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Int. J. Devl Neuroscience 21 (2003) 117–124

INTERNATIONAL JOURNAL of DEVELOPMENTAL NEUROSCIENCE

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GVS-111 prevents oxidative damage and apoptosis in normal and Down's syndrome human cortical neurons

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Received 3 December 2002; received in revised form 11 February 2003; accepted 13 February 2003

Abstract

The neuroprotective activity of a novel *N*-acylprolyl-containing dipeptide analog of the nootropic 2-oxo-1-pyrrolidine acetamide (Piracetam) designated as GVS-111 (DVD-111/Noopept) was tested in two in vitro models of neuronal degeneration mediated by oxidative stress: normal human cortical neurons treated with H_2O_2, and Down's syndrome (DS) cortical neurons. Incubation of normal cortical neurons with 50 μM H_2O_2 for 1 h resulted in morphological and structural changes consistent with neuronal apoptosis and in the degeneration of more than 60% of the neurons present in the culture. GVS-111 significantly increased neuronal survival after H_2O_2-treatment displaying a dose-dependent neuroprotective activity from 10 nM to 100 μM, and an IC_{50} value of 1.21 ± 0.07 μM. GVS-111 inhibited the accumulation of intracellular free radicals and lipid peroxidation damage in neurons treated with H_2O_2 or $FeSO_4$, suggesting an antioxidant mechanism of action. GVS-111 exhibited significantly higher neuroprotection compared to the standard cognition enhancer Piracetam, or to the antioxidants Vitamin E, propyl gallate and *N-tert*-butyl-2-sulpho-phenylnitrone (s-PBN). In DS cortical cultures, chronic treatment with GVS-111 significantly reduced the appearance of degenerative changes and enhanced neuronal survival. The results suggest that the neuroprotective effect of GVS-111 against oxidative damage and its potential nootropic activity may present a valuable therapeutic combination for the treatment of mental retardation and chronic neurodegenerative disorders.

Keywords: Alzheimer's disease; Down's syndrome; Oxidative stress; Lipid peroxidation; Neuroprotection; Neurodegeneration; GVS-111; Dipeptides

1. Introduction

The term nootropics or "cognition enhancers" is applied to a group of psychoactive substances that appear to selectively stimulate neuronal function and to enhance cognitive performance (Giurgea, 1972). Three main activities have been described as features of nootropics in experimental conditions: (1) enhancement of learning and memory; (2) facilitation of associative processes in the neocortex; and (3) ability to increase neuronal resistance to injury (Gouliaev and Senning, 1994). 2-Oxo-1-pyrrolidine acetamide (Piracetam) is the best known member of this family of compounds. A novel substituted prolyl-containing dipeptide (*N*-phenyl-acetyl-L-prolyl-glycine ethyl ester) designated as GVS-111 (DVD-111/Noopept) was designed and synthesized at the Institute of Pharmacology of the Russian Academy of Medical Sciences, as part of a new series of *N*-acylprolyl-containing dipeptides (Gudasheva et al., 1996a). GVS-111 exhibits strong neuroprotective and memory-restoring properties in three different models of brain damage: frontal lobectomy (Ostrovskaya et al., 1997), cortical compression (Romanova et al., 1996) and photochemically induced cortical thrombosis (Ostrovskaya et al., 1999). These experimental brain lesions are associated with massive release of glutamate, elevation of intracellular calcium levels and increased production of free radicals. The same three pathogenic mechanisms are related to various age-related neurodegenerative conditions including Alzheimer's, Parkinson's and Huntington's disease (Beal, 1995; Busciglio et al., 1998; Mattson and Chan, 2001). Interestingly, previous reports indicate that GVS-111 may have beneficial effects on each of these three processes. It blocks voltage-activated calcium channels in isolated neurons (Solntseva et al., 1997); it improves the

Abbreviations: DCF, 2,7-dichlorofluorescein; DCFDA, 2,7-dichlorofluorescin diacetate; DIV, days in vitro; DS, Down's syndrome; s-PBN, *N-tert*-butyl-2-sulpho-phenylnitrone; TBA, 2-thiobarbituric acid

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doi:10.1016/S0736-5748(03)00031-5

survival of cerebellar granule cells in culture after exposure to glutamate (Ostrovskaya et al., 1998); and it diminishes the accumulation of oxidation by-products in blood and brain of mice genetically predisposed to stress and in rats immobilized for 24 h (Lysenko et al., 1997). These results suggest that GVS-111 is a neuroprotective agent with potential therapeutic use. In this study, the neuroprotective properties of GVS-111 were investigated in two in vitro models of neurodegeneration associated with oxidative stress: normal human cortical neurons exposed to H_2O_2, and Down's syndrome (DS) neurons in culture. Both models present intracellular accumulation of free radicals and increased lipid peroxidation leading to neuronal apoptosis (Busciglio and Yankner, 1995). The results indicate that, in both experimental paradigms, GVS-111 significantly increased neuronal survival and prevented the accumulation of intracellular free radicals, peroxidative damage and the development of neurodegenerative changes.

2. Experimental procedures

2.1. Human fetal cortical cultures

Brain tissue samples were obtained from the Department of Pathology, Albert Einstein College of Medicine. Primary human cortical cultures were established from cortical tissue of 17–21-week-gestation normal and DS fetal abortuses. The protocols for obtaining post-mortem fetal brain tissue complied with federal and institutional guidelines. Informed consent was obtained from all participants. Normal and DS fetal cortical cultures were generated as previously described (Busciglio and Yankner, 1995; Busciglio et al., 1993). Briefly, cortical tissue was washed 3× in Hank's balanced salt solution, freed of meninges, cut in small pieces and incubated in 0.025% trypsin for 20 min. The cells were dissociated by repeated passages through a Pasteur pipette, washed 3× and plated on poly-D-lysine-coated 24-well multiwells at a density of 400,000 cells per well. The plating media was D-MEM (Life Technologies), supplemented with 10% iron-supplemented calf serum (HyClone). Two hours after plating, the media was changed to serum-free media consisting of D-MEM + N2 supplements (Life Technologies). Under these conditions, 70–80% of the cells present in the culture were neurons. Partial medium changes were performed every 3 days.

2.2. Treatment with H_2O_2

A fresh stock of 1 mM H_2O_2 was prepared in D-MEM for each experiment. At 7 days in vitro (DIV), H_2O_2 was added to the culture medium at the indicated concentrations. After 1 h, the cultures were washed with fresh medium and maintained in D-MEM + N2 for an additional 24 h. After this period, the cultures were fixed and processed for analysis.

2.3. Neuroprotective treatments

GVS-111 was designed and synthesized at the Institute of Pharmacology of the Russian Academy of Medical Sciences (Gudasheva et al., 1996a). A fresh 10 mM stock solution of GVS-111 was prepared in D-MEM for each experiment. Normal cortical neurons at 7 DIV were pre-incubated in GVS-111 for 30 min before the addition of H_2O_2. Thus, GVS-111 was present in the media during and after the treatment with H_2O_2. The cultures were fixed 24 h later and processed for analysis. Similar treatments were performed with *N-tert*-butyl-2-sulpho-phenylnitrone (s-PBN). A 2 mM stock of s-PBN was prepared in D-MEM for each experiment. Piracetam and *N*-propyl gallate were dissolved as 100× stock solutions in sterile distilled water. Vitamin E was dissolved in ethanol and diluted 1:200 in culture medium. This concentration of ethanol had no effect on neuronal viability when added alone (Busciglio and Yankner, 1995).

For neuroprotective assays on DS neurons, DS cortical cultures were continuously exposed to GVS-111 starting at 2 DIV. Partial medium changes including fresh GVS-111 were performed twice per week. DS cultures were fixed and analyzed at 11 DIV.

2.4. Immunocytochemistry

Cultures were fixed in 4% paraformaldehyde/120 mM sucrose in phosphate buffered saline (PBS) at 37 °C for 30 min, permeabilized 5 min with 0.02% Triton X-100 in PBS and blocked with 5% albumin/PBS for 1 h. After this, the cultures were incubated overnight at 4 °C with an antibody to Class III β-tubulin (1:2000; Sigma), washed 3× with PBS, incubated for 1 h at room temperature with biotin-conjugated secondary antibody followed by avidin-peroxidase (Vector Laboratories). The color reaction was developed using a metal-enhanced diaminobenzidine reaction kit (Pierce).

2.5. Assessment of intracellular free radicals

Generation of intracellular free radicals in cultured neurons was determined using the redox-sensitive fluorescent dye 2,7-dichlorofluorescin diacetate (DCFDA; Molecular Probes) as previously described (Busciglio and Yankner, 1995; Shi et al., 1998). Briefly, 10 μM DCFDA was added to the culture media for 1 h at 37 °C. DCFDA is cell permeable and interacts with reactive oxygen species (ROS) to generate the de-acetylated fluorescent product 2,7-dichlorofluorescein (DCF) that can be visualized by fluorescence microscopy (Cathcart et al., 1983).

2.6. Analysis of neuronal survival

Neuronal viability was assessed by scoring the number of viable neurons present in the culture as described (Busciglio and Yankner, 1995; Grace et al., 2002). Neuronal cells were labeled with anti-Class III β-tubulin, a neuron-specific

marker and nuclei were labeled with 10 μg/ml propidium iodide. Living neurons in early apoptotic stages were visualized by fluorescence microscopy after labeling with Annexin V following the vendor's protocol (Annexin V-EGFP kit; Clontech). The number of viable neurons was assessed in 10–12 microscopic fields per well, in quadruplicate wells, using a 20× objective (final magnification 200×). The identity of the cultures was coded to avoid experimental bias. An average of 200 neurons was counted in control cultures in each experiment. Digital images were obtained with a CCD color camera (SPOT). All experiments were replicated at least four times utilizing cultures generated from different fetal tissue specimens.

2.7. Lipid peroxidation assay

Relative levels of lipid peroxidation in individual cortical neurons were quantified using a 2-thiobarbituric acid (TBA) assay as described previously (Juurlink and Husain, 1994). Briefly, the cells were fixed after the corresponding treatments in an aldehyde-free fixative containing 50% methanol, 10% glacial acetic acid, 2 mM EDTA and 38 mM TBA. Cultures were heated to 85 °C for 45 min, fixative was removed, antifade solution was added, and cells were imaged using a fluorescent microscope (excitation at 488 nm). Images of cells receiving different treatments were acquired with a CCD camera and quantitative comparisons of relative fluorescent intensities among treatment groups were performed. Values for average staining intensity per cell were obtained using NIH image software. For some experiments, lipid peroxidation was induced by treatment of cortical cultures with 10 mM $FeSO_4$ as previously described (Goodman et al., 1996; Goodman and Mattson, 1996).

2.8. Statistical analysis

All experiments were repeated at least three times using cultures derived from different tissue samples. Each individual experiment was performed in quadruplicate. In some cases, the data were analyzed by ANOVA followed by post-hoc Student–Newman–Keul's test, in order to make all possible comparisons. Two-way ANOVA was used for comparing GVS-111 and s-PBN neuroprotective effects. Data were expressed as mean ± S.E.M. and significance was assessed at $P < 0.05$.

3. Results

The neuroprotective effect of GVS-111 was initially evaluated in normal human cortical neurons exposed to H_2O_2. The cultures were incubated with 25, 50 or 75 μM H_2O_2 for 1 h and neuronal survival was assessed 24 h later. A 75 μM concentration of H_2O_2 induced death of more than 90% of the neurons, while more than 80% of the neurons survived in cultures treated with 25 μM H_2O_2 (Fig. 1A). A 1 h exposure to 50 μM H_2O_2 resulted in 38 ± 3% neuronal survival after 24 h (Fig. 1A). Thus, a 50 μM H_2O_2 dose, which causes moderate cell death, was chosen and utilized for further studies. Degenerative changes in H_2O_2-treated cultures became evident from 2 h after initiation of the treatment and gradually increased over the next 24 h. Degenerating neurons displayed chromatin condensation and fragmentation (Fig. 1C, arrows), consistent with neuronal apoptosis. To confirm the presence of an apoptotic process in H_2O_2-treated neurons, Annexin V was utilized. Annexin V has a strong binding affinity for phosphatidylserine in cellular membranes. In apoptotic cells, phosphatidylserine looses its normal localization in the inner plasma membrane and translocates to the outer layer of the membrane where it is exposed to the extracellular environment and can bind Annexin V (Fadok et al., 1992; Martin et al., 1995). In cultures exposed to 50 μM H_2O_2, a progressive increase in Annexin V staining was observed starting 2 h after the initiation of the treatment (Fig. 1E). Control cultures did not exhibit changes in neuronal survival and showed negative Annexin V staining (Fig. 1D). Degenerative changes, chromatin condensation and positive Annexin V staining indicated that 50 μM H_2O_2 induced neuronal apoptosis. We tested the neuroprotective activity of GVS-111 (Fig. 2D) in this system. When added to H_2O_2-treated cultures, GVS-111 prevented the appearance of neurodegenerative changes in a dose-dependent manner (Fig. 2C and E). GVS-111 significantly increased neuronal survival from 35 ± 6% (mean ± S.E.M.) in cultures treated with H_2O_2 alone, to 47 ± 5% in cultures treated with H_2O_2 + 10 nM GVS-111, up to 85 ± 9% in cultures treated with H_2O_2 + 100 μM GVS-111 (Fig. 2E). The IC_{50} value for GVS-111 was 1.7 ± 0.07 μM. The neuroprotective effect of GVS-111 was compared with that of the antioxidant free radical spin trap s-PBN, which is neuroprotective on Down's syndrome neurons in culture (Busciglio and Yankner, 1995). s-PBN protected normal human cortical neurons against H_2O_2-mediated damage at final concentrations of 100 and 10 μM, increasing neuronal survival to 71 ± 8 and 69 ± 5%, respectively. In contrast, no significant protection was observed with 1 μM s-PBN (Fig. 3E). Thus, GVS-111 exhibits higher efficacy and potency against H_2O_2 than s-PBN.

To determine whether GVS-111 neuroprotective effect is related to its antioxidant properties (Lysenko et al., 1997), we analyzed its ability to prevent intracellular free radical accumulation and lipid peroxidation. Accumulation of intracellular free radicals was assessed with DCFDA. Fluorescence microscopy analysis showed a marked increase in DCF fluorescence 2 h after exposure to H_2O_2, indicating the presence of intracellular free radicals (Fig. 3B, arrows). In contrast, 1 μM GVS-111 prevented the appearance of intracellular DCF fluorescence (Fig. 3D). s-PBN (100 μM) was also effective to prevent the increase in DCF fluorescence induced by H_2O_2, but neither 1 nM GVS-111 nor 1 μM s-PBN prevented the increase in DCF fluorescence after H_2O_2

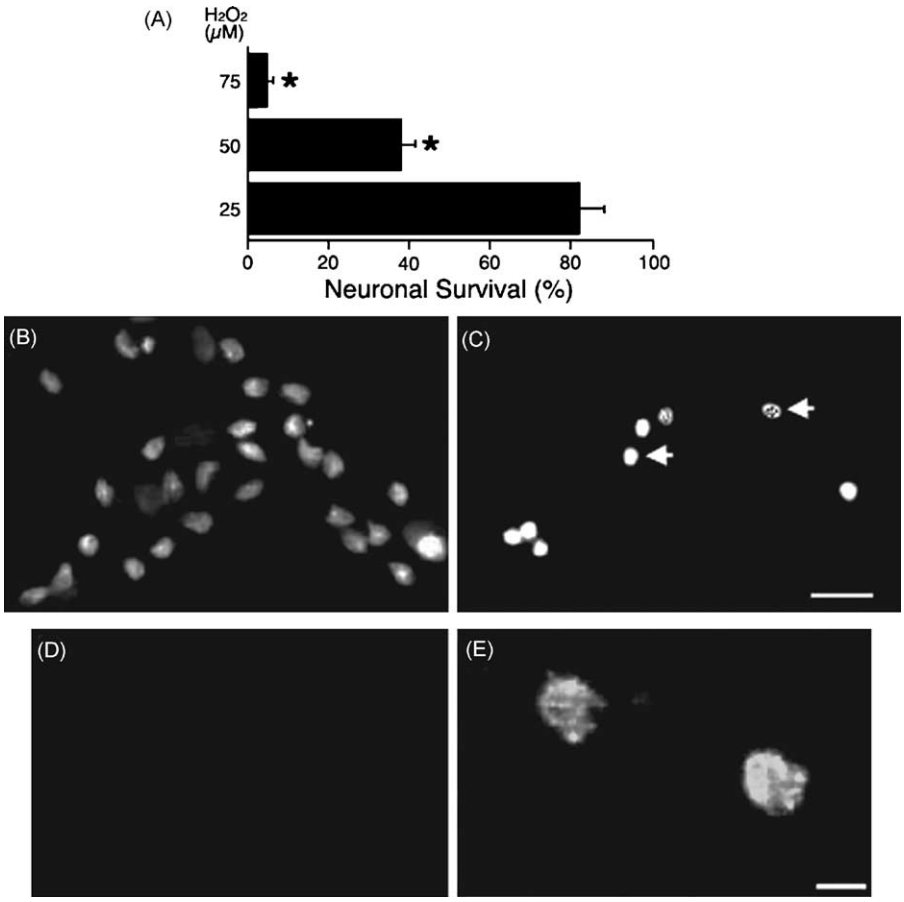

Fig. 1. H_2O_2 induces apoptosis of human cortical neurons in vitro. (A) Neuronal survival after H_2O_2 treatment. Survival is expressed as a percentage compared to vehicle-treated cultures (100%). Values are the mean ± S.E.M.; $n = 4$ independent experiments; more than 200 neurons were scored per condition in each individual experiment. *$P < 0.01$ relative to vehicle by Student's t-test. Cultures were incubated with H_2O_2 for 1 h, then the medium was changed and survival was assessed 24 h later. (B and C) H_2O_2 induces apoptotic changes in human neurons. Neurons were treated at 7 DIV with vehicle (A) or with 50 μM H_2O_2 for 1 h (B). The cultures were fixed and stained with propidium iodide 24 h after treatment. Control cultures show normal nuclear morphology (A). H_2O_2-treated neurons exhibit chromatin condensation and fragmentation (B). Scale bar for (A and B): 40 μM. (D and E) Living cultures were labeled with Annexin V and observed by fluorescence microscopy 4 h after treatment with H_2O_2. Vehicle-treated neurons showed negative Annexin V staining (D). Annexin V fluorescence is evident on the cell surface of H_2O_2 treated neurons (E). Scale bar for (D and E): 20 μM.

exposure (data not shown). These results suggest that GVS-111 neuroprotection is related to its antioxidant activity.

The neuroprotective effect of GVS-111 against oxidative damage was further analyzed using an assay based on the fluorescence generated by TBA in the presence of lipid peroxides (Juurlink and Husain, 1994). Incubation of cortical neurons with 50 μM H_2O_2 or 10 mM $FeSO_4$ resulted in significant increases of TBA fluorescence. Treatment with GVS-111 reduced significantly TBA fluorescence in cultures treated with H_2O_2 and $FeSO_4$ (Fig. 4A), further suggesting that GVS-111 possesses antioxidant activity. The neuroprotective effect of GVS-111 was compared with that of antioxidants Vitamin E and propyl gallate (Busciglio and Yankner, 1995), and the nootropic agent Piracetam, which has also been reported to be neuroprotective (Brandao et al., 1995, 1996; Vaglenova and Vesselinov Petkov, 2001). All these compounds enhanced survival of H_2O_2-treated neurons (Fig. 4B), but 10 μM GVS-111 exhibited significantly higher neuroprotection (Fig. 4B).

Finally, the neuroprotective properties of GVS-111 were assessed in DS neurons. DS cortical cultures exhibit good viability after plating and develop in a similar way to normal neurons during the first 5–7 DIV. After this period, DS neurons exhibit progressive degenerative changes and during the second week in culture approximately 50% of the neurons are lost (Busciglio and Yankner, 1995). Degenerating DS neurons show increased intracellular levels of ROS, membrane blebbing, chromatin condensation and positive TUNEL staining consistent with apoptotic cell death (Busciglio and Yankner, 1995). DS cultures were continuously treated with GVS-111 from days 2 to 11 in vitro, fixed and analyzed. At 11 DIV, untreated DS cultures showed clear signs of neurodegeneration including formation of neuronal clumps, neuritic retraction and fragmentation (Fig. 5A). In contrast, DS neurons treated with GVS-111 exhibited normal morphology (Fig. 5B). Analysis of cell viability revealed that GVS-111 increased neuronal survival from 100 ± 17% on untreated DS cultures, to 160±20% on 1 μM GVS-111- and

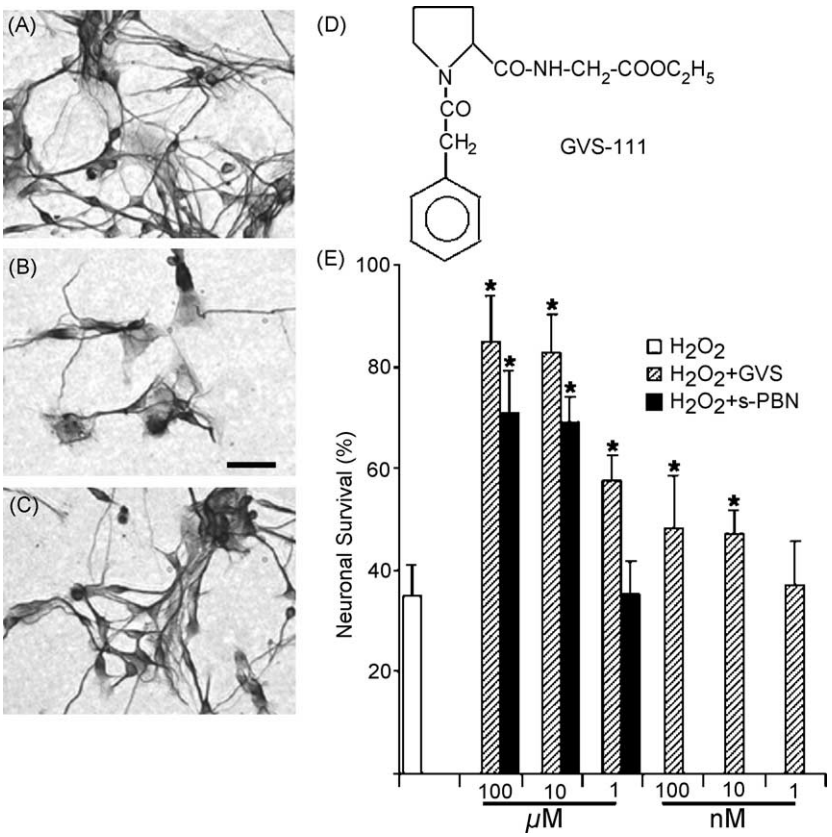

Fig. 2. GVS-111 protects human cortical neurons from H_2O_2. (A–C) Control cultures treated with vehicle show normal neuronal morphology with intact processes (A). Fifty micromolar H_2O_2 induce extensive neuritic retraction, clumping of cell bodies and degeneration (B). Cultures incubated with H_2O_2 + 100 μM GVS-111 show normal morphology with good preservation of processes (C). Scale bar: 40 μM. (D) Molecular structure of GVS-111. (E) GVS-111 and s-PBN induce dose-dependent increase in neuronal survival in H_2O_2-treated cultures. Survival is expressed as a percentage of survival in vehicle-treated cultures (100%). Values are the mean ± S.E.M.; $n = 4$ independent experiments; more than 200 neurons were scored per experimental condition in each individual experiment. Significantly different from H_2O_2-treated cultures by ANOVA (*$P < 0.01$).

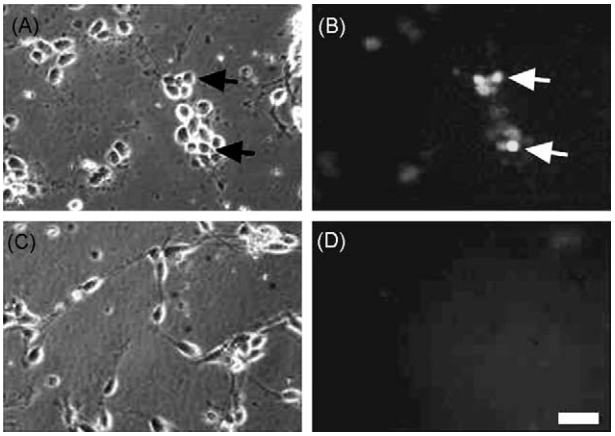

Fig. 3. GVS-111 prevents intracellular free radical accumulation after exposure to H_2O_2. A DCFDA assay was performed 2 h after treatment with 50 μM H_2O_2. DCF fluorescence was visualized in living cultures. (A and C) are phase contrast images of the fields shown in (B and D), respectively. DCF fluorescence denotes the presence of intracellular free radicals in H_2O_2-treated cells (arrows, B). Background cellular DCF fluorescence is observed in cultures treated with H_2O_2 + 100 μM GVS-111 (D). Scale bar: 40 μM.

183 ± 32% on 100 μM GVS-111-treated cultures (Fig. 5C). GVS-111 also inhibited intracellular free radical accumulation and lipid peroxidation in DS cultures (data not shown). Taken together, these results indicate that GVS-111 possesses strong neuroprotective activity against oxidative damage in cortical human neurons.

4. Discussion

This report describes the neuroprotective effect of the novel prolyl-containing dipeptide, GVS-111 on normal and DS cortical neurons in culture. GVS-111 prevented intracellular free radical accumulation, lipid peroxidation and the appearance of degenerative/apoptotic changes in normal neurons treated with 50 μM H_2O_2, resulting in a marked increase in the number of viable neurons present in the culture. The ability to prevent intracellular free radical accumulation and lipid peroxidation suggests that GVS-111 acts as a potent antioxidant that can prevent neuronal degeneration at concentrations in the nM range. Indeed, significant

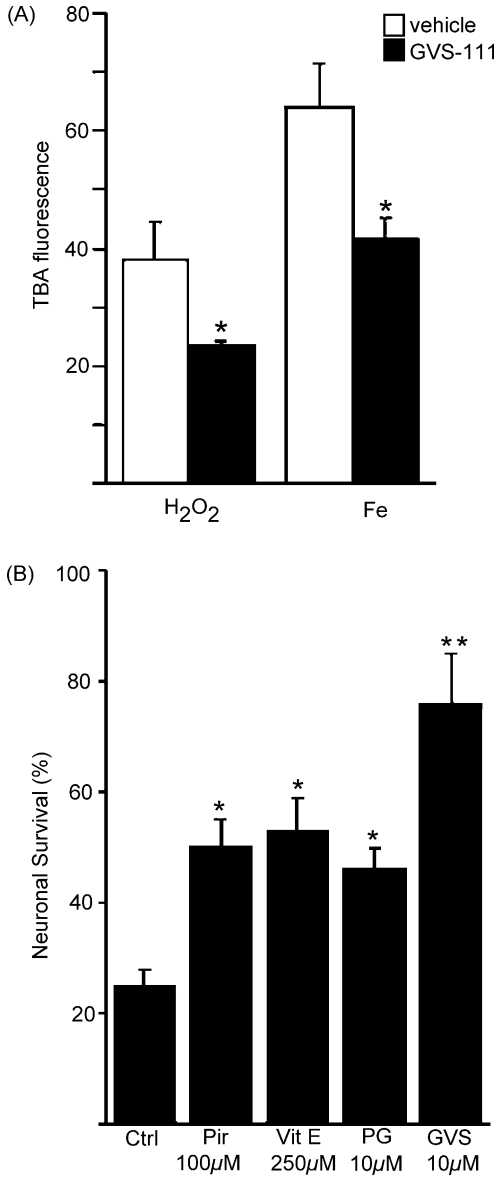

Fig. 4. GVS-111 inhibits H_2O_2-induced lipid peroxidation. (A) The graphic shows a significant decrease in lipid peroxidation expressed as TBA fluorescence in normal neurons exposed to H_2O_2 and $FeSO_4$ (Fe) and treated with 1 μM GVS-111. TBA fluorescence is expressed as arbitrary units derived from the image analysis (see Section 2). Values are the mean ± S.E.M.; $n = 4$ independent experiments; more than 20 microscopic fields were analyzed per experimental condition in each individual experiment. Significantly different from H_2O_2- or Fe-treated cultures by ANOVA (*$P < 0.01$). (B) Neuroprotective activity of Piracetam (Pir), Vitamin E (Vit E), propyl gallate (PG) and GVS-111 (GVS) on cortical neurons treated with 50 μM H_2O_2. Drugs were added at the indicated concentrations, which have been shown to produce maximal neuroprotective effect on DS neuronal cultures (Busciglio and Yankner, 1995). Neuronal survival is expressed as a percentage of survival in control cultures (100%). Values are the mean ± S.E.M.; $n = 4$ independent experiments; more than 200 neurons were scored per experimental condition in each individual experiment. Significantly different from H_2O_2-treated cultures by ANOVA ($P < 0.01$). (**) Significantly different from H_2O_2 + Pir-, H_2O_2 + Vit E- and H_2O_2 + PG-treated cultures.

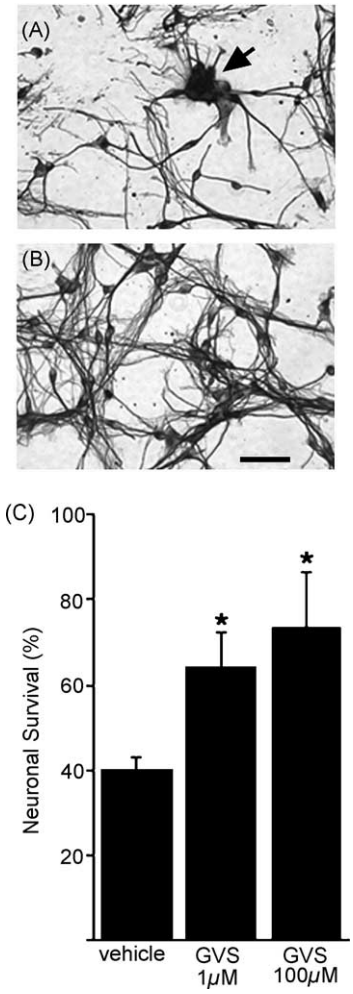

Fig. 5. GVS-111 enhances DS neuronal survival. (A) By 11 DIV, DS cortical cultures exhibit clear signs of degeneration including neuritic fragmentation and clumping of cell bodies (arrow). (B) DS cultures treated with 1 μM GVS-111 show a significant increase in neuronal survival and good preservation of neuronal processes. Scale bar: 40 μM. (C) Increase in neuronal survival in DS cultures treated with GVS-111 at the indicated doses. Results are expressed as a percentage of survival in DS cultures at 7 DIV (100%), before the onset of degeneration (Busciglio and Yankner, 1995). Results are the mean ± S.E.M. for four experiments. Significantly different from untreated cultures by ANOVA (*$P < 0.01$).

protection was observed at 100 and also at 10 nM. Analysis by ANOVA of the effect of GVS-111 and s-PBN at 100 and 10 μM showed a significant difference between the two groups, indicating that GVS-111 is more efficacious as a neuroprotective agent than s-PBN. GVS-111 also promoted higher survival than Vitamin E, propyl gallate and Piracetam, the best-known nootropic compound.

Oxidative stress is considered to play a role in the pathogenesis of several age-related neurodegenerative diseases, including Alzheimer's disease (Beal, 1995; Busciglio et al., 1998; Mattson and Chan, 2001). Interestingly, GVS-111 exhibited limited neuroprotection after amyloid β (Aβ) treatment (data not shown), supporting the notion that Aβ

induces neurotoxicity through different molecular pathways (Behl et al., 1994; Yankner, 1996; Grace and Busciglio, 2003). Oxidative stress is also a potential contributory factor in the neuropathology associated with DS (Busciglio et al., 1998; de Haan et al., 1997; Kedziora and Bartosz, 1988). DS neurons in culture exhibit increased intracellular free radicals, increased lipid peroxidation, mitochondrial dysfunction, intracellular amyloid β accumulation and neuronal degeneration (Busciglio and Yankner, 1995; Busciglio et al., 2002). In this regard, the degenerative changes observed in DS cultures are similar to those observed in normal cultures treated with 50 μM H_2O_2, including increased level of intracellular free radicals and lipid peroxidation. Moreover, DS neuronal degeneration can be blocked by different antioxidants including free radical spin traps, free radical scavengers, glutathione precursors and catalase (Busciglio and Yankner, 1995). A significant two-fold increase in neuronal survival was observed in DS neurons continuously treated with GVS-111, indicating that GVS-111 protected DS cultures against oxidative damage and that its continuous presence in the medium for 10 days was well tolerated by the cells. Previous studies have reported a diminished number of neurons in the DS brain (Ross et al., 1984), raising the possibility that neuroprotective therapies may improve neuronal survival and cognitive function in DS patients. The neuroprotective mechanism of action of GVS-111 appears to result from the above described antioxidant effect together with its ability to prevent intracellular Ca^{2+} increases (Solntseva et al., 1997), and to attenuate glutamate neurotoxicity (Ostrovskaya et al., 1998), which are pathological mechanisms closely associated with the loss of neuronal homeostasis and neurodegeneration (Busciglio et al., 1998). These effects of GVS-111 may be associated with its metabolic processing. GVS-111 main metabolic product is the dipeptide cycloprolyllglycine (cPG), which has been characterized as a naturally occurring, endogenous cyclic dipeptide in the brain (Gudasheva et al., 1996b). Recent studies revealed that GVS-111 possesses additional properties that may contribute to its potential effectiveness on Alzheimer's patients. GVS-111 improves neuronal responses to acetylcholine microiontophoretic application and restores mnestic performance in rats after treatment with scopolamine (Ostrovskaya et al., 2001a). In addition, GVS-111 showed anti-inflammatory activity in various experimental models of inflammation (Kovalenko et al., 2002). The experimental results obtained with GVS-111 combined with its high bioavailability after oral administration and its low toxicity (Ostrovskaya et al., 2001b), has led to the clinical assessment of GVS-111 in patients suffering from mild cognitive impairment, a study which is now in progress. The multiple neuroprotective properties displayed by GVS-111 along with its potential nootropic influence on cognitive function may provide a promising therapeutic approach for the treatment of mental retardation and chronic neurodegenerative conditions.

Acknowledgements

Supported by NIH grant HD38466 (J.B.) and grants from Neuropeptide Dynamics and Saegis Pharmaceuticals. C.H.-V. was Visiting Professor of Neuroscience at University of Connecticut Health Center.

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