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SHORT COMMUNICATION

Resveratrol Exacerbates Both Autoimmune and Viral Models of Multiple Sclerosis

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The polyphenol compound resveratrol is reported to have multiple functions, including neuroprotection, and no major adverse effects have been reported. Although the neuroprotective effects have been associated with sirtuin 1 activation by resveratrol, the mechanisms by which resveratrol exerts such functions are a matter of controversy. We examined whether resveratrol can be neuroprotective in two models of multiple sclerosis: experimental autoimmune encephalomyelitis (EAE) and Theiler's murine encephalomyelitis virus-induced demyelinating disease (TMEV-IDD). EAE was induced in C57BL/6 mice, which were fed a control diet or a diet containing resveratrol during either the induction or effector phase or through the whole course of EAE. SJL/J mice were infected with TMEV and fed a control diet or a diet containing resveratrol during the chronic phase of TMEV-IDD. In EAE, all groups of mice treated with resveratrol had more severe clinical signs than the control group. In particular, resveratrol treatment during the induction phase resulted in the most severe EAE, both clinically and histologically. Similarly, in the viral model, the mice treated with resveratrol developed significantly more severe TMEV-IDD than the control group. Thus, surprisingly, the resveratrol treatment significantly exacerbated demyelination and inflammation without neuroprotection in the central nervous system in both models. Our findings indicate that caution should be exercised in potential therapeutic applications of resveratrol in human inflammatory demyelinating diseases, including multiple sclerosis. *(Am J Pathol 2013, 183: 1390–1396; http://dx.doi.org/10.1016/j.ajpath.2013.07.006)*

Resveratrol is a polyphenol compound found in a variety of foods and beverages, including red grapes, peanuts, and red wine.[1] Resveratrol has been reported to have multiple functions with anti-inflammatory, antioxidant, anti-aging, and antiviral properties that could be beneficial in diabetes and cardiovascular diseases.[2,3] Some of the effects of resveratrol have been proposed to be exerted via activation of sirtuin 1 (SIRT1), which prevents axonal degeneration.[4] Increased SIRT1 activity by resveratrol was associated with the suppression of apoptotic pathways and promoting neuronal survival, although the activation by resveratrol has lately been disputed.[5] Three research groups demonstrated that, in the SIRT1 assay, resveratrol activates SIRT1 when Fluor de Lys peptide substrate with fluorophore is used but has no effect on SIRT1 activation when the Fluor de Lys peptide substrate without fluorophore is used.[6–8] In all three reports, the authors concluded that resveratrol-mediated SIRT1 activation is an experimental artifact.

Multiple sclerosis (MS) is an inflammatory demyelinating disease with axonal degeneration in the central nervous system (CNS).[9,10] We examined the neuroprotective effects of resveratrol in an autoimmune and a viral model of MS, namely, experimental autoimmune encephalomyelitis (EAE) and Theiler's murine encephalomyelitis virus-induced demyelinating disease (TMEV-IDD).[11,12] To our surprise,

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resveratrol was not neuroprotective, but instead exacerbated both EAE and TMEV-IDD. The lack of neuroprotective effects of resveratrol in our study is consistent with the above reports of failure to confirm previously reported interactions between resveratrol and SIRT1.[6–8] Our findings indicate that caution should be exercised in potential therapeutic application of resveratrol in human diseases, including MS.

Materials and Methods

Resveratrol Treatment

Although an intake of resveratrol may be less constant via chow than via oral gavage, resveratrol is more stable in chow than in water,[13] and we therefore administered the resveratrol in chow. A diet containing resveratrol (Sigma-Aldrich, St. Louis, MO) was prepared according to Banerjee et al.[14] First, 50 g of a powder diet was weighed, then 55 mL of double-distilled H_2O were added to the powder diet and mixed well. Next, 15 mL of 100% ethanol (control diet) or 100% ethanol containing 0.02 g of resveratrol (for 0.04% in the diet) was added to the chow and mixed well. For ethanol evaporation, the chow was placed in a vacuum oven at 50°C overnight. Because resveratrol is sensitive to light,[15–17] the chow was kept away from light whenever possible and stored in the dark at 4°C.[18] The mice were fed a control diet or a diet containing resveratrol at various time points as described below. A diet containing 0.04% resveratrol provided approximately 20 mg/kg per day,[2] which is the most standard and effective dose reported by others.[19–21] Although some research groups have used a higher dose (100 mg/kg per day) in animals, this higher dose corresponds to approximately 5 g/kg per day for humans, at which level kidney failure was reported in a clinical trial for multiple myeloma.[22] One concern is that food intake of mice with full-blown EAE might be lower, which would falsify the resveratrol dosage; however, in the present study, the EAE mice fed a diet containing resveratrol did not lose weight during the full-blown EAE. We therefore concluded that there was no significant change in food intake at the peak of EAE.

Animal Experiments

EAE was induced in 6-week-old C57BL/6 mice (Harlan Laboratories, Indianapolis, IN), using myelin oligodendrocyte glycoprotein $(MOG)_{35-55}$ peptide (United Peptide, Rockville, MD), as described previously.[23] The mice were fed a control diet or a diet containing resveratrol during the induction (days −1 to 8; Early) or effector (days 14 to 23; Late) phase, or through the whole course (days −1 to 63; Whole) of EAE. Clinical scoring of EAE was as follows: 0, no sign; 1, paralyzed tail; 2, mild hind-limb paresis; 3, moderate hind-limb paralysis; 4, complete hind-limb paraplegia; and 5, quadriplegia or moribund state.[23]

Five-week-old SJL/J mice (Jackson Laboratory, Bar Harbor, ME) were infected intracerebrally with the Daniels (DA) strain of TMEV, as described previously.[24] The mice were fed a control diet (DA alone) or a diet containing resveratrol during the chronic phase (days 35 to 48; Chronic) of TMEV infection. Clinical scoring of TMEV-IDD was based on impairment of the righting reflex. The proximal end of the mouse's tail was grasped and twisted to the right and then to the left, with scoring as follows: 0, the mouse resists being turned over; 1, the mouse is flipped onto its back but immediately rights itself on one side; 1.5, the mouse is flipped onto its back but immediately rights itself on both sides; 2, the mouse rights itself in 1 to 5 seconds; 3, righting takes more than 5 seconds; and 4, the mouse cannot right itself.[24] All experimental procedures involving the use of animals were reviewed and approved by the Institutional Animal Care and Use Committee of Louisiana State University Health Sciences Center and performed according to the criteria outlined by the NIH.

Neuropathology

Mice were perfused with PBS followed by a 4% paraformaldehyde solution (Sigma-Aldrich) in PBS. The CNS tissues were harvested and fixed with 4% paraformaldehyde. The spinal cords were divided into 10 to 12 transversal segments and brains into five coronal slabs before being embedded in paraffin. Sections (4 μm thick) were stained with Luxol Fast Blue (Solvent Blue 38; Sigma-Aldrich) for visualization of myelin. For scoring of spinal cord sections, each spinal cord section was divided into four quadrants (the ventral funiculus, the dorsal funiculus, and each lateral funiculus), as described previously.[24,25] Any quadrant containing demyelination, meningitis, or perivascular cuffing was given a score of 1 in that pathological class. The total number of positive quadrants for each pathological class was determined and then divided by the total number of quadrants present on the slide and multiplied by 100 to give the percent involvement for each pathological class. An overall pathology score was also determined, by giving a positive score if any pathology was present in the quadrant, and was calculated as percent involvement.

Brain pathology was scored for meningitis (0, no meningitis; 1, mild cellular infiltration; 2, moderate cellular infiltration; and 3, severe cellular infiltration), perivascular cuffing (0, no cuffing; 1, 1 to 10 lesions; 2, 11 to 20 lesions; 3, 21 to 30 lesions; 4, 31 to 40 lesions; and 5, >40 lesions), and demyelination (0, no demyelination; 1, mild demyelination; 2, moderate demyelination; and 3, severe demyelination). The three scores were combined, for a maximum brain pathology score of 11 per mouse. Damaged axons were visualized by immunohistochemistry with SMI 311 (Sternberger Monoclonal; Covance, Princeton, NJ), a cocktail of monoclonal antibodies (SMI 32, 33, 37, 38, and 39) against nonphosphorylated neurofilament, using the avidin–biotin–peroxidase complex technique (Vector Laboratories, Burlingame, CA) with 3,3′-diaminobenzidine tetrahydrochloride as chromogen (Sigma-Aldrich) after antigen retrieval in double-distilled H_2O using a Digital Decloaking

Chamber I (Biocare Medical, Concord, CA) for 15 minutes at 120°C.[26] The numbers of damaged axons were counted under a light microscope using 10 to 12 transverse spinal cord segments per mouse, as described previously.[25]

Statistical Analysis

Analysis of variance (ANOVA), Fisher's exact test, and *t*-test were performed using OriginPro 8.1 (OriginLab Corporation, Northampton, MA) and StatView version 5.0 (SAS Institute, Cary, NC). Data are expressed as means ± SEM.

Results

Resveratrol Treatment Exacerbates MOG_{35-55}—Induced EAE

We first determined whether resveratrol treatment could alter EAE induced with MOG_{35-55}. EAE mice were fed a control diet or a diet containing resveratrol on days −1 to 8 (Early), days 14 to 23 (Late), or days −1 to 63 (Whole). At approximately 12 days after MOG sensitization, all groups of mice started to develop clinical signs, including tail and hind-limb paralysis; there was no significant difference in the onset of EAE between groups ($P > 0.05$, ANOVA) (Figure 1A). In all four groups, mice exhibited disease progression and reached disease peak 3 weeks after MOG sensitization. At 5 weeks after MOG sensitization, most control EAE mice recovered completely or remained mildly paralyzed during the 2 months observation period. In contrast, all three resveratrol treatment groups recovered poorly and had significantly higher clinical scores than the control group at approximately 40 days after MOG sensitization; mean clinical scores on day 39 were 0.3 ± 0.3 (Control), 3.0 ± 0 (Early; $P < 0.01$ versus Control), 1.9 ± 0.4 (Late; $P < 0.01$ versus Control), and 2.0 ± 0.5 (Whole; $P < 0.01$ versus Control). Mice in the Early group experienced almost no remission and had lasting severe EAE; mean clinical scores on day 61 were 0.3 ± 0.3 (Control), 2.5 ± 0.5 (Early; $P < 0.01$), 0.7 ± 0.4 (Late), and 1.2 ± 0.7 (Whole). During the chronic phase of EAE, the clinical scores tended to be higher in the Late and Whole groups, compared with the Control group, although the difference did not reach statistical significance (Figure 1A). Of note, a few mice in the Early group did not develop EAE [EAE incidence: 10/13 (77%) mice], whereas all of the mice without resveratrol treatment during the induction phase developed EAE [EAE incidence of 11/11 (100%) mice]. However, no statistical difference was found between the two groups ($P = 0.228$, Fisher's exact test). Thus, resveratrol might be useful only as a prophylactic, but not for therapeutic use in autoimmune-mediated demyelinating diseases.

At 2 months after EAE induction, we sacrificed symptomatic mice and compared the neuropathology of symptomatic mice between the groups. In the spinal cord of the Control group, inactive demyelinating lesions in the white matter with meningitis and axonal degeneration were detected by Luxol fast blue staining for myelin (Figure 1B) and by immunohistochemistry against nonphosphorylated neurofilament as a marker of axonal degeneration (Figure 2A). Consistent with the clinical signs, the Early group had severe

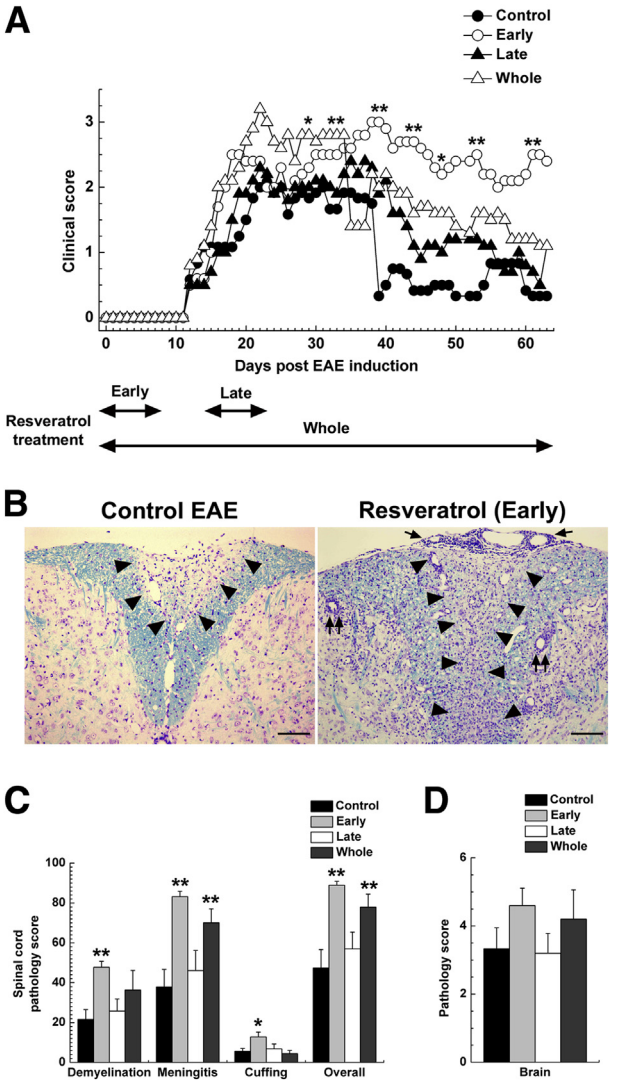

Figure 1 Adverse effects of resveratrol in the EAE autoimmune model of MS. C57BL/6 mice were sensitized with MOG_{35-55} on day 0 and were fed either a control diet or a diet containing resveratrol on days −1 to 8 (Early) or days 14 to 23 (Late) or days −1 to 63 (Whole). **A:** All three resveratrol-treated groups develop more severe EAE, compared with the Control group, although all groups had a similar disease onset. **B:** Control EAE mice have inactive demyelinating lesions in the dorsal funiculus of the spinal cord. Mice treated with resveratrol during the induction phase of EAE have more severe demyelination (**arrowheads**), meningitis (**arrows**), and active perivascular inflammation (**paired arrows**), compared with control mice. Luxol fast blue staining. **C:** Mice fed a diet containing resveratrol during the induction phase of EAE have significantly higher spinal cord pathology scores in demyelination, meningitis, perivascular cuffing (indicative of inflammation), and overall pathology, compared with mice fed a control diet. Mice treated with resveratrol during the whole course of the experiment have significantly higher scores in meningitis and overall pathology, compared with control mice. **D:** Brain pathology score does not differ significantly among the groups. Data are expressed as means (**A**) or as means ± SEM (**C** and **D**). $n = 5$ or 6 symptomatic mice per group. $^*P < 0.05$, $^{**}P < 0.01$, ANOVA. Original magnification, ×41. Scale bar = 100 μm.

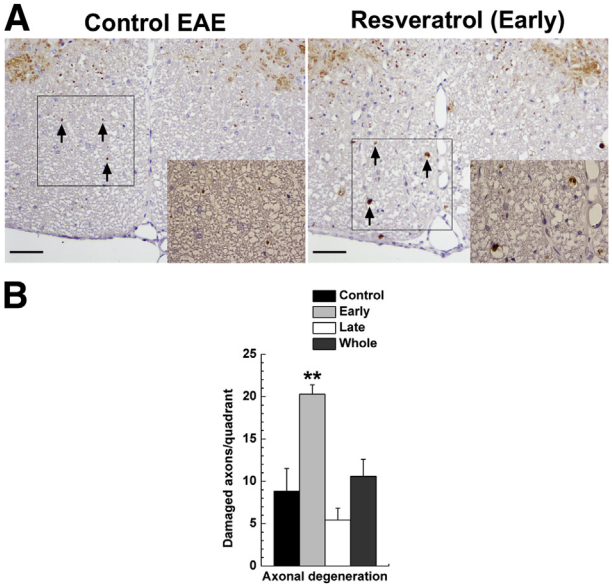

Figure 2 Axonal degeneration, 2 months after EAE induction. EAE mice were given a control diet or a diet containing resveratrol on days −1 to 8 (Early), days 14 to 23 (Late), or days −1 to 63 (Whole). **A:** Immunohistochemistry for nonphosphorylated neurofilament reveals higher levels of axonal damage (**arrows**) in the Early group than in the Control group. **Boxed regions** are shown at higher magnification in the corresponding **inset**. **B:** Higher numbers of nonphosphorylated neurofilament-positive damaged axons are detected in the spinal cord of mice fed a diet containing resveratrol during the induction phase of EAE, compared with control mice. Data are expressed as means ± SEM. $n = 5$ or 6 symptomatic mice per group. $^{**}P < 0.01$, ANOVA. Original magnification: ×83; ×115 (**insets**). Scale bars: 50 μm (**A**).

active inflammatory demyelinating lesions with axonal degeneration (Figures 1B and 2A). The pathology scores and numbers of damaged axons in the spinal cord were significantly higher in the Early group than in the Control group (Figures 1C and 2B). The Whole group had more severe demyelination and meningitis than the Control group, but the levels of axonal degeneration were similar. No significant difference was seen in neuropathology between the Control and the Late groups. Although the brain pathology scores were higher in the Early and Whole groups than in the Control and Late groups, the scores were mild in all groups, and there was no significant difference between groups (Figure 1D). We also compared the levels of MOG_{35-55}—specific lymphoproliferative responses and proinflammatory cytokine production between the groups and found no significant differences (Supplemental Figure S1). Thus, even though resveratrol has been reported to have anti-inflammatory properties,[3] in the present study resveratrol did not suppress autoimmune responses.

Resveratrol Treatment Exacerbates TMEV-IDD

We next examined whether resveratrol treatment has suppressive effects on a viral model of MS, TMEV-IDD.[27] We treated TMEV-infected mice during the chronic phase, in which mice develop inflammatory demyelinating disease at approximately 1 month after infection. Mice were infected with the DA strain of TMEV on day 0 and were fed a control diet (DA alone) or a diet containing resveratrol from day 35 to day 48 (Chronic). Beginning approximately 1 month after infection, both groups displayed impairment of righting reflexes. The Chronic group had significantly higher righting reflex scores than the DA alone group; mean clinical scores on day 48 were 1.4 ± 0.1 and 2.1 ± 0.2 for the DA alone and Chronic group, respectively ($P < 0.05$, *t*-test) (Figure 3A).

Histologically, mice treated with resveratrol developed significantly more severe demyelination, meningitis, and perivascular cuffing in the spinal cord, compared with control mice (Figure 3, B and C). Regardless of treatment, both groups of mice developed severe axonal degeneration (Figure 4A), and quantification of damaged axons revealed no significant difference between the two groups ($P > 0.05$, *t*-test) (Figure 4B). In the brain, the Chronic group had higher pathology scores than the DA alone group, although the difference did not reach statistical significance (Figure 3D). To determine whether resveratrol treatment modulated the immune responses in TMEV-IDD, we measured the levels of TMEV-specific lymphoproliferation and proinflammatory cytokine production in each group. Although no significant difference was seen in a lymphoproliferation assay between the two groups, resveratrol treatment during the chronic phase of TMEV infection resulted in approximately twofold increased IL-17 production and approximately twofold decreased interferon (IFN)-γ production (Supplemental Figure S2).

We also examined whether resveratrol has prophylactic effects on TMEV-IDD. Mice were infected with the DA strain of TMEV on day 0 and were fed a control diet (DA alone) or a diet containing resveratrol during the acute (days 0 to 14; Acute) or subclinical (days 21 to 35; Subclinical) phase of TMEV infection. Both clinically and histologically, the Subclinical group tended to have more severe disease signs than the DA alone group, although the difference did not reach statistical significance (Supplemental Figures S3 and S4). Using hyperimmune serum against TMEV, we observed similar levels and distributions of viral antigen-positive cells in the spinal cord in the three groups (Supplemental Figure S4). We also examined the effect of resveratrol treatment on immune responses in TMEV-IDD and found that the levels of TMEV-specific lymphoproliferative and antibody responses were similar in the three groups (Supplemental Figure S5). Production of both IL-17 and IFN-γ was lower in the Acute and Subclinical groups than in the DA-alone group, although the difference did not reach statistical significance (Supplemental Figure S5).

Resveratrol Treatment Is Not Neuroprotective in Infection with a Neurovirulent Strain of TMEV

In the above experiments, we did not observe a neuroprotective effect of the resveratrol treatment. One explanation could be enhancement of inflammation in the CNS, which might mask the neuroprotective effects of resveratrol.

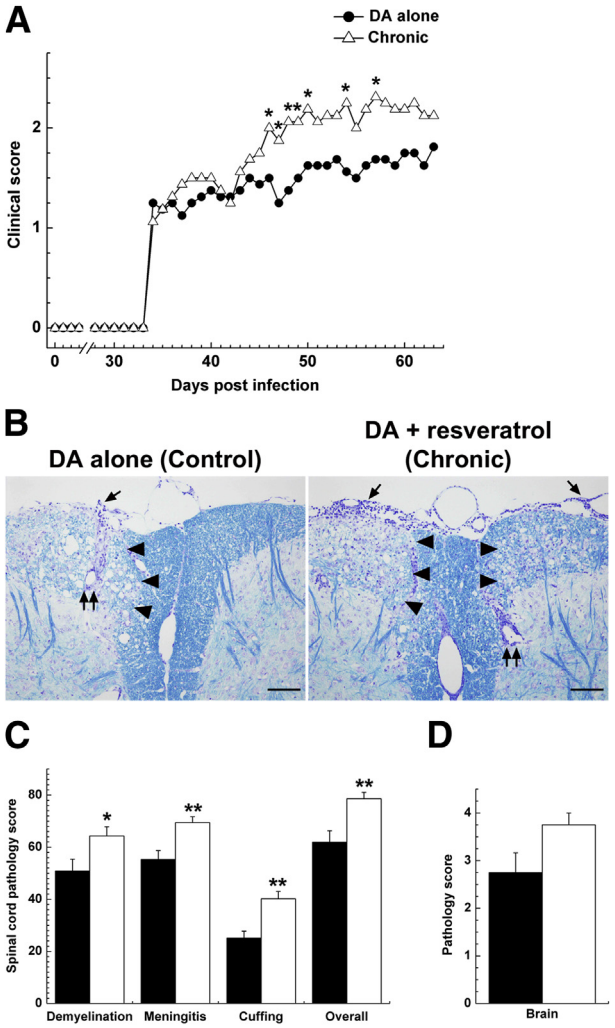

Figure 3 Adverse effects of resveratrol in the TMEV viral model of MS. SJL/J mice were infected intracerebrally with 2×10^5 PFU of the DA strain of TMEV on day 0 and were fed a control diet (DA alone, black bars) or a diet containing resveratrol from day 35 to day 48 (Chronic, white bars). **A:** The TMEV-infected mice treated with a resveratrol diet develop more severe clinical signs than mice on the control diet. **B:** Mice treated with a resveratrol diet have more severe demyelination (**arrowheads**), meningitis (**arrows**), and active perivascular inflammation (**paired arrows**) than mice on the control diet. Luxol fast blue staining. **C:** The TMEV-infected mice fed resveratrol have significantly higher pathology scores in demyelination, meningitis, and perivascular cuffing (inflammation) than mice fed a control diet. **D:** There is a small but nonsignificant difference in brain pathology scores between the two groups. Data are expressed as means (**A**) or as means ± SEM (**C** and **D**). $n = 8$ mice per group. $^*P < 0.05$, $^{**}P < 0.01$, ANOVA. Original magnification, ×42. Scale bars: 100 μm (**B**).

To address this possibility, we used the GDVII strain of TMEV, which is highly neurovirulent.[24] Unlike the DA virus, GDVII virus induces neurodegeneration without induction of TMEV-specific immune responses.[26] Thus, GDVII virus infection can be used to determine whether resveratrol can suppress neurodegeneration caused by direct viral infection (not by immunopathology). Mice were infected with 1 to 1000 plaque-forming units (PFU) of GDVII virus and were fed a control diet (GDVII alone) or a diet containing resveratrol (GDVII-resveratrol). Regardless of resveratrol treatment, all mice infected with 100 or 1000 PFU of GDVII virus started to exhibit encephalitic signs, such as weight loss, ruffled fur, and a hunched posture, at approximately day 6 and all died within 10 days of infection (Supplemental Table S1). A small number of mice infected with a low dose (1 or 10 PFU) of GDVII virus survived the infection. Both mortality and survival period were similar in the two groups. The lethal dose $(LD)_{50}$ was similar in mice fed a control diet and mice treated with resveratrol (GDVII alone, 2.6 PFU; GDVII-resveratrol, 3.8 PFU).

As an indicator of neurodegeneration, we compared the levels of neuronal apoptosis and axonal degeneration between the two groups. Using the TUNEL method, we found that all symptomatic mice had large numbers of apoptotic neurons (particularly in the cerebral cortex, thalamus, and hippocampus), regardless of treatment (Supplemental Figure S6A). Quantification of apoptotic neurons in the brain revealed no statistical difference in the number of TUNEL-positive cells between the two groups ($P > 0.05$, *t*-test) (Supplemental Table S2). Regardless of treatment, all symptomatic mice had large numbers of damaged axons in the white matter of the spinal cord (Supplemental Figure S6A). Quantification of damaged axons revealed no significant difference in the levels of axonal degeneration between the two groups ($P > 0.05$, *t*-test) (Supplemental Figure S6B). We also scored the severity of brain pathology based on the levels of meningitis and perivascular inflammation. The brain pathology scores were similar in the two groups (Supplemental Figure S6C).

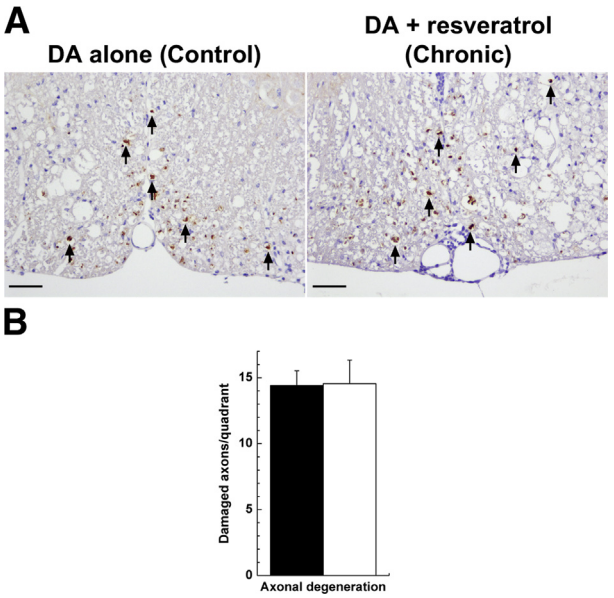

Figure 4 Axonal degeneration, 2 months after TMEV infection. SJL/J mice were infected with the DA strain of TMEV and fed a control diet (DA alone, black bar) or a diet containing resveratrol from day 35 to day 48 (Chronic, white bar). **A:** Levels of damaged axons (**arrows**) in the spinal cord are similar in the two groups. Immunohistochemistry for nonphosphorylated neurofilament. **B:** Nonphosphorylated neurofilament-positive damaged axons in the spinal cord are similar in the two groups. Data are expressed as means ± SEM. $n = 8$ mice per group. Original magnification, ×83. Scale bars: 50 μm (**A**).

Discussion

We demonstrated that the resveratrol treatment exacerbated demyelinating disease with no neuroprotection in both EAE and TMEV-IDD, although the precise mechanism is unclear. Experimentally, animal studies for cardiovascular disease suggest that resveratrol exerts a vasodilation effect by improving function of endothelial cells, which form the inner wall of blood vessels.[28] The expansion of blood vessels enhances blood flow volume and reduces blood flow velocity. Theoretically, these processes facilitate tethering, rolling, and firm adhesion of circulating inflammatory cells to endothelia, leading to the transmigration of inflammatory cells across the blood−brain barrier and into the CNS.[29] In the present study, resveratrol treatment resulted in higher pathology scores for meningitis and perivascular cuffing in both EAE and TMEV-IDD. This suggests that the vasodilation effects by resveratrol enhanced infiltration of inflammatory cells into the CNS. Inflammatory cell infiltration into the CNS has been suggested to play a key role in the pathogenesis of MS,[30] because large numbers of inflammatory cells infiltrate across the blood−brain barrier in active lesions from patients with MS.[31] Furthermore, in MS, injection of natalizumab (Tysabri; Biogen Idec, Weston, MA), a monoclonal antibody against α4-integrin [very late antigen 4 (VLA-4)], can inhibit migration of inflammatory cells into the CNS, reducing the severity of disease.[29] Similar to the case of MS, in EAE the blockade of migration of inflammatory cells into the CNS has been shown to improve clinical signs.[32,33] Thus, the exacerbation of both EAE and TMEV-IDD by resveratrol treatment observed in the present study could be due to enhanced infiltration across the blood−brain barrier of inflammatory cells, accompanied by vasodilation.

We also found no significant differences in immune responses between control and resveratrol-treated EAE mice. Although resveratrol has been tested in several different conditions in EAE,[34−36] resveratrol studies in EAE have not shown consistent outcomes. Singh et al[34] and Imler et al[35] demonstrated that EAE clinical scores were lower in mice treated with resveratrol, compared with control mice, although resveratrol treatment altered proinflammatory cytokine production either positively or negatively, depending on the disease course or the dose of resveratrol. It is unknown whether decreased clinical signs correlate with neuroprotection by resveratrol, because axonal damage was not examined in these studies. On the other hand, Fonseca-Kelly et al[36] demonstrated that a high dose (250 mg/kg per day) of resveratrol delayed the onset of EAE, but a low dose (100 mg/kg per day) had no effect[36]; however, both resveratrol treatments neither ameliorated CNS pathology nor suppressed immune responses, although resveratrol treatments protected from the loss of retinal ganglion cells, which are the neurons forming the optic nerve. The different effects of resveratrol on EAE may be due to differences in antigens and animals. For EAE induction, Imler et al[35] sensitized SJL/J mice with myelin proteolipid protein $(PLP)_{139\text{-}151}$, whereas other research groups,[33,35] including our own group, sensitized C57BL/6 mice with $MOG_{35\text{-}55}$. In MOG-induced EAE, different groups used different concentrations of MOG and pertussis toxin. Thus, further research is needed to determine the optimal concentration of resveratrol and what factors may result in deleterious effects.

Resveratrol has been shown to have antiviral effects on some viruses relevant to MS, including herpes simplex virus (HSV) and Epstein−Barr virus (EBV),[37,38] although the exact mechanisms remain unclear. In HSV infection, resveratrol inhibited viral replication by regulation of the nuclear factor-κB (NF-κB) pathway *in vitro*.[39] Resveratrol treatment modulated EBV early antigen induction *in vitro*.[40] In the present study, however, with the DA strain of TMEV infection, resveratrol-treated mice had levels of viral antigen-positive cells in the spinal cord similar to those of control mice. Furthermore, resveratrol treatment did not substantially alter LD_{50} titers in mice infected with the GDVII strain of TMEV. Thus, resveratrol did not have antiviral effects in TMEV infection.

In conclusion, we demonstrated that resveratrol treatment profoundly exacerbated demyelinating disease with enhanced CNS inflammation in EAE and TMEV-IDD, two animal models of MS. Although resveratrol has not been reported to have major adverse effects, GlaxoSmithKline reported that the safety of a proprietary formulation of resveratrol known as SRT501 was questioned in a clinical trial for patients with multiple myeloma.[22] In this trial, several patients treated with SRT501 experienced kidney failure, although it is unclear whether the kidney failure was due to SRT501 treatment or was a natural consequence of the disease. Nonetheless, resveratrol may have detrimental effects in some disease conditions and its use should be discouraged for MS patients, pending further research.

Acknowledgments

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Supplemental Data

Supplemental material for this article can be found at *http://dx.doi.org/10.1016/j.ajpath.2013.07.006*.

References

1. Jang M, Cai L, Udeani GO, Slowing KV, Thomas CF, Beecher CWW, Fong HHS, Farnsworth NR, Kinghorn AD, Mehta RG, Moon RC, Pezzuto JM: Cancer chemopreventive activity of resveratrol, a natural product derived from grapes. Science 1997, 275:218−220
2. Baur JA, Pearson KJ, Price NL, Jamieson HA, Lerin C, Kalra A, Prabhu VV, Allard JS, Lopez-Lluch G, Lewis K, Pistell PJ, Poosala S, Becker KG, Boss O, Gwinn D, Wang M, Ramaswamy S, Fishbein KW, Spencer RG, Lakatta EG, Le Couteur D, Shaw RJ,

Navas P, Puigserver P, Ingram DK, de Cabo R, Sinclair DA: Resveratrol improves health and survival of mice on a high-calorie diet. Nature 2006, 444:337–342
3. Palsamy P, Subramanian S: Ameliorative potential of resveratrol on proinflammatory cytokines, hyperglycemia mediated oxidative stress, and pancreatic beta-cell dysfunction in streptozotocin-nicotinamide-induced diabetic rats. J Cell Physiol 2010, 224:423–432
4. Borra MT, Smith BC, Denu JM: Mechanism of human SIRT1 activation by resveratrol. J Biol Chem 2005, 280:17187–17195
5. Schmidt C: GSK/Sirtris compounds dogged by assay artifacts. Nature Biotechnol 2010, 28:185–186
6. Kaeberlein M, McDonagh T, Heltweg B, Hixon J, Westman EA, Caldwell SD, Napper A, Curtis R, DiStefano PS, Fields S, Bedalov A, Kennedy BK: Substrate-specific activation of sirtuins by resveratrol. J Biol Chem 2005, 280:17038–17045
7. Beher D, Wu J, Cumine S, Kim KW, Lu S-C, Atangan L, Wang M: Resveratrol is not a direct activator of SIRT1 enzyme activity. Chem Biol Drug Des 2009, 74:619–624
8. Pacholec M, Bleasdale JE, Chrunyk B, Cunningham D, Flynn D, Garofalo RS, Griffith D, Griffor M, Loulakis P, Pabst B, Qiu X, Stockman B, Thanabal V, Varghese A, Ward J, Withka J, Ahn K: SRT1720, SRT2183, SRT1460, and resveratrol are not direct activators of SIRT1. J Biol Chem 2010, 285:8340–8351
9. Pirko I, Lucchinetti CF, Sriram S, Bakshi R: Gray matter involvement in multiple sclerosis [Erratum appeared in Neurology 2008, 71:2021]. Neurology 2007, 68:634–642
10. Martinez NE, Sato F, Omura S, Minagar A, Alexander JS, Tsunoda I: Immunopathological patterns from EAE and Theiler's virus infection: is multiple sclerosis a homogenous 1-stage or heterogenous 2-stage disease? Pathophysiology 2013, 20:71–84
11. Mecha M, Carrillo-Salinas FJ, Mestre L, Feliú A, Guaza C: Viral models of multiple sclerosis: Neurodegeneration and demyelination in mice infected with Theiler's virus. Prog Neurobiol 2013, 101–102: 46–64
12. Sato F, Omura S, Martinez NE, Tsunoda I: Animal models of multiple sclerosis. Neuroinflammation. Edited by Minagar A. London, Elsevier, 2011, pp 55–79
13. Niles RM, Cook CP, Meadows GG, Fu Y-M, McLaughlin JL, Rankin GO: Resveratrol is rapidly metabolized in athymic (nu/nu) mice and does not inhibit human melanoma xenograft tumor growth. J Nutr 2006, 136:2542–2546
14. Banerjee S, Bueso-Ramos C, Aggarwal BB: Suppression of 7,12-dimethylbenz(a)anthracene-induced mammary carcinogenesis in rats by resveratrol: role of nuclear factor-κB, cyclooxygenase 2, and matrix metalloprotease 9. Cancer Res 2002, 62:4945–4954
15. Vivekananthan DP, Penn MS, Sapp SK, Hsu A, Topol EJ: Use of antioxidant vitamins for the prevention of cardiovascular disease: meta-analysis of randomised trials [Erratum appeared in Lancet 2004, 363:662]. Lancet 2003, 361:2017–2023
16. Campos-Toimil M, Elíes J, Álvarez E, Verde I, Orallo F: Effects of trans- and cis-resveratrol on Ca2+ handling in A7r5 vascular myocytes. Eur J Pharmacol 2007, 577:91–99
17. Rius C, Abu-Taha M, Hermenegildo C, Piqueras L, Cerda-Nicolas JM, Issekutz AC, Estañ L, Cortijo J, Morcillo EJ, Orallo F, Sanz M-J: Trans- but not cis-resveratrol impairs angiotensin-II-mediated vascular inflammation through inhibition of NF-κB activation and peroxisome proliferator-activated receptor-gamma upregulation. J Immunol 2010, 185:3718–3727
18. Figueiras TS, Neves-Petersen MT, Petersen SB: Activation energy of light induced isomerization of resveratrol. J Fluoresc 2011, 21: 1897–1906
19. Gupta YK, Chaudhary G, Sinha K, Srivastava AK: Protective effect of resveratrol against intracortical FeCl3-induced model of posttraumatic seizures in rats. Methods Find Exp Clin Pharmacol 2001, 23:241–244
20. Asensi M, Medina I, Ortega A, Carretero J, Baño MC, Obrador E, Estrela JM: Inhibition of cancer growth by resveratrol is related to its low bioavailability. Free Radic Biol Med 2002, 33:387–398
21. Eybl V, Kotyzova D, Koutensky J: Comparative study of natural antioxidants—curcumin, resveratrol and melatonin—in cadmium-induced oxidative damage in mice. Toxicology 2006, 225:150–156
22. GlaxoSmithKline halts all further development of resveratrol drug SRT501. The Myeloma Beacon 2010 Nov 30 [Internet]. Available at http://www.myelomabeacon.com/news/2010/11/30/glaxosmithkline-halts-all-further-development-of-resveratrol-drug-srt501, last revised November 30, 2010
23. Tsunoda I, Tanaka T, Terry EJ, Fujinami RS: Contrasting roles for axonal degeneration in an autoimmune versus viral model of multiple sclerosis: when can axonal injury be beneficial? Am J Pathol 2007, 170:214–226
24. Tsunoda I, Tanaka T, Fujinami RS: Regulatory role of CD1d in neurotropic virus infection. J Virol 2008, 82:10279–10289
25. Tsunoda I, Wada Y, Libbey JE, Cannon TS, Whitby FG, Fujinami RS: Prolonged gray matter disease without demyelination caused by Theiler's murine encephalomyelitis virus with a mutation in VP2 puff B. J Virol 2001, 75:7494–7505
26. Tsunoda I, Kuang L-Q, Libbey JE, Fujinami RS: Axonal injury heralds virus-induced demyelination. Am J Pathol 2003, 162:1259–1269
27. Sato F, Tanaka H, Hasanovic F, Tsunoda I: Theiler's virus infection: pathophysiology of demyelination and neurodegeneration. Pathophysiology 2011, 18:31–41
28. Nicholson SK, Tucker GA, Brameld JM: Physiological concentrations of dietary polyphenols regulate vascular endothelial cell expression of genes important in cardiovascular health. Br J Nutr 2010, 103:1398–1403
29. Rice GPA, Hartung H-P, Calabresi PA: Anti-alpha4 integrin therapy for multiple sclerosis: mechanisms and rationale. Neurology 2005, 64: 1336–1342
30. Steinman L: Multiple sclerosis: a coordinated immunological attack against myelin in the central nervous system. Cell 1996, 85:299–302
31. Lassmann H, Brück W, Lucchinetti C: Heterogeneity of multiple sclerosis pathogenesis: implications for diagnosis and therapy. Trends Mol Med 2001, 7:115–121
32. Yednock TA, Cannon C, Fritz LC, Sanchez-Madrid F, Steinman L, Karin N: Prevention of experimental autoimmune encephalomyelitis by antibodies against alpha 4 beta 1 integrin. Nature 1992, 356:63–66
33. Tsunoda I, Terry EJ, Marble BJ, Lazarides E, Woods C, Fujinami RS: Modulation of experimental autoimmune encephalomyelitis by VLA-2 blockade. Brain Pathol 2007, 17:45–55
34. Singh NP, Hegde VL, Hofseth LJ, Nagarkatti M, Nagarkatti P: Resveratrol (trans-3,5,4′-trihydroxystilbene) ameliorates experimental allergic encephalomyelitis, primarily via induction of apoptosis in T cells involving activation of aryl hydrocarbon receptor and estrogen receptor. Mol Pharmacol 2007, 72:1508–1521
35. Imler TJ Jr., Petro TM: Decreased severity of experimental autoimmune encephalomyelitis during resveratrol administration is associated with increased IL-17+IL-10+ T cells, CD4(−) IFN-gamma+ cells, and decreased macrophage IL-6 expression. Int Immunopharmacol 2009, 9:134–143
36. Fonseca-Kelly Z, Nassrallah M, Uribe J, Khan RS, Dine K, Dutt M, Shindler KS: Resveratrol neuroprotection in a chronic mouse model of multiple sclerosis. Front Neurol 2012, 3:84
37. Bergström T, Andersen O, Vahlne A: Isolation of herpes simplex virus type 1 during first attack of multiple sclerosis. Ann Neurol 1989, 26: 283–285
38. Bray PF, Culp KW, McFarlin DE, Panitch HS, Torkelson RD, Schlight JP: Demyelinating disease after neurologically complicated primary Epstein-Barr virus infection. Neurology 1992, 42:278–282
39. Faith SA, Sweet TJ, Bailey E, Booth T, Docherty JJ: Resveratrol suppresses nuclear factor-κB in herpes simplex virus infected cells. Antiviral Res 2006, 72:242–251
40. Kapadia GJ, Azuine MA, Tokuda H, Takasaki M, Mukainaka T, Konoshima T, Nishino H: Chemopreventive effect of resveratrol, sesamol, sesame oil and sunflower oil in the Epstein-Barr virus early antigen activation assay and the mouse skin two-stage carcinogenesis. Pharmacol Res 2002, 45:499–505