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ORIGINAL ARTICLE

Potential adverse effects of cyclosporin A on kidneys after spinal cord injury

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Study design: Cell transplantation strategies are gaining increasing interest for spinal cord injury (SCI) with the objective of promoting spinal cord repair. To avoid allogenic graft rejection, an adequate immune suppression is required, and one of the most potent and commonly used immunosuppressives is cyclosporin A (CsA). In SCI, permanent sensory motor loss is combined with modifications of drug absorption, distribution and elimination.

Objectives: The objectives of this study were to thoroughly explore histological and functional outcomes of CsA treatment in a rat model of spinal cord compression.

Setting: Experiments were carried out at the Institute for Neurosciences of Montpellier (France), the Integrative Biology of Neurodegeneration Laboratory (Spain) and in the Novartis Institutes for BioMedical Research (Switzerland) for CsA blood concentration determination.

Methods: We first evaluated histological outcomes of CsA treatment on kidneys and spinal cord after SCI. We then investigated whether SCI modified CsA blood concentration. Finally, using behavioral analysis, we assessed the potential CsA impact on functional recovery.

Results: When spinal-cord-injured rats were treated with a CsA dose of $10\,mg\,kg^{-1}$ per day, we observed deleterious effects on kidneys, associated with modifications of CsA blood concentration. Adding an antibiotic treatment reduced kidney alteration without modifying CsA blood concentration. Finally, we showed that CsA treatment *per se* modified neither functional recovery nor lesion extension.

Conclusion: This study pinpoints the absolute requirement of careful CsA monitoring in the clinical setting for patients with SCI to minimize potential unexpected effects and avoid therapeutic failure.
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Keywords: spinal cord compression; nephrotoxicity; CsA disposition; absence of neuroprotection

Introduction

Cell transplantation approaches are promising potential therapies for central nervous system disorders. This is particularly true for spinal cord injury (SCI) that leads to permanent sensory motor loss with no current therapy. In SCI, cell grafting, among other objectives such as replacement of lost cells, may be used as a tool to provide trophic support to preserve undamaged tissues and promote spinal cord repair (for review see Barnabe-Heider and Frisen[1]). To avoid allogenic graft rejection, an immune suppression is required and one of the most commonly used immune suppressants is cyclosporin A (CsA). Besides complete or partial deprivation of sensory and motor functions, SCI induces several systemic and metabolic alterations such as reduction of hepatic microvascular blood flow.[2] Owing to modification in drug absorption, distribution and elimination, SCI alters CsA bioavailability in a complex time- and route-administration-dependent manner.[3] At an acute stage after spinal cord injury, CsA availability is either increased or decreased when delivered intraperitonealy or orally. At a chronic stage, no difference is observed whatever the administration route.[4] Increased availability of CsA may

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result from an impairment in drug clearance,[2,4] whereas decreased bioavailability may be because of modification in gastrointestinal tract absorption.[4]

Besides its effects in prevention of allograft rejection, CsA shows various adverse side effects such as nephrotoxicity[5] and leads to reduction in both renal blood flow and glomerular filtration rate. Conversely, CsA has been reported to be neuroprotective following SCI.[6] Rats subjected to spinal compression[7] or contusion[8] present a better motor outcome when treated with CsA. However, in other studies, absence of CsA neuroprotection at both behavioral and morphological levels was reported after SCI.[9–10]

In the perspective of possible clinical application of allogenic cell transplantation after SCI, we investigated outcomes of CsA treatment in a well-defined rat model of spinal cord compression.[11]

Results

Combined SCI and CsA immunosuppressive therapy induced severe kidney alterations

Male rats that underwent spinal cord compression and were treated with CsA presented exacerbated suffering. This was strikingly different to SCI-only or to uninjured rat (sham) treated with the same CsA dose. Forensic analysis revealed major kidney alterations; all 'SCI-CsA' animals developed major hydronephrosis (Figure 1a) and/or ulceration and necrosis (Figure 1b), whereas all 'sham', 'injured-only' and 'CsA-only' rats presented normal kidneys (Figures 1a and b). SCI combined with CsA treatment induced prominent glomerular (Figure 1d) and tubular (Figure 1f) changes with respect to all other groups (Figures 1c and e). Severe glomerular hydronephrosis (Figure 1d) associated with periglomerular fibrosis (arrow, Figure 1d) was observed. Moreover, tubular necrosis was associated with severe constriction due to thickening of the tubular wall (Figure 1f). Morphometric analysis of the median longitudinal section revealed dose–response kidney alterations; 70, 50 and 10% of the rats had normal kidneys in the 5, 7.5 and 10 mg kg^{-1} per day groups, respectively, whereas 0, 40 and 60% of animals presented extremely severe kidney alterations (scores 2 + 3) in the same-dose group (Table 1). Kidney alterations in the 10 mg kg^{-1} per day group were reduced by administration of gentamicin treatment and 75% of the animals had normal kidneys (Table 1).

CsA blood concentration is modified by SCI

To determine whether deleterious effects of CsA on kidneys in 'SCI-CsA' animals were correlated to a modification in drug disposition, we carried out whole-blood CsA dosage analysis. Daily subcutaneous injection of CsA started 2 days before surgery; on the day of surgery, injection was administered just after injury and CsA blood concentration was analyzed 2 and 24 h after injury and concomitant injection. Four groups of rats were used: rats with or without (sham) SCI were injected with either NaCl or CsA (10 mg kg^{-1}) (Table 2A). Sham and SCI rats injected with NaCl presented the same zero CsA baseline level (Figure 2a). At 2 h after injection, injured rats had a lower CsA blood concentration (2.0 ± 0.17 μM) than did sham animals (2.56 ± 0.21 μM) (Figure 2a). This difference was exacerbated 24 h after injection: injured rats had a CsA blood concentration of 1.34 ± 0.09 μM and sham animals 1.98 ± 0.19 μM (Figure 2a). Even if CsA levels decreased over time (2 and 24 h) in sham rats (2.56 and 1.98 μM) and injured animals (2.0 and 1.34 μM), they did not return to the baseline level (Figure 2a). In the injured group, 48 h after injection, CsA blood level almost dropped to baseline (1.98 μM) (Figure 2b). CsA dose–response analysis in both sham and injured animals using three CsA concentrations (5, 7.5 and 10 mg kg^{-1}; Table 2B) showed that, in the sham group, CsA blood concentrations correlated with CsA doses (Figure 2c). Conversely, SCI rats presented equivalent CsA blood concentrations (0.49 ± 0.07 and 0.45 ± 0.08 μM) for the 5 and 7.5 mg kg^{-1} doses (Figure 2c). At 10 mg kg^{-1}, CsA concentration was 1.25 ± 0.27 μM. Thus, CsA blood concentration was lower in SCI animals for a CsA treatment of 7.5 and 10 mg kg^{-1}, at variance with the 5 mg kg^{-1} dose (Figure 2c).

Combined antibiotic and immunosuppressive therapy reduced kidney alterations without modifying CsA disposition

To minimize urinary tract infection, we combined treatments with both CsA and gentamicin (2 mg kg^{-1}), and evaluated the outcomes on kidneys and CsA blood level (Table 2C). When gentamicin was added, 75% of animals showed normal kidneys, 16% presented minor alterations and only 9% presented more severe lesions (Table 1). CsA disposition was not modified in either group; levels were similar 24 and 48 h after injection (Figure 2d).

CsA treatment did not modify functional recovery and lesion extension after SCI

The potential neuroprotective effect of CsA in SCI is under debate.[6–10,12,13] Therefore, we conducted an analysis of functional locomotor and histopathological outcomes after SCI using various CsA concentrations and a combined treatment of CsA and gentamicin. Before injury, and for 7 (dose response) or 12 (combined treatment) days after injury, we carried out functional tests for motor, sensory and reflex recovery.[11] We did not observe differences among the Nacl-, 5- and 7.5 mg kg^{-1}-treated groups in any of the tests (Supplementary Figure). Comparison of combined CsA–gentamicin treatments with control did not reveal differences in motor and sensory recovery rates but bladder recovery was significantly improved (Figure 3). We carried out a histological analysis (Figure 4) and evaluated the lesion area on a 2.5-cm spinal cord segment centered on the lesion. For all time points analyzed (24 h, 1, 2 and 5 weeks), there was no difference in lesion extension between groups. At 24 h after SCI, approximately 90% of the tissue was injured at the epicenter in both NaCl- (88.8 ± 6.5%) and CsA (91.5 ± 3.1%)-treated groups (data not shown). No CsA dose difference was detected in the percentage of injured tissue at the epicenter (90.9 ± 4.9 and 90.5 ± 0.3% for 5 and 7.5 mg kg^{-1}, respectively; data not shown). At 2 weeks after injury, damaged areas were similar in all groups (87.7 ± 8.5,

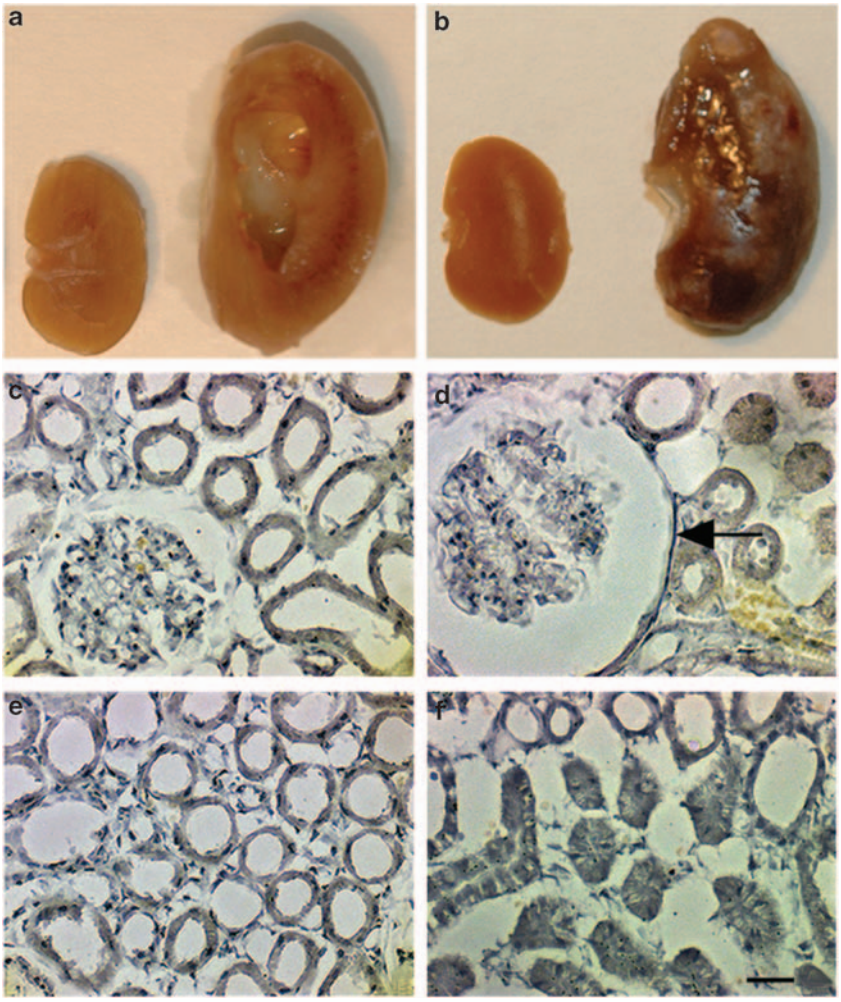

Figure 1 Combined SCI and CsA treatment induced severe nephrotoxicity. (**a**, **b**) Macroscopic kidney views. (**a**) Internal and (**b**) external sagittal view of kidneys from 'injured-only' (left) and 'SCI-CsA' rats (right). Kidneys of 'injured-only' rats appeared normal, whereas 'SCI-CsA' animals presented (**a**) major hydronephrosis and/or (**b**) ulceration and necrosis. (**c**–**f**) Light microscopic kidney sections (hematoxylin and eosin; original magnification, $\times 400$). (**c**, **e**) 'Injured-only' animals. (**d**, **f**) 'SCI-CsA'. (**c**, **d**) Renal glomeruli were normal in 'injured-only' rats (**c**), whereas those from 'SCI-CsA' rats showed severe hydronephrosis and periglomerular fibrosis (**d**, arrow). (**e**, **f**) Renal proximal tubules were normal in 'injured-only' animals. (**e**) Severe tubular atrophy and necrosis was observed in the 'SCI-CsA' group (**f**). Note that 'sham', 'injured-only' and 'CsA-only' rats presented similar normal kidneys. For clarity, only pictures from 'injured-only' animals are presented. CsA: 10 mg kg^{-1}. Scale bar = 50 μm.

Table 1 Morphometric quantification of kidney alterations

CsA (mg kg^{-1} per day)	*Score 0 (%)*	*Score 1 (%)*	*Score 2 (%)*	*Score 3 (%)*
5	70	30	0	0
7.5	50	10	30	10
10	10	30	20	40
10+gentamicin	75	16	9	0

Abbreviation: CsA, cyclosporin A.
Scores: 0, normal kidney; 1, between 5 and 40% of the increased median area without ulceration or dilatation of the pyelocalyceal cavities; 2, >40 % of the increased area without ulceration and up to a 25 % increased in pyelocalyceal cavities; 3, >40 % of the increased area associated with >25 % increase of pyelocalyceal cavities or with ulceration. Gentamicin (2 mg kg^{-1} per day).

84.3 ± 8.7 and 89.1 ± 6.6 for NaCl, CsA 10 mg kg^{-1} only and CsA and gentamicin-treated animals, respectively; data not shown). At 5 weeks after SCI, both antibiotic-only and CsA–antibiotic groups presented the same area of damaged tissue at the epicenter (75.8 ± 12.5 and 80.8 ± 8.1, respectively; Figure 4f). The total lesion extension along the rostrocaudal axis was similar in all groups for each time point (Figure 4f).

Discussion

We observed deleterious effects on kidneys when CsA was subcutaneously administered to spinal-cord-compressed rats. Nephrotoxicity is a widely described side effect of CsA treatment,[5,14–15] but increased CsA nephrotoxicity has never been reported in SCI. In our experiments, animals developed major glomerular hydronephrosis, ulceration and tubular necrosis that eventually led to death. In parallel, CsA blood concentration was modified by SCI; not only did injured rats present a lower CsA blood concentration compared with

Table 2 animal assignments

Surgery	*Sham*			*Compression*				
Treatment	*NaCl*	*CsA (10 mg kg^{-1})*		*NaCl*	*CsA (10 mg kg^{-1})*			
(A) CsA blood concentration is modified by SCI								
CsA blood concentration	2 h	2 h	24 h	2 h	Surgery	2 h	24 h	48 h
Number of animals	6	6*	6*	6	7#	7#	7#	7
Killing and histology (24 h after SCI) (kidneys and spinal cord)	Yes		Yes	Yes			Yes	

Surgery	*Sham*			*Compression*		
(B) CsA dose response						
Treatment: CsA (mg kg^{-1})	5	7.5	10	5	7.5	10
Number of animals	5	5	5	5	5	5
CsA blood concentration	24 h after the last injection					
Behavioral evaluation	Daily for 12 days					
Killing and histology (kidneys and spinal cord)	2 weeks after surgery					

Surgery	*Compression*			*Compression*	
Treatment: *CsA (10 mg kg^{-1})* *Gentamicin (2 mg kg^{-1})*	*NaCl+Gentamicin*	*CsA*	*CsA+Gentamicin*	*CsA+Gentamicin*	*NaCl+Gentamicin*
(C) CsA and gentamicin treatments					
Number of animals	6	6	6	8	7
CsA blood concentration	24 and 48 h after last injection				
Behavioral evaluation	Daily for 12 days				
Killing and histology (kidneys and spinal cord)	2 weeks after surgery			5 weeks after surgery	

Abbreviations: CsA, cyclosporin A; SCI, spinal cord injury.
*and # different time points obtained with same animals.
(A) For CsA blood concentration analysis after SCI, we analyzed 32 animals. 12 animals had undergone a sham surgery (6 rats received NaCl and 6 CsA (10 mg kg^{-1})). 13 animals had undergone a compression injury (6 rats received NaCl and 7 CsA (10 mg kg^{-1})), 7 other animals received CsA (10 mg kg^{-1}) for blood analysis at 48 h. CsA blood concentration was analyzed at different time points after injection. For histological kidneys and spinal cord analysis animals were killed 24 h after surgery. (B) For CsA dose response we analyzed 30 rats. 15 animals had undergone a sham surgery and received either different CsA doses (5, 7.5 and 10 mg kg^{-1}; 5 animals per group) or NaCl. 15 animals that had undergone a compression injury were similarly treated (CsA: 5, 7.5 and 10 mg kg^{-1} or NaCl; 5 animals per group). CsA blood concentration was analyzed 24 h after the last injection. Behavioral evaluation was carried out daily for 12 days. Animals were killed 2 weeks after surgery and histological analysis of kidneys and spinal cord were carried out. (C) For CsA and CsA–gentamicin combined treatments we analyzed 33 rats. They all had undergone spinal cord compression. 13 animals received a combined NaCl and gentamicin treatment, 6 received only CsA and 14 received a combined CsA and gentamicin treatment. CsA blood concentration was analyzed 24 and 48 h after the last injection. Behavioral evaluation was carried out daily for 12 days. 18 and 15 animals were killed 2 and 5 weeks after compression and histological analyses of kidneys and spinal cord were carried out. For all experiments, treatments (NaCl, CsA and combined CsA–gentamicin) started 2 days before surgery.

sham animals but also, and unlike with sham rats, CsA blood concentration did not correlate with CsA doses. CsA pharmakokinetics is modified by SCI.[3] in an administration-route manner.[4] We observed a decreased CsA bioavailability, as found when the drug is administered orally;[14] this may likely be because of preferential accumulation in organs, as CsA-treated mice present a higher CsA concentration peak in the spleen and in kidneys than in blood.[16] Moreover, CsA accumulates preferentially in organs susceptible to CsA toxicity (brain, kidneys, liver, thymus and spleen) than in resistant organs (heart, lungs and muscles).[17] Modification of CsA kinetics induced by SCI could therefore contribute to its toxicity by means of increased retention and/or accumulation in susceptible organs such as kidneys.

Urological infections are the most reported secondary effects for SCI patients and immunosuppressive therapies exacerbate infections. When animals were treated with gentamicin, 75% of rats presented normal kidneys as compared with 90% of kidney alterations without antibiotic treatment. Gentamicin is known for its nephrotoxicity; however, CsA–gentamicin treatments never enhanced kidney alterations. Thus, kidney alterations most likely resulted from combined urinary tract infection due to SCI and CsA treatments; however, control of the infection reduced, if not abrogated, CsA nephrotoxicity.

Cyclosporin neuroprotection after SCI has been reported in some studies (for review see Rezzani[15]) but not in others.[9–10] When CsA is administered after spinal cord contusion, improved motor function is reported.[8] After spinal cord compression, CsA treatment improves motor performance but does not increase the extent of spared tissues.[7] Conversely, no improvement in motor performance, or in the amount of spared cord tissue, is reported after spinal cord contusion when CsA is administered before, during or after injury.[9–10] In our model of compression injury, CsA treatment did not have a neuroprotective effect, as it did not modify the functional recovery and the lesion extension. The model and the severity of the lesion certainly account for some of these discrepancies in the potential neuroprotective effect of CsA. The strain of animal used, the

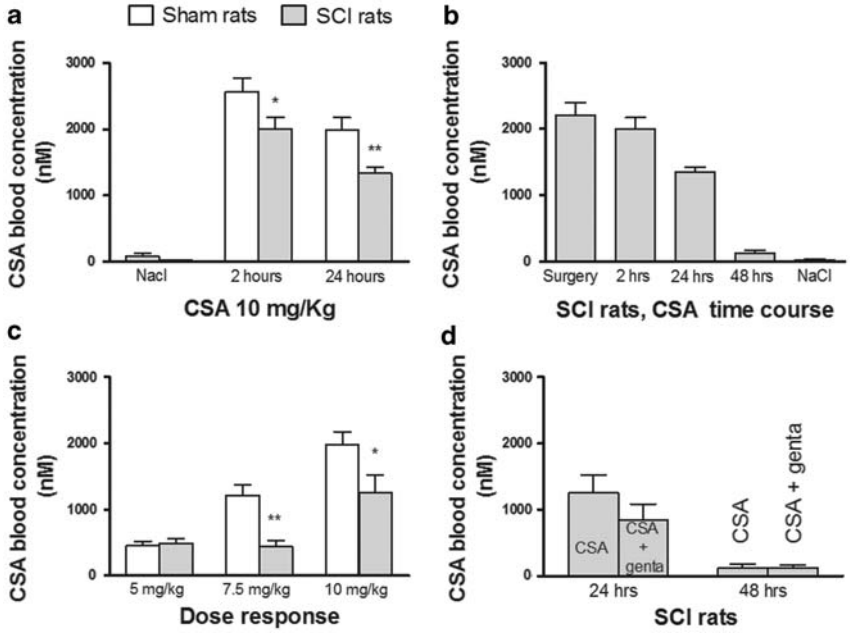

Figure 2 CsA blood concentration is modified by SCI. (**a**) Cyclosporin A blood level in sham (uninjured) and SCI rats. At 2 and 24 h after injection, CsA blood levels were lower in rats that underwent SCI. CsA treatment: 10 mg kg^{-1}. (**b**) Time course analysis of CsA blood concentrations in SCI rats. At 48 h after injection, CsA concentration almost returned to baseline. CsA treatment: 10 mg kg^{-1} (**c**) CsA dose–response comparison between sham and injured rats. Analysis was carried out 24 h after the last CsA injection. (**d**) Comparison of CsA blood concentration in injured rats that received either CsA-only (10 mg kg^{-1}) or combined CsA–gentamicin treatment (10 and 2 mg kg^{-1}, respectively). Statistical analysis: *t* test, $*P<0.05$ and $**P<0.01$; data represent the mean ± s.e.m of at least six animals per group.

level of injury, the route, dose and frequency of CsA administration may also have a significant effect.

One of the limitations of autologous grafting may be the availability of a sufficient amount of cells to be grafted. Indeed, delay in obtaining enough autologous cells to be transplanted may be incompatible with the optimal time window for treatment. In this case, heterologous grafting, which requires immunosuppression, may become the only possibility. In clinics, most drug regimens do not take into consideration the specificity of SCI patients. However, SCI influences drug disposition in a complex manner and may thus predispose patients to adverse side effects. This points to the absolute necessity not only to consider drug pharmacokinetics but also to design unique protocols for SCI patients that will require further prospective studies.

In conclusion, we show that (1) combined spinal cord compression and CsA treatment induced deleterious effects on kidneys that are reduced if not abrogated when an antibiotic therapy is added; (2) SCI induced modification in CsA blood concentration probably because of specific organ retention; (3) gentamicin treatment had no effect on CsA blood concentration; and finally (4) CsA showed no neuroprotective effect in spinal cord compression.

Materials and methods

Animal, surgery, pharmacological treatments, CsA concentration measurement

Experimental procedures followed the European legislation for animal experimentation (86/609/EEC). All applicable institutional and governmental regulations with regard to the ethical use of animals were followed during the course of this research. We used 8- to 9-week-old male Wistar rats (Charles River, Lyon, France). Surgeries were conducted with 1 liter per min O_2 supply and anesthesia was induced by 4% isoflurane and maintained at 2.5%.[11] Compression injury was carried out similarly to that described previously.[11] A 2-French Fogarty catheter (Edwards Lifesciences, Nyon, Switzerland) was introduced into the epidural space; the balloon was positioned at thoracic level 8, inflated (15 μl) and left in place for 5 min. Sham animals underwent the same protocol, except for balloon inflation. Animals were killed with an overdose of pentobarbital and perfused transcardially with 4% paraformaldehyde (Table 2). For histological studies, 2.5 cm spinal cord segments centered on the lesion and kidneys were dissected, postfixed, cryoprotected and embedded in Tissue-Tek OCT Compound (Sakura Finetech, Zoeterwoude, The Netherlands).

Pharmacological treatment consisted of daily subcutaneous injection of CsA (Novartis, Basel, Switzerland) at 5, 7.5 and 10 mg kg^{-1}. CsA injection started 2 days before surgery. Controls received the equivalent volume of NaCl (Table 2). Antibiotic therapy consisted of daily i.m. injection of gentamicin (2 mg kg^{-1}) for 7 days starting at the time of surgery.

Blood was drawn under light isoflurane anesthesia in heparin-rinsed tubes. Blood samples were spiked with a structurally closely related internal standard (cyclic peptide with one additional methyl group as compared with CsA), lysed and deproteinated using acetonitrile, centrifuged, the supernatant was evaporated and the pellet was redissolved in

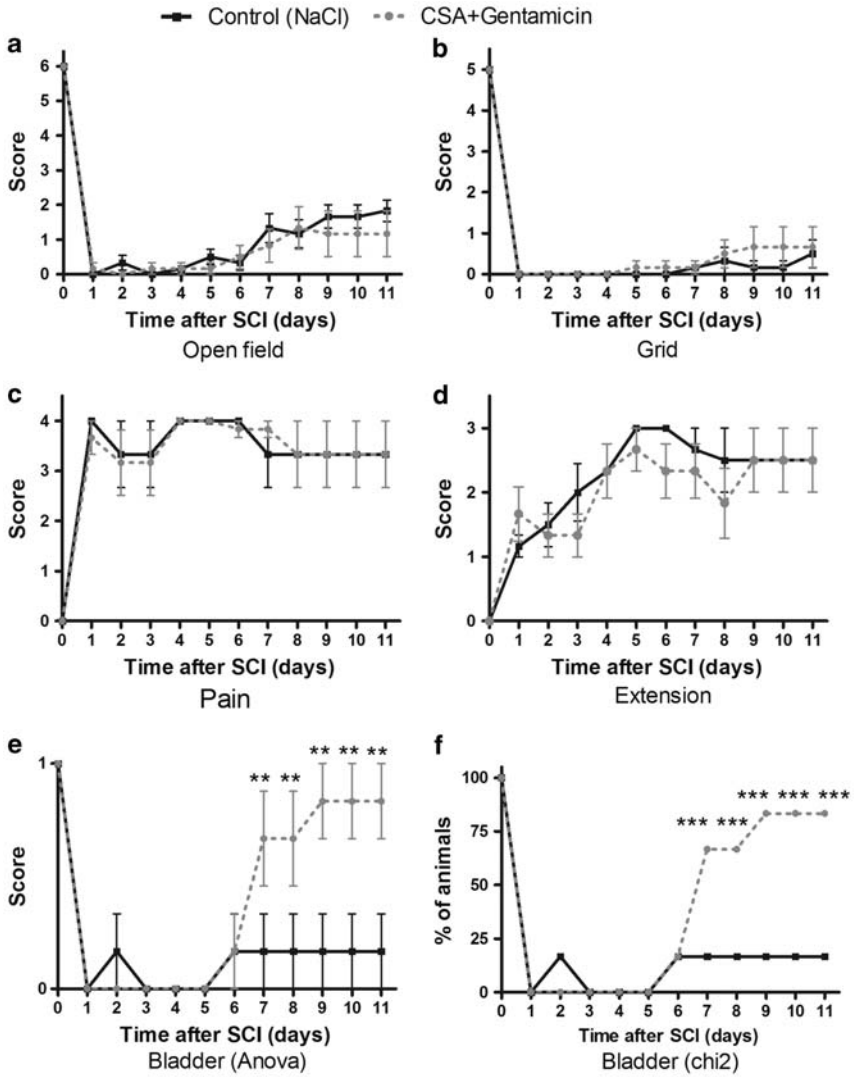

Figure 3 Combined CsA–gentamicin treatment did not modify functional recovery but improved bladder recovery after SCI. (**a**, **b**) Locomotor outcomes after spinal cord compression of injured rats treated either with NaCl or with a combination of CsA-gentamicin. (**a**) Open-field test before and during 12 days after compression for evaluation of gross motor function and (**b**) grid navigation test for evaluation of fine motor coordination over a 50-cm horizontal runaway grid. No difference was observed between groups; they were both severely impaired. Open-field walking on the horizontal plane ranges from no hind limb movement and no weight bearing (0) to normal walking (6). Scores grid: 0 corresponds to hind limb drag without foot placement and 5 to normal walking to normal course over the grid with toes gripping the wire. (**c**) Pain withdrawal was used to evaluate superficial sensory function. Scores range from normal response (0) to hyperalgesia (4). No difference was observed between the two groups, they were both hyperalgesic. (**d**) Reflexes were evaluated by hind limb withdrawal after manual extension; both groups presented similar deficits. Scores range from normal response (0) to hyper reaction (3). (**e**, **f**) Autonomic function corresponds to bladder control. (**e**) Animals treated with a combined CsA–gentamicin cocktail regained a better bladder control 7 days after injury than did control animals. (**f**) A higher percentage of animals treated with a combined CsA–gentamicin cocktail regained bladder control 7 days after injury. Scores: no bladder control (0) and bladder control (1). (**a**–**e**) Statistical analysis: two-way ANOVA, followed by Bonferroni's multiple comparison test (**$P<0.01$; ***$P<0.001$). (**f**) χ^2-Test on the percentage of animals reaching a given score was used (**$P<0.01$, ***$P<0.001$).

60% methanol. This solution was separated on a Macherey-Nagel Nucleodur Isis HPLC column (MACHEREY-NAGEL, Düren, Germany; particle size: 1.8 μm). The flow from the HPLC system was directly introduced into the ion source of a TSQ Quantum Ultra MS (Thermo Scientific, Waltham, MA, USA) and subjected to atmospheric pressure electrospray ionization. CsA was specifically detected with a parent-parent scan of its sodiated molecular ion $(M+Na)^+$ at m/z 1224.8. Quantification of blood levels of the parent compound CsA was based on a seven-level calibration curve (in triplicate) using blank rat blood samples spiked with stock solutions of external and internal standards.

Behavioral monitoring

Before injury, and for 7 or 12 days after injury, functional tests were carried out daily for motor, sensory and bladder control as previously described.[11] Motor response was evaluated by three tests: open-field walking and inclined plane for gross motor performance and a grid-navigation test

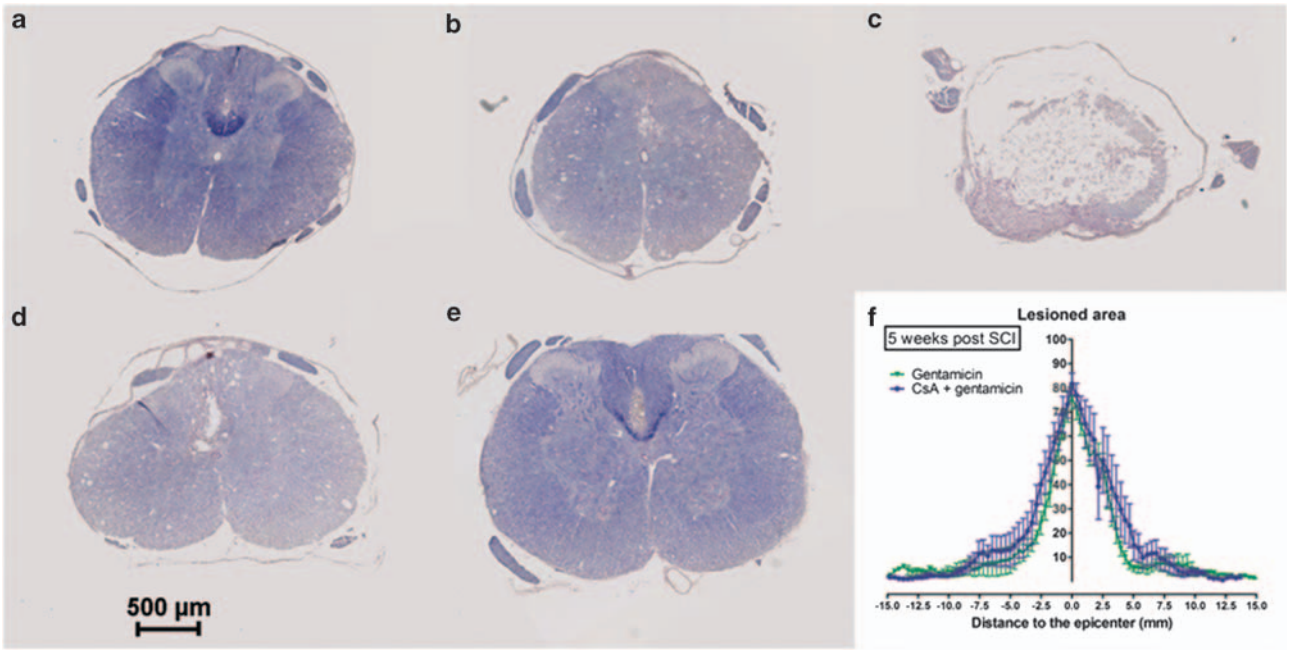

Figure 4 CsA treatment does not modify lesion extension after spinal cord compression. (**a**–**e**) Photomicrographs of Luxol fast blue-stained sections of an injured spinal cord. Transverse sections of the spinal cord taken at different levels from (**a**) rostral to (**e**) caudal. (**c**) Epicenter of the lesion. (**f**) Percentage of lesion area at the epicenter 5 weeks after spinal cord injury in gentamicin-only and CsA–gentamicin rats. At the epicenter, both groups presented lesion area of 80% and a rostrocaudal extension of 10 mm.

for fine motor performance. Evaluation of sensory response consisted of pain and heat withdrawal for superficial function and proprioception for deep function. Reflexes were evaluated by hind limb withdrawal after manual extension and hind limb and toe extension when the animal is picked up by the tail. Bladders were manually emptied until subjects regained bladder function.

Histology and lesion extension

Lesion extension was observed on 12-µm-thick Luxol fast blue-stained cryosections of the spinal cord; one section was stained each 360 µm. Morphometric quantification was carried out with MetaMorph software (MDS Analytical Technologies, Toronto, Canada). Longitudinal kidney cryosections (12 µm) were stained with hematoxylin and eosin. Morphometric quantification of the median section was performed on all kidneys and scored in four categories: 0, normal kidney; 1, between 5 and 40% of increased area without ulceration or pyelocalyceal cavities dilatation; 2, >40% of increased area and up to a 25% increase of pyelocalyceal cavities, without ulceration; 3, >40% of increased area associated with >25% increase of pyelocalyceal cavities or with ulceration. In cases of asymmetric degradation, the score of the worst kidney was considered. All volumetric analyses were performed according to the Cavalieri principle.[18]

Statistical analysis

CsA blood concentration: *t*-test, $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$. Means are presented with s.e.m. Behavioral analysis and lesion extension (area under the curve): two-way analysis of variance followed by Bonferroni's multiple comparisons; behavioral analysis: χ^2-test on the percentage of animals rising over a given score ($^{*}P<0.05$; $^{**}P<0.01$; $^{***}P<0.001$). Lesion extension: at the epicenter, *t*-test analysis was used.

Conflict of interest

The authors declare no conflict of interest.

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Supplementary Information accompanies the paper on the Spinal Cord website (http://www.nature.com/sc)