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Red Wine Ingredient Resveratrol Protects from β-Amyloid Neurotoxicity

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Key Words
Resveratrol · Red wine · Neuroprotection · β-Amyloid · Alzheimer's disease

Abstract
Background: β-Amyloid peptide (Aβ), a neutrotoxic substance, has been implicated to a great degree in cell death during the course of AD. Resveratrol, a natural polyphenol mainly found in red wine, has been shown to be cardioprotective and chemoprotective. Since a moderate wine intake correlates with a lower risk for Alzheimer disease (AD), an additional neuroprotective effect has been postulated for resveratrol. ***Objective:*** The present study aimed at elucidating the possible neuroprotective effects of resveratrol against Aβ-induced neurotoxicity. ***Methods:*** The neuroprotective capacity against Aβ-related oxidative stress was studied in a cell culture model suitable for studying such potentially neuroprotective substances. ***Results:*** Resveratrol maintains cell viability and exerts an anti-oxidative action by enhancing the intracellular free-radical scavenger glutathione. ***Conclusion:*** Our findings suggest that red wine may be neuroprotective through the actions of resveratrol.

Introduction

Resveratrol (trans-3,4′,5-trihydroxylstilbene), a natural grape-derived polyphenol present in red wine and various food products, such as peanuts and mulberries, has been reported to exert neuroprotective, cardioprotective and cancer chemopreventive activities [1–4]. Red wine ingredients such as resveratrol are considered to be related to the so-called 'French Paradox' – a low incidence of cardiovascular diseases coexisting with high fat diet and moderate wine consumption [1, 4]. There is evidence that the cardioprotective properties of resveratrol are based on counteracting atherosclerosis, platelet aggregation, proatherogenic eicosanoids and lipoprotein alterations [2, 4]. Red wine extracts have been found strongly to inhibit endothelin-1, a vasoactive peptide involved in the development of coronary atherosclerosis [5]. Furthermore, resveratrol affects mitogenesis and thereby modulates processes related to cancer cell growth [3, 6].

Although the neuroprotective effects of resveratrol are less well studied, its antioxidant properties attenuating hippocampal cell death and intracellular reactive oxygen species [1], neuroprotective activities against excitotoxic brain damage [7] and anti-apoptotic effects by caspase activation [8] suggest a broad variety of putative neuro-

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protective actions. A role as a potent antioxidant has previously been postulated for resveratrol [9, 10], but the mechanisms of its actions remain to be elucidated [11].

A possible neuroprotective effect is additionally suggested by epidemiological studies observing elderly subjects over 3 years, which showed an inverse relationship between a moderate (250–500 ml) daily red wine intake and the incidence of Alzheimer's disease (AD) [12, 13]. β-Amyloid peptide (Aβ) is the principal constituent of senile plaques in neurodegenerative disorders such as AD and contributes to cell death. Here we examined the antioxidative effects of resveratrol in human-derived SH-SY5Y neuroblastoma cell cultures [14] against oxidative stress induced by Aβ. The results indicate that resveratrol maintains call viability and thus possesses distinct neuroprotective effects.

Methods

Cell Culture Experiments

SH-SY5Y neuroblastoma cells were grown in minimum essential medium (MEM) containing 10% fetal calf serum (FCS), 100 μg/ml streptomycin sulfate, 100 U/ml penicillin G, and *L*-glutamine (designated complete medium) in a humidified air/5% CO_2 chamber 37°C, as previously described [15, 16]. Sixteen hours before treatment, FCS-medium was removed and the cells were further incubated in FCS-free MEM containing the neuroblastoma growth supplement N2 (all materials were purchased from Gibco, Life Technologies). An amount of 10 μ*M* Aβ or 50 μ*M* hydrogen peroxide (H_2O_2) was used as an inducer of oxidative stress in all experiments. H_2O_2, a known stress inducer, was chosen as a positive control to demonstrate the effect of resveratrol under cytotoxic conditions not caused by Aβ since the induction of oxidative stress in neurodegenerative disorders appears to be multifactorial. The aggregated Aβ(1–42) form was used for the neurotoxicity experiments (Bachem).

Glutathione Assay

The glutathione (GSH) assay was performed in three independent experiments in duplicate in the presence or absence of 15 μ*M* resveratrol. The following concentrations of resveratrol have been used in preceding gradient experiments to assess the optimum neuroprotective concentration: 250, 100, 50, 25, 15, 10 and 5 μ*M*. In our experiments, 15 μ*M* has been shown to be the minimum concentration of resveratrol that still shows efficacy. This concentration is less than most concentrations used in previous studies but approximates physiological concentrations present in natural products [4, 17]. GSH was measured according to the manufacturer's instructions (Clontech, ApoAlert GSH Detection Kit); detailed information has been presented elsewhere [15]. Each experiment included controls measuring GSH levels under resveratrol, H_2O_2 or Aβ effects only. In addition, GSH alterations have been assessed by adding resveratrol 12 h prior to or following the stress conditions induced by Aβ or H_2O_2. The duration of resveratrol treatment was 24 h for both pre- and post-incubation experiments.

Cell Viability Assay (MTT Reduction Assay)

Cell viability was determined under toxic conditions (Aβ and H_2O_2) using an MTT (3-[4,5 dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide, Sigma Chemical Co.) assay. Serum-free medium containing either 50 μ*M* H_2O_2 or 10 μ*M* Aβ in the presence or absence of 15 μ*M* resveratrol was added to the cells for 24 h, 12 h prior to or following the stress conditions. MTT was added to the cells, and after incubation in the dark at 37°C for 5 h followed by cell lysis, the spectrophotometric measurements were performed at 590 nm. Assays were repeated in three independent experiments performed in quadruplicate.

Statistical Analysis

In both GSH and MTT assays, the Kruskal-Wallis test was used for overall comparison and the Mann-Whitney U test (one-tailed) was used for single comparison between each stressor (Aβ or H_2O_2) and resveratrol before and after incubation in the presence of the same stressor. The level of statistical significance was set at $p < 0.05$.

Results

The measurement of intracellular changes in GSH, the main antioxidant protein within cells from a reduced to an oxidised form provides a sensitive marker of oxidative stress [18]. Both 50 μ*M* H_2O_2.and 10 μ*M* Aβ were found to induce a distinct decrease in intracellular GSH levels indicating a distinct neurotoxic activity for both substances (fig. 1b, c). The GSH suppression was more prominent for H_2O_2-induced oxidative stress than for Aβ (fig. 1b, c). The administration of 15 μ*M* resveratrol was highly effective in reversing Aβ-induced GSH reduction both in pre- and post-treatment experiments (fig. 1c). Thus, resveratrol was able to restore completely GSH levels, when compared to the control levels, indicating a resveratrol-induced increase of cellular GSH. However, when the cells were treated prior to or following the induction of oxidative stress, 15 μ*M* resveratrol did not block the H_2O_2-induced GSH attenuation (fig. 1b).

To investigate further possible neuroprotective effects of resveratrol, we performed MTT cytotoxicity assays assessing the activity of the mitochondrial respiratory chain [15]. Cell viability as assessed by MTT was distinctly suppressed by H_2O_2- and Aβ-induced oxidative stress (fig. 2b, c). When cells were pre-incubated with 15 μ*M* resveratrol they recovered from H_2O_2 neurotoxicity, whereas post-incubation with resveratrol was not protective against H_2O_2 (fig. 2b). On the other hand, resveratrol both before and after treatment was able to attenuate Aβ-induced neurotoxicity (fig. 2c). Thus, post-treatment with resveratrol was more effective in inducting MTT metabolism. Both effects were highly significant.

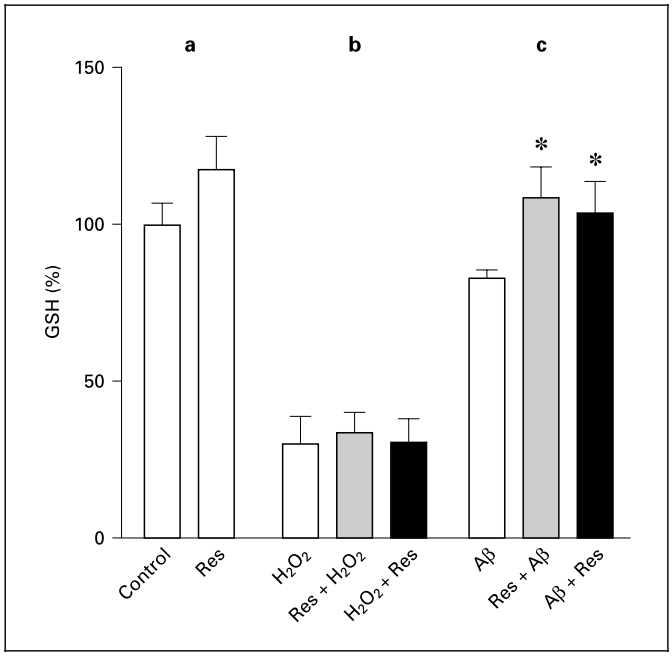

Fig. 1. Cellular GSH levels under stress conditions with H_2O_2 or Aβ and resveratrol (Res) pre- or post-incubation ($\chi^2_7 = 32.6$, $p < 0.001$). **a** Control experiments showing regular cellular GSH levels (control), and effects of resveratrol alone. **b** H_2O_2 attenuates GSH levels; this decrease is not reversed by resveratrol pre- or post-incubation. H_2O_2 vs. resveratrol H_2O_2 (pre) and vs. H_2O_2-resveratrol (post): not significant. **c** Resveratrol pre- and post-incubation counteract Aβ-induced GSH reduction. Aβ vs. resveratrol-Aβ (pre): $Z_1 = 1.9$, $p < 0.05$; Aβ vs. Aβ-resveratrol (post): $Z_1 = 1.9$. * $p < 0.05$.

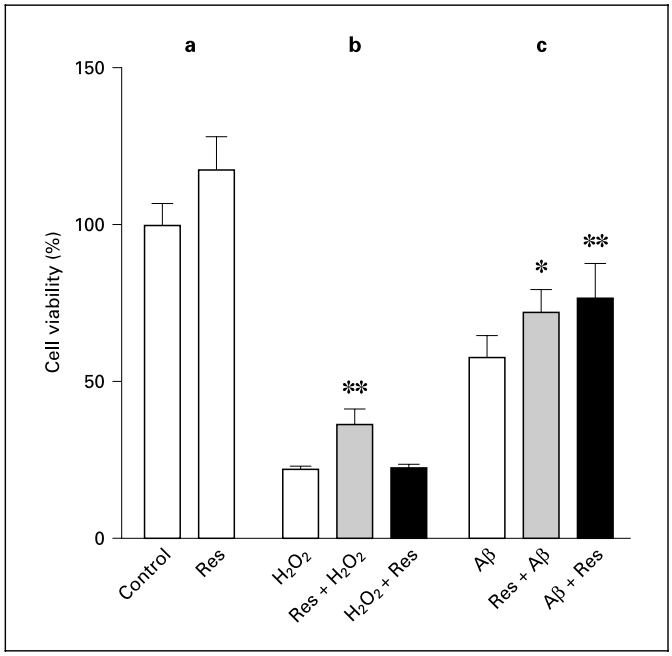

Fig. 2. Cell viability under H_2O_2- or Aβ-induced neurotoxicity and resveratrol (Res) pre- or post-incubation ($\chi^2_7 = 79.1$, $p < 0.001$). **a** MTT control experiments and in the presence of resveratrol. **b** H_2O_2 neurotoxicity is improved by resveratrol pre-incubation but not by post-incubation. H_2O_2 vs. Res-H_2O_2 (pre): $Z_1 = 4$, ** $p < 0.001$; H_2O_2 vs. H_2O_2-resveratrol (post): not significant. **c** Both resveratrol pre- and post-incubation increased cell viability suppressed by Aβ. Aβ vs. resveratrol-Aβ (pre): $Z_1 = 3.4$, * $p < 0.001$; Aβ vs. Aβ-resveratrol (post): $Z_1 = 2.6$, ** $p < 0.05$.

Discussion

The results of GSH assays underline the antioxidative properties of resveratrol. Resveratrol was highly effective in restoring intracellular GSH levels following Aβ-induced oxidative stress. Since the GSH levels in the cells were completely restored under cytotoxic conditions by resveratrol treatment, a modulatory role in inducing cellular GSH production can be postulated for resveratrol. On the other hand, resveratrol was not effective in reversing the H_2O_2-induced oxidative GSH reduction. One explanation for that may be the physiological, but low, concentration of resveratrol used in the study.

Although resveratrol was not effective in reversing the H_2O_2-induced GSH decrease, it improved cell viability in MTT assays under the same stress conditions. Independently of the recovery effects of resveratrol on GSH levels, our data suggest that resveratrol may additionally act on mitochondrial status. The direct effects of resveratrol on mitochondrial status have been previously reported. This is substantiated by the chemopreventive activity of resveratrol in colon carcinoma cell lines which is primarily based on apoptosis induced via mitochondria [19]. In those experiment, the cells were treated with a relatively high dose, 100 μ*M*, of resveratrol [19]. In experiments using the same cell line as ours, resveratrol showed anti-apoptotic effects by inhibiting caspase activation when the cells were treated with higher concentrations (50 μ*M*) of resveratrol [8]. There are some indications for a dose-dependent effect of resveratrol which is biologically active even at concentrations as low as 1 μ*M*, but the effect is reversed when higher concentrations (50–100 μ*M*) are used [20]. This may indicate that a moderate intake of resveratrol may be neuroprotective.

Besides antioxidant and cell viability-enhancing properties, resveratrol, in naturally available concentrations, induces phosphorylation of the mitogen-activated protein (MAP) kinases, ERK1 and ERK2 [20], which play a key

role in neuronal functions such as synaptic plasticity [21]. Since MPA kinases are activated in the hippocampus during associative learning tasks and are necessary for memory consolidation [22], this effect may additionally support the neuroactive capabilities of resveratrol in maintaining cognitive functions.

With the exception of red wine, its most common natural source [4], resveratrol is not widely found in appropriate concentrations in natural products. The highest concentrations are found in red wines from Cabernet-Sauvignon, Pinot noir and Merlot grapes [4]. White wines contain considerably lower resveratrol concentrations. Therefore, a moderate red wine intake may provide an adequate natural source of resveratrol [12, 13, 20], keeping in mind that excessive drinking leads to cognitive deterioration through direct toxic effects of alcohol and indirectly through nutritional deficiencies [23].

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