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BBB permeability and NDP-MSH PK study

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We use this protocol and it's working

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Abstract

This protocol is to measure the integrity of BBB and assess NDP-MSH concentrations in plasma and brain

Troubleshooting

BBB permeability

- 1 The integrity of BBB was measured through FITC-albumin (Millipore Sigma, Cat# A9771) leakage from vasculature into brain parenchyma as described previously (Chen et al. 2008). Mice were treated with MPTP+LPS and sacrificed after 6 h and 24 h after the last dose.
- 2 Mice were anaesthetized by isoflurane and perfused intracardially with heparin (100 units/kg) followed by 5 ml FITC albumin at a concentration of 5 mg/ml in PBS with a flow rate of 1.5 ml/min.
- 3 Subsequently, the brain was isolated and incubated in 4% paraformaldehyde overnight. The solution was changed to 30% sucrose in PBS. Coronal sections of striatum were mounted and analyzed under fluorescence microscope (Olympus BX51 microscope).

NDP-MSH PK study

- 4 To assess NDP-MSH concentrations in plasma and brain, male mice were treated with MPTP+LPS as described above and two concentrations of NDP-MSH (400 µg/kg and 1 mg/kg) and sacrificed after 5-, 30- and 90-min.
- 5 Blood samples were collected through cardiac puncture in 40 mM EDTA, and plasma was collected by centrifugation and stored at -80°C till further analysis.
- 6 Whole brain was dissected and homogenized in PBS.
- 7 Proteins in brain homogenate and plasma samples were crashed with 3 volumes of methanol containing internal standard (propranolol) and centrifuged.
- 8 Supernatants were analyzed by liquid chromatography/mass spectrometry (LC/MS).
- 9 NDP-MSH in plasma and brain samples was detected by LC/MS through a service contract with Cyprotex, LLC, MA, USA.