学 位 論 文 全 文 要 約

Anti-seizure effects of medicinal plants in Malawi on pentylenetetrazole-induced seizures in zebrafish larvae

(ペンチレンテトラゾール誘発性ゼブラフィッシュけいれん発作モデルによるマラウィ産薬草の抗けいれん作用)

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Background and aim

Epilepsy is a neurological disorder characterized by recurrent and unprovoked seizures. It affects 1% of the global population where up to 70% of patients can be successfully treated with anti-seizure drugs (ASDs). In Africa however, barriers such as long distance to health centers, low numbers of neurology medical personnel, high cost and unavailability of ASDs put the epilepsy treatment gap at 68.5 %. Even when ASDs are available, many patients use medicinal plants due prevailing cultural beliefs that epilepsy is caused by supernatural forces. Among the plants used in Malawi are *Margaritaria discoidea*, *Dalbergia boehmii*, *Dalbergia nitidula*, *Catunaregam spinosa*, and *Lannea discolor*. However, their biological anti-seizure efficacy remains unclear. Using the larval zebrafish (*Danio rerio*) pentylenetetrazole (PTZ) chemoconvulsant model, we screened for the anti-seizure effect of these plants

Methods

Powdered plant material was boiled to produce decoctions and maximum tolerated tests were conducted in larval zebrafish to determine experimental concentrations. To validate the seizure model, larvae pre-treated with the ASD diazepam (DZP) as positive control or vehicle were exposed to PTZ. Total distance travelled was compared between PTZ-only and vehicle groups to confirm effect of PTZ, and between PTZ-only and DZP pre-treated groups to confirm anti-seizure effect of DZP. As a primary screen of plant decoctions, larvae were pre-treated 18 hours then exposed to PTZ. Decoctions that significantly suppressed PTZ-induced total distance travelled passed the primary screen and their effects on development and progression of seizure-like behavior were further studied using seizure latency and frequency. Next, electrophysiological recordings were obtained from the tectum of paralyzed larvae to directly measure effects of decoctions on neuronal activity. To further confirm whether decoctions modified neuronal activation, expression levels of the immediate early genes *c-fos* and *npas4* were measured 0.5 and 1 hour after PTZ exposure using quantitative PCR (qPCR). Lastly, larval brains were stained for c-fos protein to explore region-specific effects of decoctions on brain activation in the telencephalon, midbrain, and hindbrain.

Results and discussion

In the primary assay, decoctions of *M. discoidea* (male) leaves, *D. boehmii* roots, and *D. nitidula* leaves significantly suppressed PTZ-induced locomotor activity without sedation. *M. discoidea* (female) leaves were excluded from further experiments because they significantly impaired spontaneous larval movement even in the absence of PTZ, suggesting

strong sedative effect. In the locomotor behavior assay, we further used seizure latency and frequency as measures to determine effects of decoctions on seizure development and progression. *D. boehmii* significantly increased the latency to first seizure and reduced seizure frequency. These results suggested that *D. boehmii* acted on seizures more predominantly that the other two decoctions.

Electrophysiological recordings from the optic tectum confirmed that PTZ elicited frequent seizure-like neural discharges not observed under baseline condition without PTZ. As anticipated, larvae pre-treated with DZP had significantly lower frequency of such discharges. Among the three decoctions, larvae pre-treated with *D. boehmii* and *D. nitidula* had a markedly lower number of seizure-like discharges with a large effect size.

As previously reported, application of PTZ to vehicle-treated larvae significantly increased expression levels of *c-fos* and *npas4* transcripts. This effect was suppressed by DZP as positive control. Similarly, both *D. boehmii* and *D. nitidula* suppressed upregulation of the two transcripts, although the effect of *D. boehmii* was longer lasting. Collectively, we observed a more robust effect of DZP, *D. boehmii*, and *D. nitidula* on PTZ-induced *npas4* than *c-fos.* This may be because *npas4* induction is more selective to excitatory neurons than *c-fos*.

To explore region-specific effects of decoctions on seizure-induced brain activation, we quantified expression of c-fos protein in the forebrain, midbrain, and hindbrain respectively. Only the midbrain had significantly higher levels of c-fos protein upon PTZ application when compared to control larvae. Comparable to DZP, all three decoctions suppressed this PTZ-induced protein expression in the midbrain. Since a previous study identified *th2*-positive dopaminergic neurons responsible for spontaneous larval movement in the hypothalamus of the midbrain, it would be interesting to examine the midbrain neuron subtypes responding to PTZ more preferentially.

Although we confirmed anti-seizure activity of three out of seven plants administered independently, two or more plants are usually prescribed in practice to increase the potency of treatment. Additionally, *M. discoidea* and *D. boehmii* used in childhood-onset epilepsy whilst *D. nitidula* is prescribed for epilepsy in adulthood. Thus, further studies are needed to address whether decoctions examined in the current analysis as monotherapy and polytherapy have a differential effect on the developmental stages of zebrafish.

Anti-seizure activities of plants have been attributed to phytocompounds such as phenols and flavonoids that also have antioxidant and anti-inflammatory effects. Oxidative stress and inflammation are known to be involved in seizure pathophysiology Previous studies have profiled phenols and flavonoids in the three decoctions showing anti-seizure

activity. It would therefore be interesting to examine the effects of such isolated phytocompounds on the larval zebrafish seizure model more specifically.

Conclusion

Our study provides evidence for anti-seizure activity in decoctions of *M. discoidea* leaves, *D. boehmii* roots and *D. nitidula* leaves from a collection of plants used in Malawian traditional medicine. Uncovering the mechanism underlying the anti-seizure effect of these three Malawian herbs would pave the way for understanding the optimal use of a combination of decoctions dependent upon the subtypes of epilepsy and complications.