Selegiline as immunostimulant — a novel mechanism of action?

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Summary. In clinical studies the MAO-B inhibitor selegiline appears to slow the progression of neurological deficits in Parkinson's disease (PD) and the cognitive decline in Alzheimer's disease (AD). The mechanisms of action remain unclear. Several lines of evidence indicate an immune-mediated pathophysiology of PD and AD. According to animal trials, selegiline increases the survival rate of immune suppressed mice. Stimulation of the immune response to bacterial or viral infection or in chronic inflammatory processes is managed by an increased synthesis of the cytokines interleukin-1β (IL-1β) and subsequent interleukin-6 (IL-6). Outcome of viral or bacterial infections in the brain highly correlates with levels of the cytotoxic cytokine tumor-necrosis-factor-alpha (TNF). The aim of our study was to characterize the influence of selegiline on the biosynthesis of IL-1β, IL-6 and TNF in human peripheral blood mononuculear cells (PBMC) from healthy blood donors. After isolation and washing PBMC were cultured without and with selegiline in three different concentrations (0.01 μmol/l, 0.001 μmol/l, 0.0001 μmol/l) in a humidified atmosphere (7% CO_2). Then cultures were centrifuged and supernatants were collected for IL-1β, IL-6 and TNF ELISA-assays. Treatment of cultured PBMC with various concentrations induced an increased synthesis of IL-1β (ANOVA $F = 9.703$, $p = 0.0007$), IL-6 (ANOVA $F = 20.648$, $p = 0.0001$) and a reduced production of TNF (ANOVA $F = 3.770$, $p = 0.040$). These results indicate, that the influence of selegiline on the cytokine biosynthesis may also contribute to its putative neuroprotective properties.

Introduction

It is still under discussion, whether the monoamine oxidase-B (MAO-B) inhibitor selegiline appears to slow the progression of neurological deficits in Parkinson's disease (PD) and the cognitive decline in Alzheimer's disease (AD) (Stoll et al., 1994; Knoll, 1995; Calne, 1995). Besides the central effect of selegiline to reduce metabolism of dopamine via MAO-B, additional mechanisms of action of selegiline independent of MAO-B inhibition have partially been demonstrated by in vitro and animal trials (for review: Knoll, 1995;

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Tatton et al., 1995; Gerlach et al., 1995). It has been suggested, that those results may be due to an altered expression of mRNAs and proteins in nerve and glial cells by selegiline (Tatton et al., 1995). Recently it has been shown, that selegiline influences the endocrine system (Thyagarajan et al., 1995) and also the immune system. Both intraperitoneal and intracerebroventricular (i.c.v.) administration of selegiline markedly attenuated restraint stress-induced gastric ulcers in rats. L-Deprenyl given i.c.v. attenuated stress ulcers in microgram doses and virtually abolished ulcer formation at a dose of 2.0 micrograms (Glavin et al., 1986). Immunosuppressed NMRI-mice were raised and kept under germ-reduced conditions and fed with a germ-reduced diet (14 animals = controls). For a further group of 14 mice 4 mg of selegiline were added to 10 kg of the diet. The 50% survival time of the latter group was 160% that of the control group measured from birth or 220% measured from the beginning of the study. The survival time in weeks of the selegiline group finally reached 350%, and the area under the curve 250% that of the control group (Freisleben et al., 1994).

Former studies revealed, that organoselenium compounds, which show similarities in the chemical structure with selegiline, have been described as anti-inflammatory, antioxidant, glutathione peroxidase-like agents and inhibitors of prostaglandin synthesis. These compounds were also inducers of the cytokines interferon-gamma (IFN-gamma) and tumor-necrosis-factor-alpha (TNF) in human peripheral blood leukocytes (Inglot et al., 1990). Cytokines have been suggested as messengers in the communication between the immune system and the nervous system, in which signals travel only short distances (Bartfai and Schultzberg, 1993; Goujon et al., 1996). Neuroimmune interactions have been discussed in view of findings that nervous signals are important for the immune response (Bartfai and Schultzberg, 1993). The occurrence of neurotransmitter receptors on lymphocytes and cytokine receptors on nerve cells or glia has initiated further studies e.g. on the localization of different cytokines in the nervous system and on long and short term actions of cytokines in the nervous system (Bartfai and Schultzberg, 1993). Interleukin-1β (IL-1β) has been studied extensively along these lines, and found to occur in the nervous and endocrine system. Furthermore, several findings indicate their role as growth promoting factors, and for example the induction of NGF production by IL-1β suggests involvement of this cytokine in regeneration and development in the nervous system (Bartfai and Schultzberg, 1993; Woodroofe, 1995; Strijbos and Rothwell, 1995). In order to characterize putative effects of selegiline on the immune system we investigated the influence of selegiline on the biosynthesis of the cytokines TNF, IL-1β and Interleukin 6 (IL-6).

Material and methods

According to Imamura et al. (1993) peripheral blood mononuclear cells (PBMC) from 3 healthy blood donors (age: 26 years male, 23 male, 28 female) were isolated,

suspended (10^6/ml) and cultured in buffered (25 mM Hepes) RPMI 1640 medium supplemented with 10% fetal calf serum and gentamycin 1.6 μg/ml at 37°C without and with selegiline in three different concentrations (10^{-8} mol/l, 10^{-9} mol/l, 10^{-10} mol/l) in a humidified atmosphere (7% CO_2). After 48 h of culturing, supernatants were collected, centrifuged and frozen in −80°C until testing. Each experiment was replicated three times.

IL-1β, IL-6 and TNF enzyme-linked immunosorbent assay (ELISA)

The levels of cytokines in the supernatants were measured using commercial available ELISA kits (Quantikine, R&D Systems, DPC Biermann GmbH, Bad Nauheim, Germany) for IL-1β, IL-6 and TNF. Collection and handling of specimens were performed and a standard curve was generated according to the manufacturers recommendations. All measurements were done in duplicate.

Statistical evaluation

ANOVA and subsequent Tukey-Kramer Multiple Comparisons Test were performed for comparison between standard (without selegiline) and selegiline treated cultures. Linear regression was used for evaluation of dose dependency of selegiline. Results are expressed as means ± SEM. Level of significance was $p < 0.05$.

Results

Treatment of cultured PBMC with selegiline induced a significant increased synthesis of IL-1β (ANOVA $F = 9.609$, $p = 0.0016$) in all three different concentrations of selegiline (Fig. 1). Regarding IL-6 a significant augmented biosynthesis (ANOVA $F = 20.648$, $p = 0.0001$) (Fig. 2) was also found, especially when treating the cultures with concentrations of 0.001 μmol/l and 0.01 μmol/l of selegiline.

In the case of TNF a significant reduced production (ANOVA $F = 3.770$, $p = 0.040$) (Fig. 3) appeared, the post test revealed, that level of significance was only reached in a concentration of 0.001 μmol/l.

No significant correlation between dose of selegiline and increased production of IL-1β ($r^2 = 0.214$, $p = 0.537$) or IL-6 ($r^2 = 0.863$, $p = 0.071$) or decreased synthesis of TNF ($r^2 = 0.000289$, $p = 0.983$) in these cultures was found.

Discussion

The results of this study indicate, that immunological properties of selegiline may cause its influence on the synthesis of cytokines IL-1β, IL-6 and TNF. These effects of selegiline were most prominent in the case of IL-1β. The elevated levels of IL-6 may be explained on the one hand by direct stimulation by selegiline. On the other hand an indirect effect caused

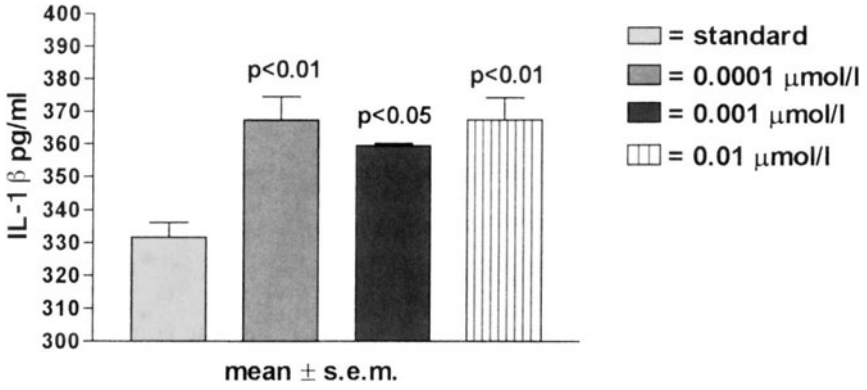

Fig. 1. IL-1β-levels in selegiline treated cultures

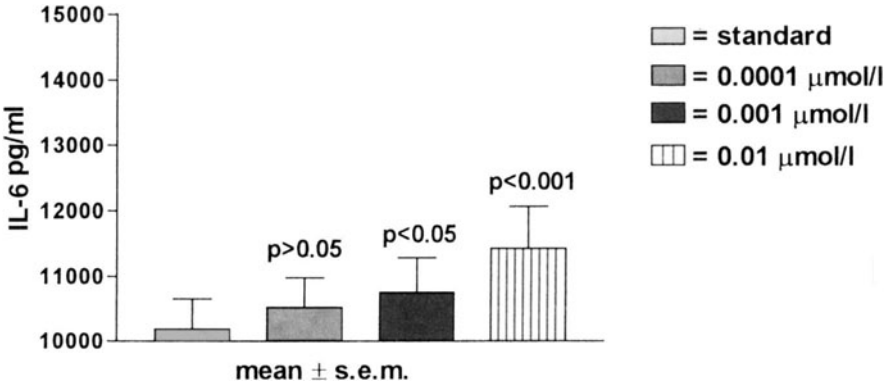

Fig. 2. IL-6 levels in selegiline treated cultures

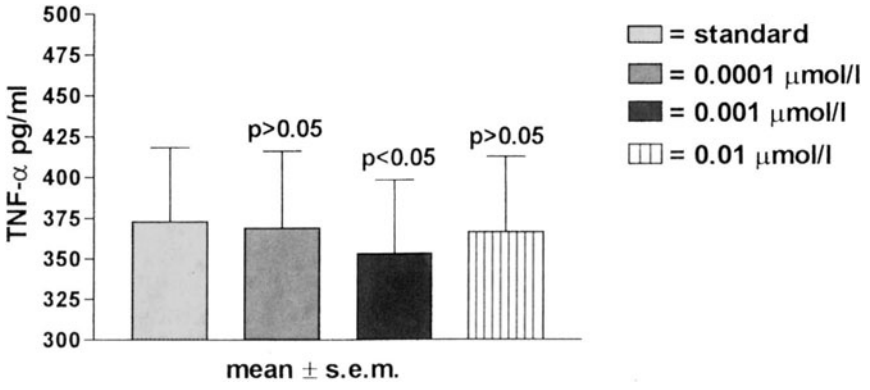

Fig. 3. TNF-levels in selegiline treated cultures

by IL-1β has to be considered, because IL-1β induces an increased release of IL-6 (Woodroofe, 1995) and subsequently gonadotropins FSH, LH (Yamaguchi et al., 1990). Our results indirectly confirm results of Thyagarajan et al. (1995), but we assume, that influence of selegiline on hormone secretion may be mediated both by its impact on cytokine synthesis and via suppression of monoamine metabolism.

There is growing evidence for a bidirectional interaction between the peripheral immune system and the brain (Gottschall et al., 1991; Goujon et al., 1996). Therefore it seems likely, that peripheral circulating cytokines may influence regenerative and degenerative processes in the brain (Wollman et al., 1992), e.g. via the organum vasculosum lamina terminalis, where the blood-brain-barrier is weak (Imura et al., 1991). In the context of AD (for review: McGeer and McGeer, 1995; Blum-Degen et al., 1995) and PD (for review: Kuhn and Müller, 1995) immune-related phenomena are also involved in the etiology of both diseases. Alterations in the content of several cytokines, e.g. IL-1β, IL-6 or TNF, and growth factors in the brain parenchyma, serum and/or cerebrospinal fluid or AD and PD patients were found (Griffin et al., 1989; Cacabelos et al., 1991; Mogi et al., 1994a,b, 1995; Blum-Degen et al., 1995). IL-1β and IL-6 are released from microglial cells and astrocytes (Lee et al., 1993). IL-1β stimulates the proliferation of astrocytes, and regulates the synthesis of nerve growth factor (Woodroofe, 1995). In the brain IL-6 induces acute phase protein synthesis, differentiation of neuronal cells and improves catecholaminergic and cholinergic cell survival (Hama et al., 1991; Bauer et al., 1991; Bartfai and Schultzberg, 1993; Blum-Degen et al., 1995). A recent study showed elevated IL-1β and IL-6 levels in the cerebrospinal fluid of de-novo-Parkinson's disease patients (Blum-Degen et al., 1995), which may reflect the original condition at the probable beginning of disease. Moreover due to this study and the influence of IL-1β and IL-6 on regenerative processes in the brain (Woodroofe, 1995) one may also speculate, that this cytokine status represents the regenerative potential of the brain in the fight against the neurodegenerative process. In contrast, increased levels of TNF in the striatum, substantia nigra and CSF of parkinsonian patients (Brosnan et al., 1988; Boka et al., 1994; Mogi et al., 1994b; Blum-Degen et al., 1995) may represent the cytotoxic and neurodegenerative disease inducing action of TNF, because in vivo intracerebral TNF levels highly correlate with the outcome in the case of viral and bacterial infections in the brain (Hofman et al., 1989; Poli et al., 1990; Gatti and Bartfai, 1993), and in vitro TNF may destroy myelin and oligodendrocytes (Selmaj and Raine, 1988a,b; Selmaj et al., 1988a,b, 1995).

On the background of these studies on cytokines and their possible impact on degenerative and regenerative mechanisms in the course of chronic neuronal death the results of our study fit to the postulated neuroprotective effects of selegiline, indicating that these mechanisms are possibly mediated by cytokines. But further in vitro and in vivo trials are necessary to provide evidence for the demonstrated influence of selegiline on cytokine biosynthesis.

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