Susceptibility of PINK-1 deficient mice to 6-OHDA induced oxidative stress determined with in vivo salicylate trapping

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Introduction:  
Mutations in the mitochondrial phosphatase and tensine homologue-induced kinase 1 (PINK-1) cause autosomal recessive early-onset Parkinson’s disease (PD). PINK-1 is a protein kinase with a mitochondrial signal sequence and confers protective effects to mitochondria, where it is primarily located. How PINK-1 causes dopaminergic dysfunction and degeneration in PD patients is unknown. PINK-1 protects mitochondria from both intrinsic stress [e.g. dopamine (DA) metabolism] and environmental insults. Loss of PINK-1 results in mitochondrial dysfunction in mice. Given that pharmacological inhibition of mitochondrial complex I recapitulates key features of PD, it is conceivable that defects in mitochondrial respiration caused by mutations in PINK-1 could contribute to PD pathogenesis by a similar mechanism [1,2,3,4,5].

PINK-1 deficiency may be correlated with an increased susceptibility to oxidative stress that can be defined as an imbalance between the production and elimination of reactive oxygen species (ROS). The most reactive and destructive free radicals are the OH radicals (•OH), which have an important role in the pathogenesis of PD [6]. In order to investigate if there is a correlation between PINK-1 deficiency and oxidative stress in vivo, we analyzed the •OH formation after administration of 6-hydroxydopamine (6-OHDA) in the striatum of female PINK-1 knock down (KD) mice by using the in vivo salicylate trapping microdialysis technique. Furthermore, we evaluated the DA content in these KD mice as well as in PINK-1 knock out (KO) and enhanced green fluorescent protein (eGFP) mice of approx. 5 weeks and 20 months old.

Methods:  
PINK-1 KD, KO and eGFP expressing mice were created by using a lentiviral vector system for RNA interference. The in vivo salicylate trapping method was used to monitor extracellular •OH in the striatum of these mice of approx. 5 months old in baseline conditions and after local administration of the neurotoxin 6-OHDA. Direct measurement of •OH in vivo is difficult because of their short half life and high reactivity. In this indirect method, salicylate as a trapping agent present in the perfusate reacts with •OH and forms 2,3-dihydroxybenzoic acid (2,3-DHBA) and 2,5-DHBA, based on the principle of aromatic hydroxylation. Only the 2,3-DHBA content is quantified, as 2,5-DHBA can be formed endogenously, while all 2,3-DHBA is derived from the reaction [6,7]. The 2,3-DHBA content was measured by HPLC with electrochemical detection.  

Guide cannulas (CMA/7 guide cannulas, CMA Microdialysis AB, Solna, Sweden) were stereotaxically implanted into the left striatum of the anaesthetized mice (AP:+0.6, L:-1.8,V:2.0, relative to bregma, according to the atlas of K. Franklin and G. Paxinos) [8]. After surgery, microdialysis probes (CMA/7/2 mm membrane length/molecular weight cut-off of 6000 Daltons, CMA Microdialysis AB, Solna, Sweden) were introduced via the cannula and perfused with modified Ringer’s solution (composition: 147 mM NaCl, 4 mM KCl, 1.1 mM CaCl$_2$·6H$_2$O). The perfusate was delivered at a constant flow rate of 2 µl/min via a CMA/100 Microdialysis Pump (CMA Microdialysis AB, Solna, Sweden). Approximately 16-20 hours (~equilibration time) after implantation of the probes, microdialysis sampling was started on freely moving mice. Samples (40 µl) were collected every 20 min at a flow rate of 2 µl/min into vials containing 10 µl of an antioxidant solution (composition: 3.3 mM L-cystein, 0.3 mM Na$_2$-EDTA, 0.1 M acetic acid). Determination of the 2,3 DHBA concentrations in the dialysates was performed using a HPLC method as previously described by Yuan et al. (2004), with slight modifications [9].
Results and discussion:
The perfusion with salicylate (500µM) produced stable and similar baseline levels of 2,3-DHBA for eGFP expressing mice and PINK-1 KD mice, respectively: 9.2 ± 2.3 nM (eGFP, mean ± S.E.M., n= 5) and 7.7 ± 0.9 nM (PINK-1 KD, mean ± S.E.M., n= 6). After perfusion with 6-OHDA (5µM) locally into the striatum of the freely moving mice for approximately 1 hour, the 2,3-DHBA concentration significantly increased above the baseline values and returned back to baseline values after switching back to salicylate as perfusate, (Figure 1). The increase in 2,3-DHBA dialysate concentration after administration of the neurotoxin was about 2.5 fold for both groups. No significant differences were observed in baseline values or 6-OHDA induced 2,3-DHBA levels between both groups. These data show that PINK-1 KD mice are not more susceptible than eGFP expressing mice for 6-OHDA induced oxidative stress.

![Figure 1. In vivo effects of 6-OHDA (5µM) on the extracellular concentration of 2,3-DHBA in striatal dialysates. The dialysate fraction 1 represents the basal concentration. The following fractions 3-5 show the values after switching the perfusate to 6-OHDA (5µM) to stimulate •OH formation. From dialysate fraction 6 the perfusion liquid was switched back to salicylate. Data are presented as mean values ± S.E.M (eGFP: n=5, PINK-1 n=6) [** p < 0.01 against baseline value represented as dialysate fraction 1, Repeated measures ANOVA followed by Dunnett’s post test.]

The striatal DA levels in PINK-1 KD and KO mice were not significantly different from those in the control mice of approx. 5 weeks and 20 months old. We also observed that the DA levels in the striatum of eGFP expressing mice are not significantly different from those in the control group, suggesting that eGFP expressing mice can be used as a control.

Conclusion:
Although, PINK-1 deficiency is linked with a higher sensitivity to oxidative stress, our data implicate that PINK-1 KD mice do not have a higher endogenous extracellular •OH concentration in the striatum and are not more susceptible to extracellular •OH generation after local administration of 6-OHDA than eGFP expressing mice. Furthermore, our data indicate that the DA levels in the striatum are similar in both control and PINK-1 deficient (KD/KO) mice of different ages.

References