Safety of Dietary Supplements: Chronotropic and Inotropic Effects on Isolated Rat Atria

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We investigated the effects of dietary supplements on atria isolated from male Wistar rats. The examined supplements, which are increasingly used in Japan, those were Ginkgo biloba extract (GBE), catechins, isoflavones, sodium iron chlorophyllin and sodium copper chlorophyllin. GBE at 100—1000 µg/ml significantly increased the beat rate and the contractile force. Catechins at 1—100 µg/ml significantly potentiated the contractile force but did not effect the beat rates. However, isoflavones, sodium iron and sodium copper chlorophyllins did not change the contractile force or the beat rates. To identify the active ingredient of GBE, ginkgolide B, quercetin and amentoflavone on the atria were tested. Ginkgolide B weakened the contractile force. Quercetin potentiated the contractile force at only 30 µg/ml. Amentoflavone significantly increased the beat rate. From these findings, amentoflavone and quercetin were considered to be the principal ingredients of GBE producing the positive chronotropic and inotropic actions, respectively. In the case of catechins, (−)-epigallocatechin gallate (EGCg), one of the principal ingredients, produced inotropic actions. These findings suggest that there are some dietary supplements which affect cardiac function, such GBE and catechins.

Key words Ginkgo biloba extract; catechin; atria; chronotropic action; inotropic action

Dietary supplements are being used by an increasing number of people and patients in Japan. Ginkgo biloba extract (GBE) has been also used as a dietary supplement in Japan, although it is among the prescribed drugs for peripheral vascular diseases in European countries.1) Recently, catechins, extracts of green tea, isoflavones, extracts of soybean, iron chlorophyllin and copper chlorophyllin have also begun to be sold and used as dietary supplements. In general, GBE is well known to prevent ischemia-induced oxidation,2—4) improve cerebral blood flow5) and antagonize the action of platelet-activating factor.6) Catechins have been shown to produce an antioxidative action,7) antibacterial8—10) and hypotensive effect.11) It was reported that isoflavones produce a prevention of osteoporosis, reduction of menopausal disorders12) and antioxidative action.13) Sodium iron chlorophyllin and sodium copper chlorophyllin appear to produce a prevention of halitosis and antiulcerative action.14,15) These supplements are used for health purposes, but accurate information about the safety and efficacy of the supplement is lacking. Presently, more people suffer cardiovascular diseases, and also tend to take dietary supplement. Little is known about the influence of dietary supplements on heart function. Accordingly, it is important to examine the effect of the dietary supplements described above on cardiac function. As an initial approach to a better clarification of whether the dietary supplements affect cardiac function, we carried out a screening test of the supplements, using atria isolated from rats. Furthermore, particular attention was focused on the active ingredients of GBE and catechins, because these two compounds showed positive chronotropic and inotropic actions.

MATERIALS AND METHODS

Experiments Male Wistar rats (SLC, Hamamatsu, Japan; ranging from 8 to 11 weeks old) were anesthetized with pentobarbital sodium (60 mg/kg, i.p.) and exsanguinated. The isolated heart was rapidly removed and immediately placed in a Krebs–Henseleit solution of the following composition (mM): NaCl 118.4, KCl 4.7, MgSO 4 1.2, CaCl 2 2.5, NaHCO 3 25.0, KH 2PO 4 1.2 and glucose 10.0. After excess blood vessels and the ventricle were removed from the heart, the atria preparation which consists of auricular nodes and left-right atrium were mounted in a 5 ml organ bath filled with Krebs–Henseleit solution. The chronotropic and inotropic changes were measured with a force-displacement transducer (Model T-7, NEC San-ei Instruments, Ltd., Tokyo, Japan) coupled to a PowerLab/800 (ADInstruments Pty Ltd., NSW, Australia) under a resting tension of 1.0 g. The bath solution was maintained 32 °C and bubbled with a 95% O 2—5% CO 2 gas mixture. Each preparation was allowed to equilibrate for at least 60 min prior to initiation of experimental procedures, and during this period the incubation medium was changed every 20 min. After the equilibration period, catechins, isoflavones, sodium iron chlorophyllin, sodium copper chlorophyllin, ginkgolide B, quercetin, amentoflavone and (−)-epigallocatechin gallate (EGCg) were cumulatively added to the bath solution. The study protocols were performed according to the guidelines of the Laboratory Animal Care and Use of Mukogawa Women’s University.

Materials The powder forms of GBE, crude catechins extracted from green tea, crude isoflavones extracted from soybean, sodium iron chlorophyllin and sodium copper chlorophyllin were supplied by TAMA BIOCHEMICAL Co., Ltd. (Tokyo, Japan). The GBE contained 24.2% flavonoids (12.4% quercetin) and 9.4% terpenes (1.1% ginkgolide B). The crude catechins contained 90% catechins (31.2% EGCg). The crude isoflavones contained 20% isoflavones (daidzein and genistein). The sodium iron chlorophyllin and sodium copper chlorophyllin contained 50% sodium iron chlorophyllin and sodium copper chlorophyllin, respectively. Ginkgolide B, quercetin, amentoflavone and EGCg were obtained from BIOMOL Research Laboratories, Inc. (Plymouth.
Meeting, PA, U.S.A.), Wako Pure Chemical Ltd. (Osaka, Japan), EXTRASYNTHESE (Lyon, France) and Kurita Industry Inc. (Tokyo, Japan), respectively. R(−)-isoproterenol (−)-bitartrate was purchased from Research Biochemicals International (Natick, MA, U.S.A.). The other reagents were purchased from Wako Pure Chemical Ltd. Isoflavones, ginkgolide B, quercetin and amentoflavone were diluted in deionized water containing 20, 10, 50 and 50% dimethyl sulfoxide, respectively. The amount of dimethyl sulfoxide used (final concentrations below 50 µl in 5 ml organ bath) did not influence the heart rate or the contractile force. All other materials were diluted in deionized water (Milli-Q Jr., MILLIPORE, Tokyo, Japan).

Statistics All values are represented as the means ± S.E.M. The findings were evaluated for statistical significance between controls (vehicle) and supplements using Student's unpaired t test. When the variances of two groups were different, the Welch test was used. A probability of less than 0.05 was considered significant. The statistical analyses were carried out using a computer program (Stat View 4.5, Abacus Concepts, Inc., CA, U.S.A.).

RESULTS

Effects of Various Dietary Supplements Figure 1 shows the effect of GBE in the atria preparation isolated from rats. GBE at 30 µg/ml showed no significant effect on beat rate. At higher concentrations (100—1000 µg/ml), however, GBE significantly increased the beat rate in a concentration-dependent manner. On the contractile force, GBE showed a potentiating action at 30—300 µg/ml. Figure 2 shows the effect of catechins on the atria isolated from rats. Catechins (1—100 µg/ml) significantly potentiated the contractile force but did not change the beat rate. Isoflavones (1—100 µg/ml), sodium iron chlorophyllin (1—30 µg/ml) and sodium copper chlorophyllin (1—30 µg/ml) did not change the beat rate or the contractile force (data not shown).

Effects of Ingredients of Ginkgo Biloba Extracts and Catechins Figure 3 shows the effects of ginkgolide B on the atria preparation isolated from rats. Ginkgolide B (30—100 µg/ml) significantly reduced the contractile force but did not change the beat rate. Figure 4 shows the effect of quercetin on the atria isolated from rats. Quercetin (1—100 µg/ml) significantly increased the contractile force but did not change the beat rate. Figure 5 shows the effect of amentoflavone on the atria isolated from rats. Amentoflavone (1—30 µg/ml) significantly increased the contractile force but did not change the beat rate.

Fig. 1. The Concentration Related Effect of GBE on the Heart Rate (Left) and the Contractile Force (Right) in the Atria Isolated from Rats
Open and closed circles indicate GBE and controls (vehicle), respectively. Each point represents the mean ± S.E.M. (n=6). *p<0.05 and **p<0.01 vs. control.

Fig. 2. The Concentration Related Effect of Tea Catechins on the Heart Rate (Left) and the Contractile Force (Right) in the Atria Isolated from Rats
Open and closed circles indicate tea catechins and controls (vehicle), respectively. Each point represents the mean ± S.E.M. (n=6). *p<0.05 and **p<0.01 vs. control.

Fig. 3. The Concentration Related Effect of Ginkgolide B on the Heart Rate (Left) and the Contractile Force (Right) in the Atria Isolated from Rats
Open and closed circles indicate ginkgolide B and controls (vehicle), respectively. Each point represents the mean ± S.E.M. (n=5). *p<0.05 and **p<0.01 vs. control.

Fig. 4. The Concentration Related Effect of Quercetin on the Heart Rate (Left) and the Contractile Force (Right) in the Atria Isolated from Rats
Open and closed circles indicate quercetin and controls (vehicle), respectively. Each point represents the mean ± S.E.M. (n=6). **p<0.01 vs. control.

Fig. 5. The Concentration Related Effect of Amentoflavone on the Heart Rate (Left) and the Contractile Force (Right) in the Atria Isolated from Rats
Open and closed circles indicate amentoflavone and controls (vehicle), respectively. Each point represents the mean ± S.E.M. (n=5). *p<0.05 and **p<0.01 vs. control.
of quercetin on the isolated atria. Quercetin significantly potentiated the contractile force at only 30 μg/ml but did not change the beat rate. Figure 5 shows the effect of amentoflavone on the isolated atria. Amentoflavone (10—50 μg/ml) significantly increased the beat rate but did not change the contractile force. Figure 6 shows effect of EGCG on the isolated atria. EGCG (3—30 μg/ml) significantly potentiated the contractile force but did not change the beat rate.

DISCUSSION

In the present study, we examined dietary supplements; GBE, catechins, isoflavones, iron chlorophyllin and copper chlorophyllin on the rat atria. All supplements except the first two did not affect the beat rate or contractile force of atria isolated from rats. In our preliminary experiment, the blood pressure and heart rate of rats fed the latter three dietary supplements for 4 weeks were measured, but there was no significant difference between the two parameters of control rats and rats fed each supplement. Therefore, isoflavones, iron chlorophyllin and copper chlorophyllin appeared not to have a direct action on the cardiovascular system of rats.

GBE (100—1000 μg/ml) significantly enhanced the contractile force and beat rate in rat atria. Previously, we observed that the heart rate increased when GBE (3.5 g/kg) was orally administrated to the rats once daily during three days. GBE contains various substances, such as flavonoids (24.2%) and terpenes (9.2%). To better understand the active ingredients of GBE that produce positive chronotropic and inotropic effects, we examined the effects of three ingredients of GBE on the atria. Interestingly, the concentration—contractile response curves for GBE and quercetin were bell shape. Gingkolide B reduced the contractile force of atria in a concentration dependent manner. Quercetin was reported to produce a positive inotropic effect in guinea-pig papillary muscle. As the content of quercetin (12.4%) in GBE is higher than that of gingkolide B (1.1%), the positive inotropic action by GBE appears to be due to the action of quercetin. Although the reason why GBE did not increase heart rate at higher concentrations (1000 μg/ml) is unclear, the negative action of gingkolide B and the elimination of the positive action of quercetin may be involved. Gingkolide B and quercetin did not affect the heart rate of rat atria, but amentoflavone significantly increased it. Biflavones are also known to be contained in GBE and inhibit phosphodiesterase in the bovine heart. The order of potency of the inhibitory effect on the enzyme was amentoflavone > bilobetin > sequoiavlanone > ginkgetin > isogingetin. We also observed that Ro 20-1724, a phosphodiesterase inhibitor, increased the beat rate by about 112% at a concentration of 10^-8 M, but did not change the contractile force. Quercetin has also been reported to inhibit phosphodiesterase, but the IC50 for quercetin is about 700-fold less than that of amentoflavone. Taken together, amentoflavone and quercetin may be one of candidates in GBE that produce the positive chronotropic and inotropic actions, respectively.

Catechins (1—100 μg/ml) and EGCG (3—30 μg/ml) significantly potentiated the contractile force but did not affect the beat rate. One microgram per milliliter of catechins contains approximately 0.5 μg/ml EGCG. The potency of the inotropic effect by catechins and EGCG at 10 μg/ml was almost equal. Therefore, one of ingredients of catechins that produces the positive inotropic action appears to be EGCG. The concentration—response curve for catechins was also bell shape. The crude catechins extracted from green tea used in the present study contains other substances, such as (−)-epicatechin, (−)-epicatechin gallate and (−)-epigallocatechin. Catechins may contain some ingredients producing positive and negative inotropic actions, which produce such bell shaped concentration—response curves. It is necessary to elucidate the specific component in catechins that produces the positive inotropic effect in future studies.

In conclusion, we found that among the tested dietary supplements, GBE showed positive chronotropic and inotropic actions and catechins showed a positive inotropic action in rat atria. These findings suggest that GBE and catechins, particularly at high doses, affect the cardiovascular system. In healthy volunteers, it was reported that the quercetin and EGCG concentrations of plasma after ingestion of 311 μmol quercetin glucoside and 800 mg EGCG was 3.5 μM (1.2 μg/ml) and 0.44 μg/ml, respectively. Since dietary supplements are generally sold as a tablet or concentrated solution, it is feasible to consume large quantities of them.

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REFERENCES