Proceedings of the British Pharmacological Society at http://www.pA2online.org/abstracts/Vol10Issue4abst103P.pdf.

Efficacy of TDZD-8 (GSK-3β Inhibitor) Against Kainic Acid Induced Excitotoxicity In Mice

M Bhowmik[1], N Saini[1,2], S Chowdhary[1,2], R Khanam[1], D Vohora[1]. [1]Hamdard University (Jamia Hamdard), New Delhi, India, [2]Institute of Genomics and Integrative Biology, New Delhi, India

Background: There is increasing concern that activated glycogen synthase kinase-3β (GSK-3β) is involved in neurodegenerative processes observed in an array of CNS diseases (Rayasam *et al.,* 2009) including temporal lobe epilepsy (Lohi *et al.,* 2005; Lee *at al.,* 2012). We hypothesized that the behavioural changes and consequent molecular and anatomical changes in hippocampal tissue in a kainic acid (KA) induced excitotoxic model in mice might be sensitive to inhibition of GSK-3β by TDZD-8 (4-Benzyl-2-methyl-1,2,4-thiadiazolidine-3,5-dione), a selective inhibitor of GSK-3β.

Objective(s): The aim of this study was to investigate the impact of TDZD-8 on KA-evoked behavioural and neurodegenerative changes in mice hippocampus as well as to unravel the underlying affection of GSK-3β signaling pathway for the characteristic hallmarks of apoptosis.

Methods: The project (Project no. 593) was undertaken with prior approval from the Institutional Animal Ethics Committee (IACE) of Hamdard University and was conducted in compliance with the guidelines of the committee. Male albino Swiss mice (n=6) weighing 18–30 g received intraperitoneal (i.p.) infusions of TDZD-8 (5 and 10 mg/kg), or DMSO prior to an i.p. infusion of KA (10 mg/kg). Seizure episodes were rated according to Racine scale (1972) for 120 minutes post-infusion, and neuronal excitotoxicity biomarkers like Bax, Bcl-2, Cleaved caspase-3 (CC3), $pAkt_{Ser473}$ and $pGSK\text{-}3\beta_{Ser9}$ expression were measured by western blotting in mice hippocampi (n=3) isolated after 48 hours. Again, mice (n=3) from each group were transcardially perfused under anaesthesia. 10μ coronal sections of the hippocampal region were obtained and Nissl-stained for microscopic examination of pyramidal neurons in the CA1, CA3 and DG regions. Results are expressed as mean±SEM and were compared using one-way analysis of variance with Tukey's post-hoc test.

Results: TDZD-8 in both the doses failed to reduce the typical behavioural seizures such as wet dog shakes and limbic seizures elicited by KA. However, TDZD-8 pre-treatment significantly antagonized ($p<0.05$) KA mediated elevation in CC3 levels. Similarly, robust restoration ($p<0.05$) of $pAkt_{Ser473}$ and $pGSK\text{-}3\beta_{Ser9}$ expression was evidenced when compared with KA treatment alone. TDZD-8 had no influence on Bax and Bcl-2 expression. Cell counting in CA1, CA3 and DG revealed that KA treatment alone caused destruction of pyramidal cells. Conversely, mice pretreated with the TDZD-8 showed attenuated cell damage in a dose dependent manner.

Conclusion: The western blot results in conjunction with histopathological findings suggest that activated GSK-3β orchestrated neurodegenerative alterations following KA treatment. In agreement with these observations, GSK-3β inhibition by TDZD-8 afforded a distinct neuroprotective profile in KA induced excitotoxicity that is unrelated to seizure suppression which could be mediated through Akt/GSK-3β axis, suggesting that GSK-3β could be a promising target in temporal lobe epilepsy.

Lee, C.Y. *et al.* (2012) *PLoS One,* **7,** e38789.

Lohi, H. *et al.* (2005) *Human Mol. Genet.,* **14,** 2727-2736.

Racine, R.J. (1972) *Clin. Neurophysiol.*, **32,** 281-294.

Rayasam, G.V. *et al.* (2009) *Br. J. Pharmacol.*, **156,** 885–898.

We thank University Grants Commission, New Delhi, India, for financial support.