The main chemical difference between entacapone and tolcapone is that entacapone is more hydrophilic. Entacapone was designed to be peripherally acting to prevent the O-methylation of levodopa in the gut and other peripheral tissues. Tolcapone due its more lipophilic nature also penetrates into the brain and is in general in vivo more effective COMT inhibitor than entacapone. However, due its higher lipid solubility, tolcapone may interfere with mitochondrial functions and this has been suggested to cause its liver toxic properties.

However, the clinical use of nitrocatechols, especially entacapone, during the past 10 years has proven that if COMT inhibitors are chemically properly designed and both biochemically and pharmacologically shown to be effective without side effects, they can be safe adjuncts in levodopa carbidopa combination therapy for the treatment of Parkinson's disease. In fact the clinical success in entacapone therapy has lead to the development of the triple combination therapy: entacapone–carbidopa–levodopa: Stalevo®, which has become the leading levodopa therapy in the treatment of Parkinson's disease.

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L3

Physicochemical and pharmacokinetic properties of COMT inhibitors

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Clinically available second generation COMT inhibitors, entacapone and tolcapone, as well as a novel experimental compound, nebicapone (BIA 3-202), are equally potent COMT inhibitors but their physicochemical and pharmacokinetic properties vary. Also the treatment strategies are different.

Physicochemical properties such as aqueous solubility and apparent partition coefficient of COMT inhibitors are dependent on pH. At acidic pH, the aqueous solubilities of entacapone and tolcapone are equally low but at physiological pH, the solubility of entacapone is 15-fold better than that of tolcapone. In contrast, tolcapone is clearly more lipophilic than entacapone. Nebicapone data is not available.

After an oral dose, COMT inhibitors are rapidly absorbed. The C_{max} values of nebicapone and tolcapone are clearly higher than that of entacapone. All COMT inhibitors are rapidly eliminated from plasma, $t_{1/2\beta}$ ranging between 2.0 and 3.4 h at recommended doses. Both non-clinical and clinical studies have shown that bioavailability of entacapone is lower than that of tolcapone (~35% versus ~60%; nebicapone data not available). Intravenous data also indicates that the total clearance of entacapone is higher than that of tolcapone.

Tolcapone penetrates better into the brain than entacapone, which has mainly peripheral effect at therapeutic doses. Nebicapone resembles entacapone. These differences could be explained by their different lipophilicities but also other factors, e.g. efflux transporters in the BBB may play a role. However, the clinical relevance of central effect is unclear.

All COMT inhibitors are extensively metabolized. The main entacapone metabolite is the glucuronide. Tolcapone and nebicapone are also O-methylated and oxidized or reduced. Large proportion of the orally administered drug and its metabolites is excreted in bile and feces.

Entacapone and tolcapone have similar EC_{50} values in vivo. As assessed by the COMT inhibition in various tissues, tolcapone has the longest duration of action and entacapone the shortest. This may be due to different pharmacokinetic properties and thus to the different amounts of drug available for the target. However, for tight binding inhibitors, the half-life of enzyme-inhibitor complex is also an important factor determining the duration of action.

New COMT inhibitors are still needed to achieve optimal benefits as an adjunct to LD-DDCI therapy.

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L4

Selegiline mimicks preconditioning induction of the redox protein thioredoxin against MPP^+-induced neurotoxicity

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Therapeutics for managing Parkinson's disease are developed mostly for symptomatic relief. Accumulated results indicate that selegiline, rasagiline and pramipexole might delay the need of L-DOPA for treating early patients. Selegiline and rasagiline also prevent neurotoxicity in animals following the administration of MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine). Our prior in vivo studies indicate that neuroprotective mechanism of selegiline may be mediated by antioxidant properties rather than its classic pharmacological inhibition of MAO-B. Unexpectedly, selegiline directly protected against 1-methyl-4-phenylpyridinium ion (MPP^+)-induced neurotoxicity in human SH-SY5Y cells via the induction of the redox protein thioredoxin (Trx) since selegiline's action can be blocked by pre-incubating cells with Trx antisense. This notion was confirmed by a recent study that exogenously administered Trx (200 nM) blocked neurotoxic effects of MPP^+ in vitro. Preconditioning up-regulation of Trx mRNA and protein significantly reduced mitochondra-mediated apoptosis caused by MPP^+. Trx-enriched SH-SY5Y Cells are more resistant against oxidative injury caused by MPP^+ while other report suggests that mice over-expressed hTRX are less vulnerable following MPTP. Additional results indicate that the observed multifaceted neuroprotective mechanisms of Trx may mediate preconditioning stress/hormesis, which is known to protect cells and animals from life threaten stimuli.

Regarding multifaceted mechanisms of Trx, earlier reports suggest that Trx is a redox protein that modifies protein-SH for redox chain reactions. Our data indicate that antioxidative property of Trx is more potent than trolox, GSNO, ascorbic acid and GSH. Interestingly, Trx is a potent inhibitor of caspases and thus blocks mitochondria-induced apoptotic chain of events. Moreover, Trx also activates the transcription factor cMyc leading to the induction of mitochondrial Bcl-2 and MnSOD for enhancing cell survival. Our unpublished molecular biological results revealed that Trx plays pivotal roles in

neuronal plasticity such as neurite outgrowth and synaptogenesis. Additional experiments of knock down of Trx levels by antisense confirmed such an important role for Trx in neuronal plasticity as well. In conclusion, the redox protein Trx may mediate a multifaceted neuroprotection and thus Trx-inducing agents such as selegiline may be useful in slowing progressive degenerative process of brain neurons and cells.

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L5

CDNF is a novel neurotrophic factor for dopaminergic neurons in vivo

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In Parkinson's disease (PD), dopaminergic neurons in the Substantia Nigra pars compacta (SNpc) are progressively degenerated. Neurotrophic factors such as glial cell line-derived neurotrophic factor (GDNF) and neurturin have a significant therapeutic potential in the treatment of PD. Conserved dopamine neurotrophic factor (CDNF) is a novel evolutionary conserved trophic factor. CDNF is a secreted protein with eight conserved cysteine residues. Since its close homolog, mesencephalic astrocyte-derived neurotrophic factor (MANF) promotes the survival of embryonic dopaminergic neurons in vitro, we studied a possible protective and restorative effect of CDNF for dopaminergic neurons in a rat unilateral 6-hydroxydopamine (6-OHDA) lesion model of PD. In neuroprotection studies, rats were given intrastriatal injections of either vehicle, GDNF (10 μg) or CDNF (10 μg) 6 h prior to intrastriatal injection of 6-OHDA (8 μg). D-Amphetamine (2.5 mg/kg, i.p.) induced rotational activity of the rats was measured at 2 and 4 weeks postlesion. In CDNF-treated rats, the functional properties of nigro-striatal dopaminergic nerves in the lesion side were improved since the amphetamine-induced ipsilateral turning behaviour was significantly reduced, when measured at 2 and 4 weeks post lesion. Immunohistochemical analyses of the brain revealed that CDNF was able to prevent the 6-OHDA-induced degeneration of dopaminergic neurons at least as efficiently as GDNF. Indeed, it almost completely rescued tyrosine hydroxylase positive cells in the SNpc. In line with this, the density of TH-positive fibers in the striatum was significantly higher in rats pretreated with GDNF or CDNF compared to the vehicle-pretreated rats. The protective effect of CDNF was dose-dependent, at doses 1, 3 and 10 μg of CDNF.

Neurorestorative activity of CDNF was studied by injecting the animals with 6-OHDA (20 μg) and 4 weeks later with CDNF (10 μg), GDNF (10 μg) or vehicle into the striatum. CDNF and GDNF significantly reduced the amphetamine-induced rotational behaviour as compared with the control group. There was also a partial recovery of TH-positive cells in the SNpc of rats treated with CDNF and GDNF. These results suggest that CDNF may have therapeutic potential in the treatment of PD.

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L6

Neuroprotective effects of coadministration of HB-GAM and GDNF in a rat model of Parkinson's disease

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Heparan sulphate proteoglycans (HSPGs) in the nervous system may play an important role in the signalling of glial cell line-derived neurotrophic factor (GDNF). Binding to heparan sulphate keeps GDNF locally concentrated which seems to be needed for receptor activation and RET phosphorylation, and it may even protect GDNF from proteolytic degradation. On the other hand, the binding may restrict the diffusion of GDNF when delivered to the brain. Syndecan-3 is a receptor for heparin-binding growth-associated molecule (HB-GAM) having a HSPG by structure. Therefore its heparan sulphate side chains, which also act as ligand binding sites, may have influence on GDNF signalling. The aim of our experiments was to study the neuroprotective effects of HB-GAM-GDNF-coadministration in a unilateral rat model of Parkinson's disease.

Rats received intrastriatal infusions of vehicle, 17 or 50 μg HB-GAM followed by vehicle or 10 μg GDNF 18 h later. Six hours after second infusion altogether 28 μg of 6-hydroxydopamine was infused to four striatal locations. Turning response to 2.5 mg/kg D-amphetamine was measured 2, 3, 7 and 8 weeks post lesion. Tyrosine hydroxylase (TH) immunohistochemistry of the brains was studied.

Statistically significant reduction in total ipsilateral turns was seen at 8 weeks postlesion in both groups that received HB-GAM combined with GDNF. However, the reduction in turning behaviour was more pronounced in animals that received vehicle and GDNF. HB-GAM alone did not have a significant neuroprotective effect in this model. Number of TH-positive cells in the substantia nigra also correlates with the behavioural data.

These results suggest that GDNF has neuroprotective effects even when the heparan sulphate side-chains of syndecan-3 are occupied with HB-GAM, but the effects are weaker than with GDNF alone. Thus, the undisturbed interaction of GDNF with syndecan-3 seems to be beneficial for the therapeutic effects.

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