Selenium activates mitochondrial biogenesis, preserves mitochondrial function and reduces infarct volume after focal cerebral ischemia

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Abstract

**Objective.** To explore the effects of selenium on oxidative stress, mitochondrial biogenesis regulation, and mitochondrial function in HT22 cells exposed to glutamate or hypoxia and in mice subjected to 60-min focal cerebral ischemia.

**Materials and Methods.**
Sodium selenite- 100 nM in culture medium for 24h prior to glutamate exposure or hypoxia; IP injection (0.2mg/kg) to C57BL/6J mice daily for 7 days prior to induction of MCAO
Cell viability- MTT assay
ROS production- dihydroethedine
Mitochondrial membrane potential- TMRM
Mitochondrial network- MitoTracker Red
Mitochondrial oxygen consumption- Oxygraph
Brain infarct volume- anti-NeuN and Fluoro-Jade immunohistochemistry
Target protein levels- Western blotting
**Results.** The cell culture studies showed that selenium significantly attenuated cell death induced by either glutamate toxicity or hypoxia. The protective effects were associated with reduction of glutamate-induced ROS production, prevention of mitochondrial hyperpolarization, amelioration of mitochondrial dynamic imbalance that favors mitochondrial fission and alleviation of hypoxia-induced suppression of mitochondrial respiratory complex activities. The animal studies demonstrated that selenite ameliorated cerebral infarct volume and reduced DNA oxidation. Furthermore, selenite increased protein levels of peroxisome proliferator-activated receptor-γ coactivator 1α (PGC-1α) and nuclear respiratory factor 1 (NRF1), two key nuclear factors that regulate mitochondrial biogenesis. Finally, selenite normalized the ischemia-induced activation of Beclin 1 and microtubule-associated protein 1 light chain 3-II (LC3-II), markers for autophagy.

**Conclusion.** Selenium protects neurons against glutamate, hypoxia- and ischemia induced damage by reducing oxidative stress, stabilizing mitochondrial membrane potential, preventing mitochondrial fission, restoring mitochondrial functional activities, and activating mitochondrial biogenesis.
Neuroprotective effect of Se on glutamate cytotoxicity and hypoxia
Se reduces glutamate-induced cell death-long term effect and posttreatment
Glutamate induces mitochondrial hyperpolarization
Se prevents glutamate-induced mitochondrial membrane hyperpolarization and ROS production
Se increases activities of mitochondrial respiratory complexes
Se prevents hypoxia-induced reduction of mitochondrial respiratory complex activities
Se inhibits glutamate-induced mitochondrial fission
Mitochondrial fragmentation induced by glutamate
Se reduces glutamate-induced mitochondrial fragmentation
Se reduces number of cells containing fragmented mitochondria after glutamate exposure
Se normalizes autophagy inducers after glutamate-exposure
Colocalization of autophagy marker LC-3 and fission marker Drp1
Infarct area demarked by PI staining and anti-NeuN immunohistochemistry
Se reduces neuronal death and infarct volume
Se reduces ischemia-induced DNA oxidation
Mitochondrial biogenesis signaling pathways

- cAMP → PKA
- CaMK ← Ca^{++}
- CREB
- MEF2
- NRF1,2
- PGC-1α
- AMPK
- NO
- cGMP
- ERRα
- PPAR
- Protein translation
- Respiratory chain
- mtDNA transcription/Replication

Modified from Scarpulla 2008
Se elevates mitochondrial biogenesis factors in cultured cells and in brain tissue after 1h MCAO
Se increases mitochondrial proteins
Se activates Akt-CREB pathway
Inhibition of PKA or Akt blocks the stimulating effects of Se on CREB and PGC-1α
Conclusions

- Se provides neuroprotection against glutamate, hypoxia and ischemia induced neuronal death.
- Se prevents glutamate-induced mitochondrial membrane potential hyperpolarization and ROS production.
- Se improves mitochondrial complex I, II-III and IV under normal and hypoxic conditions.
- Se reduces glutamate-induced mitochondrial fission and fragmentation.
- Se normalizes autophagy markers after glutamate exposure.
- Se activates mitochondrial biogenesis through activation of Akt-CREB and PKA-CREB pathways.
Acknowledgements:

Dr. Li’s laboratory is supported by a grant from National Institute of Health (7R01DK075476). The BRITE is partially funded by the Golden Leaf Foundation.