. Tomasevic G, Kamme F, Stubberöd P, Wieloch M, Wieloch T (1999) The tumor suppressor p53 and its response gene $p^{21WAF1/Cip1}$ are not markers of neuronal death following transient global cerebral ischemia. Neuroscience 90:781–792
40. Komjáti K, Mabley JG, Virág L, Southan GJ, Salzman AL, Szabó C (2004) Poly(ADP-ribose)polymerase inhibition protects neurons and the white matter and regulates the translocation of apoptosis-inducing factor in stroke. Int J Mol Med 13:373–382
41. Parsons JL, Dianova II, Allinson SL, Dianov GL (2005) Poly(ADP-ribose)-polymerase-1 protects excessive DNA strand breaks from deterioration during repair in human cell extracts. FEBS Lett 272:2012–2021
42. Toumpoulis IK, Anagnostopoulos CE, Drossos GE, Malamou-Mitsi VD, Pappa LS, Kattritsis DG (2003) Early ischemic preconditioning without hypotension prevents spinal cord injury caused by descending thoracic aortic occlusion. J Thorac Cardiovasc Surg 125:1030–1036
43. Toumpoulis IK, Anagnostopoulos CE, Drossos GE, Malamou-Mitsi VD, Pappa LS, Katritsis DG (2003) Does ischemic preconditioning reduce spinal cord injury because of descending thoracic aortic occlusion? J Vasc Surg 37:426–432
44. Toumpoulis IK, Papakostas JC, Matsagas MI, Malamou-Mitsi VD, Pappa LS, Drossos GE, Derose JJ, Angnostopoulos CE (2004) Superiority of early relative to late ischemic preconditioning in spinal cord protection after descending aortic occlusion. J Thorac Cardiovasc Surg 128:724–730