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Effects of *Ginkgo Biloba* Extract on Impairment of Learning Induced by Cerebral Ischemia in Mice

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Abstract: The effect of *Ginkgo biloba* extract (GbE) on cerebral ischemia induced by 10-min bilateral occlusion of the carotid arteries in mice was studied. Severe impairment of memory was apparent when the passive avoidance test was carried out 48 hr after bilaterally induced ischemia. When GbE at doses of 50 and 100 mg/kg was given p.o. 1 hr before the 10-min occlusion, there was a significant improvement in memory. The i.p. injection of ifenprodil (30 mg/kg) also showed improvement on learning tasks. The p.o. administration of flavonoid, a fraction isolated from GbE, showed high step-through latency on scopolamine-induced amnesia. All these findings indicate that GbE is beneficial for clinical use in amnesia accompanied with cerebral vascular disease.

Preparations of an extract from *Ginkgo biloba* leaves have been used therapeutically for centuries. *Ginkgo,* like *Ginseng* or *Epimedii,* is referred to the traditional Chinese pharmacopeia. The Chinese make tea from parts of the *ginkgo* tree that is used for the treatment of respiratory diseases such as asthma and bronchitis. In European countries, extracts from the leaves are given as film-coated tablets or as liquids for intravenously administration (Kleijnen and Knipschild, 1992a). In Germany and France, the therapeutic use of *Ginkgo biloba* extract (GbE) in treatment of vascular and cerebral disorders is well established (Meyer, 1986; Eckmann, 1990). It has been reported that the action mechanism of GbE on clinical effects is the result of free radical scavenging and platelet-activating factor (PAF) antagonism (DeFeudis, 1991; Middleton, 1984). GbE induces inhibition of hydroxy-group synthesis and has an antagonistic effect on PAF receptor (Pincemail and Deby, 1988). Thus, it is suggested that GbE acts as a brain metabolism stimulant (Janssers *et al.,* 1995) and activates cerebral circulation (Kleijnen and Knipschild, 1992a). Although the therapeutic effect of GbE in humans has been reported, the pharmacological role of

GbE in the central nervous system remains unknown. The present study was undertaken in an attempt to assess the pharmacological actions of GbE, based on its effects on learning and memory in mice.

Materials and Methods

Animals

Adult male ddY mice weighing 23-26 g were used. Animals were housed in cages with free access to food and water under conditions of constant temperature (23.0 ± 0.1°C) and humidity (55 ± 5%) and a 12 hour light/dark cycle (9:00 h-21 :00 h). Ten mice were used for each treatment.

Cerebral Ischemia

Cerebral ischemia was produced as described previously by Welsh *et al.* (1987), by temporary occlusion of the bilateral middle cerebral arteries. Mice were anesthetized with pentobarbital (50 mg/kg, i.p.). Middle cerebral arteries were seen through the semitranslucent skull when the lateral aspect of the skull was exposed after reflecting the temporal muscle forward. The exposed artery was threaded through a small polyethylene tube, and the ends of the thread were ligated. The artery was occluded by pulling the artery into a tube, and securing it with a small disposable clip. The artery of each mouse was occluded following at least a week of postoperative convalescence. Under no anesthesia, the bilateral carotid arteries were occluded for 10 min. The training trial was performed 48 hr after the occlusion. GbE was intraperitoneally administered 24 hr after the acquisition trial, and the test trial was performed 1 hour after injection of GbE.

Scopolamine-induced Amnesia

Scopolamine (3 mg/kg, s.c.) was administered 20 min before the training trial. GbE flavonoid terpenoid fractions were administered intraperitoneally 24 hr after the training trial and the test trial was performed 1 hour after the injection.

Passive Avoidance Task

Mice were trained in a conventional step-through type of one-trial passive avoidance apparatus, divided into two compartments which consisted of bright (25 x 25 x 25 cm) and dark compartments (1 4 x 1 0 x 25 cm). The mouse was placed in the bright compartment. When it stepped into the dark compartment, an electric shock (1 mA) was delivered through the stainless grid floor and the mouse was then returned to the bright compartment as a learning trial. At the test trial, 24 hr after the training trial, the animal was again placed in the illuminated compartment and the latency to enter the dark compartment was measured up to a maximum of 300 sec. When the mouse stepped into the dark compartment within 5 min, it was then returned to its home cage.

Chemicals

Gingkolide biloba extract, flavonoid and terpenoid fraction were kindly provided by Sanwell Co. Tokyo and Japan Greenwave Co. Ltd., Tokyo. Scopolamine hydrochloride was obtained from Wako Junyaku Co. Ltd., Tokyo Japan. Ifenprodil tartrate was kindly supplied by Grelan Pharmaceutical Co., Tokyo, Japan. Drug doses administered are expressed as the respective salts.

Statistical Analysis

Experimental data are expressed as mean ± SEM. The data were analyzed by analysis of variance (ANOVA), and critical differences of the means were calculated by the Dunnett's test.

Results

Amnesia was induced by cerebral ischemia 48 hr before the training trial or by 3 mg/kg of scopolamine given 20 min before the training trial. Ischemia- or scopolamine-induced amnesic animals tended to shorten the step-through latency when compared to the sham or saline-treated groups of normal mice. The step-through latency in cerebral ischemic mice was significantly prolonged after treatment with GbE 50 mg/kg (266.2 ± 25) and 100 mg/kg (254.1 ± 24) in comparison to saline-treated ischemic control (110.0 ± 31). As shown in Figure 1, saline-treated ischemic mice significantly decreased step-through latency when compared with sham-operated mice.

Intraperitoneal injection of ifenprodil (30 mg/kg) significantly increased step-through latency in cerebral ischemic mice (Figure 2).

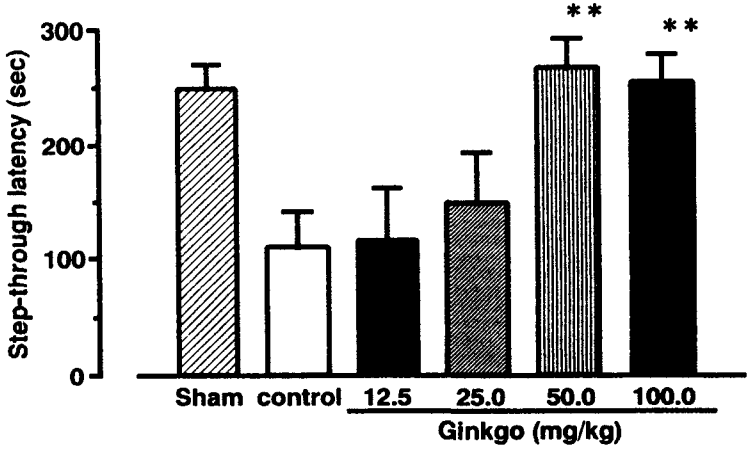

Figure 1. Effects of *Ginkgo biloba* extract on passive avoidance task in cerebral ischemic mice. **: $p<0.01$ significant difference from saline control of ischemic mice.

Furthermore, GbE also retained a high step-through latency in amnesia mice induced by scopolamine (25, 50 and 100 mg/kg, s.c.). To determine whether the improved effect on learning and memory after injection of GbE was due to the involvement of the flavonoid or terpenoid fraction isolated from GbE, the effects of these fractions in scopolamine-induced amnesia were studied. The p.o. administration of flavonoid (12.5 and 25 mg/kg) showed a significant increase in step-through latency, however, p.o. administration of terpenoid (12.5 and 50 mg/kg) failed to reverse the amnesia in comparison to the saline-treated group, respectively (Figure 3).

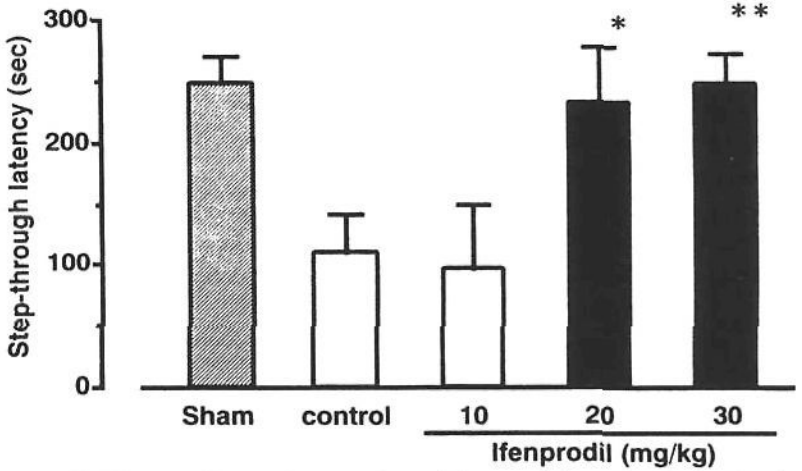

Figure 2. Effects of ifenprodil on passive avoidance task in cerebral ischemic mice.
*: $p<0.05$. **: $p<0.01$ significant difference from saline control of ischemic mice.

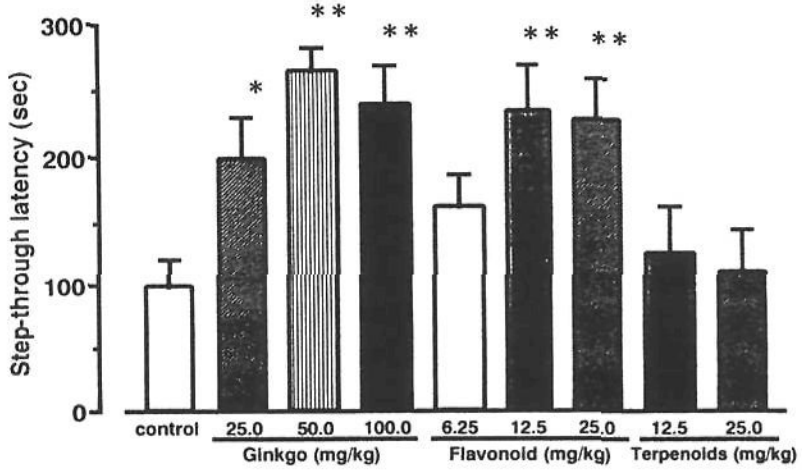

Figure 3. Effects of flavonoid and terpenoid fraction isolated from *Ginkgo biloba* extract on passive avoidance task in amnesia mice induced by scopolamine.
*: $p<0.05$, **: $p<0.01$ significant difference from saline control in scopolamine-pretreated mice.

Discussion

GbE is widely employed as a medicinal plant product in European countries. It is therapeutically used in amnesia (Kleijnen and Knipschild, 1992b), cerebrovascular insufficiency (Kleijnen and Knipschild, 1992a, b; Meyer, 1986; Eckmann, 1990), asthma (Braquet and Hosford, 1991) and related neurosensory problems (Warburton, 1986). The therapeutic effect of GbE is thought to be associated with actions of increasing glucose uptake (Rapin *et al.,* 1994) and protecting mitochondrial metabolism (Spinnewyn *et al.,* 1995) and adenosine-5'-triphosphate production (Janssers *et al.,* 1995) in various tissues. It is well known that the effect of Ginkgo biloba prepared from green leaves is due to the combined action of numerous active agents found in the extracts. The extract contains flavonoid substances, such as the Gingko-flavone glycosides and terpenoids that are characteristic of Gingko, have a unique structure (Gingkolides, Bilobalide) and are the most important substances for clinic use. GbE is standardized at 24% flavonoid glycoside (Kleijnen and Knipschild, 1992a). Flavonoids are glycosides of kaempferol, quercetin, and isorhamnetin with glucose or rhamnose. In contrast, the extract contains 6% terpenoid including gingkolides A, B and C (Kleijnen and Knipschild, 1992a). The effect of GbE on cerebrovascular disease is related to the fact that flavonoids have free radical scavenging properties (DeFeudis, 1991; Middleton, 1984), and because Gingkolides antagonize the biological action of PAF (Braquet, 1988) and have antilipoperoxidative activity (Chopra *et al.,* 1993).

In the present experiment, the injection of GbE showed improvement of learning tasks in ischemic mice since the extract prolonged step-through latency as assessed with a passive-avoidance apparatus. Similar suppression for the learning task was also found by pretreatment with scopolamine, which showed a high step-through latency. In addition to these behavioral effects, ifenprodil, an activating drug for cerebral circulation, also showed high step-through latency. Taken together, the present results suggest that the behavioral effects after an injection of GbE might primarily be the improvement of memory impairment (dysfunction) caused by brain ischemia or scopolamine.

It has been reported that GbE acts to preserve membrane from injury in the brain since the extracts decrease the accumulation of lipoperoxidative activity (Chopra *et al.,* 1993). Furthermore, GbE clinically increases cerebral bloodstream flow in cerebrovascular disease (Heiss and Zeiler, 1978). Thus, it seems reasonable to hypothesize that GbE is sufficiently effective to be clinically relevant for dementia. Additional support for this hypothesis comes from the increase in hippocampal acetylcholine receptors after injection of GbE (Taylor, 1988). As described above, the main effective components of GbE for cerebrovascular disease are flavonoids and terpenoids. This hypothesis is supported by the present findings that injection of flavonoids but not terpenoids showed high step-through latency as well as did GbE.

In conclusion, regarding the mechanism of GbE on the improvement of brain ischemia, the present findings suggest that flavonoids extracted from GbE result in activation of the central nervous system. Furthermore, these results indicate that GbE may be clinically useful for amnesia, such as memory impairment with cerebral vascular disease.

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