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Neurobiology of Disease

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Neurobiology of Disease 18 (2005) 206–217

Minocycline in phenotypic models of Huntington's disease

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Received 20 July 2004; revised 23 September 2004; accepted 30 September 2004
Available online 2 December 2004

Minocycline has been shown to be neuroprotective in various models of neurodegenerative diseases. However, its potential in Huntington's disease (HD) models characterized by calpain-dependent degeneration and inflammation has not been investigated. Here, we have tested minocycline in phenotypic models of HD using 3-nitropropionic acid (3NP) intoxication and quinolinic acid (QA) injections. In the 3NP rat model, where the development of striatal lesions involves calpain, we found that minocycline was not protective, although it attenuated the development of inflammation induced after the onset of striatal degeneration. The lack of minocycline activity on calpain-dependent cell death was also confirmed in vitro using primary striatal cells. Conversely, we found that minocycline reduced lesions and inflammation induced by QA. In cultured cells, minocycline protected against mutated huntingtin and staurosporine, stimulations known to promote caspase-dependent cell death. Altogether, these data suggested that, in HD, minocycline may counteract the development of caspase-dependent neurodegeneration, inflammation, but not calpain-dependent neuronal death.

Keywords: Huntington's disease; 3-Nitropropionic acid; Quinolinic acid; Minocycline; Striatum; Cell death

Introduction

Huntington's disease (HD) is a genetic neurodegenerative disorder characterized by motor and cognitive impairments mainly due to neuronal degeneration within the striatum and the cerebral cortex (Blum et al., 2003a; Brouillet et al., 1999). The mutation involved produces a polyglutamine expansion within the N-terminal part of the huntingtin protein (The Huntington's Disease Collaborative Research Group, 1993), which leads to many neuronal alterations such as impairments in transcription (Cha, 2000; Zuccato et al., 2003), calcium signaling (Tang et al., 2003), or axonal transport (Gunawardena et al., 2003; Szebenyi et al., 2003) and promotes mitochondrial complex II dysfunction (Beal, 2000), mitochondrial Ca^{2+} defects (Panov et al., 2002), NMDA receptor sensitization (Song et al., 2003; Zeron et al., 2002), as well as proapoptotic and pronecrotic protease activation (Wellington et al., 2003). However, there is no efficient treatment that slows down or halts the evolution of HD.

Minocycline is an antibiotic of the tetracycline family that displays beneficial activity in various models of neurodegeneration (Parkinson disease, amyotrophic lateral sclerosis, spinal cord injury, ischemia) (Brundula et al., 2002; Kriz et al., 2002; Lee et al., 2003; Stirling et al., 2004; Tomas-Camardiel et al., 2004; Wu et al., 2002; Yrjanheikki et al., 1999; see also Blum et al., 2004, for review), although recent studies pointed out that it may also be detrimental (Diguet et al., 2004a; Tsuji et al., 2004; Yang et al., 2003; see also for review Blum et al., 2004; Diguet et al., 2004b). Beneficial activity of minocycline has been related to its ability to inhibit both mitochondrial mechanisms leading to cell death and inflammatory processes (Blum et al., 2004; Scarabelli

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Available online on ScienceDirect (www.sciencedirect.com).

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doi:10.1016/j.nbd.2004.09.017

et al., 2004; Tikka et al., 2002; Wang et al., 2003, 2004; Zhu et al., 2002).

Minocycline is being tested in HD patients (http://www.huntington-study-group.org), and preliminary observations are encouraging (Bonelli et al., 2003; Thomas et al., 2004). However, the potential of minocycline for treating HD is far from clear (for a review, see Blum et al., 2004). While a former study (Chen et al., 2000) demonstrated that i.p. injections of minocycline improve motor alterations in the R6/2 transgenic mouse model of HD—that is characterized by very limited neuronal loss even at very late stages (Turmaine et al., 2000)—Smith et al. (2003) failed to reproduce this effect in the same strain following continuous oral administration. Additionally, although the inhibitory effect of minocycline on caspase activation has been well described (Wang et al., 2003), it is not known whether the antibiotic would be effective against striatal degeneration involving brain calpains. Indeed, increased calpain activity has been reported not only in the striatum of HD patients, but also in transgenic and nontransgenic rodent models of the disease (Bizat et al., 2003a; Gafni and Ellerby, 2002; Gafni et al., 2004). In the same way, the ability of minocycline to modulate the development of striatal inflammation, another important pathological feature of HD (Sapp et al., 2001), is ill-defined. Hence, to further analyze the neuroprotective potential of minocycline for HD, we tested whether it could attenuate striatal neurodegeneration in phenotypic models of HD based on complex II inhibition (Beal et al., 1993) or NMDA receptor overactivation (excitotoxicity; Beal et al., 1986), both known to mimic numerous behavioral, histological, neurochemical, and biochemical events seen in HD patients (Brouillet et al., 1999).

Material and methods

Animals

We used adult male Lewis rats, 12 weeks of age, for the 3-nitropropionic (3NP) model and adult Wistar rats (250 g) for the quinolinic acid (QA) model and microdialysis experiments. Animals were housed three per cage and maintained in a temperature- and humidity-controlled room on a 12-h light/dark cycle with food and water ad libitum. The number of animals was kept to a minimum, and all efforts to avoid animal suffering were made in accordance with the standards of the Institutional Ethical Committees.

Treatments

3NP (Fluka, Belgium) was dissolved in 0.1 M PBS, pH 7.4, adjusted to pH 7.3–7.4 with 5 N NaOH and quinolinic acid (Sigma, Belgium) was dissolved in 2 N NaOH, the pH was adjusted to 7.4, and the volume completed with PBS (pH 7.4) as previously described (Bensadoun et al., 2001; Ouary et al., 2000). Minocycline and doxycycline were dissolved in NaCl 0.9%. Fresh solutions were prepared each day before injections. The volume injected was adjusted according to the body weight of each rat (200 μl/100 g, i.p).

Chronic 3NP delivery (Fig. 1A)

Chronic treatments with 3NP (56 mg/kg/d) using 2mL1 (10 μl/h, 7 days) Alzet minipumps and neurological scoring were performed as previously described (Blum et al., 2002a,b, 2003b; Mittoux et al., 2002; Ouary et al., 2000). Rats were anesthetized with a mixture containing xylazine hydrochloride (Rompun, Bayer; 4.5 mg/kg) and ketamine hydrochloride (Imalgene, Merial; 90 mg/kg). An incision was made below the base of the neck and a 2mL1 Alzet osmotic minipump (delivering 10 μl/h for 7 days; IFFA Credo, Belgium) containing 3NP (Fluka) was positioned under the skin. The final concentration of 3NP in the pump was adjusted to the weight of the rats on the day of implantation in order to exactly deliver 56 mg/kg/d. Sham rats and animals treated with pharmacological compounds alone underwent all the surgical procedures (without minipump implantation). Thirty-nine rats (sham/vehicle, $n = 5$; 3NP/vehicle, $n = 8$; sham/minocycline 10 mg/kg, $n = 5$; 3NP/minocycline 10 mg/kg, $n = 8$; sham/minocycline 50 mg/kg, $n = 5$; 3NP/minocycline 50 mg/kg, $n = 8$) were challenged for the neuroprotective ability of minocycline. Animals were injected with vehicle or minocycline just before minipump implantation and each day until sacrifice. Controls and 3NP-treated animals were evaluated every day for motor impairments. Briefly, behavioral abnormalities were determined according to the presence and severity of motor symptoms consisting of dystonia, gait abnormalities, recumbency and also grasping and the ability to remain on a small platform for >10 s. The final neurological score was assessed as described (Mittoux et al., 2002; Ouary et al., 2000; minimum = 0, normal animal; score = 8, animal showing near-death recumbency). All rats were killed after 5 days of 3NP subcutaneous infusion (10 h after the last minocycline injection) according to the known kinetics of striatal lesion occurrence in this model as we previously reported (Bizat et al., 2003a; Blum et al., 2001, 2002a,b, 2003b; Ouary et al., 2000).

Chronic 3NP delivery and minipump removal (Fig. 1B)

We developed another 3NP protocol in which inflammation was induced in addition to the striatal lesion (see also the Results section) and determined the effect of minocycline on its development. In this model, minipumps were positioned under the skin as indicated above and removed under light anesthesia with halothane 5 days after implantation. Then, animals were injected daily with vehicle or 10 mg/kg minocycline until the sacrifice for 5 days. Thirty-two rats were used: sham/vehicle, $n = 6$; 3NP/vehicle, $n = 10$; sham/minocycline 10 mg/kg, $n = 6$; 3NP/minocycline 10 mg/kg, $n = 10$. All rats were killed 5 days after minipump removal.

Quinolinic acid-induced striatal lesions (Fig. 1C)

Animals were anesthetized with xylazine/ketamine mixture and received an intrastriatal stereotaxic injection of QA (1 μl; 180 nmol) using the following coordinates: 1.0 rostral to bregma, 3.5 mm lateral to midline and 5 mm ventral from the dural surface. The toxin was injected over 4 min, and the needle was left in place for an additional 2 min. Eighteen rats were used: QA/vehicle, $n = 6$; QA/minocycline 10 mg/kg, $n = 6$; QA/doxycycline 10 mg/kg, $n = 6$. All rats were killed 7 days after surgery.

Tissue postprocessing

All animals were killed by decapitation, and their brains were quickly removed and frozen in 2-methylbutane cooled by dry ice (−40°C). The tissue was cut at 20-μm thickness on a cryostat (Leitz), and serial coronal sections were mounted onto poly-L-lysine and stored at −20°C until use. In 3NP-treated rats, since lesions are bilateral with similar extents (Mittoux et al., 2002; Brouillet et al., unpublished results), hemispheres were separated at