Epoxyeicosatrienoic Acids Activate PPARγ and Akt and Improve Neuronal Survival after Ischemia

Lasarzik I, Wang J, Koerner IP

Department of Anesthesiology & Perioperative Medicine, OHSU, Portland, OR

Background

Epoxyeicosatrienoic acids (EETs), cytochrome P450 metabolites of arachidonic acid, reduce infarct size after experimental stroke [1] and protect cultured neurons [2] and glia [3] from in vitro ischemia. The mechanisms involved in this cytoprotection remain unclear. In endothelial cells, EETs can activate the transcription factor peroxisome-proliferator activated receptor (PPAR)γ, which up-regulates expression of the anti-apoptotic protein Bcl-2. Moreover, EETs cause activation of the anti-apoptotic protein kinase B/Akt [4]. We therefore tested the hypothesis that neuroprotection by EETs involves activation of PPARγ and Akt pathways in neurons.

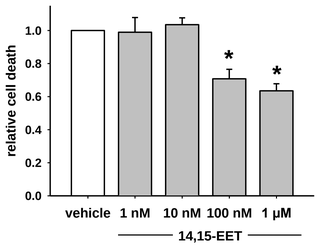
Methods

- Primary neuronal cultures are started from cortex of embryonic day 16 C57/Bl6 mice / embryonic day 18 Sprague-Dawley rats.
- Cells are plated at a density of 1.5 x 10^5 cells/cm^2 onto poly-D-lysine (100g/ml) coated 24-well plates (Corning) and grown in Neurobasal medium without phenol red supplemented with 2% B27,1% Glutamax, and 1% penicillin/streptomycin (Invitrogen).
- Cells are exposed to oxygen glucose deprivation (OGD) for 2 hours on day in-vitro (DIV)9. 14,15 EET (10 nM - 1 µM, Cayman Chemical) is added 60 minutes before OGD, and maintained during OGD and reoxygenation.
- Neuronal death is assessed 24 hours after reoxygenation by lactate dehydrogenase (LDH) release into the culture medium.
- PPARγ binding activity in neuronal nuclear extracts is measured by ELISA (Panomics) 0.5 hours after 14,15-EET treatment. Akt phosphorylation is measured by Western blot in cytosolic extracts harvested 6 hours after 14,15-EET treatment.
- RNA is extracted 4 or 24 hours after 14,15-EET treatment or OGD. Bcl-2 expression is quantified by TaqMan® real-time RT-PCR.
- Groups are compared using ANOVA. Data are mean +/- SEM.

[1] Zhang W et al. Stroke 2008;39:2073
[2] Koerner IP et al. J Neurosci 2007;27:4642
[3] Liu M & Alkayed NJ. J Cereb Blood Flow Metab 2005; 25: 939
[4] Liu Y et al. Proc Natl Acad Sci USA 2005; 102: 16747-52

Results

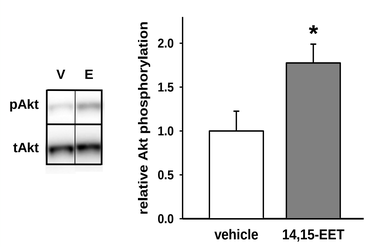
EET dose-dependently reduces neuronal death



Cell death 24 hours after 2 hours OGD is reduced in cultured cortical neurons treated with 100 nM or 1 µM 14,15-EET.

n=5; *=p<0.05

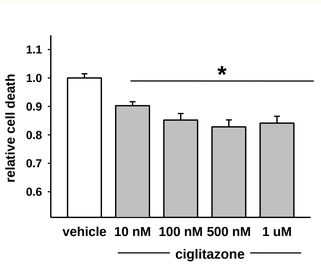
EET increases phosphorylation of Akt in neurons



Phosphorylated Akt assessed by Western blot is increased in neurons 6 hours after treatment with 1 µM 14,15-EET, whereas total Akt remains unchanged.

n=4; *=p<0.05

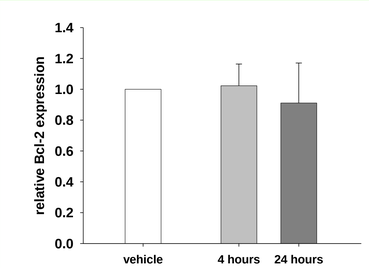
Ciglitazone dose-dependently reduces neuronal death



Cultured cortical neurons treated with the synthetic PPARγ-agonist ciglitazone show a dose-dependent reduction of OGD-induced cell death after 24 hours.

n=6; *=p<0.05

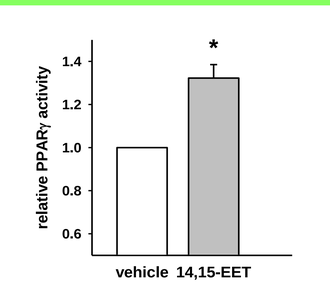
EET has no effect on bcl-2 expression



Bcl-2 mRNA expression (assessed by real-time quantitative RT-PCR) does not change 4 or 24 hours after treatment with 1 µM 14,15-EET.

n=6

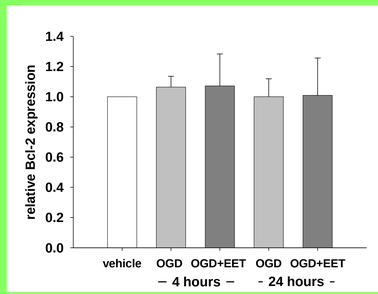
EET increases PPARγ binding to PPAR-response element



PPARγ binding to its PPAR-response element (assessed by ELISA in nuclear extracts) is increased in neurons 30 minutes after treatment with 1 µM 14,15-EET.

n=3; *=p<0.05

OGD has no effect on bcl-2 expression



Bcl-2 mRNA in cultured neurons is unchanged 4 or 24 hours after OGD.

Treatment with 1 µM 14,15-EET does not affect bcl-2 mRNA after OGD.

n=4

Summary and Conclusion

Treatment with 14,15-EET reduces neuronal death after OGD. A neuroprotective dose of 14,15-EET rapidly activates the transcription factor PPARγ in cultured neurons. However, transcription of apoptosis-inhibitor bcl-2, which is regulated by PPARγ, is not altered after 14,15-EET treatment. In contrast, the same dose of 14,15-EET induces phosphorylation and activation of anti-apoptotic Akt in cultured neurons. 14,15-EET activates several potentially protective pathways in primary neurons. However, the exact signaling pathways involved remain unclear. Future studies will focus on the role of additional PPARγ-regulated genes for neuroprotection by 14,15-EET. Further understanding of the mechanisms involved in neuroprotection by 14,15-EET may help develop new therapeutic strategies for prevention of neuronal death after brain ischemia.

korneri@ohsu.edu