論　文　内　容　要　旨

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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主指導教員：相澤 秀紀教授
（医系科学研究科　神経生物学）
副指導教員：酒井 規雄教授
（医系科学研究科　神経薬理学）
副指導教員：橋本 浩一教授
（医系科学研究科　神経生理学）

Mayeso Naomi Victoria Gwedela

（医歯薬保健学研究科 医歯薬学専攻）

Background and aim

Epilepsy is a neurological disorder that 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 %. Furthermore, prevailing cultural beliefs that epilepsy is caused by supernatural forces contribute to the use of medicinal plants even when ASDs are available. Among the plants used in Malawi are *Margaritaria discoidea*, *Dalbergia boehmii*, *Dalbergia nitidula*, *Catunaregam spinosa*, and *Lannea discolor*. However, their anti-seizure efficacy has not been documented. This study aimed at screening for anti-seizure effects of these plants using the larval zebrafish (*Danio rerio*) pentylenetetrazole (PTZ) chemoconvulsant model which is characterized by seizure-like locomotor behavior, seizure-induced gene expression, abnormal local field potential burst discharges, and sensitivity to ASDs.

Methods

To validate the PTZ-induced seizure model, larvae pre-treated with the ASD diazepam (DZP) as positive control or vehicle were exposed to the chemoconvulsant. 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. In subsequent experiments, larvae were incubated in vehicle as negative control, plant decoctions or DZP as positive control at pre-determined maximum tolerated concentrations for 18 hours then exposed to PTZ. Since application of PTZ to vehicle-treated larvae significantly increased total distance travelled, plant decoctions that significantly suppressed this effect passed the primary screen and their effects on development and progression of seizure-like behavior were further studied using seizure latency and frequency. Next, local field potentials were recorded from the tectum of paralyzed larvae to directly measure effects of decoctions on neural activity. Since neuronal activation is characterized by an increased expression of the immediate early genes *c-fos* and *npas4*, their transcripts were measured 0.5 and 1 hour after PTZ exposure using quantitative PCR (qPCR) to determine whether decoctions modified neuronal activation. 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

Upon confirming that PTZ-induced distance travelled in larvae was significantly suppressed by DZP, we used the model as a primary screen for anti-seizure activity of

medicinal plants. Because the locomotor assay may lead to the conclusion of anti-seizure activity for compounds with strong sedative effects, we also compared distance travelled between larvae pre-treated with decoctions and with vehicle without PTZ. Decoctions of *M. discoidea* (male) leaves, *D. boehmii* roots, and *D. nitidula* leaves significantly suppressed PTZ-induced locomotor activity without sedation. Furthermore, *D. boehmii* suppressed seizure development and progression by significantly increasing the latency to first seizure and reducing seizure frequency comparable to DZP.

Local field potential recordings from the tectum confirmed that PTZ elicited frequent seizure-like neuronal discharges not observed under baseline condition without PTZ. Predictably, 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.

qPCR results confirmed that application of PTZ to vehicle-treated larvae significantly increased expression levels of *c-fos* and *npas4* transcripts, an effect which was suppressed by DZP. 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*, likely because *npas4* expression is more selective to excitatory neurons.

Histological staining of larval brains revealed that, among the three regions examined, the midbrain had significantly higher levels of c-fos protein upon PTZ application when compared to control larvae. Like DZP, all three decoctions also suppressed this PTZ-induced protein expression in the midbrain. Since c-fos was more predominantly expressed in the midbrain, it would be interesting to examine the 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. 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.

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.