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Evaluation of canthinone alkaloids as cerebral protective agents

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ABSTRACT

Considerable attention has been paid to cerebral protective drugs as a potential therapy for dementia. Screening of a natural compound library here resulted in identification of five canthinone alkaloids, viz., picrasidine L (**1**), picrasidine O (**2**), eurycomine E (**3**), 3-ethyl-canthin-5,6-dione (**4**), and 3-ethyl-4-methoxy-canthin-5,6-dione (**5**), as novel cerebral protective agents. The structure–activity relationship indicated that C-4, C-9, and N-3 substitutions greatly affected their cerebral protective effect. Among these, compound **2** exhibited a cerebral protective effect through suppressing neuronal hyperexcitability due to an increase in the excitatory neurotransmitter glutamic acid. Furthermore, compound **2** did not affect heart rate and mean systolic blood pressure. This investigation suggests that compound **2** has potential for further development as a cerebral protective drug.

Dementia is a clinical syndrome characterized by a cluster of symptoms and signs manifested by memory difficulties, language disturbances, psychological and psychiatric changes, and impairment in the activities of daily living. Alzheimer's disease is the most common type of dementia, followed by vascular dementia, and Lewy body dementia.[1] Clinical drugs for the treatment of dementia include acetylcholinesterase inhibitors, such as donepezil, galantamine, rivastigmine, and an *N*-methyl-D-aspartate (NMDA) receptor antagonist, memantine.[2]

Picrasma quassioides and other plants in the *Picrasma* genus of the Simaroubaceae family have been used as bitter stomachics for gastritis, loss of appetite, and indigestion in Chinese and Japanese traditional medicine. From these plants, a number of β-carboline and canthinone type alkaloids have been isolated, which have been reported to exert a variety of biological activities, including PTP1B-inhibition, anti-inflammatory activity, 3′,5′-cyclic adenosine monophosphate phosphodiesterase inhibition, and cytotoxicity.[3] In the present study, we report five canthinone alkaloids (**1**–**5**) that are potential new cerebral protective agents (Fig. 1).

Natural or chemical synthetic canthinone alkaloids (**1**–**16**) and β-carboline alkaloids (**17**–**22**) were screened for their cerebral protective effects at 100 mg/kg (p.o.) in ischemic animal, which provided a disease model of dementia. Measurement of the latency time in a step-down passive avoidance test and the density of surviving neurons of these animals were used to assess the cerebral protective effects. The experimental scheme is summarized in Figure 2.[4]

Cerebral ischemia was induced with bilateral carotid ligation in Mongolian gerbils (*Meriones unguiculatus*).[5] Gerbils were divided into groups: (1) Normal group, a group of gerbils that underwent no treatment; (2) Control group, a group of gerbils in which cerebral ischemia was induced, but which was given no compound; (3) Compound groups, groups of gerbils that underwent cerebral ischemia and were given compounds **1**–**22**, individually; and (4) the vinpocetine group, a group of gerbils that underwent cerebral ischemia and were given vinpocetine.[6]

A step-down passive avoidance test is widely used as a standard test for evaluation of learning/memory in gerbils. In this test, electrical stimulation was provided when the gerbils stepped downed from the platform. The step-down latency time, which was defined as the length of time that gerbils stayed on the platform, was used as a parameter for accessing learning and memory ability.[7]

Cerebral ischemia led to selective necrosis of neurons in specific brain regions. The CA1 subfield of the hippocampus is a brain region that is particularly sensitive to ischemia.[8] Thus, in this study, the density of surviving neurons in the CA1 subfield of the hippocampus was measured to examine the cerebral protective effect.

We identified five canthin-5,6-dione alkaloids, namely, picrasidine L (**1**), picrasidine O (**2**), eurycomine E (**3**), 3-ethyl-canthin-5,6-dione (**4**), and 3-ethyl-4-methoxy-canthin-5,6-dione (**5**), which resulted in a longer step-down latency time and greater density of surviving neurons than in the control animals (Figs. 3 and 4). Notably, picrasidine L (**1**) and picrasidine O (**2**) treatment resulted in virtually the same results as the normal group (Figs. 3 and 4). How-

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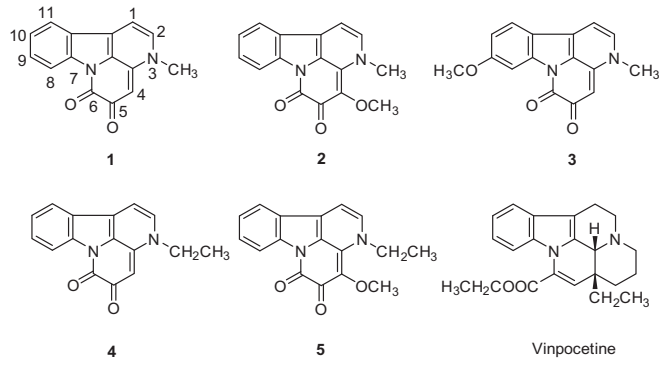

Figure 1. Five canthinone alkaloids and vinpocetine.

ever, the other 11 canthinone alkaloids (**6**–**16**) and six β-carboline alkaloids (**17**–**22**) showed no cerebral protective effects in either measurements (Supporting information). Vinpocetine, a structurally related carboline alkaloid, which is currently prescribed for the treatment of disorders arising from cerebrovascular and cerebral neurodegenerative diseases that ultimately lead to dementia in the elderly,[6] showed a much weaker effect than compounds **1**–**5** in this assay model.

In terms of step-down latency time, compounds containing an *N*-3-methyl moiety were approximately twice as potent as those containing an *N*-3-ethyl moiety (**1** vs **4**, and **2** vs **5**). Compounds containing a C-9-methoxy moiety showed a remarkably reduced activity (**3** vs **1**) (Fig. 3). In terms of the measurement of density of surviving neurons, a C-4-methoxy moiety decreased the cerebral protective activity [**1** vs **2** (p <0.05), and **4** vs **5**] (Fig. 4).

The mechanisms underlying the cerebral protective effects of compound **2** were investigated further. Benzodiazepines have been reported to have a cerebral protective effect.[9] β-carbolines, such as β-carboline-carboxyl-ethylester and harmane, were reported to bind to rat brain benzodiazepine receptor.[10] Compound **2** had β-carboline backbone skeleton in the molecular, and the cerebral protective activity of **2** was predicted to show by binding with benzodiazepine receptors. Thus, the ability of compound **2** to bind to the benzodiazepine receptor was investigated using an in silico docking study.[11] However, compound **2** showed much weaker

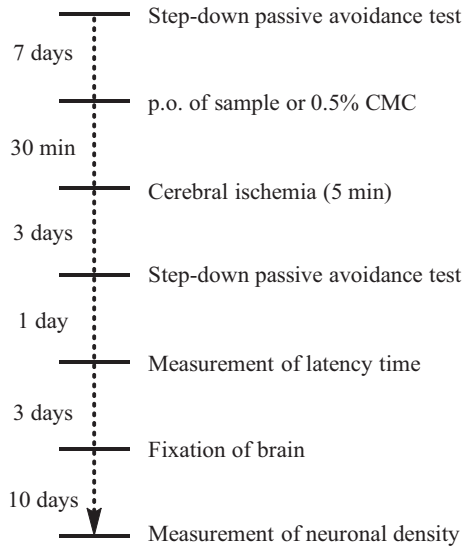

Figure 2. Experimental scheme of measurement of step-down latency time and neuronal density.

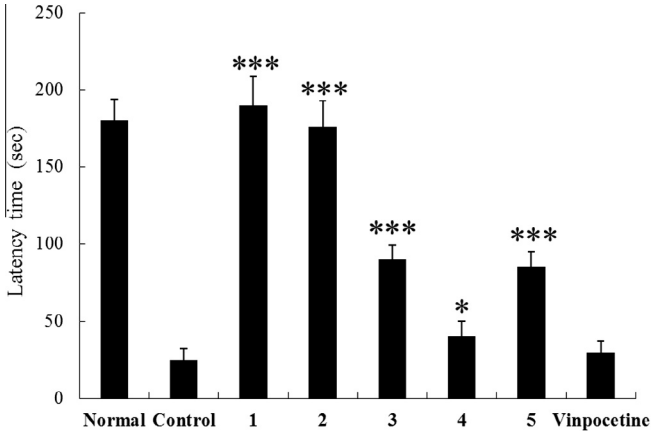

Figure 3. Step-down latency time (s) in ischemic gerbils (n = 10) treated with vehicle or with either of five canthinone alkaloids or vinpocetine administered before bilateral carotid ligation. Results are presented as the mean ± standard error of the mean. Normal: Normal group; Control: Control group; **1**, **2**, **3**, **4**, **5**: groups administered compound **1**, **2**, **3**, **4**, or **5**; Vinpocetine: group administered vinpocetine. One asterisk (*) indicates a p-value smaller than 0.05 (p <0.05), compared to the control group, three asterisks (***) indicate a p-value smaller than 0.001 (p <0.001), compared to the control group.

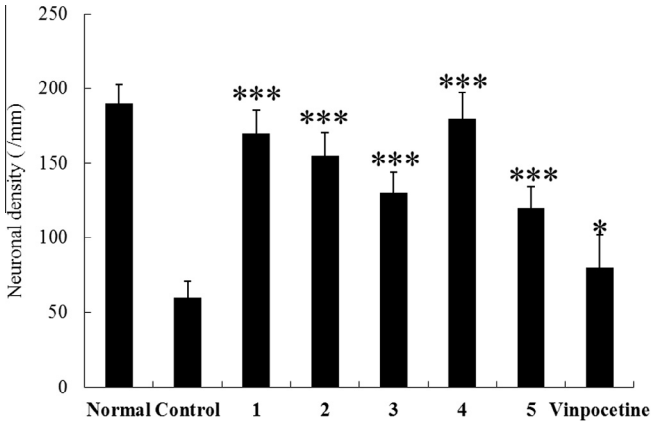

Figure 4. The density (/mm) of surviving neurons in the CA1 subfields of the hippocampus in ischemic gerbils (n = 10) treated with vehicle or either of five canthinone alkaloids or vinpocetine, administered before bilateral carotid ligation. Results are presented as the mean ± standard error of the mean. Normal: Normal group; Control: Control group; **1**, **2**, **3**, **4**, **5**: groups administered compound **1**, **2**, **3**, **4**, or **5**; Vinpocetine: group administered vinpocetine. One asterisk (*) indicates a p-value smaller than 0.05 (p <0.05), compared to the control group, three asterisks (***) indicate a p-value smaller than 0.001 (p <0.001), compared to the control group.

binding (−11.90 kcal/mol) than diazepam (binding energy: −72.64 kcal/mol), suggesting that it exerts its cerebral protective effect by other mechanisms.

Glutamic acid is the principal excitatory neurotransmitter in the brain. Endogenous glutamic acid may contribute to acute brain damage occurring after status epilepticus, cerebral ischemia, or traumatic brain injury, by activating NMDA, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid, or metabotropic glutamate receptor 1 receptors.[12] Canthinone alkaloids might exert a cerebral protective effect through suppression of neuronal cell death due to the hyperexcitability caused by a cerebral ischemia-induced elevation in the glutamic acid concentration. To test this mechanism, kainic acid was administered peripherally to gerbils to provoke over-excitement and neuronal cell death, and the gerbils were then given compound **2** orally.