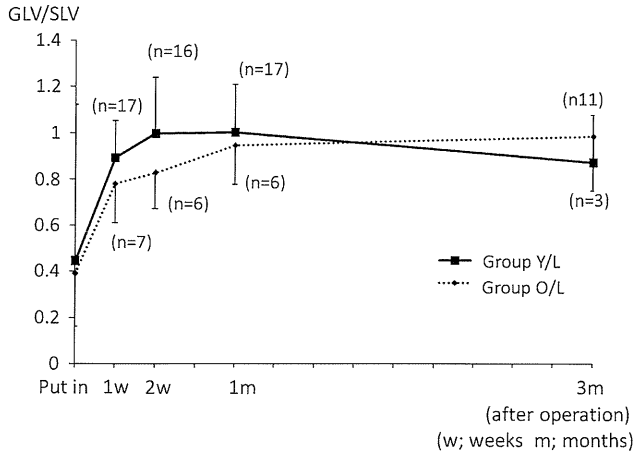

Fig. 4 The changes in recipient's liver regeneration after left lobe LDLT. There were no significant differences in GVL/SLV at all time points between the two groups

In our study, donor age affected liver regeneration during the late period in donors, but during the early period in recipients. Haga et al. [41] also compared the differences of liver regeneration between the graft liver and the donor remnant liver. They found that recipient graft volume increased more rapidly than donor remnant liver volume, which is consistent with our study. Liver regeneration is highly associated with changes of hepatic portal flow and regulation of hepatocyte proliferation, although the precise mechanism of liver regeneration is still unknown [42]. The rapid increase of recipient liver volume may be related to the immediate and significant increase in portal blood flow after LDLT because of persistence of a hyperdynamic state. In contrast, the gradual increase of donor remnant liver volume may be related to the fact that portal blood flow in the remnant liver after major hepatectomy (left or right lobe) is not significantly increased [43]. Moreover, some immunosuppression drugs, such as cyclosporine or tacrolimus, promote liver regeneration [44, 45]. From these findings, we speculate that the influence of donor age on liver volume was prominent in the early period in recipients and in the late period in donors.

In conclusion, when using older donor livers, we should pay attention to the liver volume for recipients as well as donors, considering the GRWR and remnant liver volume rate, because older donor livers might have impaired regenerative ability.

Conflicts of interest None.

References

1. Busuttil RW, Tanaka K (2003) The utility of marginal donors in liver transplantation. Liver Transpl 9:651–663
2. Cuende N, Miranda B, Cañón JF et al (2005) Donor characteristics associated with liver graft survival. Transplantation 79:1445–1452
3. Feng S, Goodrich NP, Bragg-Gresham JL et al (2006) Characteristics associated with liver graft failure: the concept of a donor risk index. Am J Transplant 6:783–790
4. Selzner M, Kashfi A, Selzner N et al (2009) Recipient age affects long-term outcome and hepatitis C recurrence in old donor livers following transplantation. Liver Transpl 15:1288–1295
5. Valadao RM, Terrault NA (2009) Older donors: mounting risks for the hepatitis C-infected liver transplant recipient? Liver Transpl 15:677–681
6. Santori G, Andorno E, Morelli N et al (2005) Impact of different cadaveric donor age cut-offs on adult recipient survival after liver transplantation: a single-center analysis. Transplant Proc 37: 2576–2581
7. Gastaca M, Valdivieso A, Pijoan J et al (2005) Donors older than 70 years in liver transplantation. Transplant Proc 37:3851–3854
8. Borchert DH, Glanemann M, Mogl M et al (2005) Adult liver transplantation using liver grafts from donors over 70 years of age. Transplant Proc 37:1186–1187
9. Cescon M, Grazi GL, Cucchetti A et al (2008) Improving the outcome of liver transplantation with very old donors with updated selection and management criteria. Liver Transpl 14: 672–679
10. Japanese Liver Transplantation Society (2009) Liver Transplantation in Japan—Registry by the Japanese Liver Transplantation Society 44:559–571
11. Ikegami T, Taketomi A, Ohta R et al (2008) Donor age in living donor liver transplantation. Transplant Proc 40:1471–1475
12. Akamatsu N, Sugawara Y, Tamura S et al (2007) Impact of live donor age (≥50) on liver transplantation. Transplant Proc 39: 3189–3193
13. Selzner M, Selzner N, Chen L et al (2009) Exaggerated up-regulation of tumor necrosis factor alpha-dependent apoptosis in the older mouse liver following reperfusion injury: targeting liver protective strategies to patient age. Liver Transpl 15:1594–1604
14. Suzuki A, Sakaguchi T, Inaba K et al. (2010) Impact of cell cycle disruption on impaired hepatic regeneration in aged livers with ischemic insult. J Surg Res. doi:10.1016/j.jss.2010.10.012
15. Chen WD, Wang YD, Zhang L et al (2010) Farnesoid X receptor alleviates age-related proliferation defects in regenerating mouse livers by activating forkhead box m1b transcription. Hepatology 51:953–962
16. Soejima K, Mimura N, Hirashima M et al (2001) A novel human metalloprotease synthesized in the liver and secreted into the blood: possibly, the von Willebrand factor-cleaving protease? J Biochem 130:475–480
17. Levy GG, Nichols WC, Lian EC et al (2001) Mutations in a member of the ADAMTS gene family cause thrombotic thrombocytopenic purpura. Nature 413:488–494
18. Zheng X, Chung D, Takayama TK et al (2001) Structure of von Willebrand factor-cleaving protease (ADAMTS13), a metalloprotease involved in thrombotic thrombocytopenic purpura. J Biol Chem 276:41059–41063
19. Plaimauer B, Zimmermann K, Völkel D et al (2002) Cloning, expression, and functional characterization of the von Willebrand factor-cleaving protease (ADAMTS13). Blood 100:3626–3632
20. Furlan M, Robles R, Solenthaler M et al (1997) Deficient activity of von Willebrand factor-cleaving protease in chronic relapsing thrombotic thrombocytopenic purpura. Blood 89:3097–3103
21. Moake JL (2002) Thrombotic microangiopathies. N Engl J Med 347:589–600
22. Okano E, Ko S, Kanehiro H et al (2010) ADAMTS13 activity decreases after hepatectomy, reflecting a postoperative liver dysfunction. Hepatogastroenterology 57:316–320
23. Kobayashi T, Wada H, Usui M et al (2009) Decreased ADAMTS13 levels in patients after living donor liver transplantation. Thromb Res 124:541–545

24. Kokame K, Sakata T, Kokubo Y et al (2011) von Willebrand factor-to-ADAMTS13 ratio increases with age in a Japanese population. J Thromb Haemost 9:1426–1428
25. Hashikura Y, Ichida T, Umeshita K et al (2009) Donor complications associated with living donor liver transplantation in Japan. Transplantation 88:110–114
26. Kuramitsu K, Egawa H, Keeffe EB et al (2007) Impact of age older than 60 years in living donor liver transplantation. Transplantation 84:166–172
27. Yokoi H, Isaji S, Yamagiwa K et al (2005) Donor outcome and liver regeneration after right-lobe graft donation. Transpl Int 18: 915–922
28. Urata K, Kawasaki S, Matsunami H et al (1995) Calculation of child and adult standard liver volume for liver transplantation. Hepatology 21:1317–1321
29. Hirata M, Harihara Y, Kitamura T et al (2001) The influence of donor age to graft volume increase rate in living donor liver transplantation. Transplant Proc 33:1416–1417
30. Ikegami T, Nishizaki T, Yanaga K et al (2000) The impact of donor age on living donor liver transplantation. Transplantation 70:1703–1707
31. Iwamoto T, Yagi T, Umeda Y et al (2008) The impact of donor age on the outcome of adult living donor liver transplantation. Transplantation 85:1240–1245
32. Veteläinen R, van Vliet AK, van Gulik TM (2007) Severe steatosis increases hepatocellular injury and impairs liver regeneration in a rat model of partial hepatectomy. Ann Surg 245:44–50
33. Shah SA, Cattral MS, McGilvray ID et al (2007) Selective use of older adults in right lobe living donor liver transplantation. Am J Transplant 7:142–150
34. Soejima Y, Shimada M, Suehiro T et al (2003) Use of steatotic graft in living-donor liver transplantation. Transplantation 76:344–348
35. Dayangac M, Taner CB, Yaprak O et al (2011) Utilization of elderly donors in living donor liver transplantation: when more is less? Liver Transpl 17:548–555
36. Yagi S, Iida T, Taniguchi K et al (2005) Impact of portal venous pressure on regeneration and graft damage after living-donor liver transplantation. Liver Transpl 11:68–75
37. Pomfret EA, Pomposelli JJ, Gordon FD et al (2003) Liver regeneration and surgical outcome in donors of right-lobe liver grafts. Transplantation 76:5–10
38. Kwon KH, Kim YW, Kim SI et al (2003) Postoperative liver regeneration and complication in live liver donor after partial hepatectomy for living donor liver transplantation. Yonsei Med J 44:1069–1077
39. Kim SJ, Kim DG, Chung ES et al (2006) Adult living donor liver transplantation using the right lobe. Transplant Proc 38: 2117–2120
40. Kawasaki S, Makuuchi M, Ishizone S et al (1992) Liver regeneration in recipients and donors after transplantation. Lancet 339:580–581
41. Haga J, Shimazu M, Wakabayashi G et al (2008) Liver regeneration in donors and adult recipients after living donor liver transplantation. Liver Transpl 14:1718–1724
42. Lautt WW (2007) Regulatory processes interacting to maintain hepatic blood flow constancy: vascular compliance, hepatic arterial buffer response, hepatorenal reflex, liver regeneration, escape from vasoconstriction. Hepatol Res 37:891–903
43. Kawasaki T, Moriyasu F, Kimura T et al (1990) Portal hemodynamic changes from partial hepatectomy—quantitative analysis of portal flow before, during and after hepatectomy, using an Doppler ultrasound system. Nihon Shokakibyo Gakkai Zasshi 87:979–988
44. Morii Y, Kawano K, Kim YI et al (1999) Augmentative effect of cyclosporin A on rat liver regeneration: influence on hepatocyte growth factor and transforming growth factor-beta(1). Eur Surg Res 31:399–405
45. Starzl TE, Porter KA, Mazzaferro V et al (1991) Hepatotrophic effects of FK506 in dogs. Transplantation 51:67–70

J. Cell. Mol. Med. Vol 16, No 8, 2012 pp. 1792–1802

Erythropoietin attenuates the sequels of ischaemic spinal cord injury with enhanced recruitment of CD34$^+$ cells in mice

Koji Hirano [a, b], Klaus Wagner [c], Peter Mark [a], Erik Pittermann [a], Ralf Gäbel [a], Dario Furlani [a], Wenzhong Li [a, *, §], Brigitte Vollmar [d], Tomomi Yamada [e], Gustav Steinhoff [a, #], Nan Ma [a, #, *, §]

[a] *Department of Cardiac Surgery, University of Rostock, Rostock, Germany*
[b] *Department of Thoracic and Cardiovascular Surgery, Mie University Graduate School of Medicene, Tsu, Japan*
[c] *Department of Anesthesia, Klinikum Südstadt, Rostock, Germany*
[d] *Institute for Experimental Surgery, University of Rostock, Rostock, Germany*
[e] *Department of Translational Medical Science, Mie University Graduate School of Medicine, Tsu, Japan*

Received: March 30, 2011; Accepted: September 21, 2011

Abstract

Erythropoietin has been shown to promote tissue regeneration after ischaemic injury in various organs. Here, we investigated whether Erythropoietin could ameliorate ischaemic spinal cord injury in the mouse and sought an underlying mechanism. Spinal cord ischaemia was developed by cross-clamping the descending thoracic aorta for 7 or 9 min. in mice. Erythropoietin (5000 IU/kg) or saline was administrated 30 min. before aortic cross-clamping. Neurological function was assessed using the paralysis score for 7 days after the operation. Spinal cords were histologically evaluated 2 and 7 days after the operation. Immunohistochemistry was used to detect CD34$^+$ cells and the expression of brain-derived neurotrophic factor and vascular endothelial growth factor. Each mouse exhibited either mildly impaired function or complete paralysis at day 2. Erythropoietin-treated mice with complete paralysis demonstrated significant improvement of neurological function between day 2 and 7, compared to saline-treated mice with complete paralysis. Motor neurons in erythropoietin-treated mice were more preserved at day 7 than those in saline-treated mice with complete paralysis. CD34$^+$ cells in the lumbar spinal cord of erythropoietin-treated mice were more abundant at day 2 than those of saline-treated mice. Brain-derived neurotrophic factor and vascular endothelial growth factor were markedly expressed in lumbar spinal cords in erythropoietin-treated mice at day 7. Erythropoietin demonstrated neuroprotective effects in the ischaemic spinal cord, improving neurological function and attenuating motor neuron loss. These effects may have been mediated by recruited CD34$^+$ cells, and enhanced expression of brain-derived neurotrophic factor and vascular endothelial growth factor.

Keywords: thoracoabdominal aortic aneurysm (TAAA) • spinal cord ischaemia • erythropoietin (EPO) • CD34 positive cell • brain-derived neurotrophic factor (BDNF) • vascular endothelial growth factor (VEGF)

Introduction

Paraplegia caused by spinal cord ischaemia (SCI) is a devastating complication following thoracoabdominal aortic surgery. Although the incidence of paraplegia is low, ranging between 2.6% and 13.2%, because of the advancement of surgical techniques and the development of various adjuncts [1, 2], patients with paraplegia have poor survival rates [3], and quality of life is significantly impaired. Recently, thoracic endovascular aortic repair (TEVAR) demonstrated favourable results over open surgical repair as a treatment option for descending thoracic aortic aneurysms [4]. However, TEVAR is not a standard treatment option for thoracoabdominal aortic aneurysms. Accordingly, paraplegia remains a critical problem associated with thoracoabdominal aortic surgery.

Erythropoietin (EPO) is known to be a hormone that regulates proliferation and differentiation of erythroid precursor cells. In the last decade, EPO receptors have been shown to be expressed in many other tissues besides the haematopoietic system, and they

[#]These authors contributed equally to this work.
[§]Current address: BCRT & CBD, Institute of Polymer Research, Helmholtz Zentrum, Geesthacht, Germany.
*Correspondence to: Dr. Nan MA, Dr. Wenzhong LI,
Department of Cardiac Surgery, University of Rostock,
Schillingallee 35, 18057 Rostock, Germany
Tel.: +49 381 494 61 00
Fax: +49 381 494 61 02
E-mail: nanma001@med.uni-rostock.de, wenzhong.li@hzg.de

doi:10.1111/j.1582-4934.2011.01489.x

are considered to be related to the cytoprotective effects of EPO against ischaemia-reperfusion (I/R) injury in various organs including the spinal cord [5, 6]. More interestingly, EPO has been reported to promote tissue regeneration in a variety of disorders *via* activation of local stem cells or recruitment of bone marrow stem/progenitor cells [7, 8].

There have been some reports in which exogenous EPO has been tested in animal models of SCI [9–11]. These studies demonstrated that EPO improved neurological function and reduced histological injury, conferred by the anti-apoptotic effect of EPO. However, these reports only focussed on the short-term effect of EPO, predominantly within 2 days of treatment. This timeframe is too narrow to evaluate the outcome of EPO treatment after SCI because critical events caused by I/R, such as inflammation, cell death and the repair process, can occur in a delayed manner, sometimes over days and weeks after the initial insult [12]. Indeed, post-operative spinal cord deficits have been reported to occur 12 hrs to some months after surgery, despite the emergence from anaesthesia with intact spinal cord function [3].

Therefore, we investigated the effect of EPO on ischaemic spinal cord injury over a longer term using a mouse model of SCI and sought the underlying mechanism, particularly focusing on the implication of stem cells.

Materials and methods

Mice

All animal experiments were performed in accordance with the guidelines published in the 'Guide for the Care and Use of Laboratory Animals' published by the United States National Institutes of Health. Surgical and animal care protocols were reviewed and approved by the local animal care committees of Mecklenburg/Vorpommern in Germany. Adult male C57BL/6 mice (8–12 weeks old; 22–27 g) were purchased from Charles River Laboratories, Sulzfeld, Germany.

Anaesthesia and surgical preparation

Mice were anaesthetized by intraperitoneal injection of ketamin/xylazin (75/25 mg/kg) and placed on a heating table set at 37°C. Rectal temperature was monitored during the surgery. Mice were intubated and mechanically ventilated with room air (300 μl/stroke, 130 strokes/min.). For anti-coagulation, heparin (400 IU/kg) was injected subcutaneously 5 min. before the surgery. Abdominal aortic blood pressure and heart rate were monitored through a catheter inserted into the right femoral artery. The data obtained were automatically recorded every minute with a Millar Pressure-Volume System [Ultra-Miniature Pressure-Volume Catheter (Model SPR-838), Millar Pressure Conductance Unit (Model MPCU-200) and Millar PowerLab data acquisition hardware; Emka Technologies, Paris, France].

Surgical procedure for SCI

A thoracotomy at the second intercostal space on the left side was used to approach the descending thoracic aorta. The left thymus was removed and the left recurrent nerve was carefully separated from the aortic arch. After isolation from the surrounding tissue, the descending thoracic aorta distal to the origin of the left subclavian artery was cross-clamped with a small aneurysm clip (Fine Science Tools GmbH, Heidelberg, Germany) for 7 or 9 min. Complete cessation of blood flow was confirmed by disappearance of blood pressure waves on the monitor screen. Mice that maintained abdominal aortic blood pressure with small waves or above 20 mmHg during the clamping were excluded from this study. After release of the clip, mice were observed for 10 min. until blood pressure returned to stable levels. Afterwards, the chest was closed and the blood pressure catheter was removed. Mice were kept on a heating table until they awoke and were extubated.

Post-operative care

Mice received an intraperitoneal injection of isotonic saline (1 ml) to compensate for fluid loss during surgery before extubation. A subcutaneous injection of 0.9% saline (1.5 ml) was administered daily until animals were killed. Manual bladder expression was performed twice a day for mice with neurological bladder.

Experimental groups

All mice (n = 148) were divided into three groups: sham (n = 5), 7-min. ischaemia (n = 72), and 9-min. ischaemia (n = 71). Exposure of the descending thoracic aorta was only applied to the sham group. The 7- and 9-min. ischaemia groups were subjected to 7 and 9 min. of cross-clamping, respectively. The 7- and 9-min. ischaemia groups were further divided into two treatment groups, which were control groups (7-min. ischaemia = 7C, n = 37; 9-min. ischaemia = 9C, n = 33) and EPO groups (7-min. ischaemia = 7E, n = 35; 9-min. ischaemia = 9E, n = 38). Groups 7E and 9E received a single dose of 5000 IU/kg of recombinant human EPO (Epoetin-α/Erypo®; Ortho Biotech, Neuss, Germany) intravenously 30 min. before ischaemia. Groups 7C and 9C received an equal volume of saline.

Blood gas analysis

Blood gas analysis was performed with three mice from each group at two different time points, which were 10 min. before ischaemia and 10 min. after the onset of reperfusion. Blood samples (70 μl) were drawn from the right femoral artery and pH, PaO_2, $PaCO_2$, base excess and haematocrit of each sample were measured with a blood gas/pH analyser (RAPIDLab 348, Siemens, Germany).

Behavioural test

Mice were subjected to a behavioural test at 3 hrs, 6 hrs, and daily thereafter up to 7 days (days 1–7) after onset of reperfusion. A following paralysis score system [13] was used to grade hind limb neurological functions: (a) walking with hind limbs: 0, normal; 1, toes flat under body when walking but ataxia is present; 2, knuckle-walking; 3, movement in hind limbs but unable to walk; 4, no movement, drags hind limbs; (b) placing/stepping reflex: 0, normal; 1, weak; 2, no stepping. Each grade was obtained by adding the scores of (a) and (b); a grade of 0 was considered normal and 6 was considered complete paralysis. Both hind limbs were assessed separately and a final grade was expressed as an average.

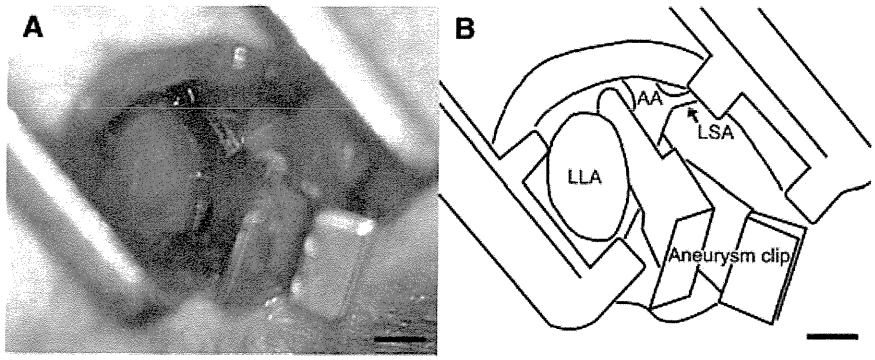

Fig. 1 A mouse model of SCI was developed by cross-clamping the descending thoracic aorta through left thoracotomy. A small aneurysm clip was placed across the descending aorta just distal to the left subclavian artery (**A**). Schematic diagram of A (**B**). LAA: left atrial appendix; LSA: left subclavian artery; AA: aortic arch; Bar = 1 mm.

Histological evaluation of ischaemic spinal cord injury

Histological evaluation was performed at day 2 (7C, $n = 10$; 7E, $n = 10$; 9C, $n = 8$; 9E, $n = 12$) and day 7 (7C, $n = 13$; 7E, $n = 11$; 9C, $n = 12$; 9E, $n = 12$) after SCI. Mice were killed using an over dose of ketamine/xylazine (150/50 mg/kg), and the thoracic and lumbar part of the spinal cord was harvested en bloc and fixed in formafix 4% (v/v) (Grimm Med. Logistik GmbH, Germany) overnight. The samples were then kept in ethylenediaminetetraacetic acid (EDTA) decalcification solution (pH 7.4) for 2 weeks, embedded in paraffin and sectioned at 4 μm thickness. Twelve cross sections from T6-L5 level of the spinal cord in each mouse were stained with haematoxylin/eosin (HE) and observed under a light microscope (Leica, Hamburg, Germany). Ischaemic injury was quantified by counting the number of normal motor neurons in the bilateral ventral horn of each cross section [14]. The final value of each sample was given as a sum of 12 cross sections.

Immunohistochemistry

Deparaffinized cross sections were incubated in boiling citric buffer (pH 6.0) for 10 min. and allowed to cool down for 20 min. Non-specific binding was blocked by protein blocking reagent (DAKO, Hamburg, Germany). Subsequently, the cross sections were incubated with goat polyclonal anti-CD34 antibody (1:20 dilution; Santa Cruz Biotechnology, Santa Cruz, CA, USA) or rabbit polyclonal anti-brain–derived neurotrophic factor (BDNF) antibody (1:50 dilution; Santa Cruz Biotechnology) at 4°C overnight. Cross sections were incubated with donkey anti-goat or goat anti-rabbit Alexa-Fluor 488–conjugated secondary antibody (1:350 dilution; Invitrogen, Darmstadt, Germany) for 2 hrs at room temperature. Nuclei were counterstained with TOPRO3 (5 μM; Invitrogen). The cross sections were mounted in Fluosaver (Merck, Germany). The labelled cross sections were observed with a Leica SP2 confocal microscope (Leica). The number of $CD34^+$ cells in the entire transverse section from the L1-2 level of the spinal cord was counted under a 400× magnification lens and final values were given as an average of three cross sections. For detection of vascular endothelial growth factor (VEGF) expression, deparaffinized cross sections treated with citric buffer were incubated with hydrogen peroxide for 5 min. and subsequently with 5% (v/v) normal donkey serum for 20 min. The cross sections were then incubated with rabbit polyclonal anti-VEGF antibody (1:50 dilution; Santa Cruz Biotechnology) at 4°C overnight. Immunoreactivity to VEGF was visualized with a linked streptavidin–biotin method using a goat ImmunoCruz™ Staining Kit (Santa Cruz Biotechnology). Cross sections were then counterstained with haematoxylin, dehydrated and mounted in Pertex (Medite, Germany).

Statistical analysis

Values are expressed as the mean ± S.D. After confirmation whether the data were approximated to the normal distribution, statistical analysis for neurological and histological data between control and EPO groups at different time points were performed by Student's *t*-test with Bonferroni correction. Survival rates were expressed using the Kaplan–Meier survival curve and the significance of these differences was analysed using the Log-rank test. Student's *t*-test was applied for physiological parameters. Differences between groups were considered significant at $P < 0.05$.

Results

Mouse model of SCI

The mouse model of SCI was first described in 2000 by Lang-Lazdunski et al. [13]. They used two aneurysm clips, one of which was placed across the aortic arch between the left common carotid artery and the left subclavian artery and the other across the left subclavian artery through a cervical mediastinotomy. In our initial experience of this procedure, it was somewhat difficult to reach both clamping sites through the cervical mediastinotomy. Instead, we performed single clamping across the descending aortic artery through a thoracotomy. We found that this procedure made it easier to reach the descending thoracic aorta and place an aneurysm clip precisely across it, allowing abdominal aortic blood pressure to drop to a sufficient level for developing SCI without additional clamping on the left subclavian artery (Fig. 1). On preliminary examination, 5-min. ischaemia developed no paralysis whereas 12-min. ischaemia resulted in complete paralysis, which is referred to as grade 6 on the paralysis score, in all mice (data not shown). Therefore, 7-min. and 9-min ischaemia were applied in this study.

Table 1 Physiological parameters

	7-min. ischaemia			9-min. ischaemia		
	Control, $n = 34(3^*)$	EPO, $n = 32(3^*)$	*P* value	Control, $n = 30(3^*)$	EPO, $n = 35(3^*)$	*P* value
HR (beats/min.)						
Pre-ischaemia	265 ± 68	286 ± 60	0.187	265 ± 36	270 ± 50	0.685
Reperfusion	269 ± 67	315 ± 50	0.003†	266 ± 45	298 ± 48	0.008†
MAABP (mmHg)						
Pre-ischaemia	61.2 ± 18.2	62.8 ± 12.9	0.671	68.1 ± 12.2	68.6 ± 14.5	0.881
Ischaemia	11.6 ± 2.0	12.0 ± 2.8	0.545	11.3 ± 4.6	10.7 ± 3.7	0.622
Reperfusion	53.5 ± 9.9	57.5 ± 10.8	0.125	59.0 ± 11.6	62.3 ± 8.1	0.199
RT (°C)						
Pre-ischaemia	36.5 ± 0.7	36.5 ± 0.4	0.679	36.4 ± 0.4	36.4 ± 0.4	0.962
Ischaemia	35.8 ± 0.5	36.0 ± 0.3	0.224	35.8 ± 0.3	35.8 ± 0.3	0.821
Reperfusion	36.6 ± 0.5	36.5 ± 0.4	0.325	36.6 ± 0.4	36.6 ± 0.5	0.657
BGA parameters						
pH						
Pre-ischaemia	7.36 ± 0.02	7.38 ± 0.04	0.496	7.38 ± 0.05	7.35 ± 0.07	0.578
Reperfusion	7.28 ± 0.00	7.30 ± 0.03	0.368	7.22 ± 0.01	7.25 ± 0.06	0.480
PaO_2 (mmHg)						
Pre-ischaemia	76.5 ± 0.1	75.4 ± 4.5	0.713	78.2 ± 3.8	80.1 ± 4.2	0.592
Reperfusion	71.0 ± 0.2	74.6 ± 3.7	0.234	79.6 ± 5.2	77.7 ± 0.2	0.592
$PaCO_2$ (mmHg)						
Pre-ischaemia	44.6 ± 2.1	40.3 ± 3.3	0.130	40.8 ± 3.8	40.4 ± 4.3	0.910
Reperfusion	38.1 ± 2.1	34.6 ± 2.1	0.111	36.2 ± 0.4	36.5 ± 3.0	0.879
BE						
Pre-ischaemia	−0.8 ± 0.2	−0.8 ± 0.9	0.935	−0.2 ± 1.0	−0.9 ± 1.2	0.481
Reperfusion	−9.2 ± 0.7	−10.0 ± 0.5	0.183	−11.9 ± 1.6	−11.7 ± 1.8	0.893
HCT (%)						
Pre-ischaemia	45.3 ± 1.5	43.5 ± 0.7	0.164	42.7 ± 2.3	47.0 ± 1.7	0.060
Reperfusion	46.0 ± 3.5	45.5 ± 0.7	0.830	45.5 ± 4.9	48.0 ± 1.4	0.476

Values are given as mean±S.D. EPO: erythropoietin; HR: heart rate; MAABP: mean abdominal aortic blood pressure; RT: rectal temperature; BE: base excess; HCT: haematocrit; pre-ischaemia, 10 min. before ischaemia; ischaemia, at the end of ischaemia; reperfusion, 10 min. after the onset of reperfusion. *Numbers for BGA analysis. †Significant values.

There was no significant difference in physiological parameters between the control (7C and 9C) and EPO (7E and 9E) group during SCI surgery except for heart rate at 10 min. after onset of reperfusion, which was higher in the EPO groups than in the control groups (Table 1). These data indicate that SCI surgeries were performed under similar physiological conditions in both groups.

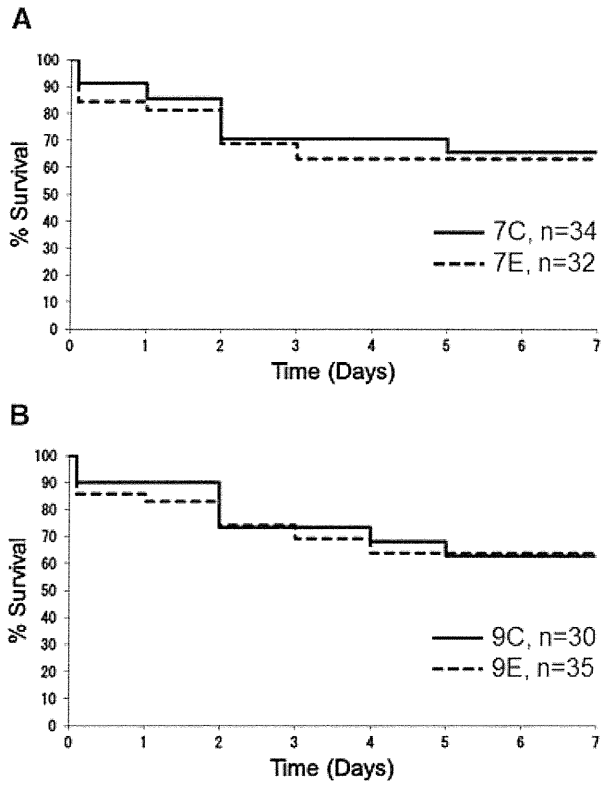

Fig. 2 Survival rates of groups subjected to 7-min. ischaemia (**A**) and 9-min. ischaemia (**B**) are illustrated using Kaplan–Meier survival curves. 7C: control group subjected to 7-min. ischaemia; 7E: EPO group subjected to 7-min. ischaemia; 9C: control group subjected to 9-min. ischaemia; 9E: EPO group subjected to 9-min. ischaemia. Survival rates at day 2 after SCI were 70.6%, 68.8%, 73.3% and 74.3%, and 65.5%, 63.0%, 62.9% and 63.7% at day 7 in the 7C, 7E, 9C and 9E groups, respectively. There were no significant differences in survival rate between groups 7C and 7E ($P = 0.797$) or between groups 9C and 9E ($P = 0.971$), according to the Log-rank test.

Survival

Survival rates at day 2 after SCI were 70.6%, 68.8%, 73.3% and 74.3%, and those at day 7 were 65.5%, 63.0%, 62.9% and 63.7% in the 7C, 7E, 9C and 9E groups, respectively. There was no significant difference in the survival rate between the 7C and 7E groups ($P = 0.797$) or between the 9C and 9E groups ($P = 0.971$) (Fig. 2). At necropsy, bowel necrosis was detected in three mice from group 7C, three from 9C and one from 9E, indicating that there was no evidence that EPO exacerbated thromboembolism.

Outcome of neurological function

In general, average paralysis score in every group improved during the first 24 hrs after SCI. However, scores deteriorated after day 2. Within the first 2 days after SCI, there was no significant difference between groups 7C and 7E or between groups 9C and 9E (Fig. 3A and C). Neurological functions at day 2 were revealed to clearly separate into normal to minor deficit (less than grade 3, defined as 'mild paralysis') and complete deficit (grade 6, defined as 'severe paralysis') in every group. The percentages of mild paralysis in groups 7C, 7E, 9C and 9E were 47.8%, 47.6%, 20.0% and 25.0%, respectively. There was no significant difference in the percentage of mild paralysis between groups 7C and 7E ($P = 0.989$) or between groups 9C and 9E ($P = 0.974$). After day 2 until day 7, mice with mild paralysis and those with severe paralysis were separately analysed in each group (7C-mild, $n = 6$; 7C-severe, $n = 7$; 7E-mild, $n = 5$; 7E-severe, $n = 6$; 9C-mild $n = 2$, 9C-severe $n = 10$; 9E-mild, $n = 3$; 9E-severe, $n = 9$; Fig. 3B and D). As a result of the separate analysis, the paralysis score for group 7E-severe significantly improved by day 7, compared to group 7C-severe (4.83 ± 0.98 *versus* 5.86 ± 0.38 at day 7, respectively, $P < 0.05$ with Bonferroni correction). A similar improvement in paralysis score was observed for group 9E-severe compared to 9C-severe (5.22 ± 0.67 *versus* 5.80 ± 0.42 at day 7, respectively, $P < 0.05$ with Bonferroni correction). Mice with mild paralysis (7C, 7E, 9C and 9E) had maintained relatively good neurological function for the last 5 days and finally recovered to normal.

Histological outcome

In cross sections from mice with mild paralysis, typical motor neurons, which contained Nissl substance in the cytoplasm, loose chromatin and prominent nucleoli, were distributed in grey matter. There was no remarkable evidence of ischaemic injury seen in grey matter for groups 7C-mild, 7E-mild, 9C-mild and 9E-mild at day 2 (Fig. 4A, B, E and F), and a small number of inflammatory cells were occasionally located in the grey matter at day 7 (Fig. 4C, D, G and H). The number of motor neurons in these groups was similar at days 2 and 7 and close to that of the sham group (Fig. 4Q and R). In contrast, motor neurons in the ventral horn showed ischaemic changes with shrunken cell bodies, eosinophilic cytoplasm and pyknotic nuclei in cross sections from mice with severe paralysis. The presence of ischaemic motor neurons and vacuolization was pronounced in the grey matter of groups 7C-severe, 7E-severe, 9C-severe and 9E-severe at day 2. A few motor neurons were present only peripherally in grey matter (Fig. 4I, J, M and N). At day 7, affected areas represented by ischaemic motor neurons and vacuolization were replaced with a high density of inflammatory cells including microglia, especially in groups 7C-severe and 9C-severe (Fig. 4K and L). However, such areas were limited in groups 7E-severe and 9E-severe (Fig. 4O and P). There was no significant difference in the number of motor neurons at day 2 between groups 7C-severe and 7E-severe (235 ± 21 *versus* 240 ± 19, respectively, $P = 0.703$) or between groups 9C-severe and 9E-severe (190 ± 30 *versus* 207 ± 28, respectively, $P = 0.290$). However, there was a significant difference at day 7 between groups 7C-severe and 7E-severe (115 ± 28 *versus* 195 ± 36, respectively, $P < 0.05$ with Bonferroni correction), and

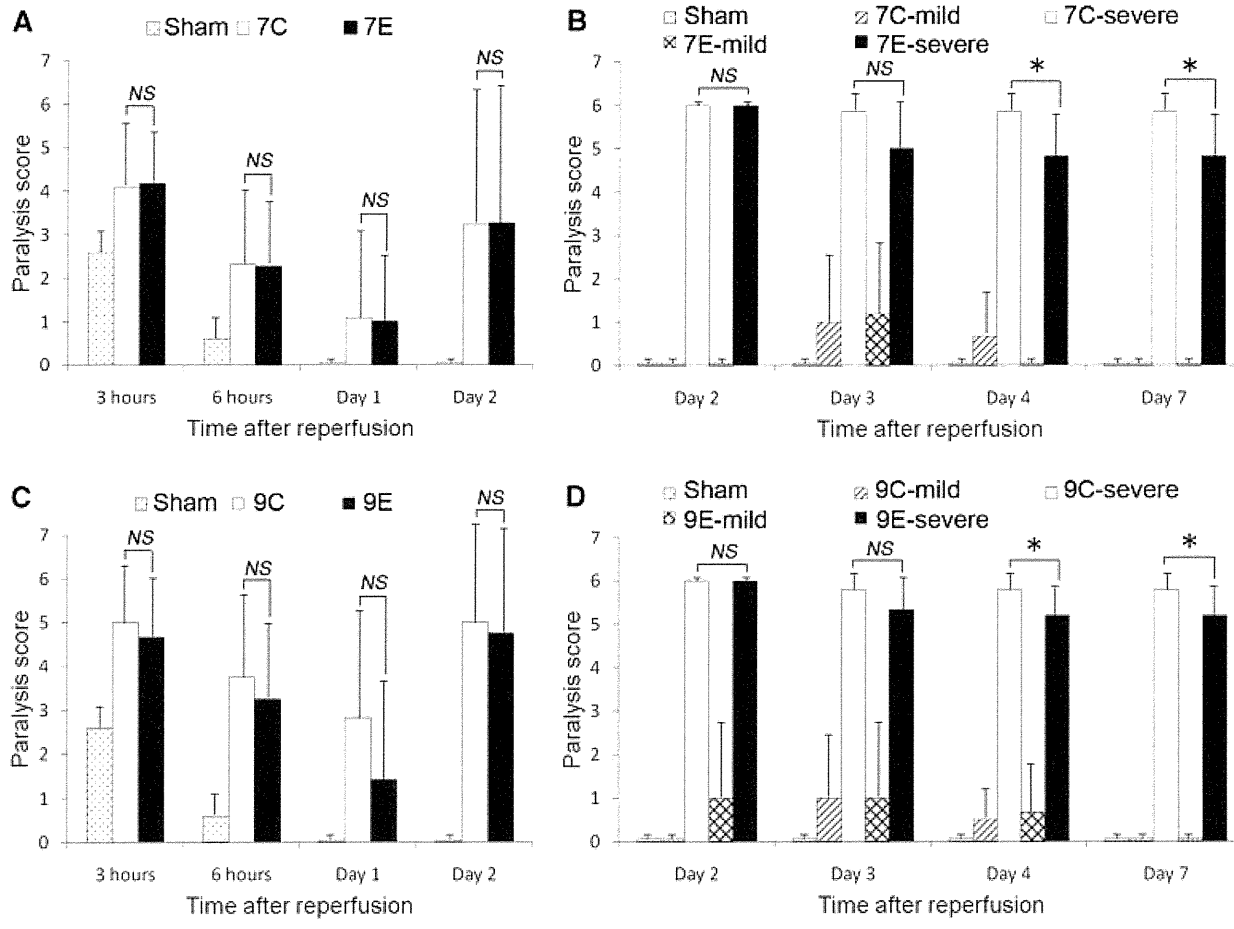

Fig. 3 Neurological outcome assessed by paralysis score from 3 hrs to day 2 after SCI with 7-min. ischaemia (**A**) and 9-min. ischaemia (**C**) and from day 2 to 7 after SCI with 7-min. ischaemia (**B**) and 9-min. ischaemia (**D**). 7C: control group subjected to 7-min. ischaemia; 7E: EPO group subjected to 7-min. ischaemia; 9C: control group subjected to 9-min. ischaemia; 9E: EPO group subjected to 9-min. ischaemia; mild: subgroup including mice with mild paralysis; severe: subgroup including mice with severe paralysis. There were no significant differences in paralysis score between groups 7C and 7E or between groups 9C and 9E until 2 days after SCI (**A** and **C**). However, group 7E-severe exhibited significant improvement between day 2 and 7, compared to group 7C-severe (**B**). A similar tendency was observed in group 9E-severe between day 2 and 7, compared to group 9C-severe (**D**). Paralysis scores in group 7C-mild, 7E-mild, 9C-mild and 9E-mild all returned to normal (grade 0) at day 7 (**B** and **D**). (**B** and **D**) $*P < 0.05$ with Bonferroni correction.

between groups 9C-severe and 9E-severe (102 ± 21 *versus* 142 ± 18, respectively, $P < 0.05$ with Bonferroni correction, Fig. 4Q and R). In summary, in groups 7E-severe and 9E-severe, motor neuron loss from day 2 to 7 was attenuated and the damaged areas in the grey matter were reduced, compared to groups 7C-severe and 9C-severe. Mice with mild paralysis exhibited only minor damage in histology regardless if they were treated with EPO.

Immunohistochemical findings

$CD34^+$ cells could be recognized in cross sections, frequently in grey matter of the lumbar spinal cord in every group (Fig. 5A and B). The number of $CD34^+$ cells in group 7E-severe was higher than that of group 7C-severe at day 2 (28.3 ± 9.5 *versus* 13.8 ± 5.1, respectively, $P < 0.05$ with Bonferroni correction), and that of group 9E-severe was also higher than that of group 9C-severe at day 2 (44.8 ± 22.4 *versus* 22.7 ± 11.8, respectively, $P < 0.05$ with Bonferroni correction, Fig. 5C and D). No significant difference was observed in $CD34^+$ cells from groups 7C-mild, 7E-mild, 9C-mild and 9E-mild. Expression of BDNF was distributed in the ventral grey matter of groups 7E-severe and 9E-severe, whereas expression was rarely detected in groups 7C-severe and 9C-severe (Fig. 5E and F). Similarly, expression of VEGF could be detected only in the ventral or ventrolateral white matter of the spinal cord in groups 7E-severe and 9E-severe, but not in groups 7C-severe and 9C-severe (Fig. 5G and H).

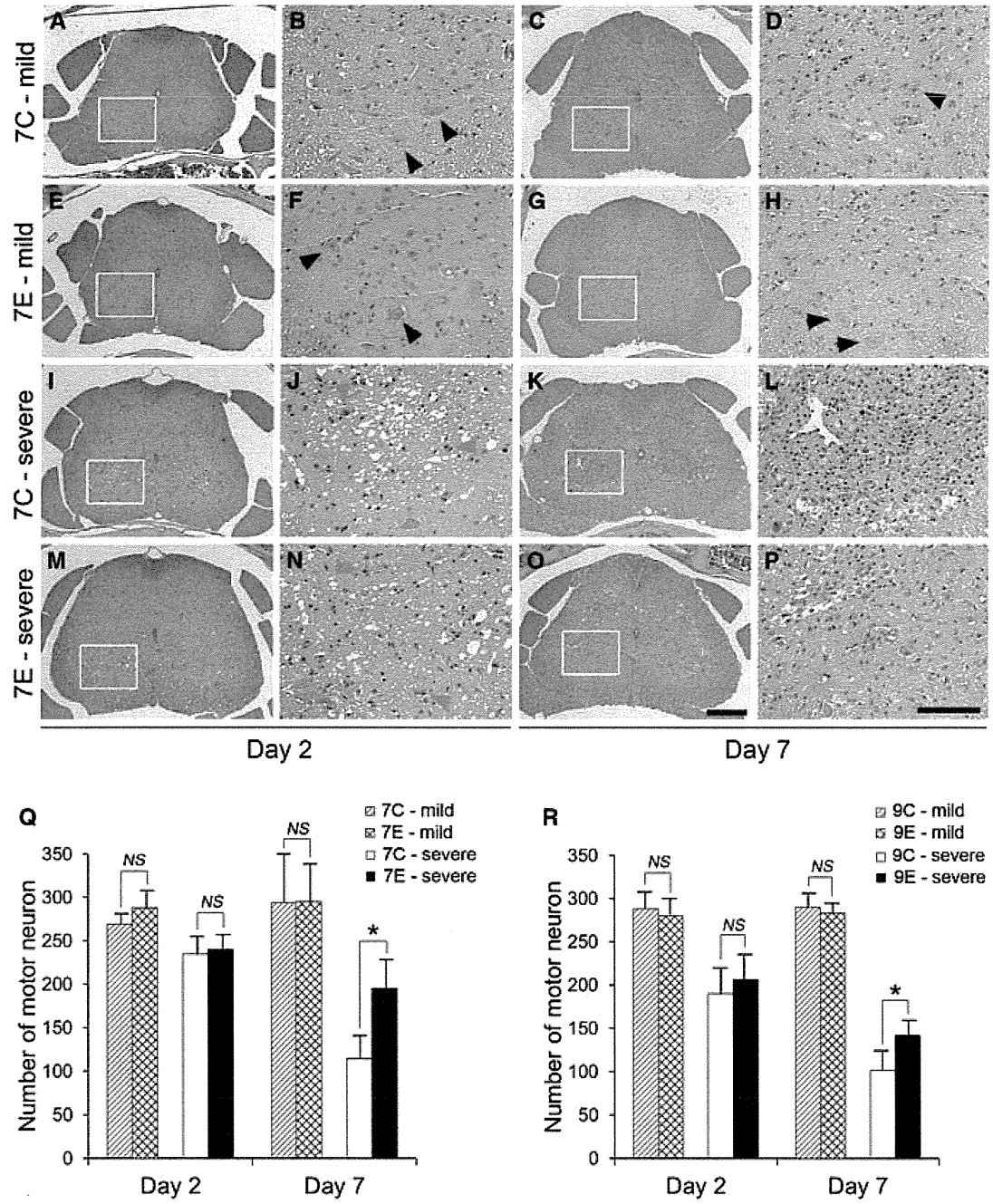

Fig. 4 Representative cross sections stained with HE from the L1-2 level of the spinal cord after 7-min. SCI (**A–P**). An enlargement of the boxed regions is provided on the right side of each cross section. Almost normal morphology was maintained in lumbar spinal cords from group 7C-mild and 7E-mild (**A–H**). Normal motor neurons are distributed in ventral grey matter (block arrow heads). In contrast, ventral grey matter in the lumbar spinal cord from group 7C-severe and 7E-severe were largely affected at day 2 with ischaemic motor neurons (grey arrow heads) and many vacuolated regions (**I**, **J**, **M** and **N**). At day 7, affected regions in grey matter were replaced with a high density of inflammatory cells including microglia (**K**, **L**, **O** and **P**). Note that lesion area was limited in group 7E-severe at day 7 (**O** and **P**), compared to group 7C-severe (**K** and **L**). Scale bar = 250 μm in low-magnification images and 100 μm in high-power images. Bar graphs illustrating the number of normal motor neurons in the thoracolumbar spinal cord in each group with 7-min. ischaemia (**Q**) and 9-min. ischaemia (**R**). Motor neurons were well preserved in groups 7C-mild, 7E-mild, 9C-mild and 9E-mild, whereas the loss of motor neurons was evident in groups 7C-severe, 7E-severe, 9C-severe and 9E-severe. However, higher numbers of motor neurons were preserved in groups 7E-severe and 9E-severe at day 7, compared to groups 7C-severe and 9C-severe. (**Q** and **R**) $^{*}P < 0.05$ with Bonferroni correction.

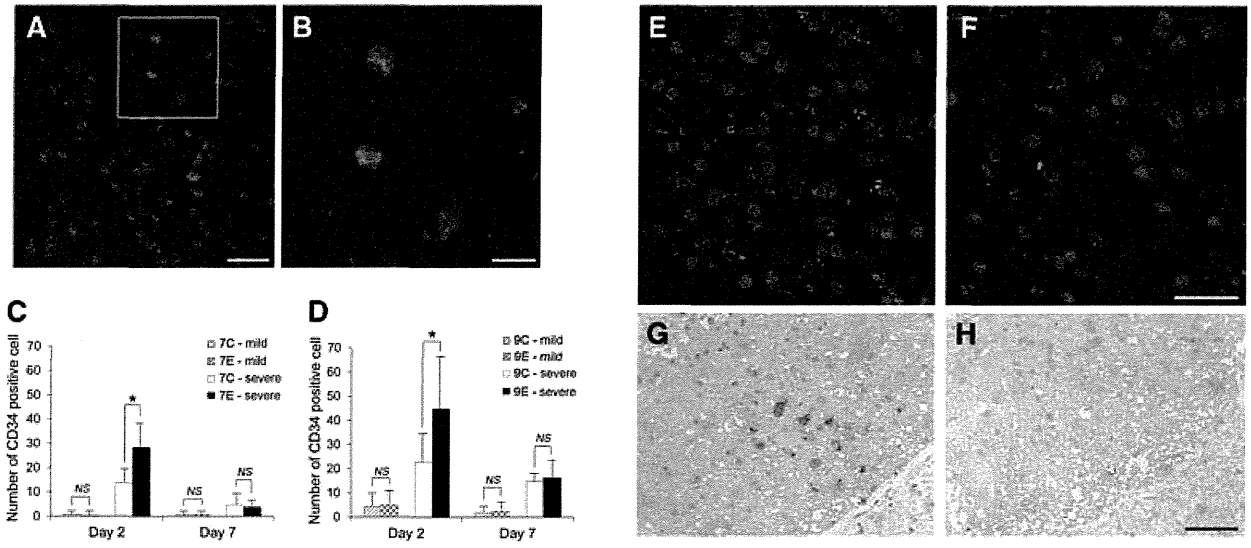

Fig. 5 Representative pictures of $CD34^+$ cells in ventral grey matter (**A**) and enlargement of boxed area (**B**). Blue: nuclei and red: CD34. (**A**) Scale bar = 25 μm; (**B**) Scale bar = 10 μm. The number of $CD34^+$ cells in the lumbar spinal cord after 7-min. (**C**) and 9-min. SCI (**D**). $CD34^+$ cells were more abundantly recruited in the spinal cord in groups 7E-severe and 9E-severe than those in groups 7C-severe and 9C-severe at day 2 (**C** and **D**, $*P < 0.05$ with Bonferroni correction). $CD34^+$ cells were not apparent in groups 7C-mild, 7E-mild, 9C-mild and 9E-mild. Representative pictures of immunofluorescence for BDNF in ventral grey matter from groups 9E-severe (**E**) and 9C-severe (**F**) at day 7. Blue: nuclei and green: BDNF. BDNF expression in group 9E-severe (**E**) was more evident than that in group 9C-severe (**F**). Scale bar = 25 μm. Representative pictures of VEGF expression in the left ventrolateral white matter of the spinal cord from groups 9E-severe (**G**) and 9C-severe (**H**) at day 7. VEGF was detectable in the white matter of group 9E-severe and vacuolization in white matter was limited (**G**). In contrast, no VEGF expression and advanced vacuolization was apparent in the white matter of group 9C-severe (**H**). Scale bar = 50 μm.

Discussion

Major findings of this study are as follows. First, our mouse model of SCI was successfully performed and resulted in stable physiological conditions during surgery and standardized neurological and histological outcome following various durations of SCI, which made investigation of EPO treatment reliable. Second, neurological function of EPO-treated mice improved when compared to control mice between day 2 and day 7, and loss of motor neurons was attenuated in EPO-treated mice at day 7 after SCI. Finally, $CD34^+$ cells were densely recruited, and expression of BDNF and VEGF was enhanced in the lumber spinal cord of EPO-treated mice after SCI.

Generally, mouse models are advantageous to study therapeutics, specific genes and molecular signalling pathways because a variety of genetically modified mice are now widely available. Previously, ischaemic models of spinal cord injury have been often described in rabbits and rats. Compared to such animals, mice are less expensive and are equally as easy to operate on. In our model, a skilled surgeon can complete the procedure within 60 min. Rabbit models have been widely used to test the effects of neuroprotective drugs and to examine the pathophysiology of spinal cord injury [15, 16]. However, little is known about the genome of rabbits, and transient occlusion of the infrarenal segment of the aorta causes paraplegia, which implies differences in vascular anatomy because infrarenal aortic cross-clamping rarely causes paraplegia in human beings. In contrast, the vascular anatomy of the spinal cord and distribution of pathology are similar to that in human beings whose spinal cord receives blood flow from one anterior spinal artery and two posterior spinal arteries [13]. In addition, some mice exhibit delayed paralysis between 24 and 48 hrs after an ischaemic insult [17]. This also replicates one aspect of neurological deficit seen after aortic surgery in human beings. However, mice are so small that surgical results are sometimes influenced by only a small change in the surgical procedure, settings or conditions. Therefore, it is crucial to monitor blood pressure down to levels that induce SCI (20 mmHg) during clamping, and to standardize body temperature, bodyweight and age in the mouse SCI model. Another important feature of this mouse SCI model is that neurological outcome is clearly separated into minor deficits and complete paralysis after a 48-hr interval [17]. From this point of view, it is difficult to detect the effect of therapeutics on neurological outcome and it is possible to misjudge the results if both patterns of neurological outcome are analysed together as a uniform result. Accordingly, we looked at results from two perspectives: One compared the percentage of patients who had mild paralysis per total patients between groups. The other, followed the two patterns of neurological outcome separately. Using this method, we revealed that EPO did not decrease the percentage of

mice with mild paralysis, but improved neurological function of severely paralysed mice between day 2 and 7.

A possible discrepancy in this study is that neurological function of severely paralysed mice improved between day 2 and 7 whereas the number of motor neurons decreased from day 2 to 7 after SCI even in the EPO-treated groups. However, a similar finding was also reported by Sakamoto *et al.*, which indicated that neurological function gradually recovered despite persistent loss of motor neurons up to 2 weeks after SCI in the rat [18]. These findings indicate that functional outcome depends not only on the number of motor neurons but also on other factors such as functional compensation by viable neuromuscular units or attenuation of inflammation in the injured spinal cord.

Neuroprotection provided by EPO in various pathophysiologies has been well documented, with its action mediated by EPO receptors expressed in many kinds of central nervous system (CNS) cells including neural progenitor cells, neurons, glial cells and endothelial cells [19]. In the rabbit model of SCI, systemic administration of human recombinant EPO reduced apoptosis of motor neurons, leading to favourable neurological outcome compared to saline controls [20]. Dame *et al.* also described the neuroprotective and neurotrophic properties of EPO observed in the CNS with *in vivo* models of ischaemic, traumatic or inflammatory brain injury. They also demonstrated that such neuroprotective and neurotrophic effects of EPO were mediated through expression of genes that are related to anti-apoptotic proteins in neuronal cells, such as NF-Ib, *IKKa*, *akt* and several Bcl 2 family members [21]. Erythropoietins' vascular actions have also been demonstrated, one of which is preserving blood–brain barrier (BBB) function, which may contribute to the prevention of brain oedema [22].

Based on the possible anti-apoptotic properties or vascular actions of EPO, we expected to observe the effects of EPO much earlier. However, a 48-hr interval was necessary before the effects of EPO were apparent. This is inconsistent with previous reports that demonstrate the neuroprotective effects of EPO within 2 days after SCI in the mouse, rat and pig [9–11]. However, it is possible that this difference in timing can be attributed to the difference in dosage, timing and route of administration of EPO used. In addition, competitive results in clinical studies have been reported. One such study indicates that EPO improves cardiac function and reduces infarct size after acute myocardial infarction treated with percutaneous coronary intervention [23], whereas EPO failed to do so in another report [24]. Therefore, further investigations would be necessary to clarify this issue.

In our findings, EPO appeared to be more effective at later timepoints, at least 2 days after SCI. This result is supported by literature in which EPO has been shown to have a wide therapeutic window of up to 6 weeks after administration in the case of traumatic spinal cord injury in rats [25]. In addition, EPO is considered to promote neurogenesis and angiogenesis in animal models of acute stroke [26]. This commitment of EPO to tissue regeneration can lead to a wide therapeutic window and delayed neuroprotection. Taguchi et al. have shown that systemic administration of $CD34^+$ cells after stroke accelerated angiogenesis and neurogenesis with functional recovery in mice, suggesting that homing of $CD34^+$ into an ischaemic region was crucial for functional recovery in the CNS [27]. This is consistent with our finding of abundant accumulation of $CD34^+$ cells and improvement of neurological function in EPO-treated groups.

There have been many reports that have addressed the local or systemic delivery of EPO-promoted migration of bone marrow stem cells, such as $CD34^+$ and c-kit^+ cells, to damaged tissue [7, 28]. An interaction between the chemokine receptor CXCR-4 and its ligand stromal cell–derived factor-1 (SDF-1) is considered to be a pivotal element in bone marrow stem cell mobilization and homing. The expression of CXCR-4 on the surface of stem cells facilitates their migration along a gradient of SDF-1 [29]. In animal models of myocardial infarction, EPO promotes the recruitment of bone marrow stem cells in the infarct heart with enhancing local SDF-1 expression [7, 28]. A similar mechanism may have been stimulated in the current study to recruit $CD34^+$ cells in the ischaemic spinal cord. In addition to SDF-1, local inflammatory cytokines in response to hypoxic stress are also involved in stem cell migration. Kaminski *et al.* demonstrated that adhesion of circulating c-kit^+ cells onto vascular endothelium, which is the first step of stem cell infiltration into injured tissue, was intensified only when SDF-1 was present together with tumour necrosis factor α (TNF-α) [30]. Therefore, little tissue damage and a subsequent low inflammatory response could account for low numbers of $CD34^+$ cells in mice with mild paralysis, even in the EPO-treated groups.

However, how accumulated $CD34^+$ cells contribute to the improvement of neurological function and preservation of motor neurons is unclear. One possible explanation is that the interaction of $CD34^+$ cells with local parenchymal cells produces trophic factors that support the recovery of neurological function [31]. In addition, BDNF is a growth factor, which is important in the development and maintenance of the nervous system and has a neuroprotective role [32]. Consistent with the present study, Wang *et al.* demonstrated that expression of BDNF was enhanced after EPO treatment in a rat model of embolic stroke, with functional improvement [26]. Brain-derived neurotrophic factor is considered to be expressed in motor neurons, microglia and endothelial cells in the CNS [26, 32]. Therefore, EPO may induce the expression of BDNF directly or indirectly in these cells through interaction with recruited $CD34^+$ cells. Vascular endothelial growth factor is an endothelial cell-specific growth factor that can be secreted from endothelial progenitor cells (EPCs), which are considered to be $CD34^+$, and mediate angiogenesis together with EPCs [27]. A recent study also revealed that VEGF had a direct neuroprotective effect on motor neurons independently of blood circulation, promoting cell survival and neuronal outgrowth [33]. These reports suggest that enhanced VEGF secretion from recruited $CD34^+$ cells in the spinal cord of the EPO-treated groups may also contribute to functional recovery and neuroprotection.

In conclusion, systemic administration of EPO improved neurological function with preservation of motor neurons in the ischaemic spinal cord after SCI in the mouse. Such beneficial effects may have been provided by recruitment of $CD34^+$ cells and

enhanced expression of BDNF and VEGF. This evidence supports EPO as a treatment option that may ameliorate paraplegia after thoracoabdominal aortic aneurysm (TAAA) surgery.

Acknowledgements

This work was supported by the German Research Foundation, Sonderforschungsbereich/Transregio 7, B5, B2 and A4, German Helmholtz Association, Mecklenburg-Vorpommern (Nachwuchsgruppe Regenerative Medizin Regulation der Stammzellmigration 0402710), Bundesministerium für Bildung und Forschung (BMBF), and Referenz- und Translationszentrum für kardiale Stammzelltherapie (RTC). The authors thank Ms. Margit Fritsche, Department of Cardiac Surgery, Ms. Berit Blendow and Ms. Doris Butzlaff, Institute for Experimental Surgery, University of Rostock, for their technical assistance.

Conflict of interest

The authors confirm that there are no conflicts of interest.

References

1. **Wong DR, Parenti JL, Green SY, *et al.*** Open repair of thoracoabdominal aortic aneurysm in the modern surgical era: contemporary outcomes in 509 patients. *J Am Coll Surg.* 2011; 212: 569–79.
2. **Conrad MF, Crawford RS, Davison JK, *et al.*** Thoracoabdominal aneurysm repair: a 20-year perspective. *Ann Thorac Surg.* 2007; 83: 856–61.
3. **Wong DR, Coselli JS, Amerman K, *et al.*** Delayed spinal cord deficits after thoracoabdominal aortic aneurysm repair. *Ann Thorac Surg.* 2007; 83: 1345–55.
4. **Bavaria JE, Appoo JJ, Makaroun MS, *et al.*** Endovascular stent grafting *versus* open surgical repair of descending thoracic aortic aneurysms in low-risk patients: a multicenter comparative trial. *J Thorac Cardiovasc Surg.* 2007; 133: 369–77.
5. **Maiese K, Li F, Chong ZZ.** New avenues of exploration for erythropoietin. *JAMA.* 2005; 293: 90–5.
6. **Goldman SA, Nedergaard M.** Erythropoietin strikes a new cord. *Nat Med.* 2002; 8: 785–7.
7. **Klopsch C, Furlani D, Gäbel R, *et al.*** Intracardiac injection of erythropoietin induces stem cell recruitment and improves cardiac functions in a rat myocardial infarction model. *J Cell Mol Med.* 2009; 13: 664–79.
8. **Shingo T, Sorokan ST, Shimazaki T, *et al.*** Erythropoietin regulates the *in vitro* and *in vivo* production of neuronal progenitors by mammalian forebrain neural stem cells. *J Neurosci.* 2001; 21: 9733–43.
9. **Simon F, Scheuerle A, Calzia E, *et al.*** Erythropoietin during porcine aortic balloon-occlusion-induced ischemia/reperfusion injury. *Crit Care Med.* 2008; 36: 2143–50.
10. **Smith PD, Puskas F, Fullerton DA, *et al.*** Attenuation of spinal cord ischemia and reperfusion injury by erythropoietin. *J Thorac Cardiovasc Surg.* 2011; 141: 256–60.
11. **Sönmez A, Kabakçi B, Vardar E, *et al.*** Erythropoietin attenuates neuronal injury and potentiates the expression of pCREB in anterior horn after transient spinal cord ischemia in rats. *Surg Neurol.* 2007; 68: 297–303.
12. **Stevens RD, Bhardwaj A.** Eryrthropoietin and the promise of ischemic multiorgan protection. *Crit Care Med.* 2008; 36: 2446–7.
13. **Lang-Lazdunski L, Matsushita K, Hirt L, *et al.*** Spinal cord ischemia. Development of a model in the mouse. *Stroke.* 2000; 31: 208–13.
14. **Kurita N, Kawaguchi M, Horiuchi T, *et al.*** An evaluation of white matter injury after spinal cord ischemia in rats: a comparison with gray matter injury. *Anesth Analg.* 2005; 100: 847–54.
15. **Lafci B, Yasa H, Ilhan G, *et al.*** Protection of the spinal cord from ischemia: comparative effects of levosimendan and iloprost. *Eur Surg Res.* 2008; 41: 1–7.
16. **Yamauchi T, Sakurai M, Abe K, *et al.*** Ubiquitin-mediated stress response in the spinal cord after transient ischemia. *Stroke.* 2008; 39; 1883–9.
17. **Awad H, Ankeny DP, Guan Z, *et al.*** A mouse model of ischemic spinal cord injury with delayed paralysis caused by aortic cross-clamping. *Anesthesiology.* 2010; 113: 880–91.
18. **Sakamoto T, Kawaguchi M, Kurita N, *et al.*** Long-term assessment of hind limb motor function and neuronal injury following spinal cord ischemia in rats. *J Neurosurg Anesthesiol.* 2003; 15: 104–9.
19. **Noguchi CT, Asavaritikrai P, Teng R, *et al.*** Role of erythropoietin in the brain. *Crit Rev Oncol Hematol.* 2007; 64: 159–71.
20. **Celik M, Goekmen N, Erbayraktar S, *et al.*** Erythropoietin prevents motor neuron apoptosis and neurologic disability in experimental spinal cord ischemic injury. *Proc Natl Acad Sci USA.* 2002; 99: 2258–63.
21. **Dame C, Juul SE, Christensen RD.** The biology of erythropoietin in the central nervous system and its neurotrophic and neuroprotective potential. *Biol Neonate.* 2001; 79: 228–35.
22. **Chi OZ, Hunter C, Liu X, *et al.*** Effects of erythropoietin on blood-brain barrier disruption in focal cerebral ischemia. *Pharmacology.* 2008; 82: 38–42.
23. **Taniguchi N, Nakamura T, Sawada T, *et al.*** Erythropoietin prevention trial of coronary restenosis and cardiac remodeling after ST-elvated acute myocardial infarction (EPOC-AMI): a pilot, randomized, placebo-controlled study. *Circ J.* 2010; 74: 2365–71.
24. **Ott I, Schulz S, Mehilli J, *et al.*** Erythropoietin in patients with acute ST-segment elevation myocardial infarction undergoing primary percutaneous coronary intervention: a randomized, double-blind trial. *Circ Cardiovasc Interv.* 2010; 3: 408–13.
25. **Coleman T, Brines M.** Science review: recombinant human erythropoietin in critical illness: a role beyond anemia? *Crit Care.* 2004; 8: 337–41.
26. **Wang L, Zhang Z, Wang Y, *et al.*** Treatment of stroke with erythropoietin enhances neurogenesis and angiogenesis and improves neurological function in rats. *Stroke.* 2004; 35: 1732–7.
27. **Taguchi A, Soma T, Tanaka H, *et al.*** Administration of $CD34^+$ cells after stroke enhances neurogenesis *via* angiogenesis in a mouse model. *J Clin Invest.* 2004; 114: 330–8.

28. **Brunner S, Winogradow J, Huber BC, *et al.*** Erythropoietin administration after myocardial infarction in mice attenuates ischemic cardiomyopathy associated with enhanced homing of bone marrow-derived progenitor cells *via* the CXCR-4/SDF-1 axis. *FASEB J.* 2009; 23: 351–61.
29. **Petit I, Szyper-Kravitz M, Nagler A, *et al.*** G-CSF induces stem cell mobilization by decreasing bone marrow SDF-1 and up-regulating CXCR4. *Nat Immunol.* 2002; 3: 687–94.
30. **Kaminski A, Ma N, Donndorf P, *et al.*** Endothelial NOS is required for SDF-1a/CXCR4-mediated peripheral endothelial adhesion of c-kit^{+} bone marrow stem cells. *Lab Invest.* 2008; 88: 58–69.
31. **Lu CZ, Xiao BG.** G-CSF and neuroprotection: a therapeutic perspective in cerebral ischaemia. *Biochem Soc Trans.* 2006; 34: 1327–33.
32. **Thoenen H.** Neurotrophins and neuronal plasticity. *Science.* 1995; 270: 593–8.
33. **Rosenstein JM, Krum JM, Ruhrberg C.** VEGF in the nervous system. *Organogenesis.* 2010; 6: 107–14.

Int J Clin Oncol (2012) 17:610–615
DOI 10.1007/s10147-011-0332-1

ORIGINAL ARTICLE

Non-surgical approach to small cell carcinoma of the esophagus: does this rare disease have the same tumor behavior as SCLC?

Yuki Nakajima · Sadamoto Zenda · Keiko Minashi · Tomonori Yano · Makoto Tahara · Toshihiko Doi · Masakatsu Onozawa · Keiji Nihei · Satoshi Fujii · Atsushi Ohtsu

Received: 8 July 2011 / Accepted: 20 September 2011 / Published online: 27 October 2011

Abstract
Background We conducted a retrospective analysis to clarify the clinical profile of a nonsurgical approach to small cell carcinoma of the esophagus (SCEC).
Patients and methods SCEC patients in our database were reviewed. Consistent with the standard approach to small cell carcinoma of the lung (SCLC), chemoradiotherapy was the first choice for limited disease (LD)-SCEC in our institution while chemotherapy was the first choice for extensive disease (ED)-SCEC. Our strategy did not include prophylactic cranial irradiation.
Results Eighteen patients were treated between January 1996 and December 2006, of whom 10 had LD-SCEC and 8 had ED-SCEC. Regarding response to chemoradiotherapy in patients with LD-SCEC, CR rate at the primary site was 90% (9/10) and total CR rate was 80% (8/10). With a median follow-up period of 55.3 months, median survival time in LD-SCEC and ED-SCEC patients was 17.3 and 13.9 months, respectively, showing no significant difference ($p = 0.57$). Brain metastases occurred in only one patient. On follow-up, eight patients with LD-SCEC and seven with ED-SCEC died of disease. Only 2 patients died of local progression, while the remaining 13 died of disease progression of distant metastases.
Conclusion Despite providing good local control, chemoradiotherapy appeared to have insufficient potential to cure LD-SCEC. Prophylactic brain irradiation for SCEC is unnecessary.

Keywords Chemoradiotherapy · Small cell carcinoma of the esophagus

Introduction

Small cell carcinoma of the esophagus (SCEC) is an extremely rare disease. In his 1952 paper, McKeown [1] reported only 2 esophageal oat-cell tumors among 9,000 postmortem examinations of deaths of all causes, and only a few case reports or small case series have appeared since then. SCEC is described as an aggressive condition with a poor prognosis. As no specific staging system is available, disease stage is presented according to two staging systems [2, 3], the American Joint Committee on Cancer (AJCC) tumor–node–metastasis (TNM) staging system for esophageal cancer and the Veterans' Administration Lung Study Group (VALSG) for primary small cell carcinoma of the lung (SCLC). This second system consists of two staging categories, limited disease (LD) and extensive disease (ED); LD is defined as tumor confined within a localized anatomical region with or without regional lymph node involvement, and ED as a tumor outside locoregional boundaries.

Chemotherapy with hyperfractionated radiotherapy [4, 5] is the standard treatment for LD-SCLC, while chemotherapy

Y. Nakajima
Division of Digestive Endoscopy, Oitatohbu Hospital,
7-6-5 Tanigasako Shimura, Oita 870-0261, Japan

S. Zenda (✉) · M. Onozawa · K. Nihei
Division of Radiation Oncology,
National Cancer Center Hospital East,
6-5-1 Kashiwanoha, Kashiwa, Chiba 277-8577, Japan
e-mail: szenda@east.ncc.go.jp

K. Minashi · T. Yano · M. Tahara · T. Doi · A. Ohtsu
Division of Gastrointestinal Oncology and Digestive Endoscopy,
National Cancer Center Hospital East, 6-5-1 Kashiwanoha,
Kashiwa 277-8577, Japan

S. Fujii
Division of Pathology, National Cancer Center Hospital East,
6-5-1 Kashiwanoha, Kashiwa 277-8577, Japan

is the first choice for ED-SCLC [6–8]. In our institution, the therapeutic strategy for SCEC was established in accordance with the tumor staging used for SCLC.

Here, we conducted a retrospective analysis to clarify the clinical profile of a nonsurgical approach for SCEC.

Patients and methods

Patients

Patients in our database treated from January 1996 and December 2006 for histologically proven small cell carcinoma of the esophagus who had not received a surgical approach as initial treatment were selected for review. In the current study, the tumors were diagnosed and classified in accordance with the WHO 2000 classification, because the individual therapy for each patient was decided in accordance with that classification. Therefore, we used the pathological-diagnostic term "small cell carcinoma of the esophagus" in this study and did not comply with the updated classification such as NET (neuroendocrine tumor) introduced in the new WHO 2010 classification.

Pretreatment evaluation

Pretreatment clinical evaluation was performed using cervical, chest, and abdominal computed tomography (CT), and/or positron emission tomography (PET)–CT. Tumor staging in the present study was based on both the section on the esophagus in the TNM classification (6th edition) and the staging system of VALSG for primary SCLC. The reason why the TNM classification (6th edition) was used in the current study is the same as above.

Radiological evaluations for staging were jointly reviewed by radiologists, thoracic surgeons, and medical oncologists at our institution.

Treatment

Consistent with the standard approach for SCLC, chemoradiotherapy was the first choice for LD-SCEC in our institution. Surgery is considered only when patients refuse chemoradiotherapy. On the other hand, if chemoradiotherapy fails, salvage surgery becomes a promising choice as a second approach.

For ED-SCEC, chemotherapy was the first choice.

Our strategy did not include prophylactic cranial irradiation. Because data were collected over a 10-year period, some treatment variation in our data is present.

With regard to radiotherapy for LD-SCLC, gross tumor volume (GTV) was determined as the region judged on endoscopic or radiographic examination to contain the gross primary tumor or metastatic lymph nodes. Clinical target volume (CTV) was defined as the GTV plus volumes considered at risk of containing microscopic disease. CTV was further categorized into two volumes, a CTV boost (CTVb), which included the primary tumor with a 3-cm margin craniocaudally, and a CTV subclinical (CTVs), which included the CTVb plus regional nodes. Until December 1999, anterior- and posterior-opposed beams were given to the CTVs at a dose of up to 40 Gy, and a booster dose of 20 Gy was given to the CTVb using bilateral oblique fields to shield the spinal cord, for a total dose of 60 Gy. From January 2000, only the CTVb was irradiated at up to 50–60 Gy with conventional fractionation.

With regard to chemotherapy, platinum-based regimens, mainly etoposide and cisplatin (EP) or irinotecan and cisplatin (IP), were used as an initial approach both for LD-SCEC and ED-SCEC. In LD-SCEC, chemotherapy was continued for four cycles or until tumor progression after concurrent chemoradiotherapy.

Efficacy

For patients receiving chemoradiotherapy, response of the primary tumor was evaluated using the Guidelines for Clinical and Pathological Studies on Carcinoma of the Esophagus by the Japan Esophageal Society (10th edition). CR for the primary tumor was defined on endoscopic observation of the entire esophagus and satisfied the following criteria: (1) disappearance of the tumor lesion, (2) disappearance of ulceration, and (3) absence of cancer cells in biopsy specimens [9]. Evaluation was repeated every 2 or 3 months after the completion of CRT [9]. For lymph node and distant metastases, response evaluation was determined with reference to the Response Evaluation Criteria in Solid Tumors (RECIST 1.1) [10].

Overall survival was calculated as the time from the start of treatment to the date of death or last confirmed date of survival. Progression-free survival (PFS) was defined as the time from the day of initiation of treatment to the first day of confirmation of disease progression or death by any cause. Local control was defined as the lack of disease progression at the primary site. The pattern of treatment failure was defined from the first site of failure, with local failure indicating recurrence or persistent disease after treatment at the primary site; regional failure indicating regional lymph node metastases after treatment; and distant failure indicating recurrence at any site beyond the primary site and regional lymph nodes.

Statistical analysis

Overall and progression-free survival time were estimated by the Kaplan–Meier product–limits method with the log-

rank test using commercially available statistical software (StatView version 5.0, SAS Institute, Cary, NC, USA).

Results

Patient characteristics

Patient characteristics are listed in Table 1. From January 1996 through to December 2006, 18 patients were reviewed, of whom 10 had LD-SCEC and 8 had ED-SCEC. Most patients had good performance status and three patients had N0M0 disease. Median age was 67 years (range 40–77 years). Regarding initial treatment of LD-SCEC, all patients received chemoradiotherapy, and the most frequent regimen was IP. For ED-SCEC, all patients received platinum-based chemotherapy, with the most frequent regimen being IP.

Table 1 Patient characteristics

No. of patients	18	
Age (years), median (range)	67 (40–77)	
Sex (M/F)	13/5	
Performance Status (0/1/2)	11/6/1	
Location		
Upper	4	
Middle	6	
Lower/GE junction	8	
Extent	LD	ED
	10	8
TNM classification (6th)		
T stage		
T1	0	0
T2	2	1
T3	5	5
T4	3	2
N stage		
N0	3	1
N1	7	7
M stage		
M0	9	0
M1	1	8
Initial treatment		
Radiotherapy	10	0
30 Gy/10 fractions	1	
50 Gy/25 fractions	4	
60 Gy/30 fractions	5	
Chemotherapy	10	8
Cisplatin, etoposide	2	1
Cisplatin, irinotecan	5	7
Cisplatin, 5-fluorouracil	3	0

Efficacy

With regard to the response to chemoradiotherapy in LD-SCEC patients, CR rate at the primary site was 90% (9/10) and total CR rate was 80% (8/10). Median progression-free survival was 9.0 months, which was significantly better than the 6.0 months in those with ED-SCEC ($p = 0.04$) (Fig. 1).

Median survival time of all patients was 16.1 months and 3-year overall survival rate was 16.7%, with a median follow-up period 55.3 months. Median survival in LD-SCEC and ED-SCEC patients was 17.3 and 13.9 months, respectively, showing no significant difference ($p = 0.57$) (Fig. 2).

Failure pattern

A total of nine LD-SCEC patients were confirmed to have tumor progression, consisting of one (11.1%), none (0%), and eight (89.9%) patients with local, regional, and distant failure, respectively. One patient has survived over 4 years without any disease progression.

Of those with ED-SCEC, in contrast, three patients (37.5%) had local progression, two (25.0%) had regional disease progression, and the remaining three (37.5%) had confirmed distant failure. The most frequent site of distant failure was the liver, while brain metastases occurred in only one case.

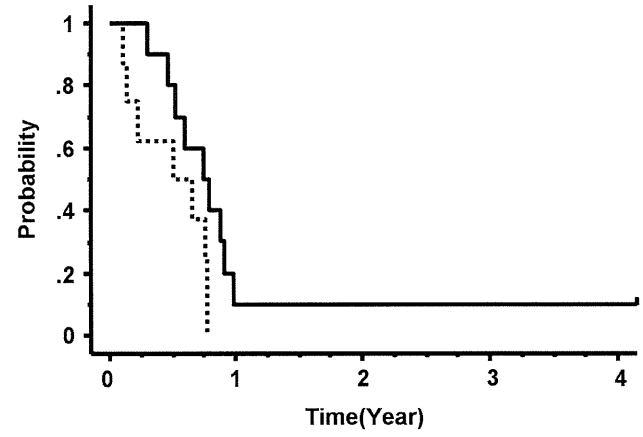

Fig. 1 Progression-free survival in LD-SCEC and ED-SCEC. *Solid line* indicates progression-free survival curve in LD-SCEC; *broken line* indicates progression-free survival curve in ED-SCEC. Median progression-free survival in LD-SCEC patients was 9.0 months, significantly better than that of ED-SCEC patients (6.0 months) ($p = 0.04$)

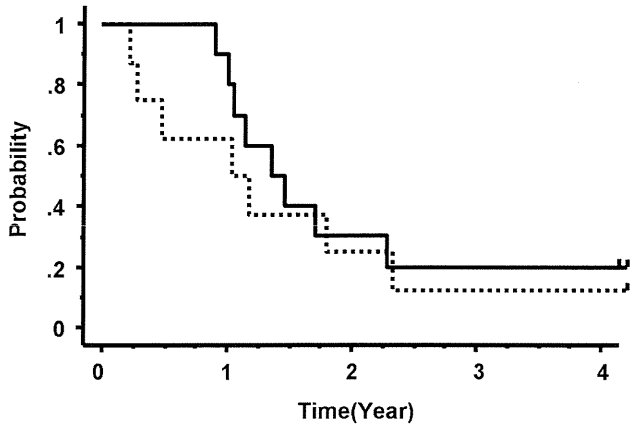

Fig. 2 Overall survival in LD-SCEC and ED-SCEC. *Solid line* indicates overall survival in LD-SCEC; *broken line* indicates overall survival in ED-SCEC. Median survival time in LD-SCEC and ED-SCEC patients was 17.3 and 13.9 months, respectively

Second-line treatment

After the failure of initial treatment, seven patients received best supportive care, including palliative radiotherapy. Second-line treatment for the other patients consisted of photodynamic therapy (PDT) in one, platinum-based chemotherapy in three, docetaxel in four, and irinotecan in two.

Cause of death

On follow-up, eight patients with LD-SCLC and seven with ED-SCLC died of disease. Only 2 patients died of local progression, with the remaining 13 dying of disease progression of distant metastases. The clinical course is summarized in Table 2.

Two LD-SCEC patients have survived long-term (54.2 and 76.5 months). One patient received photodynamic therapy (PDT) as a salvage treatment for primary residual lesion after CRT and he has continued disease-free. Another patient received chemoradiotherapy (IP-RT) and has continued disease-free.

One ED-SCEC patient has also survived. She has received palliative chemotherapy because of liver metastases and has achieved long-term progression-free survival over 4 years.

Discussion

In this retrospective analysis, we provide a specific clinical profile for SCEC. Results showed that SCEC has a more aggressive potential than other esophageal cancers, and different tumor behavior from SCLC. Furthermore, although chemoradiotherapy is effective in primary disease, it appears still to lack potential for the treatment of LD-SCEC with curative intent.

Only 0.9% (23 of 2449) of all esophageal carcinomas treated at our institution between January 1996 and December 2006 were small cell carcinoma, a similar ratio to those reported previously [11–14]. With regard to LD-SCEC, several previous reports [12, 15, 16] suggested that combined modality therapy using platinum-based combination chemotherapy and radical RT may allow a nonsurgical approach to management, and thereby avoid the morbidity of esophagectomy in LD-SCEC. In the present study, chemoradiotherapy were effective in all T-stage primary tumors (CR rate 90%). Median survival time was only 17.3 months, however, and only two patients remain alive at the time of writing. Moreover, 70% of deaths were due to metastatic disease. These results indicate that local control did not necessarily contribute to an improvement in cure rate, and that chemoradiotherapy is still insufficient for treatment with curative intent.

The role of surgery as a part of local treatment remains controversial in patients with LD-SCEC. There is a case report that radical surgery alone can result in long-term disease-free survival [19]. Nonetheless, most patients who received surgery alone developed systemic recurrence rapidly, so patients with LD-SCEC should be recommended not only resection but also chemotherapy [12, 15]. Recently, surgery has been more commonly performed as part of multimodality treatment for LD-SCEC [14, 20].

With regard to ED-SCEC, previous reports primarily used platinum-based chemotherapy, but median survival time was less than 1 year [12, 15–17]. Noda et al. [6] first reported a phase III study of IP compared with an EP regimen in 154 patients with ED-SCLC. Their interim analysis revealed significant differences in OS and toxicity profile in favor of the irinotecan arm, which led to the premature termination of the study. In contrast, Hanna et al. [18] failed to confirm these positive results. A recent meta-analysis [7] showed that IP might have an advantage over EP in overall survival with fewer hematological toxicities, indicating that IP regimens might represent a suitable alternative to EP regimens in the first-line treatment of ED-SCLC. Accordingly, treatment for ED-SCEC at our institution mainly involves IP regimens. Median survival time in this series was 13.9 months and three of these patients survived more than 20 months.

According to the previous reports on SCLC [19, 20], brain metastasis was found from 10 to 18% at the time of diagnosis, and the incidence of brain metastasis increased at 2 years from 50 to 80%.

Ku et al. [12] reported the occurrence of brain metastases in only 1 of 22 patients. In our present study also, brain metastases were confirmed in only one patient

Table 2 Summary of clinical course

Case no.	Age	Gender	Stage		1st Tx	Response	1st failure site	2nd Tx	F/U period (months)	Status (cause of death)
1	69	F	T3N0M0	LD	IP-RT	CR	Distant (liver)	BSC	12.2	DOD (distant)
2	70	M	T3N0M0	LD	FP-RT	NonCR	Local (primary site)	PDT	54.2	Alive with NER
3	66	M	T2N1M0	LD	IP-RT	CR	Distant (liver)	Docetaxel	27.1	DOD (distant)
4	68	F	T2N1M0	LD	IP-RT	CR	Distant (bone)	BSC	12.6	DOD (distant)
5	69	M	T3N1M0	LD	IP-RT	CR	NER	None	76.5	Alive with NER
6	70	M	T3N1M1a	LD	IP-RT	NonCR	Distant (liver)	BSC	20.3	DOD (distant)
7	74	M	T4N0M0	LD	FP-RT	CR	Distant (brain, abdominal LN)	BSC	13.6	DOD (distant)
8	73	M	T4N1M0	LD	EP-RT	CR	Distant (abdominal LN)	BSC	12.1	DOD (distant)
9	63	M	T3N1M0	LD	FP-RT	CR	Distant (liver)	Irinotecan	17.3	DOD (distant)
10	65	M	T4N1M0	LD	EP-RT	CR	Distant (liver)	Irinotecan	11.4	DOD (distant)
11	58	M	T2N1M1b	ED	IP	–	Local (primary site)	Docetaxel	21.3	DOD (Local)
12	59	F	T3N1M1b	ED	IP	–	Distant (liver)	Docetaxel	58.8	Alive with disease
13	68	M	T3N1M1b	ED	IP	–	Distant (liver)	Docetaxel	2.7	DOD (distant)
14	54	M	T3N1M1b	ED	IP	–	Local (primary site)	BSC	12.3	DOD (distant)
15	56	M	T3N1M1b	ED	IP	–	Local (regional LN)	EP	3.4	DOD (distant)
16	40	F	T3N1M1b	ED	IP	–	Local (regional LN)	CDGP + 5-FU	27.6	DOD (distant)
17	77	F	T4N0M1b	ED	IP	–	Local (primary site)	BSC	5.9	DOD (Local)
18	53	M	T4N1M1b	ED	EP	–	Distant (liver)	CDGP + 5-FU	13.9	DOD (distant)

F female, *M* male, *LD* limited disease, *ED* extensive disease, *IP* irinotecan and cisplatin, *FP* 5-fluorouracil and cisplatin, *EP* etoposide and cisplatin, *CR* complete response, *LN* lymph node, *NER* no evidence of recurrence, *BSC* best supportive care, *PDT* photodynamic therapy, *CDGP* nedaplatin, *F/U* follow up, *5-FU* 5-fluorouracil, *DOD* died of disease

(5.5%). Given this low incidence, prophylactic brain irradiation for SCEC appears unnecessary.

In conclusion, SCEC has more aggressive potential than other esophageal cancers, and different tumor behavior from SCLC. Although chemoradiotherapy is effective in primary disease, it appears still to lack potential for the treatment of LD-SCEC with curative intent. Prophylactic brain irradiation for SCEC is unnecessary. Further improvement in outcomes with this condition may require potent new strategies, such as chemotherapy or chemoradiotherapy with molecular targeting drugs.

Conflict of interest No author has any conflict of interest.

References

1. McKeown F (1952) Oat-cell carcinoma of the oesophagus. J Pathol Bacteriol 64:889–891
2. Greene FL (2002) The American Joint Committee on Cancer: updating the strategies in cancer staging. Bull Am Coll Surg 87:13–15
3. Zelen M (1973) Keynote address on biostatistics and data retrieval. Cancer Chemother Rep 3(4):31–42
4. Pignon JP, Arriagada R, Ihde DC et al (1992) A meta-analysis of thoracic radiotherapy for small-cell lung cancer. N Engl J Med 327:1618–1624
5. Turrisi AT 3rd, Kim K, Blum R et al (1999) Twice-daily compared with once-daily thoracic radiotherapy in limited small-cell lung cancer treated concurrently with cisplatin and etoposide. N Engl J Med 340:265–271
6. Noda K, Nishiwaki Y, Kawahara M et al (2002) Irinotecan plus cisplatin compared with etoposide plus cisplatin for extensive small-cell lung cancer. N Engl J Med 346:85–91
7. Jiang J, Liang X, Zhou X et al (2010) A meta-analysis of randomized controlled trials comparing irinotecan/platinum with etoposide/platinum in patients with previously untreated extensive-stage small cell lung cancer. J Thorac Oncol 5:867–873
8. Kalemkerian GP AW, Blum MG et al (2010) Small cell lung cancer. NCCN Practice Guidelines in Oncology-v.2.2009
9. Zenda S, Hironaka S, Taku K et al (2009) Optimal timing of endoscopic evaluation of the primary site of esophageal cancer after chemoradiotherapy or radiotherapy: a retrospective analysis. Dig Endosc 21:245–251
10. Eisenhauer EA, Therasse P, Bogaerts J et al (2009) New response evaluation criteria in solid tumours: revised RECIST guideline (version 1.1). Eur J Cancer 45:228–247
11. Medgyesy CD, Wolff RA, Putnam JB Jr et al (2000) Small cell carcinoma of the esophagus: the University of Texas M. D. Anderson Cancer Center experience and literature review. Cancer 88:262–267
12. Ku GY, Minsky BD, Rusch VW et al (2008) Small-cell carcinoma of the esophagus and gastroesophageal junction: review of the Memorial Sloan-Kettering experience. Ann Oncol 19:533–537
13. Kuo CH, Hsieh CC, Chan ML et al (2011) Small cell carcinoma of the esophagus: a report of 16 cases from a single institution and literature review. Ann Thorac Surg 91:373–378

14. Brenner B, Tang LH, Klimstra DS et al (2004) Small-cell carcinomas of the gastrointestinal tract: a review. J Clin Oncol 22:2730–2739
15. Bennouna J, Bardet E, Deguiral P et al (2000) Small cell carcinoma of the esophagus: analysis of 10 cases and review of the published data. Am J Clin Oncol 23:455–459
16. Hudson E, Powell J, Mukherjee S et al (2007) Small cell oesophageal carcinoma: an institutional experience and review of the literature. Br J Cancer 96:708–711
17. Craig SR, Carey FA, Walker WS et al (1995) Primary small-cell cancer of the esophagus. J Thorac Cardiovasc Surg 109:284–288
18. Hanna N, Bunn PA Jr, Langer C et al (2006) Randomized phase III trial comparing irinotecan/cisplatin with etoposide/cisplatin in patients with previously untreated extensive-stage disease small-cell lung cancer. J Clin Oncol 24:2038–2043
19. Auperin A, Arriagada R, Pignon JP et al (1999) Prophylactic cranial irradiation for patients with small-cell lung cancer in complete remission. Prophylactic Cranial Irradiation Overview Collaborative Group. N Engl J Med 341:476–484
20. Slotman B, Faivre-Finn C, Kramer G et al (2007) Prophylactic cranial irradiation in extensive small-cell lung cancer. N Engl J Med 357:664–672

Surg Endosc (2012) 26:2877–2883
DOI 10.1007/s00464-012-2273-9

Endoscopic balloon dilatation for benign fibrotic strictures after curative nonsurgical treatment for esophageal cancer

Yusuke Yoda · Tomonori Yano · Kazuhiro Kaneko · Shinya Tsuruta · Yasuhiro Oono · Takashi Kojima · Keiko Minashi · Hiroaki Ikematsu · Atsushi Ohtsu

Received: 18 October 2011 / Accepted: 24 March 2012 / Published online: 28 April 2012

Abstract
Background Endoscopic balloon dilatation (EBD) is performed to treat strictures after esophagectomy. However, little is known about using EBD for benign strictures that occur after nonsurgical treatments for esophageal cancer such as chemoradiotherapy (CRT) or endoscopic mucosal resection (EMR). The aim of this study was to evaluate the safety and efficacy of EBD for benign strictures after nonsurgical treatment compared with those after surgery.
Methods We identified 823 patients with esophageal cancer who completed definitive treatments between 2004 and 2007. Of these patients, 122 were enrolled in our study, including 60 who had surgery and 62 who did not have surgery (32 CRT, 30 EMR). The indication criteria for EBD were complaint of dysphagia and the inability to pass a conventional endoscope due to benign stricture. We retrospectively analyzed the safety and efficacy of EBD, and the measured outcomes were treatment success rate, time to treatment success, and refractory stricture rate.
Results Perforation occurred in 3 (0.3 %) of 1,077 EBD sessions, with no bleeding. Efficacy was evaluated in 110 of the 122 patients. While the treatment success rate was over 90 % in both the surgery and the nonsurgery group, there was a significant difference in the median time to treatment success between both groups (2.3 vs. 5.6 months, $p = 0.02$: log-rank test). There was a significant difference in the median time to treatment success between CRT and surgery groups (7.0 months, $p = 0.01$), with no significant difference in the EMR group (4.4 months, $p = 0.85$). A significant difference in the refractory stricture rate was evident between the nonsurgery group (75 %) and the surgery group (45 %, $p < 0.01$).
Conclusion EBD for stricture after nonsurgical treatment of esophageal cancer was safe and effective. However, patients with benign strictures after nonsurgical treatment required significantly longer time to recover from dysphasia compared to those after surgery.

Keywords Esophageal stricture · Balloon dilatation · Esophageal cancer

Various nonsurgical curative treatment modalities for esophageal cancer are increasing. For superficial esophageal cancer, endoscopic mucosal resection (EMR) and endoscopic submucosal resection (ESD) are less invasive curative treatments [1–4]. Chemoradiotherapy (CRT) is a standard nonsurgical treatment for locally advanced esophageal cancer, and salvage EMR or photodynamic therapy (PDT) can be curative for local or regional recurrence after CRT [5–7]. However, dysphagia can be caused by fibrotic stricture, even after nonsurgical treatments, with complication rates of 3.3–40 % after CRT [8, 9] and 6.0–18 % after EMR [4, 10, 11]. Severe dysphagia frequently reduces the quality of life in patients undergoing these nonsurgical treatments despite the achievement of a primary cure under organ preservation.

Esophageal dilatation has been the primary therapy for benign esophageal strictures. Common dilators can be categorized as follows: the simple bougie type (Maloney

Y. Yoda · T. Yano (✉) · K. Kaneko · S. Tsuruta · Y. Oono · T. Kojima · K. Minashi · H. Ikematsu · A. Ohtsu
Department of Gastroenterology and Gastrointestinal Oncology, National Cancer Center Hospital East, Kashiwanoha 6-5-1, Kashiwa, Chiba 277-8577, Japan
e-mail: toyano@east.ncc.go.jp

Y. Yoda
e-mail: yuyoda@east.ncc.go.jp