Modulatory effect of coffee fruit extract on plasma levels of brain-derived neurotrophic factor in healthy subjects

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(Submitted 6 July 2012 – Final revision received 30 October 2012 – Accepted 30 October 2012 – First published online 14 January 2013)

Abstract

The present single-dose study was performed to assess the effect of whole coffee fruit concentrate powder (WCFC), green coffee caffeine powder (N677), grape seed extract powder (N31) and green coffee bean extract powder (N625) on blood levels of brain-derived neurotrophic factor (BDNF). Randomly assorted groups of fasted subjects consumed a single, 100 mg dose of each material. Plasma samples were collected at time zero (T0) and at 30 min intervals afterwards, up to 120 min. A total of two control groups were included: subjects treated with silica dioxide (as placebo) or with no treatment. The collected data revealed that treatments with N31 and N677 increased levels of plasma BDNF by about 31 % under these experimental conditions, whereas treatment with WCFC increased it by 143 % (n 10), compared with baseline. These results indicate that WCFC could be used for modulation of BDNF-dependent health conditions. However, larger clinical studies are needed to support this possibility.

Key words: Brain-derived growth factor; Coffee fruit extract; Caffeine; Polyphenols; Procyanidins

Brain-derived neurotrophic factor (BDNF) is a member of the nerve growth factor-related family(31) and is a homodimeric protein that has been highly conserved in structure and function during evolution(2,5,32). BDNF is a secreted protein that, in human subjects, is encoded by the BDNF gene(33). It is involved in development, maintenance and function of the central nervous system(4,5). BDNF is found in a wide range of tissues(3) and it can be self-regulated(6,34). BDNF has several documented short- and long-term functional roles. It is now well-known that BDNF serves as a target-derived survival and differentiation factor for neuronal sub-populations in prenatal stages(8–10). Likewise, BDNF promotes long-term potentiation by potentiating excitatory neurotransmitter activity in the hippocampus(11) and α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor insertion post-synaptically(12). These actions, along with BDNF’s ability to stabilise dendritic spines(13), underline the molecule’s important role in learning, memory and behaviour(35). In addition, BDNF levels increase in response to certain forms of injury, such as ischaemic–hypoxic(14) and infectious insults(15), presumably by blocking apoptosis. Various studies have shown a link between BDNF and certain health conditions, such as depression(16,17), obsessive–compulsive disorder(18), Alzheimer’s disease(19), dementia(20) and Parkinson’s disease(21,22).

Despite its name, BDNF is found in a variety of tissues and cell types, not just in the brain. It is also expressed in cardiovascular, immune, reproductive and endocrine tissues(4,5,23). Intact BDNF readily crosses the blood–brain barrier via a high-capacity transporter system(24). Interestingly, exercise has been shown to increase the expression of BDNF in human subjects(25–27) and a similar effect was observed after caffeine application in vitro(28) and in vivo(29). Caffeine has been recently proposed as a potential candidate for maintaining physiological levels of BDNF, as it is capable of positively affecting cognition(29,30). In the present study, we tested polyphenol-rich natural products containing different amount of caffeine to see if a single dose could lead to an increase in plasma levels of BDNF. Three natural products containing varying amounts of caffeine were selected for the present pilot study: green coffee caffeine (N677), green coffee bean extract (N625) and whole coffee fruit concentrate powder (WCFC)(31). A grape seed extract (N31) containing high levels of polyphenols, but not caffeine(32), was also tested.

**Abbreviations:** BDNF, brain-derived neurotrophic factor; N31, grape seed extract powder; N625, green coffee bean extract powder; N677, green coffee caffeine powder; WCFC, whole coffee fruit concentrate powder.

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Materials and methods

Materials

Extract powders tested in the present study were provided by FutureCeuticals, Inc. WCFC is a patented extract of whole coffee fruit (coffee berries) from Coffea arabica. Chemical composition and polyphenol profiles of each tested extract appear in Table 1. Dulbecco’s PBS and water were purchased from Sigma Chemical Company. Protein low binding microtubes were obtained from Eppendorf and RC DC Protein Assay Kit II was purchased from Bio-Rad. Human BDNF Quantikine ELISA kits were from R&D Systems. Heparin Assay Kit II was purchased from Bio-Rad. Human BDNF tubes were obtained from Eppendorf and RC DC Protein Lysis Devices) at 450 and 540 nm wavelengths, and final concentrations were calculated from a standard curve.

Chemical analyses

Chlorogenic acid, procyanidins, flavanols and flavonols of WCFC, N625 and N31 were characterised by LC–MS. Chlorogenic acid, procyanidins, flavanols and flavonols of WCFC, N625 and N31 were characterised by LC–MS(16) and quantified by UV absorbance(25). Total polyphenol content was determined by spectrophotometry according to the Folin–Ciocalteau method(27), and was calibrated against gallic acid standard (Sigma-Aldrich). Results were expressed as grams of gallic acid equivalents.

Brain-derived neurotrophic factor detection and quantification

For the isolation of plasma, 100 μl finger blood were collected by finger puncture and placed in Safe-T-Fill® Capillary blood collection tubes (Ram Scientific, Inc.) and centrifuged at 1000g for 10 min. Blood was transferred to protein low binding tubes and kept at −80°C until use. BDNF was measured using a quantitative sandwich ELISA immunoassay (R&D Systems) following the instructions provided in the kit, using the buffers and calibrators specific for plasma. Final reactions were measured using a spectrophotometer (Molecular Devices) at 450 and 540 nm wavelengths, and final concentrations were calculated from a standard curve.

Study description

The present study was conducted according to the guidelines laid down in the Declaration of Helsinki, and all procedures involving human subjects/patients were approved by the Institutional Review Board at Vita Clinical S.A. Avenida Circunvalación Norte #135, Guadalajara, JAL, Mexico 44 270 (study protocol no. 12-06 ABC-BDNF). The present pilot acute clinical study was performed on healthy fasted subjects treated with at least one of the five treatments: N677, N625, N31, WCFC or placebo (silica oxide). In follow-up studies performed under the same experimental conditions, five participants received WCFC, chlorogenic acid or water as vehicle (no treatment group). In all cases, subjects fasted for 12 h prior to the first blood collection. Other than consuming one of the study materials or placebo, patients had no intake per os during the study period. Blood was collected at baseline (T0) and subsequent samples were collected at every 30 min (T30, T60, T90 and T120) after the treatment.

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The caffeine and trigonelline contents were characterised by HPLC Agilent 1100 (Agilent Technologies) equipped with a diode array detector and quantified by UV absorbance (W Mullen, B Nemzer, M Clifford, et al., unpublished results). Antioxidant capacities of coffee fruit extract, coffee bean extract and N31 were characterised by the ability of the samples to scavenge peroxyl radicals (oxygen radical absorbance capacity), hydroxyl radicals (hydroxyl oxygen radical absorbance capacity), peroxynitrite (peroxynitrite radical absorbance capacity), superoxide anions (superoxide radical absorbance capacity) and singlet oxygen (singlet oxygen radical absorbance capacity).\(^{39–41}\)

**Statistical analysis**

BDNF levels were compared with a reference standard curve and each subject was normalised to their own value measured at time zero \((T_0)\). Peak levels of plasma BDNF for each patient were used for comparisons. Results were pooled and standard error of the mean was used for each separate analysis. Plasma BDNF levels at 60 min after treatment were compared with baseline using a two-tail, independent Student’s \(t\) test. Power analyses were run using G*Power Data Analysis (Heinrich-Heine-University Düsseldorf). Statistical power was run for the whole group and it was also calculated per-pair power for individual comparison. Descriptive analysis was run in GraphPad to derive the mean and standard deviation for each group.

**Results and discussion**

Four polyphenol-rich fruit extracts were tested in healthy subjects. Three of the extracts contained caffeine in varying amounts (WCFC: 0.7 % caffeine; N677: 72.8 % caffeine; and N625: 2 % caffeine) and one extract was caffeine free (N31) (see Table 1). Of the substances tested, WCFC increased BDNF plasma levels (Fig. 1) in patients by an average of 137 % with respect to baseline (range 65–222 %; \(P=0.001\) v. placebo). N677 showed an increase of 42 %, but was not statistically significant \((P=0.49)\). N625 did not cause a significant increase in BDNF. N31 increased BDNF levels in plasma by 30 % with respect to baseline, though not significantly \((P=0.65)\).

It is important to note that a power analysis of the entire sample indicated that forty subjects would be required to reach a power of 80 %. Our sample size was twenty-five, therefore one must interpret the lack of BDNF effect of N677, N625 and N31 with caution. Larger groups are required to make definitive conclusions for these treatments. However, the result of this power analysis does not diminish the statistical significance of WCFC on plasma BDNF levels. Indeed, a separate per-pair power analysis of WCFC and placebo showed a sample size of ten to have a greater than 99 % power.

Quite unexpectedly, treatment with placebo (silica dioxide) resulted in a 34 % reduction in BDNF blood levels \((P=0.09)\). Silica oxide was used as placebo, as it is generally considered to be an inert material. It has been previously reported that stress can decrease plasma levels of BDNF in some human and animal models.\(^{42–44}\) However, the reduction of plasma BDNF due to the intake of silica/placebo has not been reported. Consequently, a second experiment was performed to verify the reproducibility of the effect of WCFC on BDNF and to test the effect of extended fasting (untreated control) on the baseline level of BDNF in blood.

A total of five additional healthy subjects who met the same inclusion criteria were treated with 100 mg WCFC. As before, all groups fasted for 12 h prior to testing, but the control group did not receive any treatment besides water as vehicle (200 ml) as it was used for treatment with WCFC. As shown in Fig. 2, BDNF plasma levels were increased in subjects treated with 100 mg of WCFC (148 % increase; \(P=0.002\)). However, blood BDNF level in the untreated group was not statistically changed (15 % increase by average).

Pooling all ten subjects from the two studies, treatment with 100 mg of WCFC caused a 143 % increase in BDNF plasma levels. The stimulatory effect of caffeine on BDNF has been previously observed\(^ {29,30,45,46} \). However, as presented in Fig. 1, single-dose treatment with caffeine-containing extracts...
resulted in an increased level of plasma BDNF in a caffeine concentration-independent manner. As shown in Table 1, N677 is mostly comprised of caffeine (72.8% by weight), yet caused only modest increases in plasma BDNF levels. The most profound increases in plasma BDNF were observed after treatment with WCFC, although this extract contains only 0.7% caffeine by weight.

Previous reports have shown that procyanidins extracted from grape seeds are capable of stimulating neurotrophic factors in aged rats(47). These compounds have also been implicated in the regulation of metabolic disorders(48,49) that are BDNF dependent. The N31 used in the present study had relatively high polyphenol levels compared with the coffee fruit, coffee caffeine and coffee seed extracts, yet failed to significantly increase BDNF in blood.

This result suggests that the stimulatory effect of WCFC on the blood level of BDNF is not associated with the amount of polyphenols or caffeine per dose. Rather, the effect may be related to either the amount of procyanidins or to the unique coffee polyphenol profile of the WCFC material. According to the present analyses, WCFC shows a significant amount of procyanidins in comparison to N31, N625 and N677 (Table 1), suggesting that acute treatment with procyanidin-rich whole coffee fruit extracts (and possibly other procyanidin-rich extracts), may increase blood levels of BDNF in human subjects. Future work could include testing other procyanidin-rich extracts for their ability to raise plasma BDNF in order to confirm this hypothesis.

The amount of trigonelline also varied in each material tested (Table 1). As presented, WCFC and N625 contain the highest amount of this compound; however, the effect of WCFC on plasma BDNF is superior to the effect of N625 under the same experimental conditions, suggesting that this is not the primary agent responsible for increased BDNF levels in blood.

As WCFC contains high amounts of chlorogenic acid, it was hypothesised that this specific polyphenolic acid may cause an increase in blood level of BDNF. Consequently, we administered 50 mg of chlorogenic acid as a single dose to five healthy subjects. As presented in Fig. 2, chlorogenic acid did not increase blood level of BDNF. Consequently, we administered 50 mg. In order to confirm the results of the present pilot study, further clinical testing in a larger group is required.

Acknowledgements

The present study was funded by Futureceuticals, Inc. T. R.-I. conducted the present study, worked as the data and led the manuscript writing. C. S., L. H. and R. A. performed serum tests and helped in the data analysis. R. K. organised and helped to execute clinical protocol of the study. B. N. designed and conducted all the chemical analysis. Z. P. designed and directed the study. We would like to thank Michael Sapko for his help in editing the manuscript. All authors declare that they have no conflicts of interest.

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