Bergamot Polyphenolic Fraction Supplementation Improves Cognitive Functioning in Schizophrenia

Data From an 8-Week, Open-Label Pilot Study

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Abstract:

Background: Novel treatment strategies for cognitive dysfunctions may prevent long-term disability in patients with schizophrenia, and polyphenolic compounds might be a promising strategy. Bergamot (Citrus bergamia), a citrus fruit characterized by a high amount of flavonoids and flavonoid glycosides, may represent a potential nutraceutical approach to cognitive dysfunction. The present study was aimed to explore the efficacy of bergamot polyphenolic fraction (BPF) supplementation on cognitive/executive functioning in a sample of patients with schizophrenia receiving second-generation antipsychotics.

Methods: Twenty outpatients treated with second-generation antipsychotics assumed BPF at an oral daily dose of 1000 mg/d for 8 weeks. Brief Psychiatric Rating Scale, Wisconsin Card Sorting Test (WCST), Verbal Fluency Task-Controlled Oral Word Association Test, and Stroop Color-Word Test were administered.

Results: At end point, (week 8) BPF supplementation significantly improved WCST "perseverative errors" ($P = 0.004$) and semantic fluency test ($P = 0.004$). Moreover, a trend for other cognitive variable (WCST "categories," phonemic fluency, and Stroop Color-Word Test) improvement was observed.

Conclusions: The findings provide evidence that BPF administration may be proposed as a potential supplementation strategy to improve cognitive outcome in schizophrenia. Further clinical trials with adequately powered and well-designed methodology are needed to better explore the BPF effectiveness on cognitive impairments in patients with schizophrenia.

Key Words: polyphenol, bergamot polyphenolic fraction, schizophrenia, cognition, executive functions

(*J Clin Psychopharmacol* 2017;37: 468–471)

Cognitive deficits have been consistently reported in patients with schizophrenia and are currently viewed as a core feature of the illness.[1] It has been evidenced that cognitive impairment seems to account for the heterogeneity of outcomes in schizophrenia more effectively than symptoms, because it has been related to functional and community outcome and other illness features, such as subjective and objective quality of life, course of illness, and relapses.[2] A substantial amount of research has mainly focused on the construct of executive functions, which includes goal-oriented processes, cognitive flexibility, abstract reasoning, problem solving, selective attention, inhibitory control, and the ability to organize and adaptively use information contained in working memory.[3] Such deficits are viewed as related to dysfunctions in the dorsolateral prefrontal cortex, and in dopamine, glutamate, and γ-aminobutyric acid systems,[4] probably reflecting a basic abnormality of "wiring" derived from abnormal neurodevelopment or from neurodegenerative processes. Moreover, emerging evidence indicates that inflammatory processes may be relevant for the impairment of cognitive functioning in schizophrenia, by a direct action of inflammatory markers (IL-1β, IL-6, tumor necrosis factor-α, and C-reactive protein), or via a secondary increase in proinflammatory activity induced by a high production of kynurenic acid.[5] Because first- and second-generation antipsychotics (SGAs) are mainly neutral toward cognitive symptoms,[6] treatment options aimed at addressing cognitive dysfunctions represent a priority. Recently, research has focused on neuroprotective agents and/or drugs that may improve neuroplasticity or neurogenesis, such as omega-3 fatty acids, anti-inflammatory agents (eg, acetylsalicylic acid and celecoxib), and antioxidants, (eg, N-acetyl cysteine).[7]

Nevertheless, none of these agents has demonstrated compelling efficacy on cognitive functioning.

Within this context, the nutraceutical approach might be a promising strategy.

In vitro and in vivo studies[8,9] have investigated the putative neuroprotective activity of polyphenols in preventing neurodegenerative diseases and the potential to promote memory, learning, and cognitive functions through a number of mechanisms including antioxidant, anti-inflammatory, and chelating activities and reduction of amyloid-β fibrils formation.[10,11]

Bergamot (Citrus bergamia), a typical fruit growing almost exclusively in Calabria (Italy), along the southern east coast, is characterized by a high amount of flavonoids and flavonoid glycosides (neoeriocitrin, neohesperidin, naringin, rutin, neodesmin, rhoifolin, and poncirin).[12] It has been shown that bergamot flavonoid reduces lipopolysaccharide-induced inflammatory response in THP-1 monocytes, through SIRT1-mediated nuclear factor-κB inhibition.[13,14] For these features, bergamot and bergamot polyphenolic fraction (BPF) may represent a potential nutraceutical approach to cognitive dysfunction.

To the best of our knowledge, no trials have been performed to examine the effect of BPF on cognitive deficits in patients with schizophrenia. On the basis of evidence from the literature, the present study was aimed to explore the efficacy of BPF treatment on cognitive/executive functioning in a sample of patients with schizophrenia receiving SGAs.

METHODS

Study Design

This was an 8-week, open-label, preliminary study aimed to evaluate the efficacy on cognition of adjunctive BPF to SGAs therapy in schizophrenia. Bergamot polyphenolic fraction (H&AD s.r.l, Bianco [RC], Italy) was administered at the oral daily dose of

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Received February 2, 2017; accepted after revision April 12, 2017.

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ISSN: 0271-0749
DOI: 10.1097/JCP.0000000000000730

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1000 mg/day (500 mg twice daily) and was maintained unchanged until the end of the trial at week 8. The concentration of 5 main flavonoids per capsule was neoeriocitrin (55,535 mg), naringin (58,903 mg), neohesperidin (62,966 mg), melitidin (7958 mg), and brutieridin (24,371 mg); excipients of any type were not present. During the study, no additional medications, including aspirin or nonsteroidal anti-inflammatory drugs, were allowed. The study was carried out at the Psychiatry Unit of the University Hospital of Messina, Italy. The protocol has been approved by the ethics committee of the University of Messina.

Subjects

Twenty outpatients, 15 men and 5 women, aged 20 to 58 years, who met Diagnostic and Statistical Manual of Mental Disorders-5 criteria for schizophrenia, were included in this study. All patients had been on SGAs therapy for at least 6 months; the dose had been stable for at least 3 months before the study and was left unchanged throughout the study. The patients did not receive any additional antidepressant or anticonvulsant drugs for a period of 2 months before the study. Patients with any significant concurrent medical illnesses, organic brain disorder, mental retardation, pregnant or lactating women, or a current diagnosis of alcohol/drug dependence were excluded. All the patients provided written informed consent after a full explanation of the protocol design, and the study was conducted according to the Declaration of Helsinki.

Assessment

Patients attended the following 4 visits: initial screening (week 1), inclusion (day 0), and 2 further visits at weeks 4 and 8. Clinical symptoms were assessed by the Brief Psychiatric Rating Scale (BPRS).[15] Although interrater reliability for these assessments was not established by formal training, all assessments were conducted by 2 senior psychiatrists with at least 5 years of clinical experience who were well versed with the use of the rating scales; each patient had the same person administering clinical and neuropsychological tests and conducting clinical interviews. Cognitive/executive functioning was assessed with the Wisconsin Card Sorting Test (WCST),[16] the Verbal Fluency Task, Italian translation (phonemic fluency: letters P/L/F; semantic fluency: fruits/animals/clothes),[17] and the Stroop Color-Word Test.[18] Data for clinical and neuropsychological assessments were collected at weeks 0 and 8; the design of inter-test intervals has been chosen for reducing possible sources of bias that may affect a person's performance on executive and cognitive tasks. It should be considered that the reliability of neuropsychological retesting may be biased by practice effects, defined as increase in a subject's test score due to recall effects, procedural learning, or growing familiarity with the testing environment.[19,20] Regarding the different cognitive domains, executive functions showed highest score increases over time as a result of a higher repetition rate or the use of less alternate forms.[20] Adverse effects and extrapyramidal symptoms, either observed or spontaneously reported, were recorded at each visit and classified in terms of onset, duration, severity, action taken, and outcome. To monitor the adherence to the study protocol, weekly telephone calls during the study period and a pill count on the last day (day 60) were carried out.

Statistical Analysis

Because this was a pilot study, no formal sample size calculation was performed. Data obtained from the study underwent check and quality control and, subsequently, to descriptive and inferential statistical analysis. Because of the small sample size, the analyses were carried out by nonparametric tests. An intention-to-treat analysis with last-observation-carried-forward was performed. Continuous data were expressed as mean (SD) and the within-group differences in efficacy ratings between baseline and final test were analyzed by the Wilcoxon rank sum test. To measure the magnitude of a treatment effect, effect size was provided by using Cohen d statistic. Taking into account that multiple correlations increase the risk of type 1 errors, a Bonferroni correction was applied, and a significance value of $P < 0.006$ was chosen. The statistical analysis was performed with SPSS 16.0 software (SPSS Inc, Chicago, Ill).

RESULTS

The study sample's baseline characteristics, duration of illness, antipsychotics type, and daily dose are detailed in Table 1. Seventeen patients completed the study (85% completion rate); there were 3 premature dropouts, 1 due to treatment-emergent adverse effects (heartburn) and 2 because of noncompliance with the visits.

Treatment Response

Table 2 shows the baseline and final scores of the different neuropsychological measures, and the effect size for the sample group. Regarding cognitive performances, at end point (week 8) BPF supplementation significantly improved WCST perseverative errors ($P = 0.004$), and semantic fluency test ($P = 0.004$). Moreover, a trend for other cognitive variable (WCST categories, phonemic fluency, and Stroop Color-Word Test) improvement was observed.

Effect sizes were moderate in WCST perseverative errors, semantic fluency, and Stroop task scores, and small in other explored cognitive dimensions.

Adverse Effects

The BPF adjunctive treatment was well tolerated at the dose of 1000 mg/d; only 1 subject (5%) presented adverse effects due to the treatment (heartburn), which regressed after BPF suspension. Moreover, there was no significant change in the BPRS total score (BPRS total score T0 vs T1: 38.05 (8.6) vs 34.20 (5.8); $z = -2.553$; $P = 0.011$), suggesting that psychopathological symptoms did not worsen during the trial and confirming previous studies on safety profile of bergamot extract administration in subjects receiving atypical antipsychotics.[21] Finally, no acute extrapyramidal effects, seizures, or cardiac events occurred.

DISCUSSION

To our knowledge, this is the first report of a clinical trial with BPF supplementation and cognition in patients with schizophrenia. In the present open-label, uncontrolled trial we found that

TABLE 1. Demographic and Clinical Features of Study Subjects

Patients entered (completers)	20 (17)
Sex (M/F)	15/5
Age, mean (SD), y	43.5 (10.1)
Educational level, mean (SD), y	10.1 (2.7)
Duration of illness, mean (SD), y	18.2 (8.9)
SGAs	**n**
Olanzapine (15–30 mg/d)	6
Clozapine (250–550 mg/d)	5
Quetiapine (250–600 mg/d)	5
Risperidone (3–4 mg/d)	4

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TABLE 2. Cognitive Functioning Changes and Effect Sizes for Efficacy Measures in Patients Receiving BPF at Baseline and Week 8 (Last-Observation-Carried-Forward)

	Baseline (T0)		Week 8 (T1)		Wilcoxon Test, T0 vs T1		
	Mean	SD	Mean	SD	z	*P*	Cohen d
WCST							
Perseverative errors	28.20	18.254	17.10	15.17	−2.855	0.004	0.7
Nonperseverative errors	23.45	19.403	19.35	21.09	−1.350	0.177	0.2
Categories	3.45	2.35	4.30	2.39	−1.922	0.055	0.3
Phonemic fluency	22.15	7.16	24.55	6.63	−1.969	0.049	0.3
Semantic fluency	30.25	8.46	34.10	7.22	−2.849	0.004	0.5
Stroop Test	64.00	42.78	42.53	17.92	−2.586	0.010	0.6

BPF administration was associated with a substantial improvement of cognitive executive functioning, particularly WCST domains, except for categories, and semantic fluency, a subtask of the verbal fluency. The lack of categories improvement is probably a function of the small sample size, because the participants got one more category correct at retest. Furthermore, a positive trend toward improvement was observed in attentional resistance to interference stimuli, (Stroop task), and phonemic fluency subtask. The examined cognitive functions may have a role in global functioning and outcome, because the processes underlying the WCST task are organized searching, strategic planning, inhibiting impulsive responses, mental sets shifting, and directing behavior toward achieving a goal.[3] The process of word generation involved in the verbal fluency task requires the integrity of attention and verbal declarative memory, and an executive ability to coordinate the word selection process, also subtended by working memory for performance monitoring. Regarding the Stroop test, attentional resistance to interference is a cognitive ability based on the maintenance of goal-oriented behaviors.

Our findings are barely comparable with the existing literature, because no studies have specifically addressed the effect of BPF on cognitive functioning in patients with schizophrenia. Bergamot is characterized by a high amount of flavonoids and flavonoid glycosides, particularly naringin and neohesperidin.[12]

In the past years, it has been suggested that flavonoids may have neuroprotective effects and slow down the progression of degenerative diseases. Naringin and neohesperidin have been reported to attenuate cognitive impairment and behavioral deficits, and to improve learning and memory in animal models.[22,23] The mechanisms by which flavonoids probably exert their neuroprotective activities are not only associated with the modulation of specific antioxidant enzymes (antioxidant capacity), but also with additional pathways, such as antiapoptotic activity,[24] induction of neurotrophic factors,[25] and modulation of different signaling pathways that influence neuronal survival, differentiation, proliferation, and apoptosis.[10,11]

These mechanisms are probably involved in the pathophysiology of cognitive deficits in schizophrenia; a neurodegenerative progression in schizophrenia is suggested by increasing cognitive decline and clinical and functional deterioration.[26] Furthermore, markers of oxidative stress, along with microglial activation associated with neuronal survival and lowered neurotrophic support, have been described in patients with schizophrenia.[27] This neurodegenerative process, although rather limited than in Alzheimer disease, involves lowered neurogenesis, brain volume loss, and cortical atrophy, and it is driven, to a certain extent, by immunoinflammation and oxidative stress pathways.[28]

Available drug treatments for schizophrenia are effective for controlling several symptomatic clusters of the disease; nevertheless, they do not seem to significantly address the long-term clinical and biological outcomes of the disease. Thus, congruently with the neuroprogressive processes of the pathogenesis of schizophrenia, new targets for treatment interventions may be identified.

The study presents several limitations, such as the small sample size, the open design, and the lack of a control group; moreover, the short observation period does not permit to definitely rule out possible learning and practice effects on cognitive improvement although, as acknowledged in the Methods section, the chosen retest interval should have limited such potential bias.

Beyond the limitations, the findings of the present study suggest for the first time that BPF supplementation may represent a promising therapeutic strategy for addressing cognitive impairment in patients with schizophrenia.

Cognitive impairment is a core feature of schizophrenia, and it is associated with poor functional outcome. Therapeutic approaches aimed at improving cognition may be critical in preventing long-term disability in these patients. Bergamot polyphenolic fraction may be proposed as a potential supplementation strategy to address cognitive dysfunctions in schizophrenia. Future long-term, adequately powered clinical trials are needed to further explore possible neuroprotective and procognitive effects of BPF supplementation in patients with schizophrenia.

ACKNOWLEDGMENTS

The authors thank all the participants, the whole staff of the Section of Psychiatry, Department of Biomedical and Dental Sciences and Morphofunctional Imaging, University of Messina, Italy, for assistance with recruitment and data collection, and Dr Francesco Chirico, Agrumaria Reggina SRL, Reggio Calabria, Italy, for technical support.

AUTHOR DISCLOSURE INFORMATION

The authors declare no conflict of interest. This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

REFERENCES

1. Schaefer J, Giangrande E, Weinberger DR, et al. The global cognitive impairment in Schizophrenia: consistent over decades and around the world. *Schizophr Res*. 2013;150:42–50.
2. Strassnig MT, Raykov T, O'Gorman C, et al. Determinants of different aspects of everyday outcome in schizophrenia: the roles of negative symptoms, cognition, and functional capacity. *Schizophr Res*. 2015;165:76–82.
3. Alvarez JA, Emory E. Executive function and the frontal lobes: a meta-analytic review. *Neuropsychol Rev*. 2006;16:17–42.

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4. Barch DM, Ceaser A. Cognition in schizophrenia: core psychological and neural mechanisms. *Trends Cogn Sci*. 2012;16:27–34.
5. Meyer U, Schwarz MJ, Müller N. Inflammatory processes in schizophrenia: a promising neuroimmunological target for the treatment of negative/cognitive symptoms and beyond. *Pharmacol Ther*. 2011;132: 96–110.
6. Young JW, Geyer MA. Developing treatments for cognitive deficits in schizophrenia: the challenge of translation. *J Psychopharmacol*. 2015;29: 178–196.
7. Goff DC, Hill M, Barch D. The treatment of cognitive impairment in schizophrenia. *Pharmacol Biochem Behav*. 2011;99:245–253.
8. Heo HJ, Kim DO, Shin SC, et al. Effect of antioxidant flavanone, naringenin, from Citrus junoson neuroprotection. *J Agric Food Chem*. 2004;52:1520–1525.
9. Dai Q, Borenstein AR, Wu Y, et al. Fruit and vegetable juices and Alzheimer's disease: the Kame Project. *Am J Med*. 2006;119:751–759.
10. Spagnuolo C, Napolitano M, Tedesco I, et al. Neuroprotective Role of Natural Polyphenols. *Curr Top Med Chem*. 2016;16:1943–1950.
11. Costa SL, Silva VD, Dos Santos Souza C, et al. Impact of plant-derived flavonoids on Neurodegenerative Diseases. *Neurotox Res*. 2016;30:41–52.
12. Picerno P, Sansone F, Mencherini T, et al. Citrus bergamia juice: phytochemical and technological studies. *Nat Prod Commun*. 2011;6: 951–955.
13. Risitano R, Currò M, Cirmi S, et al. Flavonoid fraction of Bergamot juice reduces LPS-induced inflammatory response through SIRT1-mediated NF-κB inhibition in THP-1 monocytes. *PLoS One*. 2014;9:e107431.
14. Impellizzeri D, Bruschetta G, Di Paola R, et al. The anti-inflammatory and antioxidant effects of bergamot juice extract (BJe) in an experimental model of inflammatory bowel disease. *Clin Nutr*. 2015;34:1146–1154.
15. Overall JE, Gorham DR. The brief psychiatric rating scale. *Psychol Report*. 1962;10:799–812.
16. Heaton RK, Chelune GJ, Talley JL, et al. *Wisconsin Card Sorting Test Manual: Revised and Expanded*. Odessa, FL: Psychological Resources; 1993.
17. Spreen O, Strauss E. *A Compendium of Neuropsychological Tests: Administration, Norms, and Commentary*. 2nd ed. New York, NY: Oxford University Press; 1998.
18. Trenerry MR, Crosson B, DeBoe J, et al. *Stroop Neuropsychological Screening Test*. Odessa, FL: Psychological Assessment Resources Inc; 1989.
19. Goldberg TE, Goldman RS, Burdick KE, et al. Cognitive improvement after treatment with second-generation antipsychotic medications in first-episode schizophrenia: is it a practice effect? *Arch Gen Psychiatry*. 2007;64:1115–1122.
20. Bartels C, Wegrzyn M, Wiedl A, et al. Practice effects in healthy adults: a longitudinal study on frequent repetitive cognitive testing. *BMC Neurosci*. 2010;11:118.
21. Bruno A, Pandolfo G, Crucitti M, et al. Metabolic outcomes of bergamot polyphenolic fraction administration in patients treated with second-generation antipsychotics: a pilot study. *J Nutr Biochem*. 2017;40: 32–35.
22. Kumar A, Dogra S, Prakash A. Protective effect of naringin, a citrus flavonoid, against colchicine-induced cognitive dysfunction and oxidative damage in rats. *J Med Food*. 2010;13:976–984.
23. Wang D, Liu L, Zhu X, et al. Hesperidin alleviates cognitive impairment, mitochondrial dysfunction and oxidative stress in a mouse model of Alzheimer's disease. *Cell Mol Neurobiol*. 2014;34:1209–1221.
24. Cirmi S, Ferlazzo N, Lombardo GE, et al. Neurodegenerative diseases: might citrus flavonoids play a protective role? *Molecules*. 2016; 21:E1312.
25. Rong W, Wang J, Liu X, et al. Naringin treatment improves functional recovery by increasing BDNF and VEGF expression, inhibiting neuronal apoptosis after spinal cord injury. *Neurochem Res*. 2012;37:1615–1623.
26. Khandaker GM, Cousins L, Deakin J, et al. Inflammation and immunity in schizophrenia: implications for pathophysiology and treatment. *Lancet Psychiatry*. 2015;2:258–270.
27. Monji A, Kato TA, Mizoguchi Y, et al. Neuroinflammation in schizophrenia especially focused on the role of microglia. *Prog Neuropsychopharmacol Biol Psychiatry*. 2013;42:115–121.
28. Anderson G, Maes M. Metabolic syndrome, Alzheimer disease, Schizophrenia, and depression: role for leptin, melatonin, kynurenine pathways, and neuropeptides. In: Farooqui T, Farooqui AA, eds. *Metabolic Syndrome and Neurological Disorders*. Chichester, West Sussex, UK: John Wiley and Sons; 2013:235–248.

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