# Role of DHA metabolites in protective effects of DHA supplementation in the brains of rotenone-induced rat models of Parkinson's disease


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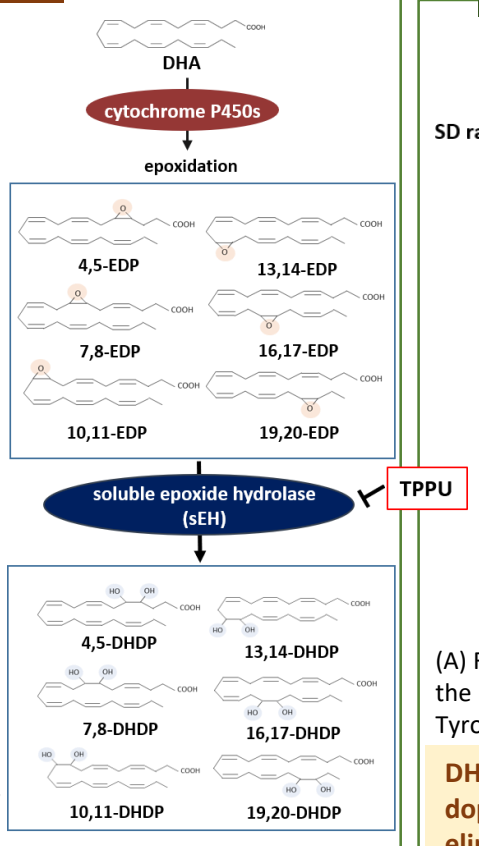
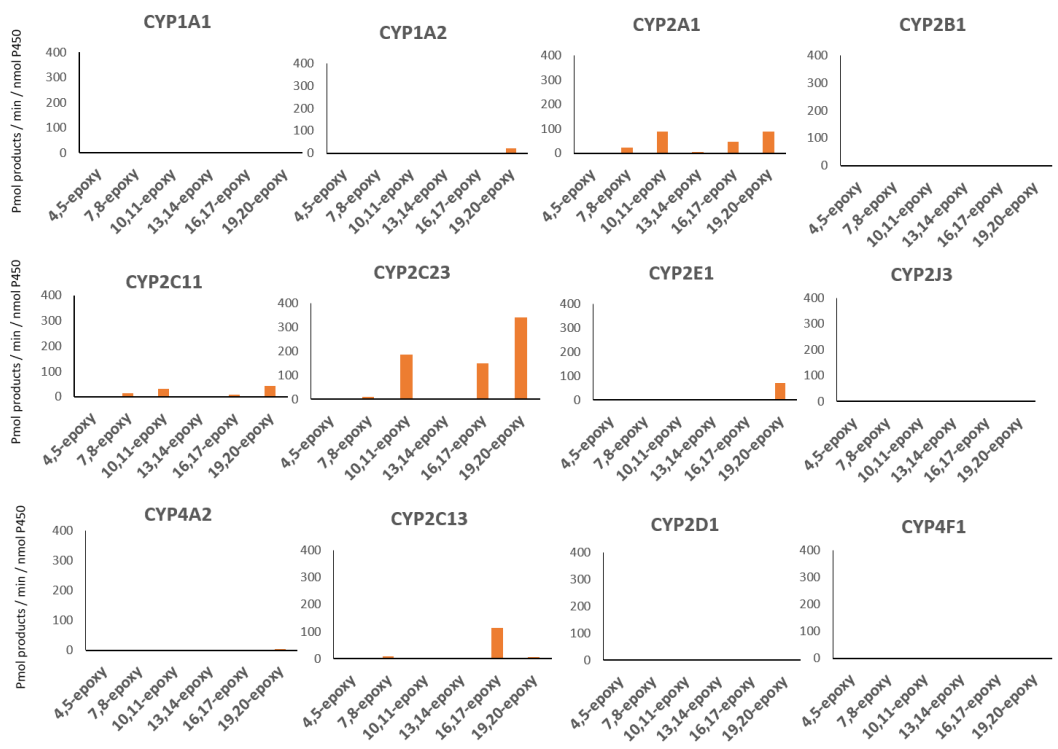
## Introduction

Docosahexaenoic acid (DHA) is an ω-3 polyunsaturated fatty acid (PUFA) enriched in the brain and essential for brain development and function. Clinical studies have indicated that supplementation or dietary intake of DHA can alleviate the symptoms of neurodegenerative disorders such as Parkinson's disease. Epidemiological studies have also shown that intake of ω-3 PUFAs was consistently associated with a low risk of Parkinson's disease. Parkinson's disease is the second most prevalent neurodegenerative disease and is characterized clinically by motor deficits. Pathological features of Parkinson's disease include loss of dopaminergic neurons projecting from the substantia nigra to the striatum. Many reports have indicated that DHA has protective effects on dopaminergic neurons, but the underlying mechanism and molecular mediators are still unclear.

In our body, DHA is metabolized to DHA epoxides, epoxydocosapentaenoic acids (EDPs) by cytochrome P450s (CYP, P450s), and EDPs are further hydroxylated to the corresponding diols, dihydroxydocosapentaenoic acids (DHDPs) by soluble epoxide hydrolase (sEH). In the present study, we investigated the roles of these DHA metabolites in the beneficial effects of DHA supplementation on a rotenone-induced rat model of Parkinson's disease.



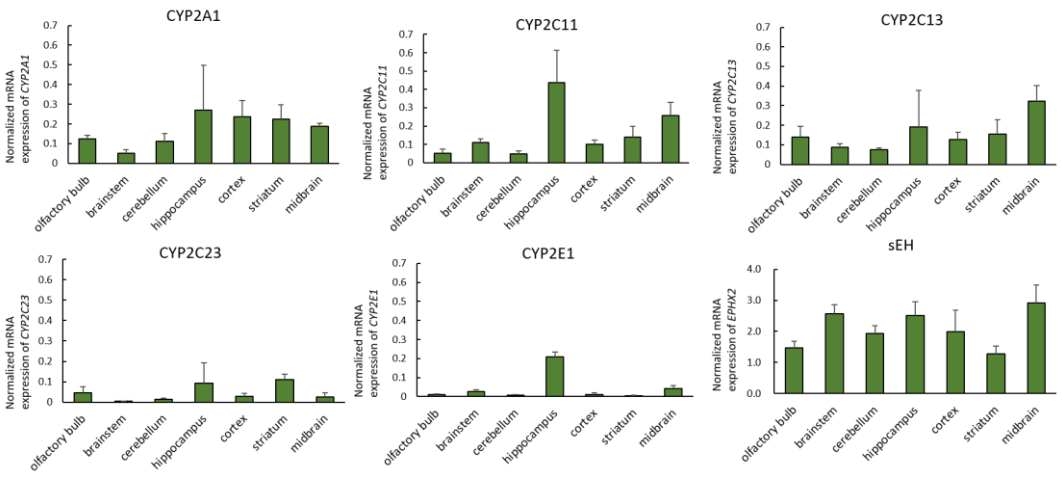

## ① Production of DHA metabolites, epoxidized forms (EDPs) by purified rat P450s




Purified rat P450 with cytochrome b5, NADPH-cytochrome P450 reductase, and dilauroylphosphatidylcholine was incubated with 100 μM DHA and NADPH for 15 min, and the metabolites were analyzed with UPLC-MS. The analytes were detected by tandem TOF monitored by total ions at m/z 343.2.

**EDPs were produced by CYP2A1, 2C11, 2C13, 2C23, and 2E1.**

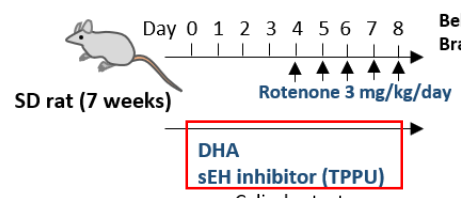
## ② mRNA expression of EDP-producing P450s and sEH in the rat brain region



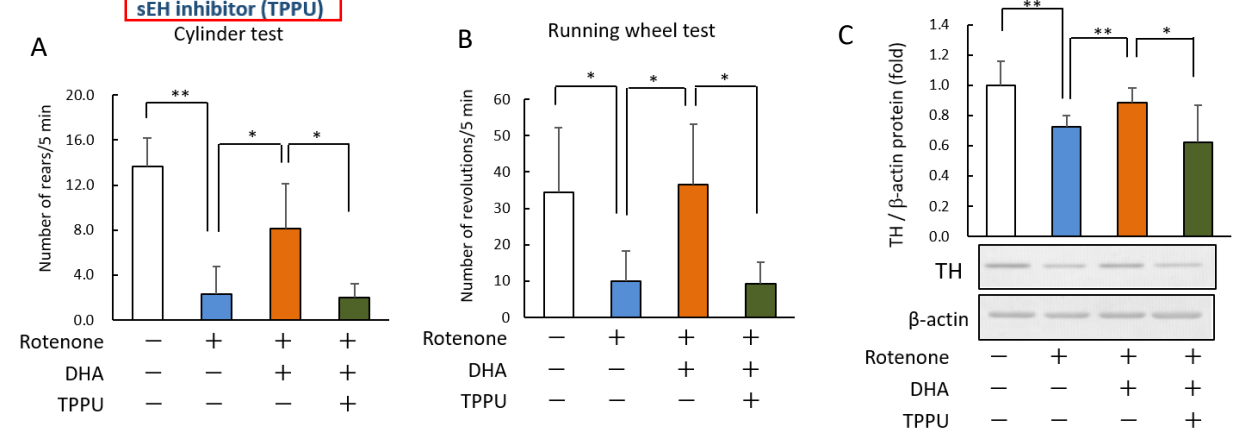

mRNA levels of EDP-producing P450s and sEH in the rat brain region were analyzed by real-time PCR. The mRNA expression levels were normalized to the expression of histone.

**The mRNA expression of EDP-producing P450s, except for CYP2E1, was detected in the striatum. sEH mRNA was broadly expressed in each region of the brain.**

## ③ Effects of DHA and sEH inhibitor supplementation on motor dysfunction and loss of tyrosine hydroxylase (TH) expression in rotenone-induced rat models of Parkinson's disease.



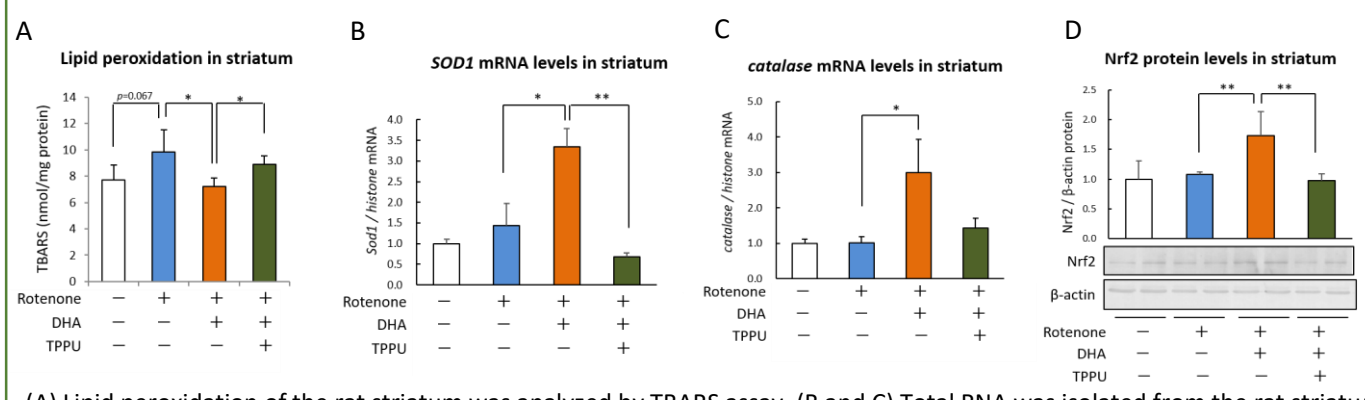

Rats were fed freely a diet (AIN-93G) containing cottonseed oil at a final concentration of 7% (w/w) with or without DHA supplementation. DHA was added to a cottonseed oil diet at a final concentration of 4% (w/w) of total fat. TPPU (5 mg/L) was added to drinking water with 0.2% PEG400.




(A) For the cylinder test, rats were placed into an open-top 10 L cylinder for 5 min, and the number of forelimb placements to the wall by rearing was quantified. (B) For the wheel running test, the number of revolutions per 5 min was measured. (C) Tyrosine hydroxylase (TH) expression in the rat striatum was analyzed by western blotting.

**DHA supplementation in rats improved the motor dysfunction and loss of TH (rate-limiting enzyme in the dopamine biosynthetic pathway) induced by rotenone. However, these effects of DHA supplementation were eliminated by cosupplementation with the sEH inhibitor TPPU, suggesting that DHA metabolites by sEH was important in the beneficial effects of DHA.**

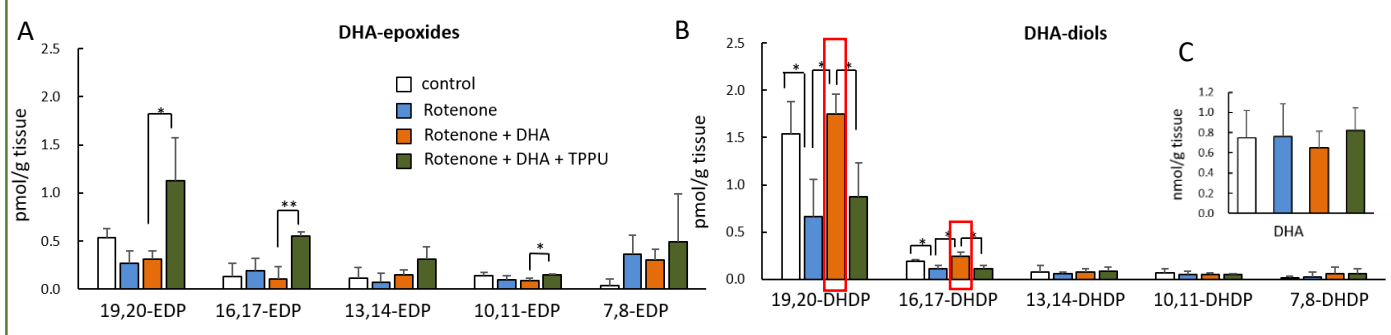
## ④ DHA supplementation, but not cotreatment with DHA and the sEH inhibitor, decreased lipid peroxidation and increased antioxidant genes in the rat striatum




(A) Lipid peroxidation of the rat striatum was analyzed by TBARS assay. (B and C) Total RNA was isolated from the rat striatum, and the mRNA levels of *sod1* and *catalase* were analyzed by real-time PCR. (D) NF-E2-related factor (Nrf2) is a transcription factor that principally regulates the induction of sod1 and catalase. The Nrf2 protein levels in the striatum of these rats were analyzed by western blotting.

**The induction of antioxidant enzymes may be one cause of the beneficial effects of DHA supplementation in decreasing oxidative stress in the striatum.**

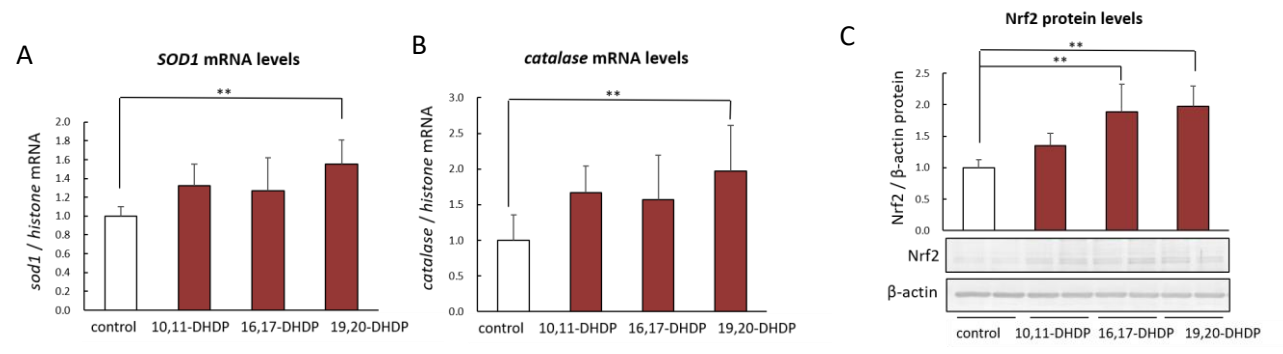
## ⑤ Quantification of endogenous DHA-epoxides (EDPs) and diols (DHDPs) in the rat brain by LC-MS/MS




Lipids were extracted by HLB cartridges from the rat brain, and EDPs (A), DHDPs (B) and DHA (C) were quantified by UPLC-tandem quadrupole mass spectrometer with MRM mode.

**DHA supplementation increased the amount of 19,20-DHDP and 16,17-DHDP in the brain , although the amount of DHA and EDPs did not change.**

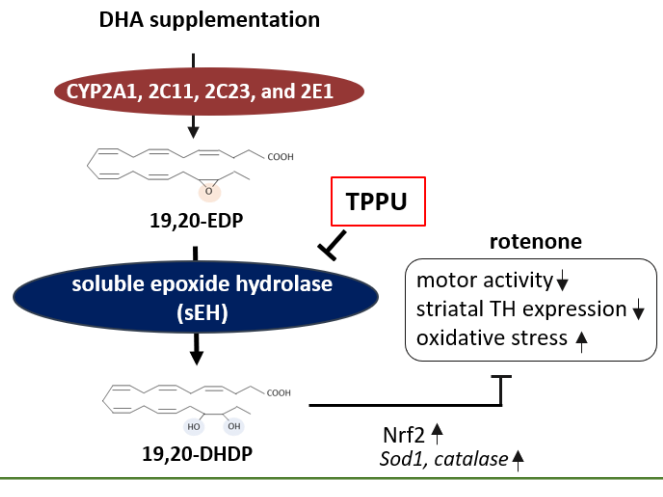
## ⑥ DHDPs induced antioxidant enzymes in PC12 cells




(A and B) Rat pheochromocytoma cell line (PC12) cells were treated with 100 nM DHDPs for 6 h, and the mRNA levels of sod1 (A) and catalase (B) were analyzed by real-time PCR. (C) Cells were treated with 100 nM DHDPs for 4 h, and the Nrf2 protein levels were analyzed by western blotting.

**P450- and sEH-mediated production of 19,20-DHDP will contribute to the induction of antioxidant enzymes by supplementation with DHA.**

## Conclusions




The present study showed that DHA metabolites (19,20-DHDP produced by P450s and sEH) have an important role in the beneficial effects of DHA supplementation in the brains of rat models of Parkinson's disease. 19,20-DHDP will reduce oxidative stress by induction of Nrf2-regulating antioxidant genes in neuronal cells.

At present, a clinical trial indicated that treatment with DHA is a valuable potential tool in the management of Parkinson's disease. The present study raise the possibility that 19,20-DHDP is more effective than DHA for improving Parkinson's disease.

## Acknowledgements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