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FRS 1000, an extract of red onion peel, strongly inhibits phosphodiesterase 5A (PDE 5A)

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Abstract

As part of our ongoing search for flavonoids that are bioactive in humans, it was determined that FRS 1000, a beverage containing flavonoids extracted from onion peel, showed unexpected improvement of male sexual function. An in vitro enzyme assay clearly showed that FRS 1000 has a strong phosphodiesterase 5A (PDE 5A) inhibitory activity, which is considered to be important for treatment of erectile dysfunction. Detailed assays of each major ingredient indicated that the antioxidative flavonoid quercetin was responsible for the activity. Results also suggested that PDE 5A inhibition is not directly related to the free radical scavenging activity of flavonoids.

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Keywords: Red onion; Bioactive flavonoids; Quercetin; PDE 5; Sexual function

Introduction

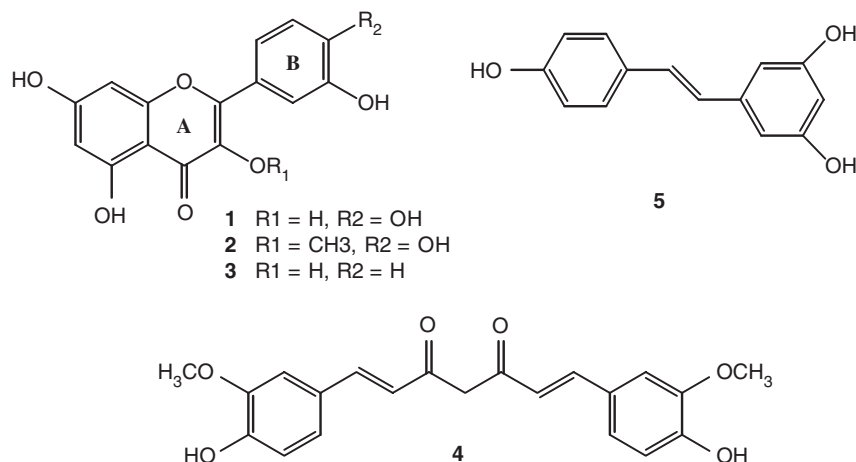
Since 1998, FRS 1000, a natural plant-based beverage, has been voluntarily provided to terminal cancer patients in South Africa, Luxembourg, and the United States to help maintain their quality of life. Since then many patients have provided feedback, by a self-administered questionnaire or by telephone interview, regarding their conditions as influenced by the long-term consumption of this beverage. A common effect was that patients felt they recovered their energy (Lines, 2003). In addition, interestingly, some male patients even confessed that they had increased sexual function

and sexual satisfaction with FRS 1000. It is known that a potent selective phosphodiesterase (PDE)-type 5A inhibitor, sildenafil, is used for treatment of sexual dysfunction (e.g., Junemann, 2003). These reports prompted us to study the influence of the beverage on enzymatic activities of PDE 4 and PDE 5A. Major chemical components of FRS 1000 are quercetin, a bioflavonoid extracted from red onion peel; vitamin C; vitamin B3; vitamin B6, caffeine and citric acid. It is formulated with water containing dietary fiber and tangerine orange flavor. We describe here the in vitro PDE activity of each ingredient of FRS 1000 with respect to explaining reports of improvement of sexual function by patients.

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Materials and methods

FRS 1000 and sample preparation

FRS 1000 was formulated according to a patented formula provided by New Sun Nutrition LLC (Carpinteria, CA, USA). FRS 1000 contains a fraction of extracts of red onion peels consisting primarily of quercetin (98.0%). The quercetin in FRS 1000 is obtained by acid hydrolysis of rutin extract from dried red onion peels, followed by treatment with charcoal and recrystallization. It requires roughly 2.0 kg of dried peels to obtain 1000 mg of quercetin with 98% purity. The amounts of rutin and isoquercitoid in the fraction were determined to be less than 1.0%, respectively, by analysis of the fraction on a reversed-phase column (Supersphere RP C18 endcapped, 250 × 4.6 mm; Merck). Chromatographic conditions: mobile phase: A: water adjusted for pH 2.8 with phosphoric acid and B: acetonitrile, 60:40 mixtures, flow rate 1.0 ml/min, with a detection wavelength of 259 nm.

The major ingredients in FRS 1000 (1000 ml) are quercetin (1000 mg), vitamin B3 (83 mg), vitamin B6 (8.3 mg), vitamin C (500 mg) and caffeine (150 mg). The FRS 1000 (5 ml) suspension was diluted to 10 ml with dimethyl sulfoxide (DMSO) to achieve a clear 50% DMSO solution. The aliquot (10 μl) was provided to serial dilution with distilled water with appropriate DMSO concentration in order to get 0.5% DMSO concentration in triplicate in the wells of a 96-well microplate. The DMSO concentration was fixed at approximately 0.5% in each well regardless of dilution rate.

In vitro assay

For SPA PDE enzyme assay (Amersham, NJ) was used for measurement of PDE 4 and PDE 5A activities in U937 cytosol (ATCC, VA) and recombinant PDE 5A

(Calbiochem, CA). The present PDE 4 and PDE 5A assays employed 2 μM of ³H-cAMP (6.75 Ci/mmol) and ³H-cGMP (3.75 Ci/mmol), respectively. Reaction buffer was composed of 50 mM Tris-HCl, pH 7.5; 8.3 mM MgCl₂; and 1.7 mM EGTA. Testing compounds were dissolved in DMSO and a 0.5-μl aliquot was added to a final reaction volume of 50 μl. Reaction was carried out at 30 °C for 30 min in a 96-well assay plate and halted by adding 25 μg of SPA yttrium silicate beads (Amersham, NJ) suspended in 25 μl of 18 mM ZnSO₄. Hydrolyzed linear nucleotides, as compared to cyclic nucleotides, bind preferentially to SPA beads. After the assay plate was shaken for 3 min and spun at 1000 rpm for 1 min, radioactivity was counted on a MicroBeta liquid scintillation counter (Perkin-Elmer, MA). Nonspecific bindings of cyclic nucleotides to the SPA beads were measured in reactions without PDEs. DMSO that carried compounds showed no effect on the reaction at the concentration used (0/5%).

Results and discussion

Fig. 1 shows the PDE 5A inhibitory profile of FRS 1000, recorded with % inhibition on the vertical axis and dilution horizontally. Because of its significant inhibition (5–20%) even at the high dilution ratio (× 10,000), the PDE 5A inhibitory activity of FRS 1000 deserves attention. The PDE 5A inhibitory activities of the four main ingredients in FRS 1000 (quercetin 1, vitamin B3, vitamin C, and caffeine) were then analyzed separately. The data are summarized in Table 1. Under our experimental conditions, only quercetin showed marked inhibitory activity against PDE 5A, with an IC₅₀ value of 1.9 μM. It is notable that this activity is comparable to that of zaprinast, which is

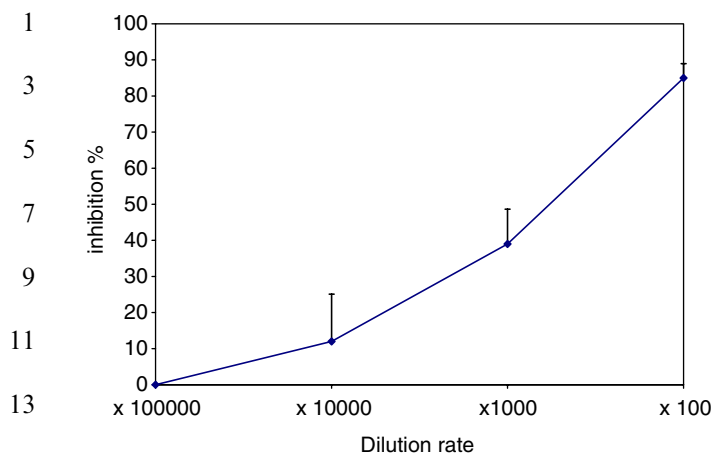


Fig. 1. PDE 5A inhibitory activity (% inhibition) of FRS 1000 diluted with water (\pm standard deviation; $n = 6$).

Table 1. IC_{50} values of various compounds against PDE 4 and 5A in vitro (μ M)

Compound	IC_{50} value (μ M)	
	PDE 5A	PDE 4
Quercetin 1	1.9	20
3- <i>O</i> -methylquercetin 2	> 30 (86.9)	25 (28.5)
Kaempferol 3	2.5	> 30
Curcumin 4	NA ^b	NA
Resveratrol 5	NA	NA
Vitamin B3	NA	NA
Vitamin C	NA	NA
Caffeine	> 30	> 30
Zaprinast ^a	1.5	NM
Rolipram ^a	NM ^c	0.9

^aPositive controls for the PDE 4 and PDE 5A inhibition assays.

^bNo activity detected at 30 μ M.

^cNot measured.

one of the few cGMP-specific PDE inhibitors (Sugita and Black, 1998).

It is known that 3-*O*-methylquercetin inhibits total cAMP and cGMP-PDE of guinea pig tracheas (Ko et al., 2002). Recently it was reported that 3-*O*-methylquercetin **2** selectively inhibits PDE subtype 3 with an IC_{50} value of 1.9 μ M, whereas it very weakly inhibits other subtypes, such as PDE 4 and 5A, with an IC_{50} value of 28.5 and 86.9 μ M, respectively (Ko et al., 2003). This provides a clear contrast between quercetin **1** and the 3-methyl derivative **2**. Because such a small substituent made a significant difference in inhibitory activities among the subtypes, several molecules with structural or functional similarities to quercetin were also analyzed, and the data summarized in Table 1.

Kaempferol **3**, which differs from quercetin **1** by a lone hydroxy group in ring B, shows a PDE 5A inhibitory activity (IC_{50} value = 2.5 μ M) comparable to that of quercetin. It is well-accepted that the catechol structure in the B ring is the major determinant for the reactive oxygen species (ROS)-scavenging capability of the flavonoids (Pietta, 2000). Recently a quantitative structure-activity relationship was proposed, linking the lipid peroxidation (LPO) inhibitory effects of flavonoids to their half-wave potentials ($E_{1/2}$) and lipophilicity (van Acker et al., 1996). In fact, the much weaker LPO inhibitory activity of kaempferol **3** is explained on the basis of the higher value of the half-wave redox potential ($E_{1/2}$) relative to that of quercetin **1** (Yang et al., 2001). Therefore the observed comparable PDE 5A inhibitory activity of quercetin **1** and kaempferol **3** is apparently not related simply to the strength of ROS-scavenging capability of flavonoids.

The naturally occurring antioxidants, curcumin **4** (Hsu et al., 2002) and resveratrol **5** (Leonard et al., 2003), which have free-radical-scavenging activity similar to that of quercetin, were also analyzed. The results, shown in Table 1, also support our observation that not all antioxidants with strong ROS scavenging activity exhibit significant PDE 5A-inhibitory activity under the conditions tested. It is well accepted that a specific PDE 5A-inhibitory activity of sildenafil elevates cGMP and leaves cAMP levels raised by PDE 4 unchanged (Kim et al., 2000). It is also confirmed that there is a 100- to 1000-fold difference in sildenafil's inhibitory activity against the two enzymes (Ballard et al., 1998).

Quercetin's PDE 4 inhibitory activity, when analyzed, was observed to be, at an IC_{50} value of 20 μ M, at least 10 times weaker than that of quercetin's PDE 5A inhibition. Together with the fact that no significant additive and/or synergistic effects for any possible combination among the major ingredients of FRS 1000 (data not shown) exhibited PDE 5A inhibition, it seems reasonable to assume that the observed PDE 5A inhibition came from quercetin. Consequently, the reported improvement in sexual function among patients might be partially explained by this newly observed specific PDE 5A-inhibitory activity of quercetin **1**, one of the major ingredients of FRS 1000.

Uncited reference

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