BIOPHARMACEUTICS & DRUG DISPOSITION, VOL. 18:3, 203–211 (1997)

ORAL PARACETAMOL BIOAVAILABILITY IN RATS SUBJECTED TO EXPERIMENTAL SPINAL CORD INJURY

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ABSTRACT

The purpose of the present study was to examine the time dependence of oral paracetamol (acetaminophen) bioavailability in an experimental model of spinal cord injury (SCI). Female Sprague–Dawley rats were subjected to spinal cord contusion at the T8–T9 level by the weight drop method producing permanent paraplegia. Oral paracetamol bioavailability after administration of a single 100 mg kg^{-1} dose was determined 1, 12, and 50 d after SCI. C_{max} and AUC were significantly decreased 1 d after SCI compared to sham-injured controls. This reduction, however, was temporary, as there was a recovery of bioavailability parameters which was partial 12 d after SCI, being complete by day 50. The present results confirm the usefulness of animal models for the characterization of the effect of SCI in drug kinetics. Data show that SCI induces significant changes in paracetamol pharmacokinetics. Nonetheless, despite the fact of a permanent loss of functions related to locomotion, pharmacokinetic alterations evolved with time. ©1997 by John Wiley & Sons, Ltd.

KEY WORDS: paracetamol; acetaminophen; spinal cord injury; bioavailability; paraplegia

INTRODUCTION

It has been reported that the oral bioavailability of several drugs, such as theophylline,[1,2] paracetamol (acetaminophen),[3] and dantrolene,[1] is reduced in patients with spinal cord injury (SCI) compared to able-bodied subjects. Moreover, an impaired elimination in SCI patients has also been reported for gentamicin,[4] amikacin,[5] lorazepam,[6] and theophylline.[7] Despite the evidence of significant pharmacokinetic alterations in SCI, the criteria and strategies for optimizing drug therapy in this type of patient are seldom based on

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CCC 0142–2782/97/030203–09$17.50

Received 15 December 1995
Accepted 7 May 1996

pharmacokinetic principles. Treatment strategies have been extrapolated, often uncritically, from clinical experience in able-bodied individuals.[1,2] It should be mentioned, however, that the information dealing with pharmacokinetics in SCI is considerably less clear than that for other patient populations. In fact, the physiological disturbances involved are still far from being understood.[1] Furthermore, it is highly possible that SCI-induced pharmacokinetic alterations evolve with time, as it is well known that functional conditions are continuously changing during the acute and subacute phases following the lesion, until a stable chronic phase is achieved.[8,9]

It is difficult to perform systematic studies in SCI patients due to an important interindividual variability in injury extent and location. Hence, experimental models appear to be the suitable alternative for the characterization of pharmacokinetic alterations induced by SCI, as well as of the physiopathological mechanisms implicated in these changes. Notwithstanding, experimental pharmacokinetic studies are scarce, although several animal models of SCI are available.[10] We have demonstrated that cyclosporin-A pharmacokinetics are altered during the acute phase following experimental SCI in the rat. Notwithstanding, these alterations appear to revert once the chronic stable phase is achieved.[11] The mechanism involved in such changes remains obscure, as the absorption and disposition of cyclosporin-A are influenced by a great number of factors.[12] We have also recently observed that oral paracetamol (acetaminophen) bioavailability is reduced during the acute phase following SCI.[13] The pharmacokinetics of paracetamol exhibit a less complex profile than those of cyclosporin-A and the physiological factors involved have been well characterized.[14] Therefore, we decided to extend our observations by studying oral paracetamol bioavailability during the acute, subacute, and chronic phases following SCI.

METHODS

Animals

Female Sprague–Dawley rats (240–260 g) were used. Twelve hours before drug administration food was withheld, but animals had free access to water.

Spinal cord injury

Animals were submitted to spinal cord contusion by the weight drop method of Allen[15] modified for rats, as described previously.[16] Briefly, animals were anaesthetized with a mixture of ketamine ($77{\cdot}5\,mg\,kg^{-1}$) and xylazine hydrochloride ($12{\cdot}5\,mg\,kg^{-1}$). Under aseptic conditions, a laminectomy was performed at the T8–T9 level. Rats were then placed on a stereotaxic device and a stainless steel cylinder weighing 15 g was dropped from a height of 4 cm

through a guided tube onto the exposed dura. Once the presence of haematoma on the dorsal aspect of the spinal cord was corroborated, the aponeurotic plane and the skin were separately sutured with 5-0 nylon thread. Post-surgical care was performed as described previously.[16,17] Sham-injured controls were only submitted to laminectomy at the same level.

Determination of paracetamol pharmacokinetics

A single oral paracetamol dose of $100\,mg\,kg^{-1}$ suspended in 0·5% carboxymethyl cellulose ($4\,mL\,kg^{-1}$) was given by gavage. 100–150 μL blood samples were drawn from the caudal artery at 0, 5, 10, 15, 20, 30, 45, 60, 90, 120, 150, 180, and 240 min after drug administration. The total blood volume extracted did not exceed 2 mL. Paracetamol concentration in whole blood was determined by a high-performance liquid chromatographic (HPLC) procedure developed in our laboratory.[18] Briefly, 100 μL blood was spiked with 10 μg 2-acetamidophenol (internal standard) and 100 μL water was added. Extraction was carried out with 1 mL ethyl acetate and the organic phase was transferred to a clean tube. The solvent was evaporated to dryness under a gentle nitrogen stream at 40 °C and the residue was redissolved in 100 μL mobile phase (*vide infra*). Portions of 50 μL were injected into an HPLC system (model 5000, Varian, Palo Alto, CA, U.S.A.) equipped with a $300\,mm \times 4{\cdot}6\,mm$ reversed-phase column (MCH-10, Varian). The column was eluted with a mobile phase consisting of a mixture of 0·02 M phosphate buffer (pH 6·0) with acetonitrile, 92·5:7·5, at a constant flow of $1{\cdot}5\,mL\,min^{-1}$ at room temperature. The effluent from the column was monitorized spectrophotometrically at 245 nm. Retention times were 5·1 and 9·1 min for paracetamol and the internal standard respectively.

Individual whole-blood paracetamol concentration against time curves were constructed and the maximal concentration (C_{max}) as well as the time to reach this maximum (t_{max}) were directly determined from these plots. The area under the curve (AUC_{0-4}) was estimated by the trapezoidal rule.

Study design

The purpose of this work was to study oral paracetamol bioavailability during the acute (necrotic), subacute, and chronic (stable) phases after SCI, i.e. 1, 12, and 50 d after experimental spinal cord contusion.

Four groups of eight rats each were studied. Animals in group 1 served as controls and were only submitted to laminectomy, paracetamol bioavailability being determined 24 h after the surgical procedure. Animals in groups 2–4 were subjected to SCI and paracetamol bioavailability was determined 1, 12, and 50 d after lesion respectively. Comparisons between the control and the SCI groups were performed by analysis of variance followed by the Newman–Keuls test. Differences were considered to reach statistical significance when $p < 0{\cdot}05$.

In order to examine whether time, by itself, had any significant effect on paracetamol bioavailability, an additional group of six rats was studied. Animals in this group were subjected only to laminectomy and paracetamol bioavailability was determined 50 d after the surgical procedure. The derived parameters were compared to those obtained with the control group 1 (1 d after laminectomy) by the Student t test for unpaired data. Differences were considered to reach statistical significance when $p < 0{\cdot}05$.

Drugs and reagents

Paracetamol and 2-acetamidophenol were purchased from Sigma Chemical Co. (St Louis, MO, U.S.A). Acetonitrile, chromatographic grade, was obtained from E. Merck (Darmstadt, Germany). All other reagents were of analytical grade. High-quality water, employed to prepare solutions, was obtained using a Milli-Q Reagent Water System (Continental Waters Systems, El Paso, TX, U.S.A.).

RESULTS

All animals exhibited normal locomotor activity before the initiation of the study. One day after SCI, rats showed complete flaccid paraplegia. There was a partial recovery of locomotor function during the 50 d following SCI. Some rats were able to support weight on hind limbs and to take one or two uncoordinated steps, but no further improvement was achieved. These results indicate that the produced lesion resulted in permanent paraplegia.[10] On the other hand, sham-injured animals exhibited normal walk once they recovered from anaesthesia.

Whole-blood paracetamol concentrations observed with a single oral $100\,mg\,kg^{-1}$ dose administered at various times after SCI and under control conditions (1 d after laminectomy) are shown in Figure 1. It can be appreciated that 1 d after SCI, paracetamol blood levels were importantly reduced compared to those observed in sham-injured animals. By day 12 after SCI, paracetamol concentrations exhibited a partial recovery, but still remained reduced, whereas at day 50 circulating drug levels were similar to those of control animals.

Oral paracetamol bioavailability was reduced by SCI, as indicated by the fact that C_{max} and AUC values were significantly lower in animals studied 24 h after lesion compared to sham-injured controls. Bioavailability, however, appeared to exhibit a time-dependent recovery. At day 12, C_{max} and AUC were higher than those observed 24 h after SCI, but remained significantly lower than control values. By day 50, AUC and C_{max} were similar to those observed in sham-injured rats. Comparable t_{max} values were observed in all the studied groups.

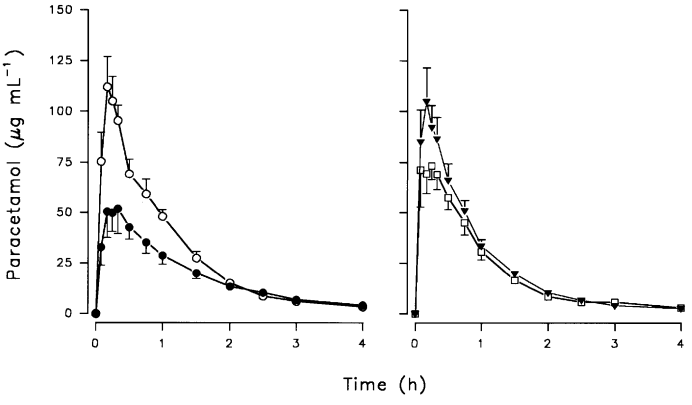

Figure 1. Whole-blood paracetamol concentrations observed in female rats subjected to spinal cord contusion which received a single 100 mg kg^{-1} oral dose: ●, animals which received paracetamol 1 d after injury; □, animals which received paracetamol 12 d after injury; ▼, animals which received paracetamol 50 d after injury; ○, sham-injured animals which received paracetamol 1 d after laminectomy. Data are presented as mean ± SEM of eight rats

Table 1. Paracetamol bioavailability parameters in rats receiving a 100 mg kg^{-1} oral dose at various times after spinal cord injury at the T8–T9 level and in sham-injured animals. Data are expressed as mean ± SEM ($n = 8$)

Group	C_{max} (μg mL^{-1})	t_{max} (min)	AUC_{0-4} (μg h mL^{-1})
Sham-injured	127 ± 13	14 ± 1	111 ± 8
1 d	60 ± 34*	14 ± 3	72 ± 8*
12 d	83 ± 22*	16 ± 2	79 ± 6*
50 d	117 ± 39	11 ± 2	93 ± 8

*Significantly different from sham-injured animals, as determined by analysis of variance followed by the Newman–Keuls test.

Sham-injured rats which received the 100 mg kg^{-1} oral paracetamol dose 50 d after laminectomy exhibited circulating concentrations similar to those observed in animals receiving the drug 1 d after the surgical procedure (Figure 2). Fifty days after laminectomy, C_{max} and AUC were (mean ± SEM) 114 ± 15 μg mL^{-1} and 135 ± 8 μg h mL^{-1} respectively, values which were not significantly different from those obtained 1 d after laminectomy.

DISCUSSION

Although SCI is a catastrophic affliction with numerous victims and a variety of pathological manifestations, the associated physiological disorders have not

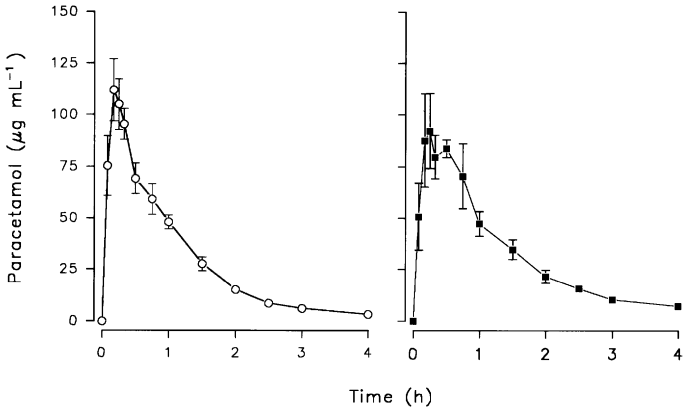

Figure 2. Whole-blood paracetamol concentrations observed in sham-injured female rats which received a single 100 mg kg^{-1} oral dose: ○, animals which received paracetamol 1 d after laminectomy, $n = 8$; ■, animals which received paracetamol 50 d after laminectomy, $n = 6$. Data are presented as mean ± SEM

yet been systematically and comprehensively studied as a probable cause for altered pharmacokinetics. SCI patients constitute an important therapeutic population, as the reported annual incidence of this disability ranges between 20 and 40 per million persons.[1] Notwithstanding, unlike other pathological entities, such as renal and hepatic dysfunction, SCI patients seldom receive a rationally designed drug therapy as a consequence of the lack of systematic pharmacokinetic information. Morbidity and mortality rates after both human[19,20] and experimental[17,21] SCI are high. Life-threatening complications such as pneumonia, urinary tract infections, and infected pressure sores are commonplace, mainly at the early stages of the lesion,[20,22,23] despite the use of standard pharmacological treatment. It is possible that such therapeutic failures are due, at least in part, to unsuitable dosing strategies which do not consider pharmacokinetic alterations in this patient population.

Since it is difficult to perform systematic studies in patients, we have undertaken the characterization of SCI-induced pharmacokinetic alterations using a well-described experimental model, i.e. the contusion of the spinal cord by the weight drop method in the rat.[10,16] This model is of clinical relevance, as it has been reported that it mimics several histopathological features of the most frequent SCI observed in humans,[10] which are due to acceleration-related fracture/dislocation of the spine producing contusion or compression of the spinal cord.[19] Paracetamol was studied since there is clinical[3] and experimental[13] evidence that the oral bioavailability of this drug is altered by SCI. Oral paracetamol bioavailability has been used as a marker of gastric emptying,[24] as it has been demonstrated in both human[25,26] and animal[27] studies that its absorption directly depends on this physiological process. Since

clinical studies have shown that SCI patients present an impaired gastric emptying,[2,28,29] the characterization of oral paracetamol bioavailability can be useful for understanding the evolution of gastrointestinal function during the different stages following SCI.

The present results confirm our previous findings on an important decrease in oral paracetamol bioavailability in the 24 h following SCI, i.e. during the acute necrotic phase.[13] This reduction, however, appears to be temporary. Twelve days after SCI, paracetamol circulating levels exhibited a partial recovery, which was complete by day 50, i.e. during the chronic stable phase of SCI. These data suggest that alterations in gastric emptying, unlike those produced on the functions related to locomotion, were reversible in this experimental model. Sham-injured animals studied 1 or 50 d after laminectomy exhibited similar paracetamol bioavailability parameters, indicating that, in the absence of SCI, a period of 50 d did not have any significant effect on paracetamol kinetics.

Clinical studies on oral drug bioavailability have been performed on patients in the chronic phase of SCI.[1] No information has been provided on the time course of SCI-induced alterations on gastrointestinal motility or drug kinetics. Furthermore, to our knowledge, no pharmacokinetic study has been performed in patients during the acute phase of SCI. It has been reported that the magnitude of gastrointestinal alterations related with drug absorption appear to depend on the level and intensity of the lesion.[28,29] The present results obtained with paracetamol, as well as those previously reported for cyclosporin-A,[11] however, clearly demonstrate that the time elapsed after SCI is also a significant determinant of oral drug bioavailability.

It should be taken into consideration that SCI is not a static state. The primary lesion produced by the mechanical trauma is followed by a secondary lesion which increases the original neural damage. This has been attributed to the presence of multiple endogenous toxic substances within the lesion area,[8–10] as well as to a disruption of the microcirculation.[30] Overlapping the pathological processes following SCI, there are several reparative changes, including scar formation[16] and plasticity processes which include both the central and autonomous nervous systems.[31–33] As a consequence of these reparative changes, a partial recovery of somatic and autonomic functions occurs. This probably explains why, under the present experimental conditions, although a permanent paraplegia was produced, alterations induced in paracetamol absorption by SCI reverted with time.

As mentioned above, there have been few attempts at the design of rational dosing regimens for SCI patients. Gilman and coworkers[4] have used a population approach to demonstrate pharmacokinetic differences between SCI patients and able-bodied individuals. If our results obtained in the rat can be extrapolated to the clinical setting, it could be expected that pharmacokinetic parameters in SCI patients change as their physiological state evolves. Therefore, in addition to the location and extent of the lesion, the time

elapsed should be considered as a covariate for dosing individualization. The development of population models will certainly yield an appropriate dosing strategy for SCI patients. However, an adequate model construction requires a minimal understanding of the numerous physiopathological factors involved.[4,34] Systematic animal studies will undoubtedly contribute to these purposes.

ACKNOWLEDGEMENTS

The authors wish to thank Drs F. J. Flores-Murrieta, I. Grijalba and A. Ibarra for discussion of the results and Mr A. Franco for drawings. This study was supported by Proyecto Camina A. C. FIIRESSIN, and CINVESTAV-IPN, Mexico City. P. García-López is a CONACYT fellow.

REFERENCES

1. J. L. Segal and S. R. Brunnemann, Clinical pharmacokinetics in patients with spinal cord injuries. *Clin. Pharmacokinet*., **17**, 109–129 (1989).
2. J. L. Segal, S. R. Brunnemann, S. K. Gordon and I. M. Eltorai, Decreased theophylline bioavailability and impaired gastric emptying in spinal cord injury. *Curr. Ther. Res*., **38**, 831–846 (1985).
3. L. S. Halstead, S. Feldman, J. Claus-Walker and V. C. Patel, Drug absorption in spinal cord injury. *Arch. Phys. Med. Rehab*., **66**, 298–301 (1985).
4. T. M. Gilman, S. R. Brunnemann and J. L. Segal, Comparison of population pharmacokinetic models for gentamicin in spinal cord-injured and able-bodied patients. *Antimicrob. Agents Chemother*., **37**, 93–99 (1993).
5. J. L. Segal, S. R. Brunnemann, S. K. Gordon and I. M. Eltorai, Amikacin pharmacokinetics in patients with spinal cord injury. *Pharmacotherapy*, **8**, 79–81 (1988).
6. J. L. Segal, S. R. Brunnemann, I. M. Eltorai and M. Vulpe, Decreased systemic clearance of lorazepam in humans with spinal cord injury. *J. Clin. Pharmacol*., **31**, 651–656 (1991).
7. J. L. Segal, S. R. Brunnemann, S. K. Gordon and I. M. Eltorai, The absolute bioavailability of oral theophylline in patients with spinal cord injury. *Pharmacotherapy*, **6**, 26–29 (1986).
8. A. Holtz and B. Nystrom, Neuropathological changes and neurological function after spinal cord compression in the rat. *J. Neurotrauma*, **7**, 155–167 (1990).
9. A. V. Krassioukov and L. C. Weaver, Episodic hypertension due to autonomic dysreflexia in acute and chronic spinal cord-injured rats. *Am. J. Physiol*., **268**, H2077–H2083 (1995).
10. G. D. Das, Perspectives in anatomy and pathology of paraplegia in experimental animals. *Brain Res. Bull*., **22**, 7–32 (1989).
11. A. Ibarra, G. Guízar-Sahagún, D. Correa, R. Kretshmer, I. Grijalba, F. J. Flores-Murrieta, G. Castañeda-Hernández, A. Odor, R. Espitia, H. Salgado-Ceballos and I. Madrazo, Alteration of cyclosporin-A pharmacokinetics after experimental spinal cord injury. *J. Neurotrauma*, **13**, 267–272 (1996).
12. J. P. Reymond, J. L. Steimer and W. Niederberger, On the dose dependency of cyclosporin-A absorption and disposition in healthy volunteers. *J. Pharmacokinet. Biopharm*., **16**, 331–353 (1988).
13. P. García-López, J. Pérez-Urizar, A. Ibarra, G. Guízar-Sahagún, F. J. Flores-Murrieta, I. Grijalba and G. Castañeda-Hernández, An experimental model for the study of pharmacokinetic alterations induced by spinal cord injury in the rat. *Pharm. Sci*., **1**, 133–135 (1995).
14. L. F. Prescott, Kinetics and metabolism of paracetamol and phenacetin. *Br. J. Clin. Pharmacol*., **10**, 2915–2919 (1980).

15. A. R. Allen, Surgery of experimental lesions of spinal cord equivalent to crush injury of fracture dislocation of spinal column. A preliminary report. *J. Am. Med. Assoc.*, **57**, 878–880 (1911).
16. G. Guízar-Sahagún, I. Grijalba, I. Madrazo, R. Franco-Bourland, H. Salgado, A. Ibarra, E. Oliva and A. Zepeda, Development of post-traumatic cysts in the spinal cord of rats subjected to severe spinal cord contusion. *Surg. Neurol.*, **41**, 241–249 (1994).
17. G. Guízar-Sahagún, I. Grijalba, I. Madrazo, R. Franco-Bourland, H. Salgado-Ceballos, A. Ibarra and J. Larriva-Sahd, Neuroprotection of completely lacerated spinal cord of adult rats by homotopic and heterotopic transplantation. *Rest. Neurol. Neurosci.*, **7**, 61–70 (1994).
18. J. Pérez-Urizar, P. García-López, F. J. Flores-Murrieta and G. Castañeda-Hernández, Determinación de acetaminofén en micromuestras de sangre de rata por cromatografía de líquidos de alta resolución. *Rev. Mexicana Ciencias Farm.*, **25**, 29–32 (1994).
19. S. L. Stover and P. R. Fine, The epidemiology and economics of spinal cord injury. *Paraplegia*, **25**, 225–228 (1987).
20. M. J. DeVivo, K. J. Black and S. L. Stover, Causes of death during the first 12 years after spinal cord injury. *Arch. Phys. Med. Rehab.*, **74**, 248–254 (1993).
21. G. D. Das, K. G. Das, J. Brasko, M. Riedl, P. Rai and V. Rajeswari, Spinal traumas: some postoperative complications in experimental animals. *Brain Res. Bull.*, **22**, 33–37 (1989).
22. K. B. Waites, K. C. Canupp and M. J. DeVivo, Phagocytosis of urinary pathogens in persons with spinal cord injury. *Arch. Phys. Med Rehab.*, **75**, 63–66 (1994).
23. A. B. Jackson and T. E. Groomes, Incidence of respiratory complications following spinal cord injury. *Arch. Phys. Med. Rehab.*, **75**, 270–275 (1994).
24. A. G. Renwick, C. H. Ashan, V. F. Challenor, R. Daniels, B. S. Macklin, D. G. Waller and C. F. George, The influence of posture on the pharmacokinetics of orally administered nifedipine. *Br. J. Clin. Pharmacol.*, **34**, 332–336 (1992).
25. R. C. Heading, J. Nimmo, L. F. Prescott and P. Tothill, The dependence of paracetamol absorption on the rate of gastric emptying. *Br. J. Pharmacol.*, **47**, 415–421 (1973).
26. J. A. Clements, R. C. Heading, W. S. Nimmo and L. F. Prescott, Kinetics of acetaminophen absorption and gastric emptying in man. *Clin. Pharmacol. Ther.*, **24**, 420–431 (1978).
27. W. E. Bagnall, J. Kelleher, B. E. Walker and M. S. Losowsky, The gastrointestinal absorption of paracetamol in the rat. *J. Pharm. Pharmacol.*, **31**, 157–160 (1979).
28. J. M. Stone, M. Niño-Murcia, V. A. Wolfe and I. Perkash, Chronic gastrointestinal problems in spinal cord injury patients: a prospective analysis. *Am. J. Gastroenterol.*, **85**, 1114–1119.
29. R. D. Fealey, J. H. Szurszewski, J. L. Merritt and E. P. DiMagno, Effect of traumatic spinal cord transection on human upper gastrointestinal motility and gastric emptying. *Gastroenterology*, **87**, 69–75 (1984).
30. E. D. Hall and D. L. Wolf, A pharmacological analysis of the pathophysiological mechanisms of posttraumatic spinal cord ischemia. *J. Neurosurg.*, **64**, 951–961 (1986).
31. F. W. L. Kerr, Structural and functional evidence of plasticity in the central nervous system. *Exp. Neurol.*, **48**, 16–31 (1975).
32. M. E. Helgren and M. E. Goldberger, The recovery of postural reflexes and locomotion following low thoracic hemisection in adult cats involves compensation by undamaged primary afferent pathways. *Exp. Neurol.*, **123**, 17–34 (1993).
33. T. H. Williams, J. Jew and S. L. Palay, Morphological plasticity in the sympathetic chain. *Exp. Neurol.*, **39**, 181–203 (1973).
34. B. Whiting, A. W. Kelman and J. Grevel, Population pharmacokinetics: theory and clinical application. *Clin. Pharmacokinet.*, **11**, 387–401 (1986).