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# The effect of the nonpeptide neurotrophic compound SR 57746A on the progression of the disease state of the *pmn* mouse

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**1** The progressive motor neuronopathy (*pmn*) mouse is an autosomal recessive mutant, in which the homozygotes suffer caudio-cranial degeneration of motor axons and die several weeks after birth. This strain provides the opportunity of testing potential therapeutic strategies for the treatment of motor neurone diseases such as amyotrophic lateral sclerosis. We have performed a study of the effects on the *pmn* mouse of SR 57746A, an orally-active, non-peptide compound which has been found to exhibit neurotrophic effects *in vitro* and *in vivo*. In order to treat the affected mice from birth, the mothers were administered 2.5 mg $kg^{-1}$, p.o., SR 57746A every two days until the weaning of the offspring (at day 20); then the offspring were given every two days a dose of 30 $\mu g\ kg^{-1}$, p.o., until their death.

**2** Affected mice treated with SR 57746A had a lifespan 50% longer than that of the vehicle-treated mice ($P=0.01$). Compared to vehicle-treated *pmn* mice, SR 57746A improved the performance of the *pmn* mice in three different behavioural tasks. SR 57746A also maintained the amplitude of the motor evoked response of the gastrocnemius muscle, reduced the distal motor latency, and delayed the occurrence of the spontaneous denervation activity in this muscle. Histological studies indicated that at 20 days of age the mean surface areas of the fibres of the sciatic nerve were higher in SR 57746A-treated than in vehicle-treated mice.

**3** At present, SR 57746A is the only orally active, nonpeptide compound known to be capable of delaying the progression of the motor neurone degeneration in *pmn* mice.

**Keywords:** SR 57746A; *pmn* mouse; motor neurone disease; neuroprotection; neurotrophic effect

## Introduction

A number of experimental approaches have improved our understanding of the processes underlying neuronal viability. Many studies have reinforced the concept that endogenous neurotrophic factors play an essential role in the outcome of degenerative processes concerning both central and peripheral nervous systems. Nerve growth factor (NGF) was the first factor found to be able to influence neuronal development and survival. NGF is now known to be only one of many endogenous factors able to influence neuronal viability, and it has gradually become clear that different neuronal systems require different neurotrophic factors in order to develop and/or survive (Hefti, 1994). For example, brain-derived neurotrophic factor (BDNF), neurotrophin-3 (NT-3) and neurotrophin-4/5 (NT-4/5), but not NGF, are able to sustain motor neurone survival and function (Sendtner *et al*., 1996; Braun *et al*., 1996). NT-3 and glial cell line-derived neurotrophic factor (GDNF), but not NGF, BDNF or NT-4/5, prevent the degeneration of the noradrenergic neurones of the locus coeruleus (Arenas & Persson, 1994; Arenas *et al*., 1995).

In the context of a potential therapeutic use, the most interesting aspect of neurotrophic factors is their ability to minimize experimentally-induced degeneration of neurones in a variety of lesion models, even if their protein nature renders their widespread clinical use highly problematic. However, substances which could enhance the effects of neurotrophic factors after oral administration would clearly be good candidates for the treatment of neurodegenerative diseases. Such a compound could limit the consequences of injuries to neurones *in vivo* by, for example, increasing the synthesis of neurotrophic factors, or potentiating or mimicking their effects on their target cells.

SR 57746A (1-(2-naphtalen-2-ylethyl)-4-(3-trifluoromethylphenyl)-1,2,3,6-tetrahydro-pyridine, hydrochloride) is an orally-active, non-peptide compound which has been found to exhibit neurotrophic effects in a variety of experimental systems both *in vitro* and *in vivo*, and this profile of activity confers on the compound considerable potential for the treatment of neurodegenerative pathologies. Among the experimental systems in which SR 57746A was found to be active were models of peripheral neuropathy. In rats subjected to controlled crushing of the sciatic nerve, SR 57746A accelerated the regrowth of regenerating sensory fibres and the recovery of sensorimotor function (Keane *et al*., 1992; Fournier *et al*., 1993). In a second model, in which rats were administered acrylamide, leading to a sensorimotor lesion which developed into a complete paralysis of the posterior limbs, SR 57746A delayed the onset of the paralysis and reduced the degree of severity and also accelerated recovery when acrylamide administration was ceased (Fournier *et al*., 1993). Finally, SR 57746A prevented cytostatic drug-induced reductions of neurite outgrowth *in vitro* (Ruigt *et al*., 1996). In view of the capacity of SR 57746A to attenuate such experimentally-induced states, it was of interest to determine whether the compound could act in other models of neuronal distress.

The progressive motor neuronopathy (*pmn*) mouse is a genetic model of a degenerative motor neurone disease, involving a dying-back process with distal axon degeneration and relative preservation of proximal axons and cell bodies (Schmalbruch *et al*., 1991). The *pmn*/*pmn* homozygotes suffer caudio-cranial degeneration of motor axons and die several

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weeks after birth, probably due to respiratory muscle denervation (Schmalbruch *et al*., 1991; Sendtner *et al*., 1992). This strain has already been used to determine the pathogenic mechanisms underlying motor neurone degeneration (Sagot *et al*., 1995), and to evaluate potential therapeutic strategies for the treatment of motor neurone diseases such as amyotrophic lateral sclerosis, including the administration of trophic factors (Sendtner *et al*., 1992; Sagot *et al*., 1996). We have now performed a study of the effects of SR 57746A on the *pmn* mouse, in order to determine whether this neuroprotective compound could be capable of improving the deficits associated with this mutation, and of extending the lifespan of the animals (Kennel *et al*., 1996).

## Methods

### *Mice*

Breeding pairs of Xt+/+*pmn* double heterozygous mice were provided by Dr J.-L. Guénet (Institut Pasteur, Paris, France) in 1994. From these pairs we developed a colony in our facilities by heterozygous brother-sister mating. Heterozygous mice have supernumerary toes, whereas homozygous +*pmn*/+*pmn* mice do not.

### *Drug administration*

SR 57746A (batch 94-01) was synthesized in the laboratories of Sanofi Recherche (Montpellier, France). The drug was suspended in an aqueous solution of 0.6% methylcellulose (metolose, Seppic, Paris, France; a generous gift of Dr G. Le Calvez). In order to treat the animals starting at their birth, it was decided to administer the compound in two stages: (i) treatment of the mothers, so that the offspring would receive the compound via the maternal milk, until their weaning (which occurs around the 20th postnatal day), (ii) from day 20 onwards, administration of a low oral dose directly to the offspring. Preliminary studies showed that the compound passed via the maternal milk into the gastrointestinal tract of the newborn mice, where it was detectable for at least 24 h after a single dose. Based on these data, the treatment schedule selected was 2.5 mg $kg^{-1}$, p.o., administered every two days to the mothers until the weaning of the offspring. Then the offspring were given every two days a dose of 30 $\mu$g $kg^{-1}$, p.o., until their death. This dose resulted in blood levels of SR 57746A in the offspring approximately equivalent to those achieved during the maternal phase of the drug administration. SR 57746A or its vehicle were administered to groups of ten *pmn* mice for the electrophysiological, behavioural and survival studies, and five per group for the histological studies. One mouse treated with SR 57746A succumbed on day 16 to the anaesthetic treatment necessary for the electrophysiological procedures; the data from this animal was eliminated from the experimental analysis.

In order to show the extent of the deficits produced by the mutation, data obtained from ten untreated healthy littermates (heterozygotes) are also shown in the Results section.

### *Survival and motor performance tests*

The age of the mice at death was recorded. The animals were subjected to the following tests at regular intervals: (a) the rotarod test (diameter of the rotating axle: 3.6 cm; speed of rotation: 13 r.p.m.) which consisted of measuring how long the mouse could maintain itself on the axle without falling, the test being stopped after an arbitrary limit of 120 s; (b) the hanging test to determine the ability of the mouse suspended on a horizontal thread by its forepaws, to reach it with its hindlegs; and (c) the grid test, which consisted in placing the animal on a grid situated 18 cm above a plane support (length of the grid: 37 cm; width: 10.5 cm; size of the mesh: 1 cm × 1 cm) and in counting the number of times the paws cross the grid during the ambulation.

### *Electrophysiological measurements*

All recordings were made with a standard EMG apparatus (Keypoint, Dantec, les Ulis, France) in accordance with the guidelines of the American Association of Electrodiagnostic Medicine. Mice were anaesthetized with 60 mg $kg^{-1}$ ketamine hydrochloride (Ketalar) and 2 mg $kg^{-1}$ diazepam (Valium). A monopolar needle electrode (Dantec, 13L70, diameter 0.3 mm) was inserted into the back of the mouse to ground the system. Throughout the procedure, mice were kept under a heating lamp to maintain a physiological muscle temperature (31°C). The temperature was verified on the surface of the tail with the contact thermometer incorporated in the Keypoint apparatus (YSI reusable temperature probe, Yellow Springs Instruments, Ohio, U.S.A.; delivered by Dantec).

*Recording of the spontaneous activity of the gastrocnemius muscle* Recordings were made with an ordinary concentric needle electrode (Dantec, 13L58, diameter 0.3 mm) inserted in the muscle explored. Each muscle was monitored for at least 2 min. Traces showing voluntary activity were discarded. Spontaneous activity (mainly fibrillations) characteristic of muscle denervation was differentiated from voluntary activity by visual and auditory inspection. Voluntary activity is characterized by rather repetitive regular discharges, which disappear with relaxtion of the muscle. Only spontaneous activity with a peak-to-peak amplitude of at least 50 $\mu$V was considered to be significant.

*Eliciting and recording of the compound muscle action potential (CMAP)* CMAPs were recorded in the gastrocnemius muscle. Amplitudes (mV) from the left and the right muscle-evoked responses were measured and averaged. Supramaximal square pulses, of 0.2 ms duration, were delivered through a needle electrode (Dantec, 13L70) to the sciatic nerve at the sciatic notch level. An anode needle electrode was inserted at the base of the tail. The active recording needle electrode was inserted in the medial part of the gastrocnemius or just behind the big toe for the plantar muscle recording. The reference recording needle electrode was inserted over the Achilles tendon. The myoelectric signal was bandpass filtered (2 Hz-5 kHz) to eliminate artifacts. An initial negative deflection and biphasic waveform indicated recording at the motor point (Dimitru & DeLisa, 1991).

*Distal motor latency* Given the short conduction distance in mice, a conventional motor nerve conduction study could not be applied to the sciatic nerve. However, determination of the time of transmission of the action potential along the motor nerve and the synapse is possible by measuring the distal motor latency. This is measured from the latency of the stimulus artifact to the onset of the negative peak of the CMAP.

### *Histological analysis*

For histological examinations, mice (5 vehicle-treated and SR 57746A-treated *pmn* mice, and 3 healthy littermates) were

first perfused with phosphate-buffered saline (PBS) containing heparin (83.3 u $ml^{-1}$; Sigma, L'Isle d'Abeau, France), injected in the left ventricle. Then, animals were perfused by the same route with a 4% solution of paraformaldehyde in PBS until the animal became rigid. Finally, sciatic nerves and spinal cord were dissected under a binocular loop and fixed as described below. Sciatic nerves were fixed overnight at 4°C with a 4% solution of glutaraldehyde (Sigma) in PBS: they were then post-fixed at room temperature with a 3% solution of osmic acid (Sigma) in PBS, dehydrated and embedded in epon. Semi-thin sections (thickness: 1.5 $\mu$m; 15 sections per nerve) were made with the aid of an ultramicrotome (LKB Instruments SA, Les Ulis, France). The sections were stained for 15–30 s with a 1% solution of toluidine blue in PBS, then dehydrated and mounted in Eukitt. Two fields from one section per animal were analysed with the aid of an image analysis programme (Alcatel, TITN Answar, Grenoble, France). The parameters measured were the surface of each fibre (i.e. the axon + its myelin sheath), and the thickness of the myelin.

Spinal cords were post-fixed overnight at 4°C with a 4% solution of paraformaldehyde in PBS. They were then immersed in a mixture of glycerol (1 v) and PBS (1 v) and thawed in isopentane (Prolabo, Fontenay-sous-bois, France). Sections (thickness: 30 $\mu$m) were made with the aid of a cryostat (Leica Jung CM 1800, Rueil-Malmaison, France) and then stained with a 0.1% aqueous solution of cresyl violet for 30 to 45 s. The sections were dehydrated and mounted in Eukitt. Only one section out of two were collected, to avoid the possibility of a given motor neurone be counted twice in two contiguous sections. Only the lumbar segment of the spinal cord was examined. With this protocol, and except for one case, 18 to 25 sections were obtained from a given lumbar segment. All the sections collected were observed using an optical microscope. Results are expressed as the mean number of motor neurones per section counted in one of the two ventral horns.

### *Statistical analysis*

Values are given as mean ± s.e.mean. Differences between drug-treated and vehicle-treated mice were analysed as follows. Survival rates of the two groups of mice were evaluated by the Kaplan-Meier method, followed by the Log Rank test. Differences in spontaneous muscle activity were evaluated with Fisher's exact test, with the Bonferroni correction for multiple comparisons. The amplitude and latency of CMAP, and data in the behavioural tests were evaluated by ANOVA and Dunnett's test when the variances were homogeneous, and by the Kruskal-Wallis test when they were not. Only data from days 4 to 20 were analysed, as the number of vehicle-treated mice surviving on day 24 was too low to enable statistical analysis. A mixed model was used to analyse the histologicals parameters, the animals being considered as the random effect and the treatment as the fixed effect. The procedure 'MIXED' of the SAS system (version 6.12) was used for this purpose.

## Results

### *Survival*

Figure 1 shows the survival curves for the *pmn* mice treated with the vehicle or with SR 57746A. Treatment with SR 57746A significantly prolonged the lifespan of the *pmn* mice ($P = 0.01$, log rank test). The mean lifespan of the vehicle-treated mice was 22.5 ± 0.9 days, while that of the animals

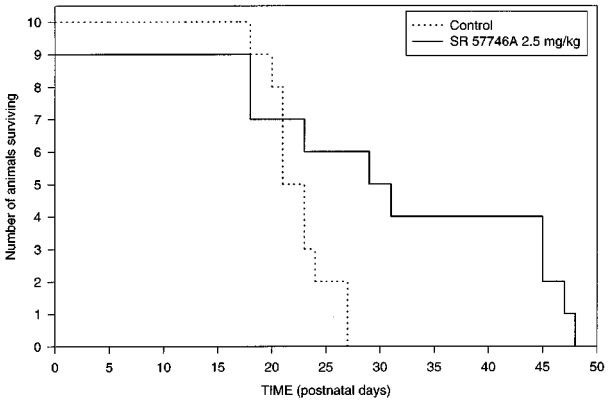


**Figure 1** Survival of *pmn* mice after administration of SR 57746A or its vehicle every two days until death. The survival of SR 57746A-treated animals was significantly different ($P = 0.01$, Log Rank test) from that of the vehicle-treated animals.

treated with SR 57746A was increased by 50% (33.8 ± 4.2 days).

### *Behavioural assessment of motor dysfunction*

The motor performance of the affected mice treated with SR 57746A was significantly improved in the three behavioural models which were evaluated, the optimal effect of the compound being observable on days 16 to 20 (Figure 2). In the grid test, at days 16 and 20, in contrast to the poor performance of the vehicle-treated animals, the mice treated with SR 57746A had performances very close to those of the healthy littermates (Figure 2a). A similar observation could be made with rotarod test: at days 16 and 20, SR 57746A-treated animals were able to maintain themselves without falling for a mean period of over 30 s, whereas the vehicle-treated animals were unable to maintain themselves on the rotating axle (Figure 2b). Finally, Figure 2c shows that at days 16 and 20, over 80% of the affected animals treated with SR 57746A succeeded in the hanging test at a time when none of the vehicle-treated animals were able to reach the thread with their hindlegs.

### *Electrophysiological measurements*

As shown in Figure 3a, the treatment with SR 57746A considerably delayed the appearance of spontaneous denervation activities (fibrillations) in the affected animals. At days 16 and 20, 80–100% of the vehicle-treated mice displayed fibrillations in the gastrocnemius muscle, whereas only one affected mouse among the animals treated with SR 57746A displayed signs of denervation. It took 28 days for 100% of the affected animals treated with SR 57746A to have fibrillations. SR 57746A treatment also produced a significant improvement in the motor evoked response (CMAP), which was maintained at an amplitude of around 30 mV between days 12 and 24 of the disease, whereas during this period the motor evoked response of the vehicle-treated mice fell to a very low level (< 10 mV) (Figure 3b). Similarly, the distal latency was significantly reduced in SR 57746A-treated animals (Figure 3c).

### *Histological evaluations*

When the surfaces of the fibres from 20 day-old vehicle-treated and SR 57746A-treated *pmn* mice were compared within a

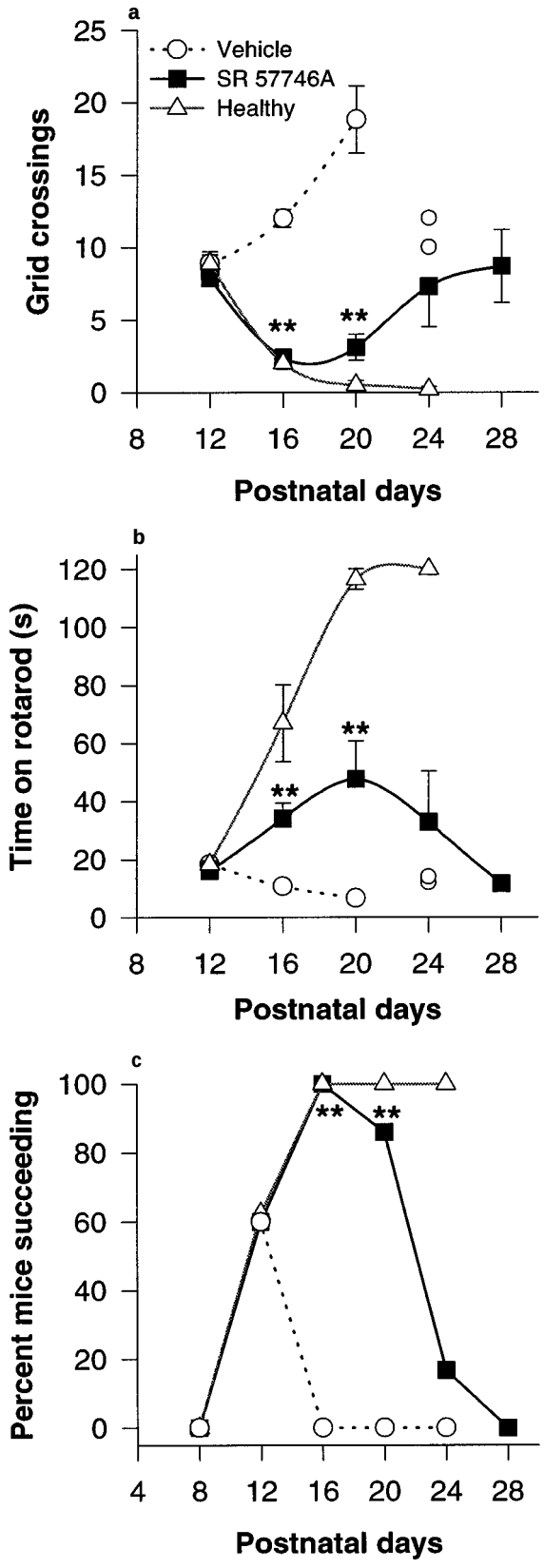


**Figure 2** Behavioural performance. (a) Grid test, (b) rotarod test, (c) hanging test, in vehicle-treated *pmn* mice, SR 57746A-treated *pmn* mice and untreated healthy littermates. Individual data are given for the vehicle group on day 24 (except for (c)), as only 2 vehicle-treated mice survived at this time. $^{**}P<0.01$ between vehicle- and SR 57746A-treated mice.

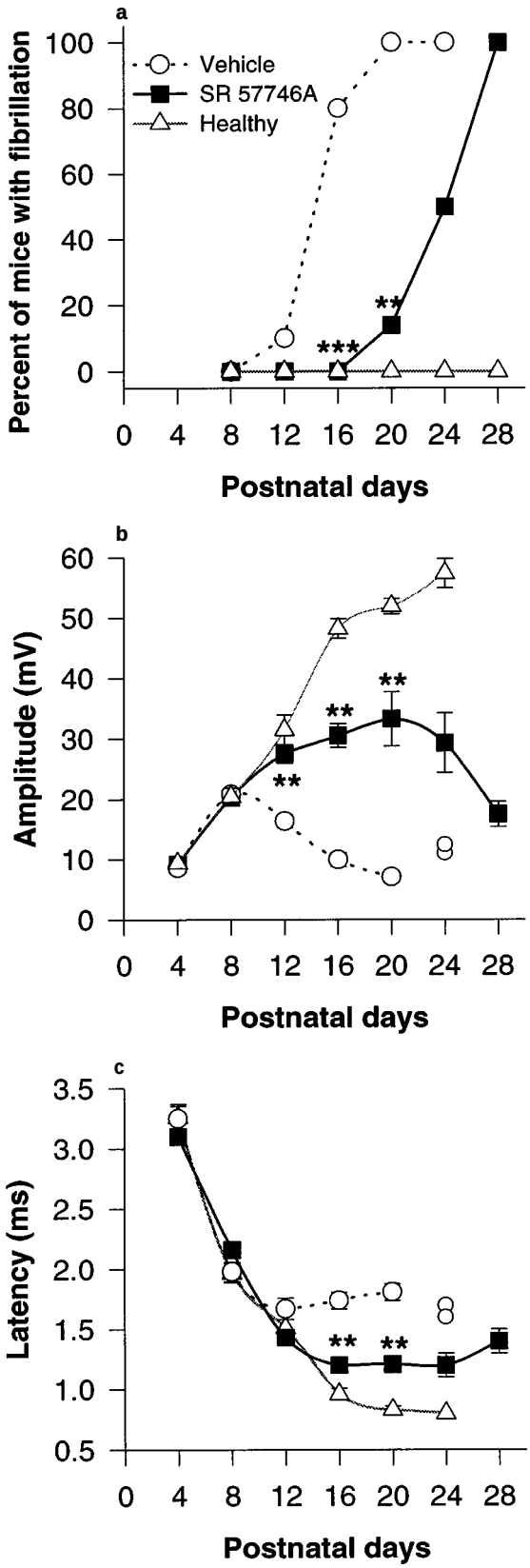


**Figure 3** Electromyographic activity of the gastrocnemius muscle. (a) Spontaneous denervation activity, (b) compound muscle action potential and (c) distal motor latency, in vehicle-treated *pmn* mice, SR 57746A-treated *pmn* mice and untreated healthy littermates. Individual data are given for the vehicle group on day 24 (except for (a)), as only 2 vehicle-treated mice survived at this time. $^{**}P<0.01$, $^{***}P<0.001$ between vehicle- and SR 57746A-treated mice.

number of fibre size subpopulations, no statistically significant differences were noted (Figure 4a). However, this analysis showed that, compared with the healthy littermates, whose fibres were distributed relatively homogeneously among the categories (about 10–20% per size group), *pmn* mice exhibited a much higher percentage (over 50%) of their fibres in the smaller surface category ($<30$ $\mu m^2$), about the same percentage of fibres in the intermediate categories (30–90 $\mu m^2$), and a much lower percentage (about 2%) in the largest surface categories ($>120$ $\mu m^2$). Treatment with SR 57746A tended to normalize the fibre distributions of the *pmn* mice, in that the percentage of fibres in the smallest-size catetory was lower in SR 57746A-treated mice, while that in the larger-size groups was higher.

The difference between the vehicle- and SR 57746A-treated mice attained statistical significance when the overall population of the fibres in each treatment group was studied. The mean surface area of the fibres in the sciatic nerve of the healthy heterozygote mice was $86.5 \pm 5.4$ $\mu m^2$. In the *pmn* mice which had been treated with the vehicle, the mean surface area of the fibres was $40.6 \pm 3.2$ $\mu m^2$, and that of the animals treated with SR 57746A was $58.0 \pm 4.0$ $\mu m^2$ (an increase of 45% compared to the vehicle-treated mice, $P<0.01$). This observation was confirmed in cumulative histograms of the surfaces of these fibres, which clearly showed that SR 57746A treatment increased the proportion of the fibres with the largest surface areas (Figure 4b). In contrast to the reduction of the surface area of the sciatic nerve fibres, the thickness of the myelin sheath of these fibres was not modified by the *pmn* mutation, nor by the administration of SR 57746A ($1.66 \pm 0.08$ $\mu m$ in the healthy littermates, $1.48 \pm 0.07$ $\mu m$ in the vehicle-treated *pmn* mice, and $1.63 \pm 0.07$ $\mu m$ in SR 57746A-treated mice).

Finally, the mean number of motorneurones counted per section in the lumbar segment of the spinal cord was about 20% lower in *pmn* mice than in healthy animals. This parameter was not affected by treatment with SR 57746A (data not shown).

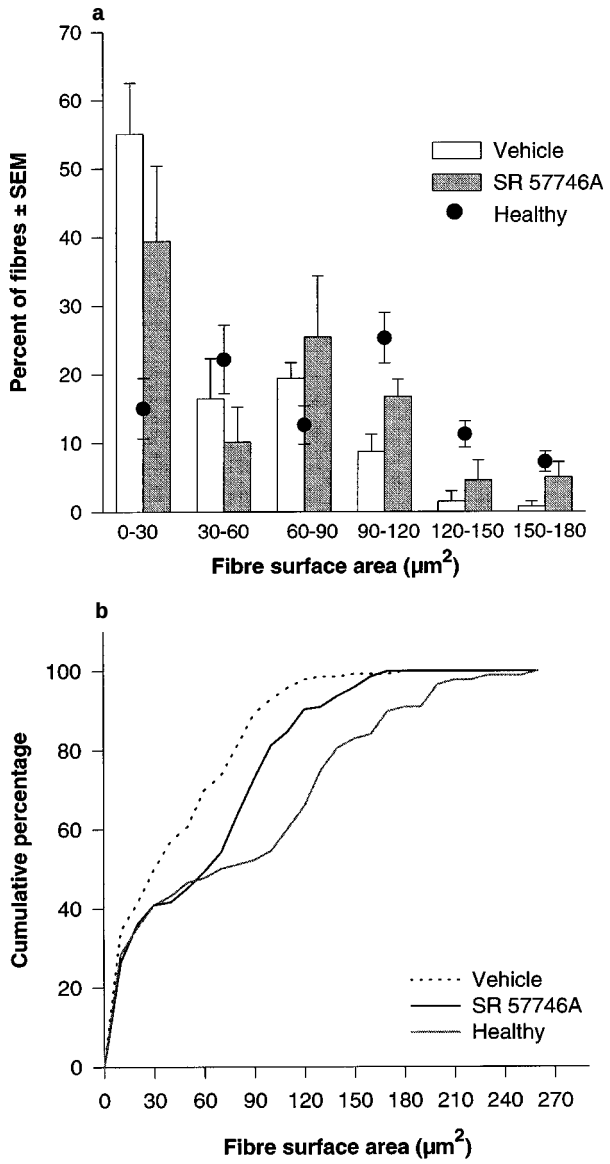


**Figure 4** (a) Histogram of the fibre areas of the sciatic nerve of vehicle-treated *pmn* mice, SR 57746A-treated *pmn* mice, and untreated healthy littermates, arbitrarily subdivided into 30 $\mu m^2$ segments of area. $n=5$ vehicle-treated and SR 57746A-treated mice, and 3 healthy littermates. (b) Cumulative histogram of the same data.

## Discussion

The mouse mutant *pmn* is an autosomal, recessively inherited mutation which causes a progressive dying-back type degeneration, principally of the larger-diameter myelinated motor axons. This degeneration leads to the apparition of spontaneous denervation activity in the hindlimb muscles, electrophysiological deficits in the motor nerves and the inability to perform simple behavioural tasks. After a few weeks the mice die, presumably due to denervation of the respiratory system (Schmalbruch *et al.*, 1991; Kennel *et al.*, 1996).

The present study has found that treatment of *pmn* mice from birth with SR 57746A, at first via the maternal milk, and then by direct oral gavage, results in an improvement of the consequences of the genetically-induced motor neurone degeneration that occurs in this strain. Affected mice treated with SR 57746A can survive much longer than the vehicle-treated mice. Measurements of motor performance and electrophysiological and histological parameters reveal an improvement of the state of the *pmn* mice treated with SR 57746A, and suggest that the progression of the disease is slowed down by the treatment. Thus, compared to vehicle-treated *pmn* mice, SR 57746A maintained the amplitude of the motor evoked response of the gastrocnemius muscles, reduced the distal motor latency, and also delayed the apparition of the spontaneous denervation activity in this muscle. The compound also improved the performance of the mice in three different behavioural tasks. In agreement with previous studies (Schmalbruch *et al.*, 1991), our histological observations indicated that, although the overall number of fibres was not reduced in the sciatic nerve of the *pmn* mouse compared to the healthy heterozygotes, the mutation resulted in a reduction of the number of large fibres in this nerve. At 20 days of age the mean surface areas of the fibres of the sciatic nerve were higher in SR 57746A-treated than in vehicle-treated mice. Finally, SR 57746A produced a highly significant prolongation of the lifespan of the *pmn* mice. All these results indicate that the compound was able to delay markedly the disease process in this genetic model of axonopathy. SR 57746A has already been shown to possess neuroprotective properties in a number of experimental conditions, both *in vitro* and *in vivo* (Fournier *et al.*, 1992; 1993; Keane *et al.*, 1992). *In vitro*, SR 57746A improves the survival of septal neuroblasts and promotes their neurite outgrowth, and potentiates the effect of NGF on the differentiation of PC12 cells. *In vivo*, the compound reduces the

histological damage and deficits in exploratory behaviour of rats subjected to transient global ischaemia, counteracts the reductions in cholinergic markers and the deficits in social memory produced by intraseptal injection of vincristine, and accelerates recovery in two peripheral models, one produced by crushing the sciatic nerve, and the other by repeated administration of acrylamide. In the present study, it was not feasible to undertake a dose-response study of the effect of SR 57746A, due to the complex dosing regimen required to ensure treatment of the *pmn* mice from birth, and the difficulty of obtaining large numbers of these mice. Nevertheless, it should be pointed out that the previous studies performed with SR 57746A have already demonstrated the dose-dependency of the neurotrophic and neuroprotective effects of the compound in both *in vitro* and *in vivo* models (Fournier *et al*., 1993; 1997; Labie *et al*., 1998).

Our initial hypothesis concerning the mechanism of action of SR 57746A was that the compound could afford neuroprotection by promoting the effects of NGF, as this factor was capable of neuroprotective activity in many of the different lesion models in which SR 57746A is active (see Fournier *et al*., 1993), and because SR 57746A increased the synthesis of NGF in the C6-2B glioma cell line (Fournier *et al*., 1992). However, the effects of SR 57746A in *pmn* mice are likely to be unrelated to NGF, as this neurotrophic factor does not seem to play a crucial role in the differentiation or survival of motor neurones (Sendtner *et al*., 1996).

A number of trophic factors are known to be capable of sustaining motor neurone survival and function. These include the neurotrophins BDNF, NT-3 and NT-4/5 (Sendtner *et al*., 1996; Braun *et al*., 1996), but also a number of other trophic factors, such as CNTF, GDNF, LIF or IGF (Sendtner *et al*., 1990; Hughes *et al*., 1993; Sagot *et al*., 1996). Whereas GDNF did not increase the lifespan of *pmn* mice (Sagot *et al*., 1996), CNTF has been found to prolong survival and improve the motor function of these animals (Sendtner *et al*., 1992). Although methodological differences preclude a detailed comparison of the activity of SR 57746A with that of CNTF (which was administered via the intraperitoneal injection of transfected, CNTF-secreting cells 21 days after birth), the effects we have observed with SR 57746A closely resemble those described with CNTF. Haase *et al*. (1997) have recently shown that a gene therapy approach to the treatment of *pmn* mice using intramuscular injections of adenoviral vectors for NT-3 can produce substantial therapeutic effects. The mean lifespan of the affected animals was considerably increased with this treatment. Moreover, this gene therapy treatment reduced the loss of motor axons in the phrenic nerve, improved the motor performance and maintained the electrophysiological parameters closer to those of the normal animal. The present results show that a noninvasive treatment using orally administered SR 57746A can exert an analogous effect on the progression of the disease.

Recently, we have found that SR 57746A can increase the synthesis of BDNF in primary cultures of rat hippocampal astrocytes (Fournier *et al*., 1997; Labie *et al*., 1998). SR 57746A could possibly act by enhancing the synthesis and release of this neurotrophin, or of other factors known to play an important role in the maintenance of motor neurones. Alternatively, the compound could have a direct, neurotrophic-like effect on the motor neurones in the *pmn* mice. SR 57746A has demonstrated a number of effects which are unlikely to be attributable to an increased synthesis of endogenous factors, notably its effects *in vitro* on PC12 cells and on septal neuroblasts (Fournier *et al*., 1993; Pradines *et al*., 1995), so that an intrinsic neurotrophic-like effect of the compound also remains a possible explanation of its pharmacological profile.

The effects of SR 57746A are currently being evaluated in worldwide clinical trials on amyotrophic lateral sclerosis. In an initial Phase I-II trial, after 32 weeks of double-blind treatment at the dose of 2 mg per day, the compound significantly slowed the evolution of the disease, as indicated by measures of functional parameters (Lacomblez *et al*., 1996). Although the *pmn* mouse cannot be considered as an exact animal counterpart of any particular human motor neurone disease (Kennel *et al*., 1996), the present results show that it may represent a useful model for the evaluation of the capacity of new drugs to alleviate the degeneration of motor neurones.

In conclusion, SR 57746A has been found to reduce the electrophysiological, behavioural and histological deficits, to delay the apparition of skeletal muscle denervation, and to increase the lifespan of the *pmn* mouse. At present, this is the only orally active, nonpeptide compound known to be capable of delaying the progression of the motor neurone degeneration in *pmn* mice.

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