ABSTRACT

„Effect of selected antiepileptic drugs on seizure activity in experimental model of seizures in adult zebrafish"

Epilepsy is one of the most common neurodegenerative disease characterized by a tendency to unprovoked and recurrent epileptic seizures. The mechanism of the transition from normal brain function to seizure is a result of an imbalance between excitatory and inhibitory activity of neural networks in central nervous system (CNS). It is estimated that this disorder affects 65 million people worldwide. Anti-epileptic drugs (AEDs) are the most commonly used treatment for epilepsy. The goal of antiepileptic therapy is to prevent seizures without adverse effects. Despite the availability of broad spectrum AEDs, clinical observations indicate that approximately 30% of patients using monotherapy are not free from seizures. For these patients, polytherapy is a standard practice. The potential advantages of rational polytherapy are to achieve better seizure control as well as control of multiple seizure types that respond to different drugs. Unfortunately, the side effects of AEDs are the main reason for stopping treatment. We still do not have the ideal medicine for epilepsy, and those available only allow to treat the symptoms, not the causes of the disease. There is a need for further systematic investigation into the development of new antiepileptic drugs that could prevent the progress of the disease and which would not cause adverse side effects. In these researches, a model organism such as zebrafish (*Danio rerio*) can be useful.

Zebrafish has recently become a very popular animal model which is often used in pharmacology and neurobiology research. Behavioral studies on fish model allow to analyse the effects of various substances on vertebrates CNS, as well as the possibility of showing their side effects. In case of assessing the antiepileptic properties of substances, an increasingly common method is to induce seizures by immersing zebrafish in a PTZ solution. The time intervals from the start of PTZ immersion to the onset of the different stages of seizures are recorded. The anticonvulsant effect of drug could be confirmed when it increases the seizure latency to one or more of the stages. Among the many advantages of this fish species, small sizes and low maintenance costs are often mentioned. Moreover, basic research on zebrafish model requires small amounts of reagents.

The aim of this study was to investigate the effect of selected AEDs administered intraperitoneally on seizure activity in an experimental convulsive model, which was induced by immersion of adult zebrafish in a PTZ solution. From classic AEDs, VPA, PHT, CBZ, DZP, PB were selected to the study, while LTG, TPM, FBM and LEV were selected from a

new generation of AEDs. The first goal was to determine the time of maximal effect of the drugs, and further to determine the effective doses of drugs in this particular experimental model. Another goal was to evaluate adverse effects of tested drugs in zebrafish by using a measuring chamber and software which analyzes the behavior of adult fish. The locomotor activity of fish and anxiety-like behavior induced by color stimulation were investigated. Furthermore, drugs concentrations in the zebrafish homogenates were determined. These results were compared with data from the scientific literature, in particular with appropriate concentrations of AEDs in rodent brains.

The time of maximal anticonvulsant effect of each tested AED was determined after their intraperitoneal injection in several points of time. For VPA the time of maximal anticonvulsant effect was 5 min, PHT – 60 min, CBZ – 30 min, DZP – 60 min, PB – 60 min, LTG – 60 min, TPM – 60 min, FBM – 60 min, LEV – 45 min.

VPA administered at doses of 75-200 mg/kg significantly increased the SI seizure latency in PTZ-seizure model. For the SII, a similar effect was observed at doses of 100-200 mg/kg, and for the SIII only at a dose of 200 mg/kg. PHT at doses of 10-30 mg/kg caused a statistically significant increase of SI seizure latency. For SII, a similar effect was observed at doses of 15 and 30 mg/kg. The anticonvulsant effect of CBZ was observed only at the dose of 50 mg/kg for SII and SIII. DZP at a dose of 1 and 2 mg/kg effectively increased the SI seizures latency. For the stage II and III, a similar effect was observed only at a dose of 2 mg/kg. PB at doses of 5-20 mg/kg significantly increased the SI seizure latency. For SII and SIII, a similar effect was observed only at a dose of 20 mg / kg. LTG at doses of 8-15 mg/kg significantly increased seizure latency for all stages of PTZ-seizure model. All TPM doses effectively increased the SI seizure latency. For SII and SIII, a similar effect was observed at doses of 20-50 mg/kg. FBM administered at 30 and 60 mg/kg significantly increased the SI seizure latency. Similarly, LEV at doses of 10-30 mg/kg significantly increased the SI seizure latency. The anticonvulsant effect of this drug was observed at doses of 15 and 30 mg/kg for SII and SIII.

Statistical analysis of the results showed that after administration of DZP and TPM a significant decrease in zebrafish locomotor activity was observed.

Results of AEDs treatment on anxiety-like behavior in adult zebrafish showed that after stimulation with red/yellow light combination, the anxiolytic effect against aversive yellow color occurred after administration of PB, FBM, TPM and PHT. Furthermore, a weak anxiolytic effect was observed after administration of single doses of VPA, DZP and LEV.

The results of HPLC analysis of whole fish homogenates showed what average concentration of AED was maintained in the fish body during maximum activity of drug. It is probable that the differences in the concentration of some AEDs in rodent and zebrafish may be due to their faster elimination by the kidneys and gills in fish.

In summary, studies show that some AEDs have different anticonvulsant efficacy in subsequent stages of PTZ-induced seizures in adult zebrafish. In addition, the used methodology allows the registration of adverse effects of some drugs in fish. Experiments presented in this work were an attempt to determine standard procedures, which could be use in the laboratory and could facilitate basic research on epilepsy with an animal model other than rodents.

Mateusz Picnog