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Effects of common antiepileptic drugs on biomarkers of oxidative stress in a human neuroblastoma cell line

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Abstract

Background: Oxidative stress is believed to play a role in the evolution of epilepsy and antioxidants have been proposed as putative antiepileptic drugs (AEDs). We explored the effects of commonly used AEDs on biomarkers of oxidative stress and on the expression of related genes and proteins in a human neuroblastoma cell-line.

Methods: SH-SY5Y cells were grown under standard culture conditions. Cells were exposed to carbamazepine (CBZ; 0-100µM), levetiracetam (LEV; 0-300µM), lamotrigine (LTG; 0-100µM) and valproic acid (VPA; 0-1000µM) for 24hrs, with or without 100µM H_2O_2 for further 24hrs. AED effects were assessed against basal oxidative stress, determined by malondialdehyde (MDA) concentration, superoxide dismutase (SOD) activity and reduced/oxidised glutathione (GSH/GSSG) ratio. The expression of nuclear factor (erythroid-derived 2)-like 2 (Nrf2), haemoxygenase-1 (HOX-1) and NADPH quinone oxidoreductase-1 (NQO-1) was determined using real-time PCR and western blot. Statistical analysis was performed by ANOVA with Dunnett correction.

Results: MDA level was elevated in a concentration-dependent manner following exposure to CBZ (up to 9.5-fold), LEV (up to 13.0-fold), LTG (up to 25.1-fold) and VPA (up to 26.7-fold) (all $p<0.01$). In contrast, SOD activity was reduced in a concentration-dependent manner, by a factor of up to 7.2 ($p<0.01$), 2.5 ($p<0.05$), 5.7 ($p<0.01$) and 4.0 ($p<0.05$) after incubation with CBZ, LEV, LTG and VPA, respectively. The GSH/GSSG ratio was also reduced following exposure to CBZ (up to 7.8-fold), LEV (up to 14.4-fold), LTG (up to 10.7-fold) and VPA (up to 17-fold) (all $p<0.01$). Further exposure to 100µM H_2O_2 amplified AED effects on MDA concentration, SOD activity and GSH/GSSG ratio in all cases. Expression of the Nrf2 gene was increased by 75% following exposure to both 100µM CBZ and 1000µM VPA, while expression of HOX-1 was increased by 66% and 63%, respectively (all $p<0.01$). NQO-1 gene expression was increased after incubation with 100µM LTG and 1000µM VPA by 56% and 52%, respectively (both $p<0.05$). Additional exposure to 100µM H_2O_2 augmented AED effects on Nrf, HOX-1 and NQO-1 gene expression. Relative expression of Nrf2, HOX-1 and NQO-1 proteins was increased by 46% ($p<0.01$), 26% ($p<0.05$) and 22% ($p<0.05$) after exposure to CBZ, LTG and VPA, respectively. There was no effect of LEV and only modest additional increases (≤10%) in protein expression were seen after further incubation with H_2O_2.

Conclusions: These findings imply that several AEDs have pro-oxidant rather than antioxidant effects, albeit at high concentrations. This could potentially limit their anti-seizure activity and/or contribute to their adverse effect profile.