Effects of resveratrol on cerebral blood flow variables and cognitive performance in humans: a double-blind, placebo-controlled, crossover investigation

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ABSTRACT

Background: The many putative beneficial effects of the polyphenol resveratrol include an ability to bolster endogenous antioxidant defenses, modulate nitric oxide synthesis, and promote vasodilation, which thereby improves blood flow. Resveratrol may therefore modulate aspects of brain function in humans.

Objective: The current study assessed the effects of oral resveratrol on cognitive performance and localized cerebral blood flow variables in healthy human adults.

Design: In this randomized, double-blind, placebo-controlled, crossover study, 22 healthy adults received placebo and 2 doses (250 and 500 mg) of trans-resveratrol in counterbalanced order on separate days. After a 45-min resting absorption period, the participants performed a selection of cognitive tasks that activate the frontal cortex for an additional 36 min. Cerebral blood flow and hemodynamics, as indexed by concentration changes in oxygenated and deoxygenated hemoglobin, were assessed in the frontal cortex throughout the posttreatment period with the use of near-infrared spectroscopy. The presence of resveratrol and its conjugates in plasma was confirmed by HPLC after the same doses in a separate cohort (n = 9).

Results: Resveratrol administration resulted in dose-dependent increases in cerebral blood flow during task performance, as indexed by total concentrations of hemoglobin. There was also an increase in deoxyhemoglobin after both doses of resveratrol, which suggested enhanced oxygen extraction, that became apparent toward the end of the 45-min absorption phase and was sustained throughout task performance. Cognitive function was not affected. Resveratrol metabolites were present in plasma throughout the cognitive task period.

Conclusion: These results showed that single doses of orally administered resveratrol can modulate cerebral blood flow variables. Am J Clin Nutr 2010;91:1590–7.

INTRODUCTION

The possibility that resveratrol (3,4’,5 trihydroxystilbene)—a phytoalexin polyphenol (1)—may offer protection against many diseases in humans, including cardiovascular (2) and neurodegenerative (3) diseases, atherosclerosis (4), and cancer (5), has been suggested by in vitro and in/ex vivo studies.

In vivo studies in rodents have shown that resveratrol can preserve brain function in aged (6) and brain-damaged (7–10) rats. Potential neuroprotective properties include resveratrol’s ability to promote antioxidant defenses by regulating a host of antioxidant enzymes (11–13). This may be partly a consequence of its ability to activate the Nrf2 transcription factor, and therefore cellular redox status (13), and modulate nitric oxide (NO) synthesis (14). Resveratrol may also benefit central nervous system function directly by improving blood flow and perfusion. In this respect, it has been shown that resveratrol beneficially modulates platelet aggregation (15) and platelet NO synthesis (16) and enhances endothelium-dependent (17) vasorelaxation by promoting endothelial NO synthase (eNOS) and/or NO synthesis. Vasorelaxation has been seen in vitro (18–21) and in vivo after oral administration to rats (18, 19). Resveratrol, in combination with other red wine polyphenols, has also been shown in vivo to increase cerebral arteriole dilation in rats (22) and improve postischemic cerebral perfusion after both acute (23) and chronic (24) doses. Tsai et al (25) observed that the in vivo protective properties of resveratrol after ischemia in rats were related to concomitant down-regulation of the expression of inducible NO synthase (iNOS) and up-regulation of vasorelaxant eNOS.

Brain function is dependent on increased delivery of blood-borne metabolic substrates to active tissue. NO is a key vasodilatory mediator of the neurovascular coupling of neuronal activity to increased blood supply in active tissue (26, 27) and is released from cortical neurons in an activity-dependent manner (28). The effects of resveratrol may therefore be more prevalent during periods of local neural demand when NO synthesis drives vasodilation.

In humans, orally administered resveratrol is rapidly metabolized into glucuronide and sulfate conjugates, with the parent molecule and metabolites initially peaking in the plasma 30–90 min after dosing (29–32). Given that resveratrol itself has low bioavailability in humans, the direct relevance of in vitro research involving high concentrations of the parent molecule has been...
RESVERATROL AND CEREBRAL HEMODYNAMICS

Participants

Nine healthy men (mean age: 24.8 y; range 21–29 y) took part in the bioavailability assessment. All participants either worked or studied at Northumbria University and had either attained, or were enrolled in, an undergraduate degree–level course. All participants attended the laboratory having had nothing to eat or drink (except water) since the previous evening and no resveratrol-containing products for 24 h. All participants reported themselves to be in good health and to not be using social drugs, alcohol, prescription medication, and herbal extracts/food supplements. Participants who had suffered a head injury, neurologic disorder, or neurodevelopmental disorder were excluded from participation, as were those who had any relevant food allergies or intolerances, smoked tobacco, drank excessive amounts of caffeine (>6 cups coffee/d (>450 mg caffeine/d)), or took illicit social drugs. One participant was excluded from the analysis of bioavailability data following the ingestion of 500 mg trans-resveratrol because of failure to observe fasting requirements.

Treatments

On each of 2 visits, the participants consumed 2 identical capsules containing either an inert placebo or 250 mg trans-resveratrol. The capsules were combined to give a dose of either 250 mg or 500 mg trans-resveratrol. The treatments were consumed on separate occasions 7 ± 2 d apart. The natural, pure trans-resveratrol was purchased from Biotivia Bioceuticals (Vienna, Austria). The purity of the extract (99.02%) had been confirmed by HPLC for the manufacturer’s certificate of analysis.

Procedure

Participants attended the laboratory at 0830 on 2 separate occasions, receiving a different treatment on each occasion. Venous blood samples were collected by using 4.7-mL monovettes (containing lithium heparin) before the day’s treatment was consumed and 45, 90, and 120 min after consuming the day’s treatment.

Bioavailability assessment

Samples were prepared by using the method described previously for human plasma (32). The HPLC system consisted of a Dionex GS50 pump, an AS50 autosampler, and an AD25 Absorbance detector with ultraviolet detection (Dionex UK Ltd, Camberley, United Kingdom) carried out at 325 nm. The HPLC system and detector were controlled by using Dionex Chromleon software. The mobile phase consisted of 5 mmol ammonium acetate/L containing 2% propan-2-ol (A) and methanol with 2% propan-2-ol (B). Chromatographic separation was accomplished by injecting the samples onto a Synergy 250 mm × 4.6 mm, 4-μm C18 column. The temperature of the column was set at 40°C with a flow rate of 1 mL/min. A gradient elution was carried out as follows: 0 min, 0% B; 4 min, 20% B; 7 min, 20% B; 18 min, 55% B; 22 min, 65% B; 24 min; then equilibration with 100% A for 6 min before the next injection. Identification of resveratrol conjugates was carried out by incubating serum samples with β-glucuronidase and sulfatase as described previously (34) and analyzed by HPLC as described above. Quantification of resveratrol was carried out by using standards ranging from 4 to 250 ng/mL. However, the quantities of resveratrol conjugates were calculated on the basis of the assumption that recovery characteristics and relation between peak area ratios and concentrations were the same as those for resveratrol. Metabolite concentrations are therefore described as “resveratrol equivalents.”

CBF and cognitive performance assessment

Participants

Twenty-four healthy adults (4 men, 20 women; mean age: 20.17 y; age range: 18–25 y; 21 right-handed, 3 left-handed) took part in the CBF/cognitive performance assessment. The inclusion/exclusion criteria were as reported for the bioavailability study (see above), with the exception that there was no restriction on resveratrol-containing products until the fasted period began the evening before testing. The data from 2 participants were excluded from the analysis of the CBF/cognitive performance data on the basis of failure to complete the study.

The study received ethical approval from the Northumbria University School of Psychology and Sport Sciences Ethics Committee and was conducted according to the Declaration of Helsinki (1964). All participants gave their informed consent before their inclusion in the study.

Treatments

During the 3 study visits, the participants received 3 single-dose treatments in an order dictated by random allocation to a counterbalancing (Latin Square) order. The 3 treatments consisted of 2 capsules, each containing either placebo or 250 mg trans-resveratrol (as described above). The capsules were combined to give the following treatments: 1) inert placebo, 2) 250 mg trans-resveratrol, and 3) 500 mg trans-resveratrol. The treatments were administered in identical size-0 vegetable capsules, which were prepared and coded by a third party who had no further involvement in any aspect of the study. No member of the investigational team was aware of the contents of the capsules until a blind-data review was completed.
NIRS

Functional NIRS is a brain-imaging technique that is predicated on the intrinsic optical absorption properties of oxygenated hemoglobin (oxy-Hb) and deoxygenated hemoglobin (deoxy-Hb) after the introduction of near-infrared light through the intact skull. When assessed by NIRS, the increase in CBF in the surface layers of the cortex during localized neural activity is seen as an increase in the total concentration of hemoglobin (total-Hb) and comparative decrease in deoxy-Hb (35), with both variables corresponding strongly with the functional MRI BOLD signal (35–37). NIRS has been used extensively as a technique for multiple-channel imaging of task-related brain activity over relevant areas of the head (38), including in groups suffering from potential decrements in CBF (39). To date, a small number of pharmacologic intervention studies have also used the technique to infer localized brain activity (40) and CBF and oxygenation (41) from changes in hemoglobin concentrations.

In the current study, relative changes in the absorption of near-infrared light were measured at a time resolution of 10 Hz by using a 12-channel Oxymon system (Artinis Medical Systems BV, Zetten, Netherlands). The system emitted 2 nominal wavelengths of light (765 and 855 nm) with an emitter/optode separation distance of 4 cm. The differential pathlength factor was adjusted according to the age of the participant. Relative concentration changes in oxy-Hb, deoxy-Hb, and total-Hb were calculated by means of a modified Beer-Lambert law (42) with the proprietary software.

In this study, given the extended recording period and the investigational aims, a simple 2-emitter/optode pair configuration was used (ie, 2 channels). The emitter/optode pairs were positioned over the left and right frontal cortex by using a standard optode holder headband, which separated the pairs from each other by 4 cm. Each pair therefore collected data from an area of prefrontal cortex that included the areas corresponding to the International 10–20 system Fp1 and Fp2 EEG positions. The NIRS data output was time stamped at the start of each task segment to ensure that data corresponded to the relevant epoch of task performance.

Cognitive tasks

The 3 tasks that were used were previously shown to activate the prefrontal cortex in brain-imaging studies (43, 44). The objective of this collection of tasks was generally to assess the effect of the treatment on speed/accuracy and mental fatigue during continuous performance of cognitively demanding or “effortful” tasks. Multiple completions of the 9-min battery of tasks (see below) was previously shown to reliably increase self-ratings of mental fatigue and to be sensitive to many natural interventions (45–48). The 9-min battery consists of 4-min Serial Subtraction, 5-min Rapid Visual Information Processing (RVIP), and a Mental Fatigue Visual Analogue Scale.

The original verbal Serial Sevens test has appeared in many forms, including as part of the Mini-Mental State Examination for dementia screening. In the current study, a modified, 4-min, computerized version of the Serial Subtraction task was used (49), which consists of 2 min of Serial 3s followed by 2 minutes of Serial 7s subtractions. At the start of each 2-min section, a standard instruction screen informed the participants to count backward in 3 or 7 s, as quickly and accurately as possible, using the keyboard’s linear number keys to enter each response. Participants were also instructed verbally at the outset that if they were to make a mistake they should continue subtracting from the new incorrect number. A random starting number between 800 and 999 was presented on the computer screen, which was cleared by the entry of the first response. Each 3-digit response was represented on screen by an asterisk. Pressing the enter key signaled the end of each response and cleared the 3 asterisks from the screen. Performance data (total number of subtractions and number of errors) were calculated for the Serial 3s and 7s elements separately. In the case of incorrect responses, subsequent responses were scored as positive if they were correct in relation to the new number.

The RVIP task has been widely used to study the cognitive effects of psychotropic drugs. The participant monitors a continuous series of single digits for targets of 3 consecutive odd or 3 consecutive even digits. The digits are presented on the computer screen at the rate of 100/min in pseudo-random order, and the participant responds to the detection of a target string by pressing the space bar as quickly as possible. The task is continuous and lasts for 5 min, with 8 correct target strings being presented in each minute. The task is scored for number of target strings correctly detected, average reaction time for correct detections, and number of false alarms.

With the mental fatigue visual analogue scale, participants rated their subjective feelings of mental fatigue via an on-screen 100-mm visual analogue scale with the endpoints labeled as “not at all” and “extremely.” The scale was scored as a percentage along the line toward “extremely.” The tasks were presented by using the COMPASS cognitive assessment system (Northumbria University, Newcastle, United Kingdom).

Procedure

Each participant was required to attend the laboratory on 4 occasions. The first of these occasions was an initial screening/training visit, which was followed within 14 d by the first active study morning. During the initial visit, participants provided written informed consent and were screened regarding the study exclusion/inclusion criteria. Training was given on the cognitive tasks, and the compliance requirements for the following visit were explained.

On the 3 active study mornings, which were conducted 7 d apart, participants attended the laboratory between 0800 and 1000 in a fasted state and provided confirmation of continued compliance with the inclusion/exclusion requirements. Before taking their treatment for that day, the participants were fitted with the NIRS headband and completed a single repetition of each of the cognitive tasks and completed one rating of their mental fatigue to establish baseline performance and subjective state. After this, the participants sat quietly for 5 min, with the last 3 min of this period used as the NIRS resting baseline measurement. Participants then consumed their treatment for that day and sat quietly, watching one of a selection of nonarousing DVDs, during a 45-min “absorption” period. They were then verbally instructed to start the period of task performance and made 4 consecutive repetitions of the Serial Subtractions and RVIP tasks (ie, 36 min of continuous performance). NIRS data were captured throughout. The timelines and running order of the testing session are shown in Figure 1.
resting phase used to baseline-adjust all posttreatment data.

spectroscopy (NIRS) data were collected throughout, with the pretreatment Information Processing (RVIP) tasks 4 times in succession. Near-infrared absorption period, they completed the serial subtraction and Rapid Visual period, they received their day’s treatment, and after a 45-min resting/absorption period before the start of the tasks, but thereafter the higher dose (500 mg) resulted in significantly higher total-Hb concentrations during the epochs spanning 46–49 min, 55–58 min, and 73–76 min (all $P < 0.01$ for all epochs, except $P < 0.05$ for 68 to 72 min). Similar differences after the lower dose (250 mg) were restricted to significantly higher total-Hb concentrations during the epochs

Any differences in hemodynamic responses to the Serial Subtractions and RVIP tasks were explored with additional within-subjects ANOVAs [task (Serial Subtractions, RVIP) × repetition (1–4) × treatment] by using data averaged over each cognitive task epoch.

Task performance data were analyzed as the change from predose baseline for each individual task (Serial 3s, Serial 7s, RVIP, and mental fatigue scales) by within-subjects ANOVA (treatment × repetition), with planned comparisons for data from each repetition as described above.

In the absence of any directly relevant data, the required sample size was calculated as the multiple of 6 (for counterbalancing purposes) that exceeded 80% power to detect the medium effect sizes, in terms of cognitive outcomes, seen previously after psychoactive phytochemical extracts. The resultant sample size of 24 (for a within-subjects, crossover design) was in excess of the typical sample sizes for NIRS investigations.

**RESULTS**

**Bioavailability**

Mean plasma concentrations of trans-resveratrol and its conjugates before dosing and 45, 90, and 120 min after dosing are shown in Figure 2.

**NIRS variables**

**Total hemoglobin**

ANOVA showed that there was a significant interaction between the postdose epoch and treatment ($P < 0.05$). Reference to the planned comparisons showed that there were no significant differences in concentrations of total-Hb during the resting/absorption period before the start of the tasks, but thereafter the higher dose (500 mg) resulted in significantly higher total-Hb during each task period epoch in comparison with placebo ($P < 0.01$ for all epochs, except $P < 0.05$ for 68 to 72 min). Similar differences after the lower dose (250 mg) were restricted to significantly higher total-Hb concentrations during the epochs spanning 46–49 min, 55–58 min, and 73–76 min (all $P < 0.05$).
Oxygenated hemoglobin

The ANOVA showed that there was no significant pattern of treatment-related effects on concentrations of oxy-Hb during either resting or task performance periods.

Deoxygenated hemoglobin

There was a significant interaction between treatment and posttreatment epoch on the initial ANOVA \((P < 0.01)\). Reference to the planned comparisons showed that both the 250-mg and 500-mg doses of resveratrol led to significantly higher deoxy-Hb concentrations, in comparison with placebo. This was evident during the 21–25-min epoch for the 500-mg dose and the last two 5-min epochs of the resting/absorption period for the 500-mg \((P < 0.05)\) and 250-mg resveratrol doses \((36–40\) min: \(P < 0.05; 41–45\) min: \(P < 0.01)\). Both doses of resveratrol also resulted in higher deoxy-Hb concentrations during each epoch of task performance \((all P < 0.01, except P < 0.05 for 59–63 min with the 500-mg dose)\). The mean \((\pm SEM)\) data and the results of the planned comparisons for those measures that evinced a significant difference on the initial ANOVA (total-Hb and deoxy-Hb) are represented in Figure 3.

Task-related differences

There were no significant differences seen in the hemodynamic response to the Serial Subtractions and RVIP tasks.

Cognitive task performance and mental fatigue

There were no significant, treatment-related differences on these measures.

DISCUSSION

In the current study, the overall pattern of task-related changes in local hemoglobin concentrations, irrespective of treatment, was as expected \((42, 50)\), with increases in total-Hb and oxy-Hb and decreases in deoxy-Hb during cognitive task performance. In comparison with placebo, the consumption of resveratrol resulted in a dose-dependent pattern of higher CBF in the prefrontal cortex.

**FIGURE 3.** Mean \((\pm SEM)\) changes in concentrations of total hemoglobin and deoxyhemoglobin during a 45-min absorption period and a subsequent 36-min period of cognitive task performance after placebo or 250 or 500 mg trans-resveratrol. The study followed a crossover design \((n = 22 per condition)\). Data are averaged across 5-min [absorption period; Rapid Visual Information Processing (RVIP)] or 4-min [serial subtraction (SS) tasks] epochs. Repeated-measures ANOVA showed a significant treatment × epoch interaction \((P < 0.05)\) for both total hemoglobin and deoxyhemoglobin. A priori planned comparisons of data from each resveratrol group with data from the placebo group for each epoch were carried out with \(t\) tests incorporating mean squares error from the ANOVA. ***Significantly different from placebo: \(*P < 0.05, **P < 0.01.***
during the cognitive tasks, as indexed by changes in the concentration of total-Hb. Both doses of resveratrol also resulted in significantly higher concentrations of deoxy-Hb than those seen in the placebo condition, with this effect becoming evident toward the end of the 45-min resting/absorption period and being sustained throughout the subsequent 36-min period of task performance. In this instance, there were no significant treatment-related differences in performance of the cognitive tasks or participants’ ratings of mental fatigue. The bioavailability data obtained from the separate cohort confirmed the presence of resveratrol and metabolites in plasma after both doses of resveratrol. Whereas the parent compound had low (but measureable) bioavailability, the glucuronidated and sulfated conjugates were present in much higher concentrations at a time point corresponding to the start of the cognitive tasks (45 min). Concentrations, particularly of the sulfated conjugate, continued to rise in a dose-dependent manner up to the 90-min postdose time point that corresponded to the end of the cognitive tasks.

With regard to the pattern of treatment-related hemodynamic changes, concomitant increases in total-Hb and deoxy-Hb were previously seen after vinpocetine (41)—a semisynthetic alkaloid treatment for stroke and vascular dementia that increases CBF (52) via many mechanisms (41). In terms of higher total-Hb concentrations, the modulation seen in the current study was likely due to the vasorelaxatory properties of resveratrol, which have been attributed to an interaction with aspects of NO synthesis (18–21). Given that NO is a key vasodilator mediator of local blood supply during activity in neural tissue (26, 27), it was suggested here that the effects of resveratrol would be seen during the task performance period, as opposed to the resting period. This was the case, with a sharp, linear, dose-related separation in concentrations of total-Hb for the ascending doses of resveratrol from the outset of the tasks. This pattern of results may suggest the involvement of NO synthesis, although it is also possible that many unmeasured variables related to, for instance, hormones and neurotransmitters that have an effect on vasodilation, could also have underpinned the effects seen here. Both of the tasks used in the study were previously shown to activate the frontal cortex in imaging studies (43, 44). Therefore, the question of the specificity of the CBF effect to periods of task performance could be investigated further by the addition of a “resting” condition with no task performance, the inclusion of “nonfrontal” tasks, additional NIRS channels covering areas of cortex that should not have been activated by these specific tasks, or the concomitant measurement of variables related to total CBF by using transcranial Doppler.

Concentrations of deoxy-Hb were also significantly higher after both doses of resveratrol than after placebo, which suggests increases in oxygen extraction and utilization (41). This observation finds some support in reports that resveratrol has a beneficial effect on mitochondrial function and biogenesis, for instance via the activation of peroxisome proliferator-activated receptor γ coactivator 1z and expression of sirtuin and AMP-activated protein kinase (53, 54) and has been shown to directly increase aerobic capacity and the consumption of oxygen in normal mice (54) and when combined with exercise in accelerated-senescence mice (55). It is also noteworthy that the measurement of deoxy-Hb with NIRS is not sensitive to the extracerebral changes in blood flow variables, such as general increases in blood pressure and modulated blood flow in the scalp, which may contribute in part to modulation of total-Hb (50). In this instance, the pattern of modulation of deoxy-Hb was somewhat more marked than that for total-Hb, which suggests that the treatment-related hemodynamic changes were taking place directly in the cortical tissue under investigation. Similarly, given that the effects on total-Hb did not become apparent until the task performance commenced, it seems unlikely that they reflect general extracerebral effects on any other blood flow variables.

Reference to the bioavailability data suggests that the metabolites (and low concentrations of the parent) would have been present in plasma at the start of the cognitive task period and that concentrations rose steeply thereafter to a peak somewhere in the vicinity of the end of the task period. This raises the possibility that the effects on CBF might have, in part, been due to the start of the tasks coinciding with the concentrations reaching levels sufficient to modulate hemodynamic variables. This interpretation is supported by the significant differences in deoxy-Hb seen in the 10 min before the start of the cognitive tasks after both doses of resveratrol. Given that the bioavailability data were collected in a separate, smaller (n = 9) cohort of healthy adults, it could certainly be argued that the data from the 2 investigations are not directly comparable. However, the contribution of increased neural activity to the effects seen here deserves further investigation.

One key issue regarding resveratrol and other polyphenols is that of the low bioavailability of the parent molecule in humans. Whereas the relevance of much of the in vitro literature to oral consumption by humans has been questioned, there is a plethora of research showing in vivo modulation of multifarious variables in animal models (33). The results here confirm that orally administered resveratrol can modulate brain function in humans. Whether this is as a consequence of the very low concentrations of the parent molecule seen here in plasma, the action of the much higher concentrations of its glucuronide and sulfate conjugates or other metabolites, or the conversion of these metabolites back to the parent form once they reach target tissues (32, 56) remains to be elucidated.

Natural aging is associated with impaired NO-mediated vasodilation, and reduced CBF (57) and the etiologies of many neurologic disorders, such as Alzheimer disease, vascular dementia, and stroke, include deficits in CBF (58). The results of the current study provide the first indication in humans that resveratrol may be able to modulate CBF variables. Thus, it seems reasonable to suggest that the potential effects of this molecule on brain function deserve a great deal more research attention with a clear focus on both healthy humans and pathologic groups.

The authors’ responsibilities were as follows—DOK, ELW, CFH, JLR, GL, EJO, and AW: actively involved in the planning of the research described herein and in writing the manuscript; EW: collected the data; GL and EJO: planned and supervised the analysis; and AW: conducted the analysis of the plasma samples. All authors contributed to and reviewed the final publication. None of the authors had any conflict of interests with regard to the research described in this article.

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