Macedonian Pharmaceutical Bulletin, 68 (Suppl 2) 177 - 178 (2022)
ISSN: 1857 - 8969
DOI: 10.33320/maced.pharm.bull.2022.68.04.081
Short communication

Identification of anti-epileptic natural products using zebrafish

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The challenge of treating epilepsy

People with epilepsy (PWE) comprise 1% of the population, and one third of PWE have treatment-resistant seizures. Over 80,000 PWE are lost worldwide each year to sudden unexpected death in epilepsy (SUDEP). Many epilepsy patients of all ages have additional – often untreated – cognitive, affective, and behavioral disorders.

Over 25 anti-seizure medications (ASMs) are currently available, but these function through a limited number of mechanisms (primarily GABA, glutamate, calcium, and sodium). Most major pharmaceutical companies have given up their epilepsy R&D programs, and compared to other major neurological diseases (e.g. Alzheimer's and Parkinson's disease), there is much less R&D funding for epilepsy despite a much larger number of patients. Another challenge is the limited utility of a classical, single-target approach for epilepsy drug discovery in an organ as complex as the human brain.

For these reasons, new approaches are needed to discover novel drugs for epilepsy, particularly medicines for patients with treatment-resistant seizures.

Natural products as a source of epilepsy drugs

Medicinal plants are an underutilized resource for developing new therapies for epilepsy that may have novel mechanisms of action. Other natural products (NPs), such as fungal, microbial, and human metabolites, are also attractive sources of neuroactive small molecules with potential for epilepsy drug discovery.

Several recently approved epilepsy drugs, and drug candidates in clinical development, are NPs or NP derivatives. Cannabidiol (Epidiolex®), the primary non-psychoactive metabolite of cannabis, was approved in 2018 as a treatment for three childhood epilepsy syndromes – Dravet syndrome, Lennox-Gastaut syndrome, and tuberus sclerosis complex (TSC). Ganaxolone (Ztalmy®), a derivative of the human metabolite allopregnanolone, was approved in 2022 for the treatment of CDKL5 deficiency disorder. Sirolimus (Rapamune®), a derivative of the microbial metabolite rapamycin, originally isolated from the soil bacterium *Streptomyces hygroscopicus*, has shown anti-seizure efficacy in clinical trials with TSC and other treatment-resistant epilepsy patients. Huperzine, derived from the Chinese medicinal plant *Huperzia serrata*, and ivermectin, derived from the soil bacterium *Streptomyces avermitilis*, are both in clinical development for epilepsy.

Zebrafish as an *in vivo* model for biodiscovery

Over the past 15 years, we and others have established zebrafish as an *in vivo*, microscale biodiscovery platform for the systematic identification of bioactive NPs from medicinal plants, marine microbes, and other NP sources (Crawford et al., 2008; Challal et al., 2012). Because of their high genetic, physiological and pharmacological similarity to humans, zebrafish are well-suited for the identification of bioactive NPs with therapeutic potential. Key advantages of zebrafish include the small size, optical transparency, and rapid development of their embyros and larvae, enabling high-throughput screening in 96-well format. Zebrafish disease models have been established for most indication areas, and many of these models can be used for *in vivo* screening of NP extracts, as well as for *in vivo* bioassay-guided fractionation.

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The larval zebrafish brain encompasses the major neuronal and non-neuronal cell types and neurotransmitters found in the human brain, and can also experience epileptic seizures. Several zebrafish models of epilepsy have been generated using both pharmacological and genetic perturbations (Gawel et al., 2020), and these exhibit seizures that can be quantified by behavioral or electrophysiological analysis.

Zebrafish-based identification of anti-seizure NPs

Using zebrafish as a biodiscovery platform, we have identified several novel anti-seizure NPs to date that could also be validated in rodent epilepsy models.

Several of these molecules were isolated from medicinal plants used in traditional medicine for the treatment of epileptic seizures, including (1) spirostane glyocosides identified from the Philippine medicinal plant *Solanum torvum* (tandang-aso), structurally similar to the recently approved epilepsy drug ganaxolone (Challal et al., 2014); (2) tanshinones, identified from the Chinese medicinal plant *Salvia miltiorrhiza* (dan shen) (Buenafe et al., 2013); (3) indirubin, a known inhibitor of the kinase GSK-3, identified from the Congolese medicinal plant *Indigofera arrecta* (kasholoza) (Aourz et al., 2018); (4) turmerones, identified from the Ayurvedic medicinal plant *Curcuma longa* (turmeric) (Orellana Paucar et al., 2012), and (5) β-boswellic acid, identified from the East African medicinal plant *Boswellia sacra* (frankincense) (Brillatz et al., 2021).

In addition, large-scale zebrafish-based screening of marine extracts, carried out as part of the EU project PharmaSea, has yielded several anti-seizure NPs to date, including isoquinoline alkaloids from the marine fungus *Aspergillus insuetus* (Copmans et al., 2019).

Perspective

Of the multiple anti-seizure NPs we have identified to date using zebrafish, turmerones have already been advanced into clinical development for treatment-resistant epilepsy. By using a target-agnostic, phenotypic screening approach, combined with the advantages of using a functioning vertebrate brain as an *in vivo* bioassay, our zebrafish-based biodiscovery platform will continue to deliver multiple novel drug leads that have the potential to help patients with treatment-resistant epilepsy.

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