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Original Article

Interleukin-6 attenuates the development of experimental diabetes-related neuropathy

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Neuropathy is the most severe and the least understood complication of diabetes. We investigated the potential neuroprotective effect of IL-6 therapy in an experimental model of diabetic neuropathy. A single i.v. injection of streptozotocin (STZ, 55 mg/kg) was used to induce experimental diabetes in adult males. IL-6 (1, 10 or 30 μg/kg) was administrated either intraperitoneally on a daily basis or subcutaneously (s.c.) on a daily, on a three times or one time per week basis, starting at day 10 post-STZ. A decrease in sensory nerve conduction velocity (SNCV), indicative of neuropathy, is seen in STZ rats as early as day 10 post-STZ, a time at which blood glycaemia is already maximal. At later time points, this electrophysiological impairment became severe and clinically apparent by affecting tail flick latency. Motor dysfunction defined by a significant increase in compound muscle action potential (CMAP) latency was also recorded. At the completion of the study (day 40 post-STZ), histological examination revealed significant axonopathy and myelin loss, along with an increase in the proportion of fibers with abnormal appearance in sciatic nerves of STZ rats. These changes were not observed in non-diabetic rats and were significantly prevented by IL-6 treatment. The optimal dose appeared to be 10 μg/kg s.c. three injections per week, which showed a better effect in most of the parameters studied than 4-methylcatechol, a NGF-like neuroprotective compound. Once weekly and three times weekly administrations of IL-6 were as effective as daily treatment. Taken together, these results support the potential neuroprotective actions of IL-6. The fact that the half-life of IL-6 is only approximately 5 h while weekly dosing was neuroprotective strongly suggests activation by IL-6 of effector molecule(s) with longer duration of action.

Key words: diabetes, IL-6, neuroprotection, peripheral neuropathy, streptozotocin.

INTRODUCTION

Diabetic polyneuropathy (DPN) is the most common chronic complication of diabetes[1] and remains probably the least understood. The underlying mechanisms are multiple and appear to involve several interrelated metabolic abnormalities consequent to hyperglycemia and to insulin and C-peptide deficiencies (see [2] for review).

The most common early abnormality indicative of DPN is asymptomatic nerve dysfunction as reflected by decreased nerve conduction velocity.[3] These changes are usually followed by a loss of vibration sensation in the feet and loss of ankle reflexes. Electrophysiological measurements often reflect fairly accurately the underlying pathology and changes in nerve conduction velocity or amplitude correlate with myelinated nerve fiber density (see [4,5] for review).

The streptozotocin (STZ) diabetic rat is the most extensively studied animal model of DPN (see [2,6] for review). It develops an acute decrease in nerve blood flow (40%) and slowing of nerve conduction velocity (20%),[7] followed by axonal atrophy of both the motor and sensory nerve fibers.[8] The reduction in axonal size progresses proximally with diabetes duration.[6] Demyelinating and degenerating myelinated fibers as well as axo-glial dysjunction are seen.[9]

In this animal model, neuroaxonal changes are prevented and/or reversed by a variety of experimental manipulations including insulin administration or pancreatic islet transplantation,[10] aldose reductase inhibition,[11] aminoguanidine treatment[12] and insulin-like growth factor

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administration.[13] In addition, distal axonal atrophy with a proximo-distal gradient, characteristic of myelinated fiber pathology in the STZ rat, is reversed following metabolic corrections,[14] correction of vascular dysfunction[15] and normalization of neurotrophic support.[16]

Interleukin-6 belongs to a cytokine family which includes ciliary neurotrophic factor (CNTF), leukemia inhibitory factor (LIF), oncostatin M (OSM), IL-11, cardiotrophin-1 (CT-1) and cardiotrophin-like cytokine (CLC), the latter also known as novel neurotrophin-1/B-cell stimulating factor-3.[17, 18]

A variety of *in vitro* and *in vivo* studies demonstrate the neuroprotective actions of IL-6. It has been demonstrated to support the survival of cortical and mesencephalic neuron survival,[19, 20] and sensory neurons.[21] Pretreatment with IL-6 protects various types of peripheral and central neuronal cultures against intoxication with glutamate[22–24] and 1-methyl-4-phenylpyridinium ion $(MPP)^+$ toxicity.[25] Supportive *in vivo* evidence comes from studies in which IL-6-deficient mice showed altered sensory function and a delayed regeneration of lesioned sensory axons.[26] Conversely, transgenic mice overexpressing IL-6 and its receptor demonstrate an accelerated regeneration of axotomized nerves, an event that is sensitive to antibody against IL-6 receptor.[27] The neuroprotective action of IL-6 has also been evidenced in different animal models of neuronal injury, such as ischemia-induced neuronal loss,[28] axotomy-induced motoneuron loss[29] and Theiler's virus-induced murine CNS demyelination.[30]

Here, we show evidence of IL-6 effects on diabetes-induced peripheral nerve injury in the STZ-intoxicated rat. We found that chronic intraperitoneal (i.p.) as well as subcutaneous (s.c.) administration of IL-6 are protective against the development of diabetic-related neuropathy. Behavioral, electrophysiological and histological analyses conducted in the present study demonstrated that IL-6 prevented both sensory and motor dysfunctions as well as their histological correlates to a greater extent than that provided by 4 methylcatechol (4-MC), an NGF inducer agent used as reference compound.[31,32]

METHODS

Animals, induction of diabetes and pharmacological treatment

The present study protocol was approved by the local veterinary ethics committee.

Diabetes was induced in 6-week-old male Sprague Dawley rats (Janvier, Le Genest-St-Isle, France) by i.v. injection of a buffered solution of STZ (Sigma, L'Isle d'Abeau Chesnes, France) in the surgically denuded left saphena magna at a dose of 55 mg/kg body weight. The drug was dissolved immediately before injection in 0.1 mol/L citrate buffer pH 4.5. The day of STZ injection was considered as day (D) 0.

At D 10, tail vein blood was assayed for glycemia in each individual animal using a glucometer (Glucotrend test, Roche, Mannheim, Germany). Only animals showing a value above 260 mg/dL (15 mmoles/L) were considered hyperglycemic as previously reported.[33] Glycemia was checked again at D 40, at the end of the experiment. Treatment (vehicle, IL-6 and 4-MC) was performed from D 11–40. IL-6 was presented as human glycosylated IL-6 produced by Chinese hamster ovary (23.3×106 IU/mL HGF Bioassay) provided by Serono International S.A. (Geneva, Switzerland)

Rats were randomly distributed in five different treatment groups comprising control animals treated with a sterile solution of saline bovine serum albumin (BSA) 0.02% (weight/volume); the STZ-intoxicated group was injected with a sterile solution of saline BSA 0.02%; three groups of STZ-intoxicated rats were injected with IL-6 at the doses of 1, 10 or 30 μg/kg. Each treatment group was divided into four subgroups (with 10 rats in each), subjected to four different types of treatment schedule: daily i.p. regimen (schedule A) and s.c. at one, three or seven administrations per week (schedule B, C and D, respectively). Additionally, a group of STZ-intoxicated rats receiving a daily i.p. injection of 4-MC 10 μg/kg was added in the schedule A.

The animals were group-housed (two animals per cage) and maintained in a room with controlled temperature (21–22°C) and a reversed light–dark cycle (12 h/12 h) with food and water available ad libitum. All experiments were carried out in accordance with institutional guidelines.

Electrophysiological studies

Electrophysiological recordings were performed using a Neuromatic 2000M electromyograph (EMG) (Dantec, Les Ulis, France). Rats were anesthetized by i.p. injection of 60 mg/kg ketamine chlorhydrate (Imalgene 500, Rhône Mérieux, Lyon, France). The normal body temperature was maintained at 30°C with a heating lamp and controlled by a contact thermometer (Quick, Bioblock Scientific, Illkirch, France) placed on the tail surface. The compound muscle action potential (CMAP) was recorded in the gastrocnemius muscle after stimulation of the sciatic nerve. A reference electrode and an active needle were placed in the hind paw. A ground needle was inserted on the lower back of the rat. The sciatic nerve was stimulated with a single 0.2 ms pulse at a supramaximal intensity. The velocity of the motor wave was recorded and expressed in ms.

Sensory nerve conduction velocity (SNCV) was also recorded. The tail skin electrodes were placed as follows: a

reference needle was inserted at the base of the tail and an anode needle placed 30 mm away from the reference needle, towards the extremity of the tail. A ground needle electrode was inserted between the anode and reference needles. The caudal nerve was stimulated with a series of 20 pulses of 12.8 mA intensity and 0.2 ms duration. The velocity was expressed in m/s.

Behavioral examination; sensory function: tail flick test

The tail of the rat was placed under a shutter-controlled lamp as a heat source (Bioseb, Paris, France). The latency before the rat flicked its tail from the heat was recorded. A sensory alteration increased the latency of flick. Two trials were performed and the mean value was calculated and retained as the characteristic value.

Histomorphometric analysis

Morphometric analysis was performed at the end of the study (D 40) on three animals per treatment group. The animals were anesthetized by i.p. injection of 100 mg/kg Imalgène 500. A 5-mm segment of sciatic nerve was excised for histology. The tissue was fixed overnight with 4% glutaraldehyde (Sigma) in PBS (pH = 7.4) and maintained in 30% sucrose at +4°C until use. The nerve sample was fixed in 2% osmium tetroxide (Sigma) in PBS for 2 h, dehydrated in serial alcohol solution, and embedded in Epon (Sigma). Embedded tissues were then placed at +70°C during 3 days of polymerization. Transverse sections of 1.5 µm were cut with a microtome, stained with a 1% toluidine blue solution (Sigma) for 2 min, dehydrated, and mounted in Eukitt (Labonord, Villeneuve d'Ascq, France). Six randomly selected slices were analyzed using a semiautomated digital image analysis software programme (Biocom, Paris France).

Two randomly selected fields per slice were studied. The proportion of myelinated fibers with abnormal and normal appearances were analyzed. Myelinated fibers without axons, redundant myelin and fibers showing sheaths with thicknesses too large in respect to their axonal diameter were considered as abnormal fibers. Abnormal fibers were not taken into account from the myelin thickness measurement.

Data recording

Body weight and survival rate were recorded every day. Tail flick and EMG testing were performed at D-2, 10, 24 and 40 (±2 days), and the morphometric analysis was performed at the end of the study (D 40).

Data analysis

Global analysis of the data was performed using ANOVA. Where appropriate, Fischer's PLSD (protected least significant difference) test was used for pairwise multiple comparison. The level of significance was set at $P = 0.05$. Results are expressed as mean ±SEM.

RESULTS

Body weight and glycemia

In contrast to control animals, which had doubled their initial weight by D 40, STZ-treated rats demonstrated a marked growth arrest. Indeed, they stopped gaining weight once the STZ-intoxication was performed, regardless of whether they were treated with IL-6, 4-MC or vehicle. It is worth noting that at D 10 post-STZ (the starting point of treatment), the body weight of different groups of STZ rats showed similar comparable body weight (265 ± 4 g).

As early as D 10 post-STZ, STZ rats showed glycemia five or six times higher than control rats. At D 40 post-STZ, the glycemia of STZ rats remained comparable to the level measured at D 10, suggesting that the plateau of hyperglycemia was reached before the onset of IL-6 therapy. In addition, it was observed that none of IL-6/4-MC treatment modalities affected the glycemia of STZ rats (Table 1).

Latency of CMAP

Streptozotocin injection induced a slight, but not significant, delay in the CMAP latency of rats at D 10 post-STZ (Fig. 1A). This impairment of motor conduction and the difference between control and STZ rats became more obvious over time with a CMAP latency in untreated diabetic rats being extended up to 40% (Fig. 2). Overall, IL-6 treatment via i.p. (Fig. 2A) or s.c. (Fig. 2B–D) routes significantly improved the motor conduction of STZ rats after 2 and 4 weeks of treatments. The best effect seemed to be obtained at doses of 10 and 30 µg/kg, administered three times per week, showing complete prevention of latency impairment throughout the study. The effect was less pronounced at 1 µg/kg when the IL-6 was administered three times or once a week.

Sensory nerve conduction velocity

In contrast to the motor conduction, SNCV was markedly slowed by about 40% as early as 10 days following STZ injection when compared to the SNCV of control rats (Fig. 1B). This alteration remained throughout the study in vehicle-treated STZ rats (Fig. 3).

Streptozotocin rats treated with IL-6 demonstrated significantly better SNCV than vehicle-treated STZ rats,

Table 1 Glycemia in each experimental group

Study groups	Treatment schedule	Doses	Glycemia at D 10 (mg/dL)	Glycemia at D 40 (mg/dL)
Control/vehicle	7× week i.p.	2 mL/kg	104 ± 3	97 ± 3
	7× week s.c.		123 ± 7	138 ± 31
	3× week s.c.		108 ± 6	103 ± 2
	1× week s.c.		115 ± 5	99 ± 7
STZ/vehicle	7× week i.p.	2 mL/kg	475 ± 37	543 ± 35
	7× week s.c.		574 ± 18	600 ± 0
	3× week s.c.		490 ± 29	594 ± 4
	1× week s.c.		577 ± 10	584 ± 10
STZ/IL-6	7× week i.p.	1 µg/kg	432 ± 24	566 ± 18
		10 µg/kg	469 ± 23	510 ± 31
		30 µg/kg	433 ± 27	593 ± 7
	7× week s.c.	1 µg/kg	592 ± 8	>600
		10 µg/kg	581 ± 10	>600
		30 µg/kg	575 ± 17	>600
	3× week s.c.	1 µg/kg	503 ± 20	>600
		10 µg/kg	528 ± 13	588 ± 8
		30 µg/kg	521 ± 24	567 ± 14
	1× week s.c.	1 µg/kg	571 ± 13	559 ± 18
		10 µg/kg	545 ± 23	583 ± 13
		30 µg/kg	533 ± 17	584 ± 13
STZ/4-MC	7× week i.p.	10 µg/kg	482 ± 26	591 ± 6

D, day; STZ, streptozotocin; i.p., intraperitoneally; s.c., subcutaneously.

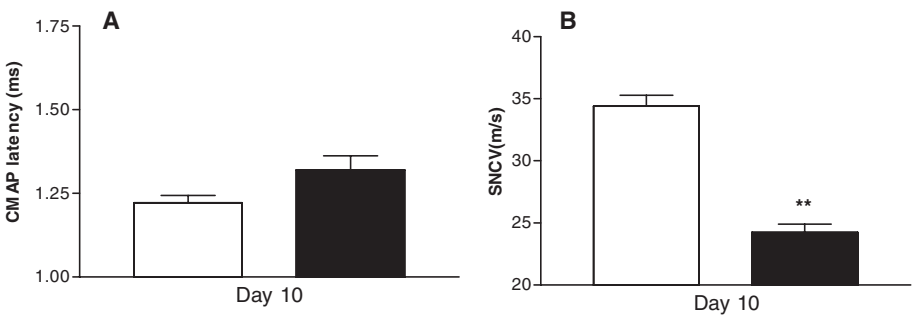

Fig. 1 Electromyograph (EMG) measurements in control and diabetic animals 10 days post administration of streptozotocin (STZ). On day (D) 10 electrophysiological recordings were performed in 10 control rats and 10 diabetic rats. The (A) compound muscle action potential (CMAP) and (B) sensory nerve conduction velocity (SNCV) were recorded using a Neuromatic 2000M electromyograph on anesthetized rats maintained at a body temperature of 30°C. Values are means ± SEM. **, $P \leq 0.01$ as compared to control group. □, control/vehicle; ■, STZ/vehicle

although full prevention of SNCV impairment was not obtained (Fig. 3). The maximal effect of IL-6 was observed with the dose of 30 µg/kg, which provides an improvement of approximately 30% as compared to the score of untreated diabetic rats (Fig. 3B).

Sensory test: tail flick

Streptozotocin injection induced a significant delay in the tail flick performance of rats when compared to controls (Fig. 4). As time progressed, this thermal perception defect increased and the difference between control and STZ rats became obvious and reached a value of 150% in the worst case (Fig. 4A). Overall, IL-6 treatment via i.p. or s.c. routes reduced by half the defect seen in untreated diabetic rats, suggesting significant improvement of pain perception (Fig. 4). However, when STZ rats received IL-6 s.c. on a daily basis, the beneficial effect of the treatment was no longer observed, except at the lowest dose 1 µg/kg on D 38 (Fig. 4B). In contrast, after three times or once a week injections, a significant decrease of the latency was observed for 10 and 30 µg/kg.

Morphometric analysis

Approximately 2606 ± 78 fibers (including abnormal fibers) per animal were analyzed.

Streptozotocin intoxication caused a significant increase in the proportion of fibers with abnormal features in vehicle-treated rats as compared to control animals

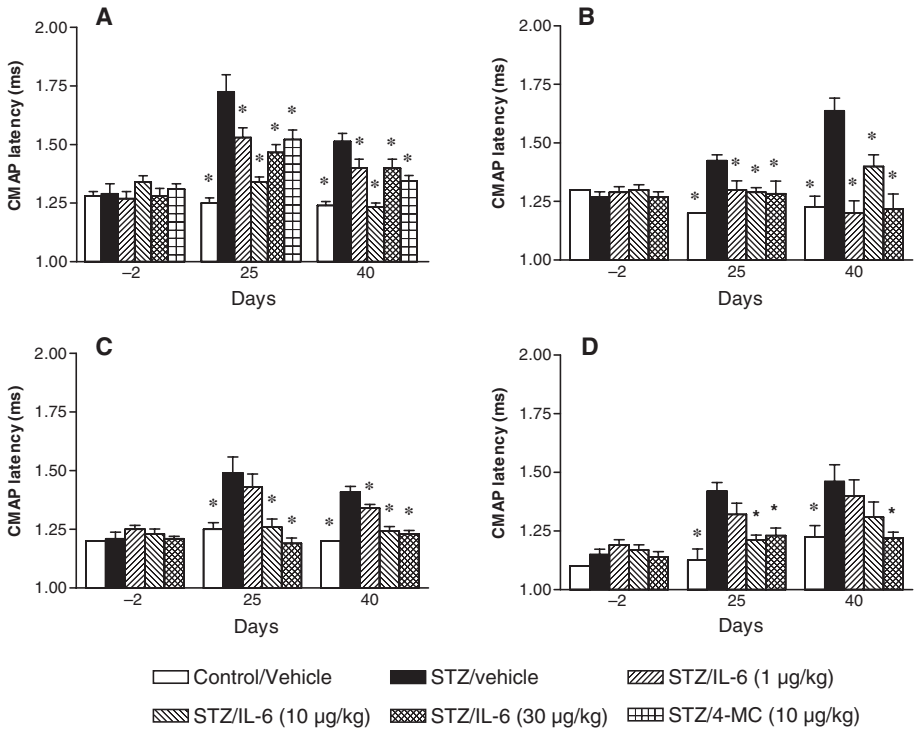

Fig. 2 Effect of (A–D) IL-6 and (A) 4-methylcathecol (4-MC) administration on latency of the CMAP in STZ rats. Animals were treated from D 11–40 with 4-MC intraperitoneally (i.p.) or IL-6 at 1, 10 or 30 µg/kg (A) i.p. daily, (B) subcutaneously (s.c.) daily, (C) three times per week, or (D) once a week. On D 2, 25 and 40, post-STZ electrophysiological recordings were performed in 10 control rats and 10 diabetic rats. Values are means ± SEM. *, $P \leq 0.05$ as compared to the STZ/vehicle group.

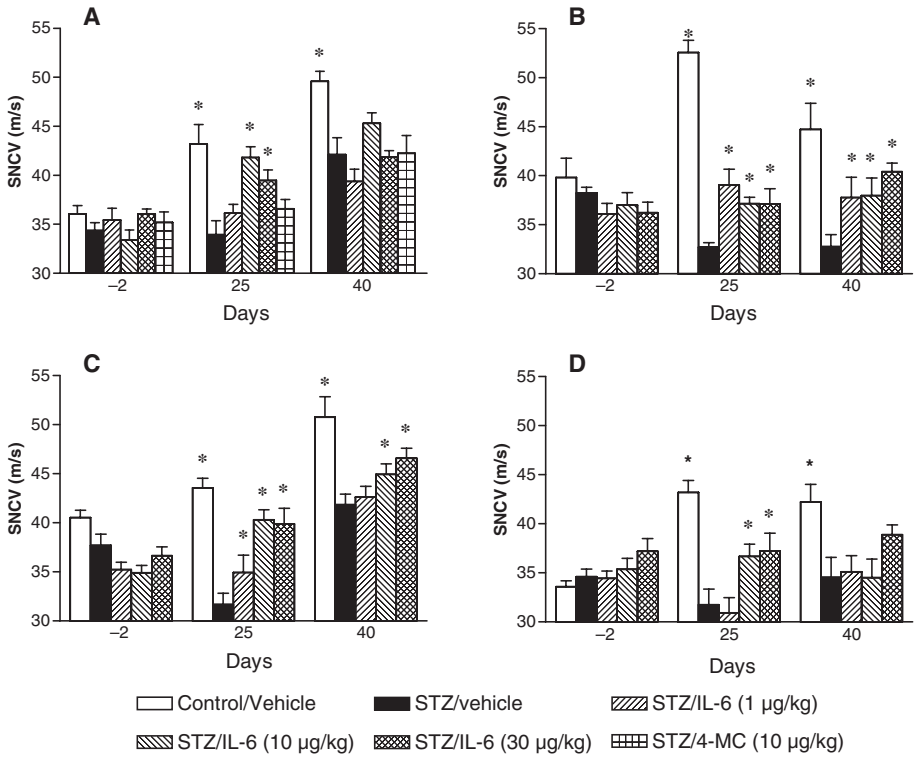

Fig. 3 Effect of (A–D) IL-6 and (A) 4-MC administration on SNCV in STZ rats. Animals were treated from D 11–40 with (A) 4-MC i.p. or IL-6 at 1, 10 or 30 µg/kg (A) i.p. daily, (B) s.c. daily, (C) three times per week or (D) once a week. On D 2, 25 and 40, post-STZ electrophysiological recordings were performed on 10 animals per group. Values are means ± SEM. *, $P \leq 0.05$ as compared to STZ/vehicle group.

(Fig. 5). In the worst case, the proportion of these abnormal fibers could reach up to 10% above the control baseline (Fig. 3B). Whereas treatment with IL-6 at the dose of 1 µg/kg did not modify the proportion of abnormal fibers in STZ rats (except when administered thrice a week), the doses 10 or 30 µg/kg induced a significant reduction in this parameter. Full normalization was observed, except when administrated daily. STZ-intoxicated animals

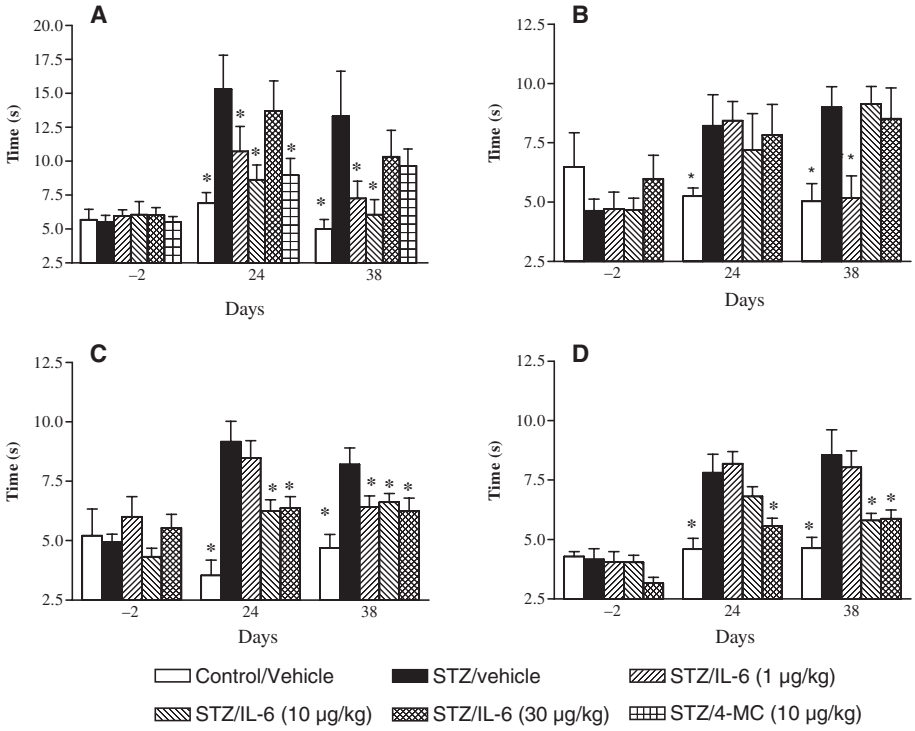

Fig. 4 Effect of (A–D) IL-6 and (A) 4-MC administration on tail flick test in STZ-intoxicated rats. Animals were treated from D 11–40 with (A) 4-MC i.p. or IL-6 at 1, 10 or 30 µg/kg (A) i.p. daily, (B) s.c. daily, (C) three times per week or (D) once a week. On D 2, 24 and 38 post-STZ, the tails of rats ($n = 10$/group) were placed under a shutter-controlled lamp as a heat source and the latency before the rats flicked their tail was recorded (s). Values are means ± SEM. *, $P \leq 0.05$ as compared to STZ/vehicle group.

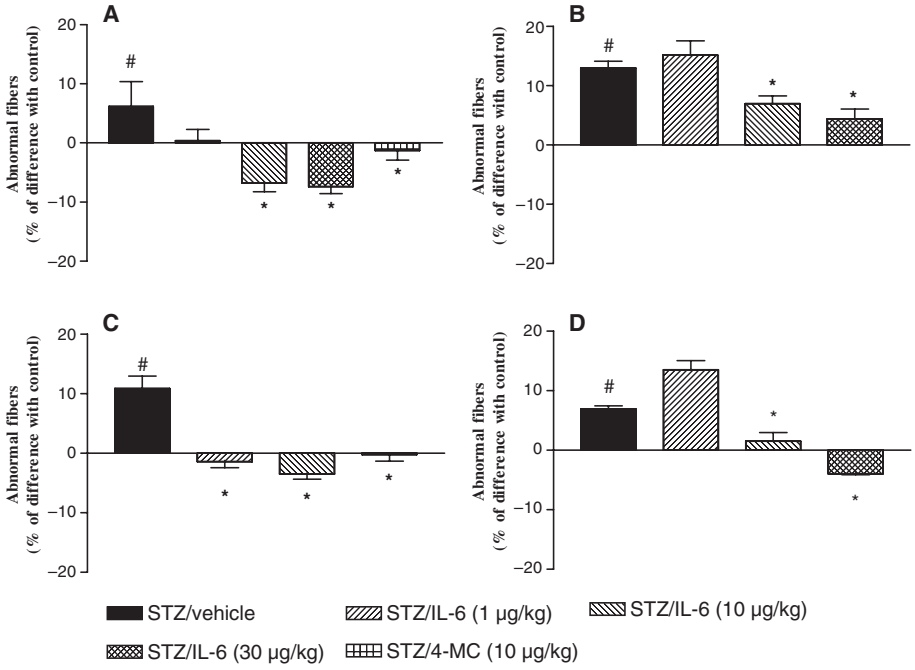

Fig. 5 Effect of (A–D) IL-6 and (A) 4-MC administration on population of abnormal fibers in STZ-intoxicated rats. Animals were treated from D 11–40 with (A) 4-MC i.p. or IL-6 at 1, 10 or 30 µg/kg (A) i.p. daily, (B) s.c. daily, (C) three times per week or (D) once a week. At the end of the study (D 40) animals were sacrificed and a fragment of their sciatic nerve was excised for histology. Myelinated fibers showing collapsed myelin sheath were counted using a semiautomated digital image analysis software. Under the present experimental conditions, the control group demonstrated approximately 25.4 ± 1.3 abnormal fibers. Results were reported as a percentage of difference with controls. Values are means ± SEM. *, $P \leq 0.05$ as compared to STZ/vehicle group. # $P \leq 0.05$, control versus STZ/Vehicle group.

receiving 4-MC treatment demonstrated a comparable proportion of fibers of abnormal appearance as control animals. These fibers show features that may suggest degeneration; an alternative explanation for their appearance could be the proximity of the histological section to a node of Ranvier. In either instance, IL-6 appears to have an effect on nerve fiber morphology, either by preventing/reversing degeneration or by increasing internodal distance.

Streptozotocin intoxication led to a significant thinning (up to 30% in the worst case) of myelin sheath of fibers in the sciatic nerve (Fig. 6). Treatment with IL-6 prevented

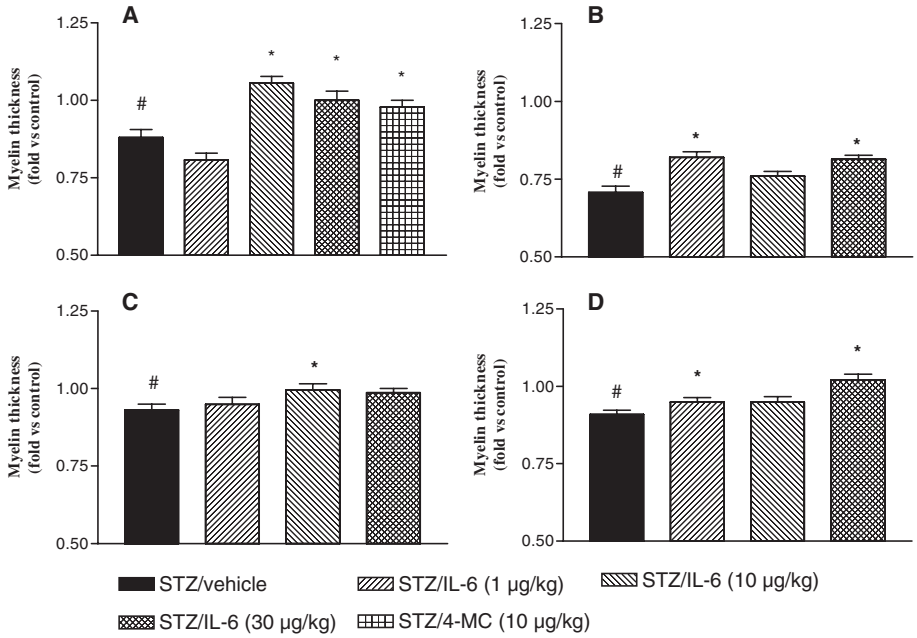

Fig. 6 Effect of (A–D) IL-6 and (A) 4-MC administration on myelin thickness in STZ-intoxicated rats. Animals were treated from D 11–40 with (A) 4-MC i.p. or IL-6 at 1, 10 or 30 µg/kg (A) i.p. daily, (B) s.c. daily, (C) three times per week or (D) once a week. At the end of the study (D 40), animals were sacrificed and a fragment of their sciatic nerve was excised for histology. Myelin thickness of fibers on six randomly slices was counted using a semiautomated digital image analysis software. The myelin thickness was reported as a fold increase and/or decrease as compared to the myelin thickness of controls (2.21 ± 0.04 µm^2). Values are means ± SEM. *, $P \leq 0.05$ as compared to STZ/vehicle group. #, $P \leq 0.05$ control versus STZ/vehicle group.

this loss of myelin in STZ rats. In the most severe case of myelin loss (Fig. 6B), IL-6 treatment prevented about 35% diabetes-induced myelin sheath thinning. The effect of IL-6 was similar whether the drug was administrated via i.p. or via s.c. routes. In addition, weekly administration was as effective as daily treatment with IL-6 at preventing the myelin sheath thinning in STZ rats.

DISCUSSION

In the present study, we sought to evaluate the neuroprotective and/or neuroreparative effect of IL-6 on the development of diabetes-related neuropathy. Investigations were conducted on STZ-induced diabetic related neuropathy in immature rats. We found that both i.p. and s.c. IL-6 administration (1, 10 and 30 µg/kg) improved behavioral (assessed by tail flick test), electrophysiological (assessed by CMAP and SNCV measurements) and histopathological signs in this model of diabetic neuropathy. The best effect was obtained with the treatment schedule of three s.c. administrations per week, which induces a clear improvement in both sensory and motor functions of STZ rats and markedly reduces the proportion of abnormal fibers in the sciatic nerve histological sections. These results demonstrate a neuroprotective and/or neuroreparative effect of IL-6 in the rat model of STZ-induced diabetic-related neuropathy. Impaired sensory nerve conduction detected as early as D 10 post-STZ is the first sign to indicate neuropathy in this model, which is in agreement with evidence of histomorphometric changes observed at later time points. In line with what has been previously reported in the literature,[34] we observed that at the time when diabetes is confirmed by the appearance of severe hyperglycemia (D 10 post-STZ), sensory nerve dysfunction is present. As time progresses, this sensory defect increases and affects a more integrative parameter such as the tail flick latency test while motor defects, which were not evident at D 10, become evident. At the completion of the study, histological signs of neuropathy, such as myelin thinning and abnormal fibers, are evident in the sciatic nerve. These observations mimic the findings in diabetic neuropathy in man. Treatment of STZ rats with IL-6 at doses (micromolar ranges) far lower than those used in other rodent models (e.g. the millimolar ranges for thrombopoiesis), slowed the progression of neuropathy without interfering with the development of diabetes, thus further supporting the previously reported neuroprotective and/or neuroreparative properties of IL-6.

Interleukin-6 is a pleiotrophic cytokine that mediates immune responses and inflammatory reactions affecting growth and differentiation of various types of cells,[35,36] including neuronal cells.[21,37] In addition, a peripheral nerve regenerative action of IL-6 has been demonstrated in various nerve axotomy models, although the underlying mechanisms remain to be established. Here, we report for the first time that IL-6 therapy improved the signs and electrophysiological evidence of nerve dysfunction associated with diabetes-related neuropathy in STZ rats. Because IL-6 treatment was initiated at a time when sensory neuropathy was already present, as demonstrated by a markedly reduced SNCV, this type of treatment could be considered

as a curative approach to sensory neuropathy. In contrast, motor dysfunction (CMAP latency) was milder at the initiation of IL-6 therapy but became severe with time in vehicle-treated animals. Therefore, improvements in the motor function of STZ rats could be considered as a consequence of the preventative action of IL-6.

Interestingly, when considering the daily treatment schedule in this model, it appears that the effect on SNCV was better when IL-6 was administered via s.c. than via the i.p. route. This profile of results is in accordance with previously documented results showing that better bioavailability of proteins is obtained with s.c. as compared with the i.p. route.[38]

On the other hand, however, this enhanced bioavailability of s.c. IL-6 appeared to impair the tail flick performance of rats when using a daily dosing schedule of 10 or 30 μg/kg, but still provides a better myelination profile than untreated diabetic rats. This is supported by findings showing the involvement of IL-6 in the alteration of animal behaviors.[39] When the daily dosage was reduced to 1 μg/kg or the dosing schedule was reduced to three times and once weekly, IL-6 treatment became effective at preventing the disturbances of the tail flick test, suggesting that the frequency of IL-6 administration is crucial at the obtaining the optimal effect of IL-6.

The diabetic rats treated with 4-MC, a compound with previously reported neuroprotective action, showed lesser degrees of sensorimotor dysfunction than untreated diabetic animals and fewer profound morphological changes. These results are in accordance with previous investigations.[40,41] Indeed, 4-MC treatment has been shown to promote fiber growth and improve the findings associated with diabetic neuropathy. 4-MC could promote endogenous production of NGF by induction of cyclooxygenase activity.[42] In the present study, i.p. IL-6 showed a greater degree of neuroprotective and/or neuroreparative effect than intraperitoneal 4-MC, no matter which parameter was considered, especially at the dose of 10 μg/kg.

Moreover, the neuroprotective effect of IL-6 demonstrated in the present study is in accordance with previously documented effects of IL-6 on sensory as well as motor neurons. Marz *et al.*[43] have shown that IL-6 in conjunction with sIL-6R administered *in vitro*, can confer IL-6 sensitivity to sympathetic neurons, resulting in enhanced neuronal survival in the absence of NGF and induction of neuropeptides and choline acetyltransferase. Moreover, IL-6 is rapidly elevated following axotomy of peripheral nerves, and, in the CNS, following brain lesioning.[44,45] Hirota *et al.*[27] have identified, on injured neurons as well as on glial cells, cellular sites of IL-6 production and local accumulation of IL-6 following nerve injury. In parallel, acceleration of functional nerve regeneration has been recorded. *In vivo*, IL-6 injections appeared to reduce demyelination in the murine encephalomyelitis model produced by Theiler's virus infection.[30]

The role of the activation of the gp130 signaling pathway is essential in the process of nerve regeneration and/or survival, as suggested by the potentiation of the neuroprotective effects of IL-6 following co-administration of its soluble receptor.[46] This concept is further supported by the fact that mutant mice lacking a functional LIF-R to trigger the activation of gp130 show a loss more than 35% of facial motor neurons, 40% of spinal motor neurons and 50% of neurons in the nucleus ambiguus.[47] In addition, gp130 is required for the integrity of the myelin sheath as conditional gp130-mutant mice show a progressive alteration of the myelin sheath structure.[48] Also, the Schwann cell covering some axon bundles in the myocardium and gut is incomplete in gp130 mutant animals,[49] suggesting that in adult animals gp130-stimulating cytokines not only mediate survival of motor neurons[47] but also affect Schwann cell covering in myelinated and unmyelinated peripheral nerves.[49] Activation of gp130 signaling by IL6R/IL6, an interleukin-6 receptor-interleukin-6 fused molecule,[50] appears comparable to cyclic AMP elevating agents such as forskolin, known to induce the myelin gene products (myelin basic protein and myelin protein zero genes) in DRG and Schwann cell cultures.

Our results are of interest with respect to the finding that administration of IL-6 without its soluble receptor induces a strong protective effect in STZ-intoxicated animals. Similarly to IL-6, sIL-6R is predominantly expressed in neurons and can be regulated by inflammatory mediators (see [51] for review). The expression of IL-6R in various neuropathies is being investigated. However, we speculate here that IL-6 works by interacting with the circulating sIL-6R present at ng concentrations.

It has been demonstrated that IL-6 enhances the mRNA expression of gp130 and the mRNA expression of the CNTF.[52] CNTF is well-known to significantly increase neurite regeneration when added exogenously. Some authors have shown that CNTF family strongly promotes myelin formation by activating the 130 kDa glycoprotein Janus kinase (gp130- JAK) pathway.[53] It has been shown in the STZ-diabetic rat that CNTF activity in the sciatic nerve was reduced to 70% of control values after 2 months of STZ diabetes and may contribute to the development of diabetic neuropathy.[54,55] CNTF is well-known to significantly increase neurite regeneration when added exogenously. IL-6 could act in our diabetic model enhancing regeneration via upregulating CNTF expression. Nevertheless, because the present study did not specifically address the mechanism(s) by which IL-6 protects against the development of diabetic neuropathy or repairs the damage induced by diabetes, additional investigations should be carried out in order to confirm and further char-

acterize modulation of gp130 signaling pathway by IL-6 in the present model.

In conclusion, despite the obvious difference between the clinical reality of diabetes-related neuropathy and the animal model used in the present study, at least some of the mechanisms instrumental in the pathology are similar, and the present findings may help design clinical applications of IL-6 for protection against the development of diabetic neuropathy.

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