Biochemical and Biophysical Research Communications 441 (2013) 318–320

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Effect of oral imperatorin on memory in mice

Steinthor Sigurdsson [a,*], Sigmundur Gudbjarnason [a,b]

[a] SagaMedica, Vatnagardar 18, IS-104 Reykjavik, Iceland
[b] Science Institute, University of Iceland, Dunhagi 3, IS-107 Reykjavik, Iceland

ARTICLE INFO

Article history:
Received 27 September 2013
Available online 16 October 2013

Keywords:
Imperatorin
Memory
Passive avoidance
Step-down latency
Step-through latency
Angelica archangelica

ABSTRACT

The aim of this study was to explore the effect of the acetylcholinesterase inhibiting mixture of extracts of *Angelica archangelica* fruit and *Geranium sylvaticum* on memory. Furthermore the effect of the main compound, the furanocoumarin imperatorin, which has been shown to affect several neurotransmitters, was studied. Passive avoidance was measured by step-down latency and step-through latency of 10 months old mice receiving 0.79 mg/kg of imperatorin daily, pure or as part of the extracts, for 14 days or longer. Step-down latency was significantly higher in both groups receiving imperatorin than in the control group. In contrast, no difference was found between treatment groups regarding step-through latency. The results indicate that the imperatorin is the main active component of the extract mixture.

1. Introduction

Angelica archangelica is one of the most respected medicinal herbs in Nordic countries, where it was cultivated during the Middle Ages, and exported to other parts of Europe. The most characteristic secondary metabolites of its fruits are essential oils and furanocoumarins [1]. *Geranium sylvaticum* (woodland geranium) is widespread in Europe, but is seldom referred to as a medicinal herb in recent literature. The extracts of *A. archangelica* fruit and *G. sylvaticum* dose-dependently and synergistically inhibit acetylcholinesterase [2].

The furanocoumarin imperatorin, the major secondary metabolite of *A. archangelica* fruit, affects various neurotransmitter systems. It has been found to inhibit acetylcholinesterase in some studies [3–5], and butyrylcholinesterase more effectively [5]. Furthermore, it has been shown to inhibit the breakdown of γ-aminobutyric acid (GABA) [6], inhibit phosphodiesterases 4A and 4B [7] and to facilitate the release of the neurotransmitter glutamate [8,9]. Two recent studies have demonstrated anxiolytic activity for imperatorin in mouse experiments [10,11], and in one of those studies memory-improvement was found using modified elevated plus maze test [10]. Imperatorin passes the blood brain barrier easily and similar levels have been found in plasma and brain in mouse experiments [12].

The aim of this study was to explore the effect of the mixture of extracts of *A. archangelica* fruit and *G. sylvaticum* on memory in mice, as well as that of imperatorin, the major secondary metabolite.

2. Materials and methods

2.1. Animals

Female Hsd:ICR (CD-1) mice (Harlan Laboratories, The Netherlands) aged 10 months were housed at a room temperature of 22 ± 1 °C with a light–dark cycle (light on 6:00 am–6:00 pm). Food, as described below, and water were available *ad libitum*.

2.2. Imperatorin

Imperatorin was precipitated from 45% ethanolic extract of fruits of *A. archangelica* growing in Iceland by adding water, and recrystallized twice from diethylether until its purity was more than 97% (monitored by HPLC).

2.3. Extracts

Dried and ground plant material was extracted for 14 days with 45% aqueous ethanol at room temperature, 10 ml/g of plant material, and subsequently filtered. The *A. archangelica* extract was found (by HPLC) to contain 0.55 mg/ml of imperatorin, and 0.71 mg/ml of other furanocoumarins. The dry weight of the *A. archangelica* fruit and *G. sylvaticum* extracts were 21.6 and 36.2 mg/ml, respectively.

2.4. Food

The mice were fed standard maintenance diet (Altromin GmbH, Lage, Germany). Special food was prepared by mixing imperatorin

* Corresponding author.
E-mail address: steinthor@sagamedica.is (S. Sigurdsson).

0006-291X/$ - see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.bbrc.2013.10.036

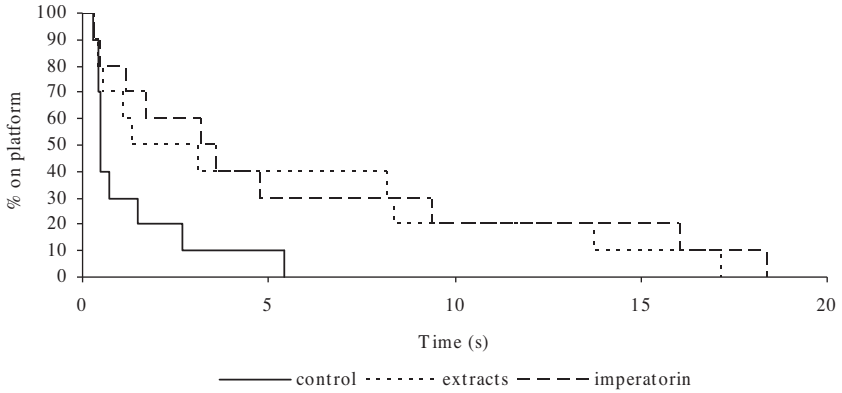

Fig. 1. Percentage of mice of each treatment group still standing on the platform at each time upon release 24 h after training.

or extracts to powdered food and making pellets of the resulting material. Prior to addition to the powder imperatorin was dissolved in 45% ethanol, 0.35 mg/ml, and 15 ml of this solution were added to 1 kg of the powder feed, which was pelleted upon drying at room temperature. In the case of the extracts, to each kg of powder 9.6 ml of *A. archangelica* fruit extract and 1.5 ml *G. sylvaticum* extract was added. Thus, both types of pellets contained 5.25 mg imperatorin/kg. Food consumption was assumed to be 15% of body weight daily, resulting in a daily dose of 0.79 mg/kg of imperatorin.

2.5. Step down latency

The experiment commenced when the mice in the experimental groups had received special pellets for 14 days. The apparatus consisted of a box with a 30 × 32 cm floor and 20 cm height. The floor was made of six stripboards ("veroboards") with a platform (10 × 10 × 2.5 cm) with a plastic surface in its center. On the day before training each mouse was allowed to acquaint itself with the surroundings for 30 s. On training day the mouse was placed on the platform. When the mouse stepped down electric shock was applied to the floor (0.5 mA, AC, 15 s). After 24 h each mouse was gently placed on the platform and the time measured from its release until it placed its front paws on the floor. The sessions were recorded by IPod Touch, 30 frames/s, and those recordings were used for evaluation of the step down latency.

2.6. Step through latency

The experiment commenced when the mice in the experimental groups had received special pellets for 21 days. The apparatus consisted of a box with a 30 × 32 cm floor and 20 cm height. Additionally to normal room illumination, the box was illuminated by 25 W light bulb 35 cm above the floor. The box was connected to a dark box, 16 × 10 cm floor and 10 cm height, by a trap door. The floor of the dark box consisted of a stripboard. On the day before training, each mouse was allowed to acquaint itself with the lit box for 30 s before the trap door was opened. In all sessions, the mouse entered the lit box facing away from the trap door. Upon entering the dark box, the trap door was closed, and the mouse was returned to its cage. On training day the mouse again familiarized itself with the lit box for 30 s before the trap door was opened. Upon entering the dark box, the door was closed and electric shock was applied to the floor (0.5 mA, AC, 2 s). On the following days the procedure was similar, except that the trap door was opened 5 s after the mouse entered the lit box. As before, the sessions were recorded by IPod Touch, 30 frames/s, and those recordings were used for evaluation. Step through latency was defined as the time from the opening of the trap door until the mouse entered the dark box.

Table 1
Step-down latency is shown as average and standard error, and *p*-values for comparison of the treatment groups with the control group by the Kaplan–Meier log rank test.

	Step down latency (s)	*p*
Control (*N* = 10)	1.29 ± 0.52	–
Extracts (*N* = 10)	5.39 ± 1.94	0.047
Imperatorin (*N* = 10)	5.88 ± 2.07	0.029
Both imperatorin-groups (*N* = 20)	5.63 ± 1.38	0.013

2.7. Statistical evaluation

The Kaplan–Meier log rank test was applied for statistical evaluation. A value $p < 0.05$ was considered statistically significant. SigmaPlot (Systat Software, San Jose, CA) was used for statistical analysis.

2.8. Approval

The study was approved by the Icelandic Laboratory Animals Committee (Tilraunadýranefnd).

3. Results

In the step down experiment, both experimental groups had average latency times that were more than four times that of the control group, as shown in Table 1. Both imperatorin-consuming groups, as well as both groups combined, were found to have significantly higher step down latency times as analyzed by the Kaplan–Meier log rank test (see Fig. 1).

In the step through experiment no significant differences were found between groups (results not shown)

4. Discussion

Those results indicate memory-enhancing activity of imperatorin, confirming previously published study [10]. In the present study, however, a different model is used and the animals receive 0.79 mg/kg daily orally instead of more than 10 times bigger single dose i.p. Furthermore, the present results indicate that the extracts are not more effective than the imperatorin contained in them, leading to the conclusion that other compounds present in the extracts do not contribute significantly to this activity.

Due to a mistake in setting up the step-down experiment some mice received electric shock immediately upon placing their front paws on the floor, instead of only after leaving the platform entirely. Thus the time from release of the mouse to placing its front paws on the floor were used as latency times, as those were the best comparable latency times available.

Survival statistics, such as Kaplan–Meier estimates or Cox proportional hazard models (when one or more covariates are considered) have recently been proposed for the analyzis of latency data [13]. In contrast to ANOVA or *t*-test they do not assume normal distribution and equal variance, which is often lacking in latency data. Furthermore, they are more powerful than non-parametric analysis such as Mann–Whitney rank sum test, that is they are less likely to reject a false null hypothesis when the samples are small, which is often the case in cognitive experiments [13].

Anxiolytic activity has been demonstrated for imperatorin [10,11]. This could affect the present methods of assessing memory, as less anxiety has been shown to reduce latency time in passive avoidance as measured by step-through latency [14].

It is curious that the two tests yielded different results, as they are both used to measure passive avoidance and assumed to be interchangeable. They are however dissimilar in some respects. In the step-down latency test the mouse can react very rapidly on its impulse to step down, whereas in the step-through test it gets 5 s to adjust itself to its surroundings before the possibility of stepping through is available, and generally the latency times are longer. It is possible that because of this difference that anxiolytic activity may affect the tests differently.

Imperatorin affects various neurotransmitter systems associated with memory. Its effect as inhibitor of the breakdown of acetylcholine and/or γ-aminobutyric acid (GABA) has previously been suggested as a mechanism for the effect of imperatorin on memory [10]. The inhibition of acetylcholinesterase by imperatorin has been found at IC_{50} at 63.7 μM [3] and higher [5]. GABA-transaminase inhibition was studied at considerably higher concentrations of imperatorin [6].

A further possibility is that the glutamate system is involved. Glutamate as a neurotransmitter is generally thought to have an important role in learning and memory [15]. A recent study found glutamate release to decline with age in subregions of rat hippocampus [16], possibly contributing to memory decline with aging. Imperatorin has been shown to markedly facilitate hippocampal glutamate release *in vitro* at concentrations above 1 μM, with 50% increased glutamate release at imperatorin concentrations between 3 and 10 μM [8]. This is in the range of imperatorin concentration found in plasma upon reception of big doses. Imperatorin concentration of 3.5 μM has been measured in plasma of rats upon receiving 10 mg/kg orally [17] and almost 12 μM in plasma of dogs which had received 5 mg/kg intravenously [18]. Furthermore, imperatorin easily passes the blood brain barrier, and 2.4 μM imperatorin has been found in the brains of mice receiving big doses of imperatorin-containing herbal extract orally [12]. Similar concentration was measured in the plasma of the animals.

5. Conclusion

Imperatorin enhances memory as measured by step-down latency but not step-through latency. The result indicate that imperatorin is the main active component of the extract mixture.

Acknowledgment

This study was supported by the Icelandic Ministry of Industries and Innovation.

References

[1] C.A. Newall, L.A. Andersen, J.D. Phillipson, Herbal Medicines. A Guide for Health Care Professionals, The Pharmaceutical Press, London, 1996.
[2] S. Sigurdsson, S. Gudbjarnason, Inhibition of acetylcholinesterase by extracts and constituents from *Angelica archangelica* and *Geranium sylvaticum*, Z. Naturforsch. C 62 (2007) 689–693.
[3] D.K. Kim, J.P. Lim, J.H. Yang, D.O. Eom, J.S. Eun, K.H. Leem, Acetylcholinesterase inhibitors from the roots of *Angelica dahurica*, Arch. Pharm. Res. 25 (2002) 856–859.
[4] S. Di Giovanni, A. Borloz, A. Urbain, A. Marston, K. Hostettmann, P.A. Carrupt, M. Reist, In vitro screening assays to identify natural or synthetic acetylcholinesterase inhibitors: thin layer chromatography versus microplate methods, Eur. J. Pharm. Sci. 33 (2008) 109–119.
[5] N. Wszelaki, K. Paradowska, M.K. Jamróz, S. Granica, A.K. Kiss, Bioactivity-guided fractionation for the butyrylcholinesterase inhibitory activity of furanocoumarins from *Angelica archangelica* L. roots and fruits, J. Agric. Food Chem. 59 (2011) 9186–9193.
[6] S.Y. Choi, E.M. Ahn, M.C. Song, D.W. Kim, J.H. Kang, O.S. Kwon, T.C. Kang, N.I. Baek, In vitro GABA-transaminase inhibitory compounds from the root of *Angelica dahurica*, Phytother. Res. 19 (2005) 839–845.
[7] F.D. Ivey, L. Wang, D. Demirbas, C. Allain, C.S. Hoffman, Development of a fission yeast-based high-throughput screen to identify chemical regulators of cAMP phosphodiesterases, J. Biomol. Screen. 13 (2008) 62–71.
[8] S.J. Wang, T.Y. Lin, C.W. Lu, W.J. Huang, Osthole and imperatorin, the active constituents of *Cnidium monnieri* (L.) cusson, facilitate glutamate release from rat hippocampal nerve terminals, Neurochem. Int. 53 (2008) 416–423.
[9] T.Y. Lin, C.W. Lu, W.J. Huang, S.J. Wang, Osthole or imperatorin-mediated facilitation of glutamate release is associated with a synaptic vesicle mobilization in rat hippocampal glutamatergic nerve endings, Synapse 64 (2010) 390–396.
[10] B. Budzynska, M. Kruk-Slomka, K. Skalicka-Wozniak, G. Biala, K. Glowniak, The effects of imperatorin on anxiety and memory-related behavior in male swiss mice, Exp. Clin. Psychopharmacol. 20 (2012) 325–332.
[11] D. Kumar, Z.A. Bhat, V. Kumar, M.Y. Shah, Coumarins from *Angelica archangelica* Linn. and their effects on anxiety-like behavior, Prog. Neuropsychopharmacol. Biol. Psychiatry 40C (2012) 180–186.
[12] W. Lili, S. Yehong, Y. Qi, H. Yan, Z. Jinhui, L. Yan, G. Cheng, In vitro permeability analysis, pharmacokinetic and brain distribution study in mice of imperatorin, isoimperatorin and cnidilin in radix *Angelicae Dahuricae*, Fitoterapia 85 (2013) 144–153.
[13] A. Jahn-Eimermacher, I. Lasarzik, J. Raber, Statistical analysis of latency outcomes in behavioral experiments, Behav. Brain Res. 221 (2011) 271–275.
[14] L.V. Loskutova, N.I. Dubrovina, The effects of the duration of adaptation to laboratory conditions on the formation of a passive avoidance reflex in rats, Neurosci. Behav. Physiol. 34 (2004) 189–192.
[15] W.J. McEntee, T.H. Crook, Glutamate: its role in learning, memory, and the aging brain, Psychopharmacology 111 (1993) 391–401.
[16] M.L. Stephens, J.E. Quintero, F. Pomerleau, P. Huettl, G.A. Gerhardt, Age-related changes in glutamate release in the CA3 and dentate gyrus of the rat hippocampus, Neurobiol. Aging 32 (2011) 811–820.
[17] S. Wang, Q. Chen, L. Wang, L. He, Development and validation of a gas chromatography-mass spectrometry method for the determination of imperatorin in rat plasma and tissue: application to study its pharmacokinetics, Anal. Sci. 25 (2009) 869–873.
[18] L. Wang, W. Lu, Q. Shen, S. Wang, H. Zhou, L. Yu, S. Wang, H. Jiang, L. He, S. Zeng, Simultaneous determination of imperatorin and its 2 metabolites in dog plasma by using liquid chromatography–tandem mass spectrometry, J. Pharm. Biomed. Anal. 70 (2012) 640–646.