ELSEVIER

Clinica Chimica Acta 339 (2004) 129–133

www.elsevier.com/locate/clinchim

Comparative vasodilating actions among terpenoids and flavonoids contained in Ginkgo biloba extract

Seiichiro Nishida, Hiroyasu Satoh*

Department of Pharmacology, Division of Crude and Herbal Medicine, Nara Medical University, Kashihara, Nara 634-8521, Japan

Received 6 August 2003; received in revised form 1 October 2003; accepted 3 October 2003

Abstract

Background: Comparative vasodilating actions of the constituents of Ginkgo biloba extract (GBE), terpenoids (bilobalide, ginkgolides A, B and C) and flavonoids (quercetin and rutin), were examined using rat aorta ring strips. *Methods*: Cumulative administrations of GBE and its constituents were followed with the pretreatment of 5 μmol/l NE. *Results*: GBE at 0.03 to 3 mg/ml had a potent concentration-dependent relaxation; by 70 ± 4.5% ($n=6$, $P<0.001$) at 3 mg/ml. Terpenoids and flavonoids at 0.1 to 100 μmol/l had potent concentration-dependent relaxation. At 100 μmol/l, bilobalide dilated by 17.6 ± 3.9% ($n=7$, $P<0.05$), and ginkgolides A, B and C also caused it to the almost same extent. Quercetin (100 μmol/l) caused a potent vasorelaxation by 49.9 ± 4.8% ($n=10$, $P<0.001$). Rutin at 100 μmol/l had weaker vasorelaxation; by 13.7 ± 3.2% ($n=6$, $P<0.01$). *Conclusions*: All constituents of GBE have the concentration-dependent vasorelaxtant effect. The potency of GBE's action was not made simply by addition of those of the constituents. Each constituent itself would contribute to the GBE-induced vasodilation, although the constituents have the complicated interactions with each other.

Keywords: Terpenoids; Bilobalide; Flavonoids; Quercetin; Ginkgo biloba extract; Vasodilation

1. Introduction

Recently, Ginkgo biloba extract (GBE) has been used worldwide as a herbal medicine [1,2]. There are many clinical applications that demonstrate the efficacy of GBE for treatment of disturbances of circulation disorders, cerebrovascular insufficiency [3], dementia [4], peripheral vascular disease such as arterial occlusive diseases [5], and age-dependent damages [6]. For the pharmacological mechanisms, it has already been shown that GBE causes an endothelium-dependent relaxation in isolated rabbit aorta strip [7] and exhibits cerebrovascular relaxation via NO releasing [8].

While GBE contains many constituents, the main pharmacologic agents are flavonoids and terpenoids [9,10]. In recent HPLC analysis, the quality of GBE has been standardized as containing 22–27% w/w flavonoid glycosides and 6% w/w terpenoids [2]. Bilobalide has a sesqiuterpen lactone, and ginkgolides A, B and C have a diterpen lactones. Quercetin, one of the flavonoids in GBE, exists as mono-, di-, tri-glycosides in GBE. Rutin (or termed as quercetin rutinoside) is also involved in GBE and is flavonol glycoside derived from quercetin.

* Corresponding author. Tel.: +81-744-29-8831; fax: +81-744-25-7657.

E-mail address: hysat@naramed-u.ac.jp (H. Satoh).

0009-8981/$ - see front matter © 2003 Elsevier B.V. All rights reserved.
doi:10.1016/j.cccn.2003.10.004

We recently demonstrated that GBE causes the vasorelaxation mainly due to both endothelium-dependent (NO releasing from endothelium cells) and -independent mechanisms (Ca^{2+} influx control of smooth muscles) induced by bilobalide [11]. Bilobalide also has potent electropharmacological effects to cardiac cells [12]. The other constituents of GBE (such as ginkgolides A, B and C, quercetin and rutin) also possess unique pharmacological actions on cardiac muscle cells [13]. These suggest that each constituent of GBE would exhibit different pharmacological effects on the ion channels or the intracellular signal transductions and also on the vascular tone control system. However, there is yet little information regarding their pharmacological mechanisms of vasorelaxation induced by each constituent of GBE. In the present study, we examined how much each constituent contributes to GBE-induced pharmacological actions, and compared the vasodilating actions induced by major constituents such as terpenoids (bilobalide, ginkgolides A, B and C) and flavonoids (quercetin and rutin).

2. Materials and methods

All experiments were carried out according to the guidelines laid down by the Nara Medical University Animal Welfare Committee, and also under the terms of the Declaration of Helsinki. Wistar male rats (4 to 15 weeks old) were anesthetized with ether, and euthanized by exsanguination. The thoracic aorta was quickly removed, and the isolated aorta was cut into 3-mm ring in length. The rings were suspended between two triangular-shaped stainless steel stirrups in a jacketed organ chamber filled with 20 ml modified Krebs–Henseleit solution. The modified Krebs–Henseleit solution was in mmol/l; 118 NaCl, 4.6 KCl, 1.2 $MgSO_4$, 1.2 KH_2PO_4, 11.1 glucose, 27.2 $NaHCO_3$, 0.03 Ethylene glyco-bis (2-aminoethylether)-*N*,*N*,*N*′,*N*′-tetraacetic acid (EGTA), and 1.8 $CaCl_2$. The chamber solution was kept at 36.5 °C and was oxygenated with 95% O_2 and 5% CO_2. The lower stirrup was anchored and the upper stirrup was attached to a force-displacement transducer (Nihon Kohden TB-652T, Tokyo, Japan) to record the isometric force. All rings were stretched to generate a resting tension of 2 g, which was optimal for contractions with α-adrenergic receptor agonist. After 40 min of resting, NE (5 μmol/l) was added into the tissue bath. After the NE-induced contractile response became steady, the compounds were cumulatively administrated into the bath solution. The changes by each concentration of compounds were measured 6–10 min after their responses became steady. The relaxation response was analyzed as a percentage decrease from the maximal contraction induced by NE.

The drugs used were GBE (Ginkgolon-24®), bilobalide, ginkgolides A, B, and C, quercetin (an aglycone type) and rutin (Tokiwa Phytochemical, Tokyo, Japan). Ginkgolon-24® was prepared as follows. Dried green leaves of Ginkgo biloba were extracted with diluted ethanol and concentrated at 50–60 °C under reduced pressure. The concentrated solution was filtered through a filter aid (diatomite), and then, purified with an absorbent resin. The fraction corrected was concentrated and dried at 70–80 °C with reduced pressure. All drugs were dissolved into DMSO (Wako, Osaka Japan). All values are represented as the means ± S.E.M. The differences of data in mean values were analyzed by the student *t*-test and ANOVA, and a $P<0.05$ was considered significant.

3. Results

The aorta ring strips of rat exhibited a strong contraction after an initial application of 5 μmol/l NE. Subsequent applications of GBE (0.03 to 3 mg/ml) potently relaxed the contraction induced by NE in a concentration-dependent manner. The significant relaxation was produced at the concentrations >0.1 mg/ml. Application of 0.1 mg/ml GBE produced a significant relaxation by 3.7 ± 1.2% ($n=6$, $P<0.05$) and GBE at 3 mg/ml decreased the contractions by 70.0 ± 4.5% ($n=6$, $P<0.001$) (Table 1). Similarly, bilobalide, ginkgolides A, B and C at 0.1 to 100

Table 1
GBE-induced vasorelaxation in rat aorta ring strips

GBE concentrations (mg/ml)					
n	0.03	0.1	0.3	1	3
6	0.61±0.29	3.7±1.2[a]	9.9±2.8[a]	26.1±4.0[b]	70.0±4.5[c]

Values (%) represent mean±S.E.M. [a]$P<0.05$, [b]$P<0.01$, [c]$P<0.001$, with respect to control value.

Table 2
Concentration-dependent vasorelaxation in the presence of terpenoids (bilobalide, ginkgolides A, B and C) and flavonoids (quercetin and rutin)

	n (μmol/l)	0.1	0.3	1	3	10	30	100
Bilobalide	7	0.30±0.62	1.1±0.75	1.4±1.0	3.0±1.7[a]	5.0±1.7[b]	9.5±2.4[c]	0.2±3.5[c]
Ginkgolide A	8	1.1±0.53	1.3±0.38[b]	3.1±0.68[c]	5.1±0.64[c]	6.7±0.86[c]	10.2±1.1[c]	15.0±1.6[c]
Ginkgolide B	9	1.6±0.45[b]	2.4±0.62[b]	3.5±0.84[c]	5.5±1.1[c]	7.3±0.86[c]	9.4±1.2[c]	6.6±2.6[c]
Ginkgolide C	8	1.1±0.35[b]	1.9±0.48[b]	2.8±0.55[c]	4.8±0.55[c]	5.8±1.2[c]	8.1±0.90[c]	17.9±3.6[c]
Quercetin	10	0.98±0.48[a]	2.0±0.66[a]	3.7±0.97[a]	5.8±1.4[b]	17.2±4.3[c]	27.7±5.7[c]	49.4±4.7[c]
Rutin	6	−0.71±0.65	−1.9±1.8	−1.3±2.2	0.28±2.3	2.8±2.2	5.0±2.8	13.6±3.2[b]

Values (%) represent mean±S.E.M. [a]$P<0.05$, [b]$P<0.01$, [c]$P<0.001$, with respect to control value.

μmol/l caused concentration-dependent relaxations. Bilobalide did not significantly exert vasorelaxation at 0.1–1 μmol/l (by approximately 0.3–1%) but did at >3 μmol/l. Bilobalide at 100 μmol/l dilated by 20.2 ± 3.5% ($n=7$, $P<0.001$). Ginkgolide A at >0.3 μmol/l, and ginkgolides B and C at >0.1 μmol/l showed similar concentration-dependent vasorelaxation. Ginkgolides A, B and C caused the significant effects at lower concentrations than bilobalide. However, no significant difference among terpenoids occurred at any concentrations. These vasodilating actions of the constituents are summarized on Table 2. At 100 μmol/l ginkgolides A, B and C dilated rat aorta by 15.0 ± 1.6% ($n=8$, $P<0.001$), 16.6 ± 2.6% ($n=9$, $P<0.001$) and 17.9 ± 3.6% ($n=8$, $P<0.001$), respectively.

On the other hand, quercetin, a type of flavonoids, showed stronger vasorelaxation than each terpenoid. Quercetin's actions behaved in a concentration-dependent fashion. Quercetin at 0.1 μmol/l produced signficant vasorelaxation and was very strong at 49.4 ± 4.7% at 100 μmol/l ($n=10$, $P<0.05$). Rutin caused the vasorelaxation, but was weaker than quercetin. Rutin at low concentrations (0.1 to 30 μmol/l) had weak contractions (not significant), and at 100 μmol/l caused a significant vasorelaxation by 13.7 ± 3.2% ($n=6$, $P<0.01$) (Table 2).

4. Discussion

The present experiments showed that (a) GBE caused a concentration-dependent vasorelaxation, (b) bilobalide and ginkgolides A, B and C also showed the concentration-dependent vasorelaxations, but their actions were weaker than GBE's action, and (c) flavonoids also showed a vasorelaxation. (d) Especially, quercetin showed the strongest action among the crude drugs in terpenoids and flavonoids.

Recently, the contents of constituents in GBE have been analyzed in the laboratory of Tokiwa Phytochemical. 1 mg/ml of GBE contains approximately 100 μmol/l bilobalide, 68 μmol/l ginkgolide A, 37 μmol/l ginkgolide B, 33 μmol/l ginkglide C, and 42 μmol/l rutin. Quercetin itself is aglycone and exists in GBE just as mono-, di-, or tri-glycosides. After a hydrolysis of quercetin glycosides, the quantity of quercetin was evaluated by HPLC analysis. The amount of quercetin glycosides containing in 1 mg/ml GBE is estimated to be approximately 300 μmol/l quercetin. In a recent German pharmaceutical analysis, the quality of GBE was standardized to contain 22–27% w/w flavonoid glycosides and 6% w/w terpenoids [2], and the similar GBE is also used in Japan. However, flavonoids contain not only quercetin-related derivative but also kaempferol and isorhamnetin. Also, other terpenoids (e.g., ginkgolide J or M) not examined in this study also contain in GBE. Furthermore, the other substances in about 70% remaining of GBE might exert some actions. But these substances would have minor effects on the vasodilating actions from the previous reports [9–11].

4.1. Comparative vasodilating actions of terpenoids

GBE produced significant vasorelaxation from concentrations >0.1 mg/ml. GBE at 0.1 mg/ml contains 10 μmol/l bilobalide, 6.8 μmol/l ginkgolide A, 3.7 μmol/l ginkgolide B, and 3.3 μmol/l ginkgolide C. These concentrations are sufficient to produce significant vasorelaxation and contribute to GBE's effect. But the dose–response curve of bilobalide was similar to that of GBE up to 1 mg/ml. In the present studies, GBE at 1 mg/ml produced 26.1%-vasorelaxation, and

bilobalide at 100 μmol/l was 20.2%. Once made a total of vasodilating potencies at concentrations of each constituent (100 μmol/l bilobalide, 68 μmol/l ginkgolide A, 37 μmol/l ginkgolide B, and 33 μmol/l ginkgolide C) containing 1 mg/ml GBE, the sum was much larger than 1 mg/ml GBE. Thus, the intricate interactions with the constituents would be involved with the GBE-induced vasorelaxation. It has been reported that bilobalide, ginkgolides A and B possess high bioavailability, when orally administrated [14,15]. Therefore, it can be suggested that terpenoids may contribute to GBE's effects sufficiently on clinical use.

4.2. Comparative vasodilating actions of flavonoid

Quercetin itself does not exist in GBE, but had a strong vasorelaxation effect in the present experiments. After hydrolysis, quercetin glycosides in 1 mg/ml GBE correspond to 300 μmol/l quercetin. However, the vasorelaxation induced by 300 μmol/l quercetin itself was too much stronger as compared with that induced by 1 mg/ml GBE. In the present experiments, thus, since quercetin exists as glycosides in GBE, quercetin might have less or no effect in vasodilation induced by GBE. Rutin showed weaker vasorelaxation than quercetin. Rutin is one of the glycosides of quercetin. When orally administrated, it is still unclear how much quercetin contributes to GBE's actions. Several studies for human have reported that quercetin and quercetin glycosides can be absorbed from intestines and are found in plasma as glucuronides or sulfates of quercetin and unconjugated quercetin aglycone [16–18]. In oral administration, rutin would be absorbed and be converted to quercetin-related metabolites and/or unconjugated quercetin aglycone. Further investigations are needed to elucidate the clinical pharmacological effects of quercetin and metabolites of quercetin (ie, using glycosidase and purified extract from hepatocytes).

4.3. Conclusion

These results suggest that all the constituents cause vasodilating action and contribute much or less to GBE-induced vasorelaxation. However, a sum of the pharmacological potencies induced by both terpenoid and flavonoids (except for quercetin) is not equal to that of GBE. Thus, GBE-induced vasorelaxation would be exhibited as a whole response mediated through the extremely complicated interactions with all the constituents.

Acknowledgements

Authors wish to thank Tokiwa Phytochemical for supplying the constituents of GBE for HPLC analysis. This work was in part supported by the grants from Tokiwa Phytochemical and Tsumura.

References

[1] Dennis JM, Kenneth J, Kerry H. Efficacy, safety, and use of Ginkgo biloba in clinical and preclinical applications. Altern Ther 2001;7:70–90.
[2] Kressmann S, Muller WE, Blume HH. Pharmaceutical quality of different Ginkgo biloba brands. J Pharm Pharmacol 2002;54:661–9.
[3] Vesper J, Hänsegen KD. Efficacy of Ginkgo biloba in 90 outpatients with cerebral insufficiency caused by old age; results of a placebo-controlled double-blind trial. Phytomedicine 1994;1:9–16.
[4] Le Bars PL, Katz MM, Berman N, Itil TM, Freedman AM, Schatzberg AF. A placebo-controlled, double-blind, randomized trials of an extract of Ginkgo biloba for dementia. JAMA 1997;278:1327–32.
[5] Peters H, Kieser M, Hölscher U. Demonstration of the efficacy of Ginkgo biloba special Extract EGb 761® on intermittent claudication—a placebo-controlled, double-blind multicenter trial. Vasa 1998;27:106–10.
[6] Diamond BJ, Shiflett SC, Schoenberger NE. Ginkgo biloba extract: mechanisms and clinical indications. Arch Phys Med Rehabil 2000;81:668–78.
[7] Delaflotte S, Augguest M, DeFeudis FV, Baranes J, Clostre F, Drieu K, et al. Endothelium-dependent relaxation of rabbit isorated aorta produced by carbachol and by Ginkgo biloba extract. Biomed Biocim Acta 1984;43(8–9):S212–6.
[8] Chen X, Salwinski S, Lee TJ-F. Extracts of Ginkgo biloba and ginsenosides exert cerebral vasorelaxation via a nitric oxide pathway. Clin Exp Pharmacol Physiol 1997;24:958–9.
[9] Huh H, Staba EJ. The botany and chemistry of Ginkgo biloba L. J Herbs Spices Med Plants 1992;1:92–124.
[10] Sticher O. Quality of Ginkgo preparations. Planta Med 1993;59:2–11.
[11] Nishida S, Satoh H. Mechanisms for vasodilations induced by Ginkgo biloba extract and its main constituent, bilobalide, in rat aorta. Life Sci 2003;72:2659–67.
[12] Satoh H. Effects of Ginkgo biloba extract and bilobalide, a main constituent, on the ionic currents in guinea pig ventricular cardiomyocytes. Arzneim-Forsch/Drug Res 2003;53(6):407–13.
[13] Satoh H. Electropharmacology of Ginkgo biloba extract and

the constituents in guinea pig ventricular cardiomyocytes. Jpn J Pharmacol 2002;88(Suppl. 1):259.

[14] Foutillan JB, Brisson AM, Girault J, Ingrand I, Decourt JP, Drieu K, et al. Phamacokinetic properties of bilobalide and ginkgolides A and B in healthy subjects after intravenous and oral administration of Ginkgo biloba extract (EGb 761). Therapie 1995;50(2):137–44.

[15] Mauri P, Simonetti P, Gardana C, Minoggio M, Morazoni P, Bombardelli E, et al. Liquid chromatography/atmospheric pressure chemical ionization mass spectrometry of terpene lactones in plasma of volunteers dosed with Ginkgo biloba L. extracts. Rapid Commun Mass Spectrom 2001;15(12):929–34.

[16] Erlund I, Kosonen T, Alfthan G, Maenpaa J, Perttunen K, Kenraali J, et al. Pharamacokinetics of quercetin from quercetin and rutin in healthy volunteers. Eur J Clin Phamacol 2000;56(8):545–53.

[17] Morand C, Manach C, Crespy V, Remesy C. Respective bioabailability of quercetin aglycone and its glycosides in a rat model. BioFactors 2000;12(1–4):169–74.

[18] Oliveira EJ, Watson DG, Grant MH. Metabolism of quercetin and kaempferol by rat hepatocytes and the identification of flavonoid glycosides in human plasma. Xenobiotica 2002; 32(4):279–87.