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Neuroscience Letters 375 (2005) 107–111
Neuroscience Letters
www.elsevier.com/locate/neulet

Tumor necrosis factor-alpha receptor ablation in a chronic MPTP mouse model of Parkinson's disease

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Received 2 July 2004; received in revised form 7 August 2004; accepted 27 October 2004

Abstract

Recently, we demonstrated that mice deficient of the pro-inflammatory cytokine tumor necrosis factor-alpha (TNF-alpha) were partly protected against 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) neurotoxicity. Here we extended the study and investigated TNF-alpha receptor 1 (−/−) (TNFR1) and TNF-alpha receptor 2 (−/−) (TNFR2) mice using a chronic MPTP dosing regimen (15 mg/kg MPTP on 8 consecutive days). One week after the last MPTP treatment, HPLC determination of striatal dopamine (DA) and immunostaining for the dopamine transporter (DAT) in the substantia nigra pars compacta (SNpc) was performed.

MPTP treatment reduced striatal DA levels significantly; nigral DAT immunoreactivity was reduced to a lower extent. However, there was no difference in DA levels and the number of DAT positive neurons between TNFR1 (−/−), TNFR2 (−/−) and wild type mice after MPTP treatment.

In contrast to TNF-alpha deficiency neither TNFR1 nor TNFR2 gene ablation showed protection against MPTP neurotoxicity, which argues for a protective mechanism of TNF-alpha not mediated by TNFR1 and TNFR2 signaling.

Keywords: TNF-alpha; MPTP; Inflammation; Parkinson's disease; Cytokine; Tumor necrosis factor

Parkinson's disease (PD) is a slowly progressing movement disorder characterized by a loss of dopaminergic cells in the substantia nigra pars compacta (SNpc) and a massive reduction in striatal dopamine (DA). The impact of inflammatory processes in PD [9] is supported by the observation that the number of activated microglia [14] and level of pro-inflammatory cytokines are elevated in PD patients [2,15,16]. These findings are also supported by animal models of PD. Activated microglia and elevated cytokine levels are present in the MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) model [13,12] and in the 6-hydroxydopamine model of PD [17]. Among the pro-inflammatory cytokines, tumor necrosis factor-alpha (TNF-alpha) is thought to play a prominent role in the pathological process of PD. TNF-alpha can induce microglia activation [19]. Neurons in the SNpc express both TNF-alpha receptors 1 and 2 [2]. Elevated levels of TNF-alpha receptors were found in PD patients [16], and TNF-alpha polymorphism has been observed in patients with sporadic PD [11,18]. Under pathological conditions TNF-alpha is mainly expressed by astroglial and microglial cells [3]. Interestingly, TNF-alpha is involved in both neurodegenerative and neuroprotective pathways [8]. The two subtypes of TNF-alpha receptors (TNFR), TNF-alpha receptor 1 (TNFR1) and TNF-alpha receptor 2 (TNFR2), and their distinct signaling pathways might be responsible for this ambivalent function [23]. The TNFR1 includes an intracellular death domain and is involved in apoptotic cell death [7]. The TNFR2 has no death domain and is considered to activate anti-apoptotic pathways [23]. However, TNFR2 can also enhance the apoptotic action of TNFR1, which can be involved in anti-apoptotic pathways (for review see [7,8,19]). In a previous study, we

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doi:10.1016/j.neulet.2004.10.077

demonstrated that TNF-alpha (−/−) mice and the TNF-alpha synthesis inhibitor thalidomide attenuated MPTP toxicity [4]. Additionally, others [20,21] and we [4] found elevated TNF-alpha mRNA levels after MPTP treatment. Consequently, we were interested whether TNFR1 or TNFR2 signaling is involved in the protective effect observed in TNF-alpha (−/−) mice against MPTP toxicity. Meanwhile two other groups performed MPTP studies using TNFR1 or TNFR2 mice [20,21] showing a protective [21] or no protective effect [20] against MPTP toxicity by genetic ablation of TNFR1 and TNFR2. Both studies used acute MPTP treatment schedules whereas in the present study a chronic MPTP dosing schedule was applied. The chronic MPTP model was selected for the present study, because a protective effect indicated by striatal DA and DAT measurements was obtained in a previous experiment using TNF-alpha (−/−) mice only after chronic MPTP treatment but not after acute MPTP administration [4].

The present study was conducted in male C57bl/6 mice and in homozygous mice deficient in the TNFR1 or TNFR2 gene. All mice were about 10–12 weeks old at the beginning of the experiment. TNFR1 (−/−) and TNFR2 (−/−) mice (Jackson Laboratory, Bar Harbor, ME) were bred at the Research Unit Schwerzenbach, Switzerland, and maintained under standard conditions (temperature, 21 ± 1.0 °C; humidity, 55 ± 5%) on a 12 h light/12 h dark cycle (lights on at 7 a.m.) with free access to standard food (Nafag 9431, Nafag Ecossan, Gossau, Switzerland) and water ad libitum. All animal studies were carried out in accordance with the European Convention for Animal Care and Use of Laboratory Animals and were approved by the appropriate institutional governmental agency (Kantonales Veterinäramt Zürich, Switzerland).

TNFR1 (−/−) and TNFR2 (−/−) mice, which have been backcrossed over 10 generations to a C57bl/6 background, were genotyped using polymerase chain reaction (PCR) amplification from genomic DNA obtained from a tail biopsy. The presence of TNFRs was tested with the following oligonucleotide primer: for TNFR1: 5′-TGT GAA AAG GGC ACC TTT ACG GC-3′ and 5′-GGC TGC AGT CCA CGC ACT GG-3′, and for TNFR2: 5′-CCT CTC ATG CTG TCC CGG AAT-3′ and 5′-AGC TCC AGG CAC AAG GGC GGG-3′ (Microsynth, Balgach, Switzerland). The PCR mix consisted of 0.2 mM dNTP, 1 mM $MgCl_2$, 1 μM primer and 0.5 units Taq DNA polymerase (Roche Diagnostics, Mannheim, Germany) in PCR buffer. One microliter of DNA extract (approximately 50 ng DNA) was used for the following PCR reaction carried out in a thermocycler (Perkin-Elmer PE 9600, Perkin-Elmer Switzerland AG, Hünenberg, Switzerland): After an initial denaturating step at 95 °C for 5 min, the cycle consisted of denaturating for 30 s at 95 °C, annealing for 30 s at 55 °C, and extending for 30 s at 72 °C. The cycle was repeated 35 times. The final extension step was done at 72 °C for 5 min. PCR products (470 bp fragment for the wild type TNF-alpha R1 allele and 200 bp fragment for the TNF-alpha R2 allele) were separated on ethidium bromide-stained 2% agarose gel using TAE buffer (40 mM Tris–acetate; 1 mM EDTA; pH 8.0) at 80 V and visualized by ultraviolet light.

MPTP (15 mg/kg, i.p., calculated as free base) was injected daily on 8 consecutive days as a chronic treatment. Seven days after the last MPTP or saline administration, the mice were sacrificed by cervical dislocation. The brains were rapidly removed and placed on an ice-cooled plate for dissection of the striatum. Immediately after dissection, the striata were weighed and placed in 1.5 ml plastic tubes containing ice-cooled perchloric acid (500 μl, 0.4 M), homogenized for 10 s using ultrasound and centrifuged for 20 min at 15000 × *g* and 4 °C. The supernatant was passed through a 0.2 μm filter and kept at 4 °C until HPLC analysis. DA and its metabolites 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA), were analyzed using reversed-phase ion-pair chromatography combined with electrochemical detection under isocratic conditions [22]. The detector potential was set at +750 mV using a glassy carbon electrode and an Ag/AgCl reference electrode. The mobile phase (0.6 mM 1-octanesulfonic acid, 0.27 mM Na_2EDTA, 0.043 M triethylamine and 50 ml acetonitrile/l, adjusted to pH 2.95 with H_3PO_4) was delivered at a flow rate of 0.5 ml/min at 22 °C onto the reversed phase column (125 mm × 3 mm with pre-column 5 mm × 3 mm, filled with Nucleosil 120-3 C18, Knauer, Berlin, Germany). Ten microliters of aliquots were injected by an autosampler with a cooling module set at 4 °C. Data were calculated by an external standard calibration.

The midbrain including the substantia nigra pars compacta (SNpc) was post-fixed for 2–3 days in cold fixative with 4% paraformaldehyde in 0.01 M phosphate-buffered saline (PBS, pH 7.4), then transferred to a 30% sucrose solution and kept at 4 °C until it sunk. Subsequently, the tissue was cut with a freezing microtome, and coronal sections (25 μm thick) were collected throughout the rostro-caudal extent of the SNpc and stored in a cryoprotectant solution. As a specific marker for dopaminergic cells the dopamine transporter (DAT) was used to estimate the extension of the MPTP lesions in the SNpc. Sections were processed using the standard peroxidase-antiperoxidase method. After 3 × 5 min rinses in PBS, the free floating sections were blocked for 1 h in PBS containing 5% normal goat serum plus 0.3% Triton X-100. The sections were then incubated in a solution of PBS and 2% normal goat serum plus 0.15% Triton X-100 containing the primary antibody rat anti-DAT (1:2000, Chemicon, Lucerne, Switzerland) for 2 days at 4 °C. Following this, the sections were rinsed and incubated for 1 h in biotinylated secondary antibodies (goat anti-rat, 1:300, Jackson Immuno Research, West Grove, USA) in a solution of 2% goat serum plus 0.15% Triton X-100 at room temperature. Subsequently, the sections were treated with avidin-biotin-horseradish peroxidase complex (Vectastain Elite, Vector Laboratories, Burlingame, CA) for 1 h at room temperature, followed by 3 min × 5 min rinses in 0.1 M Tris buffer (TB, pH 7.4). Immunoreactivity was visualized with 0.05% 3,3′-diaminobenzidine tetrahydrochloride (DAB, Sigma, Buchs, Switzerland) and 0.004% H_2O_2 in TB for 3–5 min. Nickel chloride (0.08%) was added

to the DAB solution to intensify the staining. The sections were then rinsed 3 min × 5 min in TB and mounted on slides, air dried, dehydrated through an alcohol series, cleared in xylene and coverslipped. Dilution series were run to establish the optimum staining. Controls for non-specific staining were performed, in which either the first or secondary antibody was omitted. These controls did not produce specific staining.

The total numbers of DAT positive cells were counted using the image analysis computer software Stereo Investigator (Version 4.10, Microbrightfield, Colchester, USA). DAT cell counting started at a random position, and an average of 12 sections per animal were counted using the fractionator method with a counting frame (45 μm × 45 μm) placed randomly on a virtual grid (75 μm × 75 μm). The position of the SNpc was defined according to the mouse brain atlas of Franklin and Paxinos [5]; anterior–posterior coordinates were between −2.90 mm and −3.90 mm according to bregma. Digitized bright-field images were captured using a Zeiss Axiophot microscope (Jena, Germany) in combination with a video-camera (Kodak Megaplus, Eastman Kodak, San Diego, CA, USA) and the image based analysis computer software described above. All values are expressed as mean ± S.E.M. Analysis of variance (ANOVA) was used with MPTP treatment (MPTP or saline) and genotype (TNFR1, TNFR2 or wild type) as independent factors, and followed by Fisher's least significant difference (PLSD) test.

One day after the last MPTP treatment an open field experiment was carried out: the mice were placed in circular activity cages (diameter 36 cm, height 40 cm) under dim light conditions. A video tracking system (EthoVision, Noldus, Wageningen, The Netherlands) calculated the total distance moved of each mouse during the observation time of 20 min.

Post mortem neurochemical analysis of striatal dopamine and its metabolites (for summary please see Table 1) revealed that TNFR1 and TNFR2 deficient mice were not different from wild type mice (wt) after saline treatment (DA: TNFR1 versus wt, $p = 0.8$; TNFR2 versus wt, $p = 0.5$; DOPAC: TNFR1 versus wt, $p > 0.07$; TNFR2 versus wt, $p = 0.95$; HVA: TNFR1 versus wt, $p = 0.8$; TNFR2 versus wt, $p = 0.999$). MPTP significantly reduced DA levels in the striatum [$F(1,58) = 215.78$; $p < 0.001$]. However, no effect of genotype was found [$F(2,58) = 0.03$; $p = 0.97$] with no genotype × MPTP treatment interaction [$F(2,58) = 0.91$; $p = 0.4$]. In the MPTP-treated group also no statistical difference between genetically modified animals and wild type animals (TNFR1 versus wt, $p = 0.97$; TNFR2 versus wt, $p = 0.4$).

DA metabolite levels, which were decreased by MPTP treatment (DOPAC [$F(1,58) = 122.29$; $p < 0.001$] and HVA [$F(1,58) = 53.29$; $p < 0.001$]), showed no significant difference between genetically modified animals and wild type animals (DOPAC: TNFR1 versus wt, $p = 0.1$; TNFR2 versus wt, $p = 0.8$; HVA TNFR1 versus wt, $p = 0.8$; TNFR2 versus wt, $p = 0.6$). In all groups of MPTP treated mice the DA turnover was increased due to the response to the damage [$F(1,58) = 62.09$; $p < 0.001$]. However, there was no difference in the comparison between of genetically modified animals to controls (TNFR1 versus wt, $p = 0.1$; TNFR2 versus wt, $p = 0.9$).

The number of DAT positive cells in the substantia nigra was only slightly reduced (main effect of MPTP treatment ([$F(1,34) = 5.02$; $p < 0.05$]; for summary please see Table 2). There was no significant difference between genetically modified animals and wild type animals (TNFR1 versus wt, $p = 0.14$; TNFR2 versus wt, $p = 0.7$).

Table 2
Effect of MPTP treatment and genotype on DAT-positive cells in the substantia nigra

Genotype	Treatment	*n*	DAT positive cells
Wild type	Saline	6	2661 ± 234
	MPTP	6	2308 ± 179
TNFR1 (−/−)	Saline	6	2383 ± 226
	MPTP	8	1992 ± 154
TNFR2 (−/−)	Saline	6	2632 ± 280
	MPTP	8	2215 ± 195

Substantia nigra sections were obtained 1 week after the last MPTP treatment (15 mg/kg on 8 consecutive days) and analyzed using a stereological method described in the method section. Values are mean ± S.E.M. and *n* indicates the number of mice per group. Statistical comparison was performed using ANOVA with subsequent Fisher's PLSD test (MPTP vs. respective saline control). Since no MPTP treatment × genotype interaction was obtained, post hoc tests of single groups were not carried out (MPTP vs. saline $p < 0.05$).

Table 1
Effect of MPTP treatment and genotype on striatal DA and DA metabolites levels (ng/mg wet tissue weight).

Genotype	Treatment	*n*	DA	DOPAC	HVA	DA-turnover
Wildtype	Saline	10	13.5 ± 0.8	1.01 ± 0.06	1.26 ± 0.08	0.170 ± 0.008
	MPTP	10	5.9 ± 0.7	0.53 ± 0.04	0.82 ± 0.05	0.244 ± 0.018
TNFR1	Saline	12	13.2 ± 0.5	1.17 ± 0.04	1.24 ± 0.06	0.182 ± 0.004
	MPTP	14	5.9 ± 0.5	0.60 ± 0.05	0.9 ± 0.05	0.270 ± 0.019
TNFR2	Saline	10	14.1 ± 0.8	1.00 ± 0.08	1.26 ± 0.09	0.160 ± 0.004
	MPTP	8	5.1 ± 0.5	0.50 ± 0.05	0.84 ± 0.08	0.269 ± 0.015

Striatal tissues were analyzed 1 week after the last MPTP treatment (15 mg/kg on 8 consecutive days). Values are mean ± S.E.M. and *n* indicates the number of mice per group. DA-turnover is calculated as the sum of the DA metabolites divided by DA. Statistical comparison was performed using ANOVA with subsequent Fisher's PLSD test (MPTP vs. respective saline control). Since no MPTP treatment × genotype interaction was obtained in the parameters, post hoc tests of single groups were not carried out (MPTP vs. saline in all parameters $p < 0.001$).

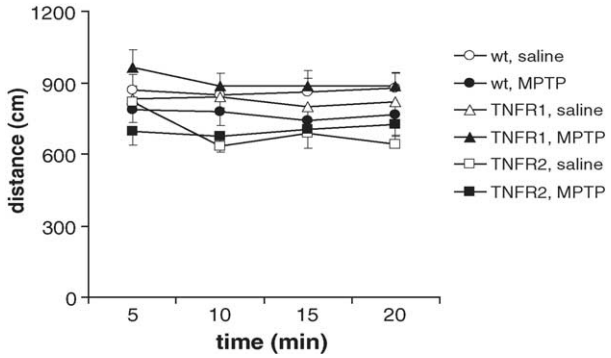

Fig. 1. Effects of genotype and MPTP on locomotor activity 1 day after the last treatment of MPTP or saline. Distance moved was measured over 20 min and divided into four 5-min time bins. Values are mean ± S.E.M. (n = 8–14 mice per experimental group).

One day after the last MPTP treatment TNFR2 (−/−) mice (MPTP and saline) showed a lower total distance moved in the open field environment than wild type ($p < 0.05$) and TNFR1 (−/−) mice ($p < 0.01$; effect of genotype [$F(2,58) = 5.73$; $p < 0.01$]; please see Fig. 1). There was no effect of time [$F(3,174) = 1.60$; $p = 0.2$] and no interaction of time and genotype [$F(6,174) = 0.42$; $p = 0.9$] or MPTP treatment [$F(3,174) = 0.19$; $p = 0.9$] or both [$F(6,174) = 1.08$; $p = 0.4$].

In the present study, neither TNFR1 nor TNFR2 gene ablation showed protection against MPTP neurotoxicity indicated by HPLC analysis of striatal DA levels, nigral DAT positive cell counts and locomotor activity.

Recently, Sriram et al. [21] reported that mice lacking the TNFR1 and R2 receptor showed attenuation of signs of MPTP toxicity. However, in this study only short-term effects up to 48 h after the injection were investigated. Eleven days after an acute treatment of MPTP Rousselet et al. [20] did not find any protective effects in TNFR1 and R2 receptor (−/−) mice either. In contrast to the published reports on acute MPTP neurotoxicity using TNFR1 and TNFR2 deficient mice [20,21] we applied a chronic MPTP treatment of TNFR1 and R2 (−/−) mice instead of an acute MPTP dosing regimen, because we showed that TNF-alpha deficiency was protective against MPTP neurotoxicity after a subacute but not after an acute MPTP treatment in a previous study [4].

From our previous findings [4] with TNF-alpha (−/−) mice, we expected a reduced MPTP toxicity in either TNFR1 or R2 (−/−) mice, suggesting a neurodegenerative role of TNF-alpha in this model. Blocking the TNF-alpha synthesis by thalidomide also led to a reduction in MPTP toxicity [4,1]. But why did the reduction of TNF-alpha alleviate MPTP toxicity while the ablation of TNF-alpha receptors did not? Obviously, there is a discrepancy in the outcome of ablation of TNF-alpha and its receptors. TNF-alpha receptors are not only receptors for TNF-alpha itself, but also for lymphotoxin, and TNF-alpha binds to other receptors belonging to the TNFR superfamily [19]. There is an intensive interaction and homeostasis between the receptors [19]. It is likely that the genetically modified mouse compensates for the lack of one of the receptor by over-expressing the other one. Furthermore, during development glial cells might change their cytokines response due to the lack of TNF-alpha signaling. It has been shown that TNF-alpha levels were reduced in mice lacking both TNFR [6]. TNF-alpha receptors are also involved in neurodegenerative as well as neuroprotective pathways [19,23], which is strongly dependent of the experimental manipulations used. Thus, the biological effects of TNF-alpha might depend on various stimuli, like induction of expression by other cytokines, oxidative stress, alteration in the cellular energy status and the severity of the MPTP-induced damage. Therefore, we cannot rule out that genetic ablation of either TNFR1 or TNFR2 or both protect neurons against a more severe neurotoxic damage as obtained after chronic MPTP treatment. In addition, TNF-alpha may act directly by a receptor-independent way to produce neurotoxicity, such as by formation of an ion permeable channel into the cell membrane [10].

We conclude that TNF-alpha receptor signaling does not play a relevant role in neuroprotection after a chronic treatment with MPTP, which produced a moderate toxicity only. This argues for a receptor-independent mechanism of TNF-alpha neurotoxicity obtained in TNF-alpha deficient mice, or reflects that the severity of the neurodegeneration determines if TNF-alpha receptor signaling is protective, neurodestructive or not involved at all.

Acknowledgements

This work was supported by a grant from the Swiss National Foundation. We thank Ms. E. Weber for genotyping of the genetically modified mice and Mr. Alan Ipekian and Ms. Misa Kuper-Yamanaka for linguistic revision of the manuscript.

References

[1] A. Boireau, F. Bordier, P. Dubedat, C. Peny, A. Imperato, Thalidomide reduces MPTP-induced decrease in striatal dopamine levels in mice, Neurosci. Lett. 234 (1997) 123–126.
[2] G. Boka, P. Anglade, D. Wallach, F. Javoy-Agid, Y. Agid, E.C. Hirsch, Immunocytochemical analysis of tumor necrosis factor and its receptors in Parkinson's disease, Neurosci. Lett. 172 (1994) 151–154.
[3] Y. Dong, E.N. Benveniste, Immune function of astrocytes, Glia 36 (2001) 180–190.
[4] B. Ferger, A. Leng, A. Mura, B. Hengerer, J. Feldon, Genetic ablation of tumor necrosis factor-alpha (TNF-alpha) and pharmacological inhibition of TNF-alpha synthesis attenuates MPTP toxicity in mouse striatum, J. Neurochem. 89 (2004) 822–833.
[5] K.B.J. Franklin, G. Paxinos, The Mouse Brain in Stereotaxic Coordinates, Academic Press, 1997, pp. xxii, [186] of plates pp.
[6] G. Guo, J. Morrissey, R. McCracken, T. Tolley, H. Liapis, S. Klahr, Contributions of angiotensin II and tumor necrosis factor-alpha to the development of renal fibrosis, Am. J. Physiol. Renal Physiol. 280 (2001) F777–F785.

[7] S. Gupta, Molecular steps of tumor necrosis factor receptor-mediated apoptosis, Curr. Mol. Med. 1 (2001) 317–324.
[8] S. Gupta, A decision between life and death during TNF-alpha-induced signaling, J. Clin. Immunol. 22 (2002) 185–194.
[9] S. Hunot, E.C. Hirsch, Neuroinflammatory processes in Parkinson's disease, Ann Neurol. 53 (3) (2003) S49–S58, discussion S58-60.
[10] B.L. Kagan, R.L. Baldwin, D. Munoz, B.J. Wisnieski, Formation of ion-permeable channels by tumor necrosis factor-alpha, Science 255 (1992) 1427–1430.
[11] R. Kruger, C. Hardt, F. Tschentscher, S. Jackel, W. Kuhn, T. Muller, J. Werner, D. Woitalla, D. Berg, N. Kuhnl, G.A. Fuchs, E.J. Santos, H. Przuntek, J.T. Epplen, L. Schols, O. Riess, Genetic analysis of immunomodulating factors in sporadic Parkinson's disease, J. Neural. Transm. 107 (2000) 553–562.
[12] I. Kurkowska-Jastrzebska, A. Wronska, M. Kohutnicka, A. Czlonkowski, A. Czlonkowska, The inflammatory reaction following 1-methyl-4-phenyl-1,2,3, 6-tetrahydropyridine intoxication in mouse, Exp. Neurol. 156 (1999) 50–61.
[13] G.T. Liberatore, V. Jackson-Lewis, S. Vukosavic, A.S. Mandir, M. Vila, W.G. McAuliffe, V.L. Dawson, T.M. Dawson, S. Przedborski, Inducible nitric oxide synthase stimulates dopaminergic neurodegeneration in the MPTP model of Parkinson disease, Nat. Med. 5 (1999) 1403–1409.
[14] P.L. McGeer, S. Itagaki, B.E. Boyes, E.G. McGeer, Reactive microglia are positive for HLA-DR in the substantia nigra of Parkinson's and Alzheimer's disease brains, Neurology 38 (1988) 1285–1291.
[15] M. Mogi, M. Harada, T. Kondo, P. Riederer, H. Inagaki, M. Minami, T. Nagatsu, Interleukin-1 beta, interleukin-6, epidermal growth factor and transforming growth factor-alpha are elevated in the brain from parkinsonian patients, Neurosci. Lett. 180 (1994) 147–150.
[16] M. Mogi, M. Harada, P. Riederer, H. Narabayashi, K. Fujita, T. Nagatsu, Tumor necrosis factor-alpha (TNF-alpha) increases both in the brain and in the cerebrospinal fluid from parkinsonian patients, Neurosci. Lett. 165 (1994) 208–210.
[17] M. Mogi, A. Togari, K. Tanaka, N. Ogawa, H. Ichinose, T. Nagatsu, Increase in level of tumor necrosis factor (TNF)-alpha in 6-hydroxydopamine-lesioned striatum in rats without influence of systemic L-DOPA on the TNF-alpha induction, Neurosci. Lett. 268 (1999) 101–104.
[18] M. Nishimura, I. Mizuta, E. Mizuta, S. Yamasaki, M. Ohta, R. Kaji, S. Kuno, Tumor necrosis factor gene polymorphisms in patients with sporadic Parkinson's disease, Neurosci. Lett. 311 (2001) 1–4.
[19] S.W. Perry, S. Dewhurst, M.J. Bellizzi, H.A. Gelbard, Tumor necrosis factor-alpha in normal and diseased brain: Conflicting effects via intraneuronal receptor crosstalk? J. Neurovirol. 8 (2002) 611–624.
[20] E. Rousselet, J. Callebert, K. Parain, C. Joubert, S. Hunot, A. Hartmann, C. Jacque, F. Perez-Diaz, C. Cohen-Salmon, J.M. Launay, E.C. Hirsch, Role of TNF-alpha receptors in mice intoxicated with the parkinsonian toxin MPTP, Exp. Neurol. 177 (2002) 183–192.
[21] K. Sriram, J.M. Matheson, S.A. Benkovic, D.B. Miller, M.I. Luster, J.P. O'Callaghan, Mice deficient in TNF receptors are protected against dopaminergic neurotoxicity: implications for Parkinson's disease, FASEB J. 16 (2002) 1474–1476.
[22] P. Teismann, B. Ferger, Inhibition of the cyclooxygenase isoenzymes COX-1 and COX-2 provide neuroprotection in the MPTP-mouse model of Parkinson's disease, Synapse 39 (2001) 167–174.
[23] L. Yang, K. Lindholm, Y. Konishi, R. Li, Y. Shen, Target depletion of distinct tumor necrosis factor receptor subtypes reveals hippocampal neuron death and survival through different signal transduction pathways, J. Neurosci. 22 (2002) 3025–3032.