Kv7ACTIVATORS AS TARGETED THERAPIES FOR EPILEPTIC ENCEPHALOPATHY CAUSED BY Kv7.2MUTATIONS

Ilaria Mosca[1], Maria Virginia Soldovieri[1], Paolo Ambrosino[2], Elena Freri[3], Barbara Castellotti[3], Cinzia Gellera[3], Di Francesco Jacopo[3], Gaetan Lesca[4], Maurizio Taglialatela[5]

[1]Dept. of Medicine and Health Science, University of Molise, Campobasso - Italy, [2]Dept. of Science and Technology (DST), University of Sannio, Benevento - Italy, [3]Fondazione IRCCS Istituto Neurologico "Carlo Besta", Milan - Italy, [4]Dept. of Medical Genetics, Hospices Civils de Lyon, Lyon - France, [5]Dept. of Neuroscience, University of Naples "Federico II", Naples - Italy

Background and aim: Epileptic Encephalopathies (EEs) are severe forms of epilepsy in which epileptiform activity contributes to a progressive cerebral dysfunction. Seizures are often drug resistant and early death can occur. In a group of patients, the genetic causes of EEs have been identified, and knowledge of the underlying genetic cause is likely to improve treatment opportunities and clinical outcome. Recently, de novo mutations in the kcnq2(Kv7.2) gene have been identified in patients affected by early-onset EEs (EOEEs; www.rikee.org). Kv7.2, together with highly-homologous Kv7.3subunits, underlie the M-current (I_{KM}), a slowly activating and deactivating K^+ current that regulates neuronal excitability. Epilepsy-causing mutations in Kv7.2often induce loss-of-function (LoF) effects, leading to the suggestion that Kv7activators may represent a precision medicine approach. Retigabine is the first-in-class Kv7activator, showing high selectivity and efficacy; however, because of safety concerns, this drug has been recently withdrawn. Gabapentin has been recently described as a potent Kv7activator. In the present study, we have characterized the functional and pharmacological properties of Kv7.2channels incorporating a de novo mutation (c.928G>A; p.G310S) found in a patient affected by EE.

Methods: CHO cells were transiently-transfected with plasmids containing the cDNA for wild-type or mutant Kv7.2or Kv7.3subunits and used 24h later for functional and pharmacological experiments.

Results: Patch-clamp recordings revealed that Kv7.2-transfected CHO cells express robust voltage-gated outward K^+ currents; by contrast, cells transfected with mutant plasmids failed to elicit measurable currents. Furthermore, when expressed in heteromeric configuration Kv7.2G310S subunits prompt a strong inhibition of currents expressed by Kv7.2, Kv7.3, or Kv7.2/Kv7.3subunits, suggesting a mutation-induced dominant negative LoF effect. Notably, exposure to the Kv7activator retigabine (10 μM) restored Kv7.2/Kv7.2G310S/Kv7.3currents to wild-type levels. In addition, preliminary in vitro experiments revealed that also gabapentin (10 μM) activated both Kv7.2/Kv7.3and Kv7.2/Kv7.2G310S/Kv7.3currents, prompting its clinical use Treatment of the EE-affected patient carrying this mutation with gabapentin (600 mg/die) revealed a clinical improvement in terms of reduction of seizures and a mild improvement of postural control, in the absence of toxic effects, allowing the withdrawal of the anti-epiletic drug levetiracetam.

Conclusion: Overall, the results obtained suggest that the clinical use of Kv7activators could improve clinical outcome of EE-affected patients carrying Kv7.2mutations causing LoF effects, thus highlighting the relevance of precision medicine approaches in the treatment of this pharmacoresistant and severe form of epilepsy.