Sigma-1 Receptor Agonists Inhibit Oligodendrocyte Cytotoxicity Induced by Molecules Involved in Cell Damage in Multiple Sclerosis

Robert P. Lisak M.D., Liljana Nedelkoska, Joyce A. Benjamins, Ph.D.
Department of Neurology, Wayne State University School of Medicine, Detroit, Michigan

ABSTRACT

Background: The sigma-1 receptor (s-1R) is an endoplasmic reticulum (ER) chaperone upregulated during ER stress; the receptor also regulates calcium homeostasis through its association with inositol triphosphate (IP3) at the ER membrane-associated mitochondrial interface. Activation of s-1R by a variety of agonists has neuroprotective effects both *in vitro* and *in vivo*. Dextromethorphan (DM) is a s-1R agonist as well as a weak N-methyl-D-aspartate receptor (NMDAR) antagonist, which in combination with quinidine sulfate is approved for treatment of pseudobulbar affect in multiple sclerosis (MS) and other neurologic diseases. We have reported that DM protects oligodendrocytes (OL) *in vitro* from the cytotoxic effects of staurosporine (inducer of apoptosis), glutamate (excitotoxicity), reactive oxygen species (ROS, induced by hydrogen peroxide, H_2O_2) and quinolinic acid (QA, a product of tryptophan indoleamine metabolism associated with inflammation) (Lisak et al. Glia, 2014). Objective: To determine if another s-1R agonist might also have OL protective properties, which might be effective in progressive MS, we examined the effects of ANAVEX2-73 on protection of OL from these cytotoxic molecules. ANAVEX2-73 is a new clinical compound, which is chemically unrelated to DM, and a s-1R agonist, NMDAR antagonist, also targeting muscarinic receptors, currently in a Phase 2a trial in Alzheimer's disease (Macfarlane et al. 2015 AAIC abstract). Methods: Glial cultures enriched in OL were prepared from newborn rat brain and cells identified by phenotypic markers (Lisak et al. Mult Scler, 2006). Cultures were incubated with various concentrations of ANAVEX2-73, 200 nanoM (nM)-20 microM, for 24 hours to assess cell death by trypan blue uptake. Since there was no toxicity noted at 200 nM, we then incubated cultures with staurosporine, glutamate, H_2O_2, QA or medium (control) and determined cell death. Results: ANAVEX2-73 reduced OL cell death induced by all 4 molecules by over 50%. Conclusion: ANAVEX2-73, which like DM is a s-1R agonist and NMDAR antagonist but differs in other activities, protects OL from cytotoxic mechanisms involved in pathogenesis of MS lesions. Studies to determine the relative roles of s-1R agonism, NMDAR antagonism and muscarinic activities are objectives for future studies. ANAVEX2-73 and DM are small molecules that enter the central nervous system and thus have potential to provide protection of OL in MS.

INTRODUCTION

Developing effective pharmacologic agents is critical for decreasing or preventing death of oligodendrocytes (OL) and OL precursors (OPC) from a wide variety of harmful agents found in inflammatory white matter and gray matter lesions in multiple sclerosis (MS), and in inflammatory responses in other neurodegenerative diseases and stroke. In the central nervous system (CNS), mature OL do not proliferate, and thus are unable to regenerate and remyelinate to support axons if the OL are injured or killed. However, the adult CNS contains OPC which can proliferate, migrate, mature and partially remyelinate axons that have been demyelinated. Normal appearing OPC have been identified in areas of MS lesions. Thus, OPC are critical not only during development, but also for remyelination after myelin has been destroyed, as occurs in multiple sclerosis. We reported that dextromethorphan (DM), a sigma-1 receptor (s-1R) *agonist,* protects OL and OPC *in vitro* from cell death induced by molecules that damage OL, OPC and neurons/axons in MS (Lisak et al. 2014). Several s-1R agonists protect neurons, both *in vivo* and *in vitro*, but little is known about the effects of s-1R agonists on OL or OPC protection, or differentiation, myelination or remyelination by OL lineage cells. The mechanisms underlying the effects of s-1R agonists are not well understood in either neurons or OL. ANAVEX2-73, chemically unrelated to DM (Figure 1) is currently undergoing a Phase 2a clinical trial in Alzheimer's disease. DM is approved as a treatment for pseudobulbar affect (PBA) when combined with quinidine sulfate (QS), as well as an over-the counter anti-tussive. In addition to acting as *agonists* on s-1R, both agents are *antagonists* of NMDA receptors, while ANAVEX2-73 is also a muscarinic receptor *agonist* and has activity at Na+ channel site 2. To date there is no evidence of clinically significant benefits of NMDA receptor antagonists in MS. Demonstrating that beneficial effects of current or future s1-R agonists are due, at least in part, to direct activation of s-1R in OL and OPC would greatly increase the therapeutic potential of DM, ANAVEX2-73 or newer s-1R agonists for protection and repair in patients with MS. In this study we sought to determine if ANAVEX2-73 protects OL from staurosporine (inducer of apoptosis), glutamate (excitotoxicity), reactive oxygen species (ROS) induced by H_2O_2, quinolinic acid (QA, a tryptophan indoleamine metabolite associated with inflammation), kynurenic acid (KA, a molecule upstream from QA), and generators of nitric oxide (NO).

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METHODS

Glial cultures enriched in OL were prepared from newborn rat brain and cells identified by phenotypic markers (Lisak et al. 2006). Cultures were incubated with various concentrations of ANAVEX2-73 (provided by ANAVEX™ Life Sciences Corp. under the SIGMACEPTOR® program) for 24 hours to assess cell death by trypan blue uptake. As noted in Results, there was minimal toxicity at 200 nM, and this concentration was more effective than lower concentrations at decreasing glutamate induced OL death. We then incubated cultures with staurosporine, glutamate (and agonists of the ionotropic glutamate receptors NMDA, AMPA and kainate), H_2O_2, KA and QA as well as NOC12 and NOC18, rapid and slow generators of nitric oxide, at previously used concentrations (Benjamins et al. 2013, Benjamins et al. 2014, Lisak et al. 2014), without and with ANAVEX2-73 at 200 nM (see Results).

RESULTS

As noted in Methods, incubation of OL enriched cultures with ANAVEX2-73 for 24 hours revealed a concentration dependent effect on OL viability with no significant toxicity at 200 nM or 1 μM compared to control medium (Figure 2). Since ANAVEX2-73 was not toxic to OL at 200 nM and protective against glutamate induced toxicity (Figure 3), we tested the protective effects of ANAVEX2-73 at 200 nM. ANAVEX2-73 inhibited OL cell death induced by glutamate, staurosporine, QA and ROS (H_2O_2) (Figure 4). Protection of OLs from glutamate-induced toxicity involved protection from activation of NMDA, AMPA and kainate receptors (Figure 5). There was no inhibition of toxicity induced by rapid (NOC12) or slow (NOC18) generation of nitric oxide or by KA (Figure 6). Results to date with the s-1 R antagonist BD1047 indicate that protection is due to signaling through s-1 R (Figure 7). ANAVEX2-73 also protects OPC from the same agents as found for OL (Figure 8A,B).

Figure 1. Structures of ANAVEX2-73 and dextromethorphan

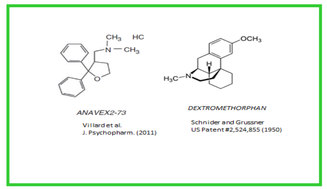


Figure 2. ANAVEX2-73 dose response for OL toxicity

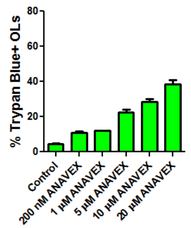


Figure 3. Optimal protection by 200 nM ANAVEX2-73 against glutamate

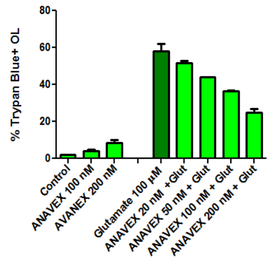


Figure 4. Protection against glutamate, staurosporine, quinolinic acid and H_2O_2

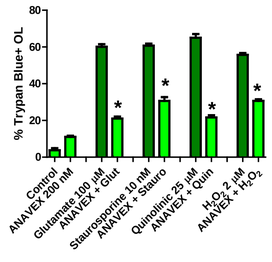


Figure 5. Protection against NMDA, AMPA and kainate

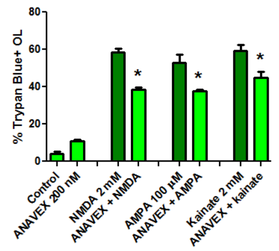


Figure 6. No protection against kynurenic acid or NO

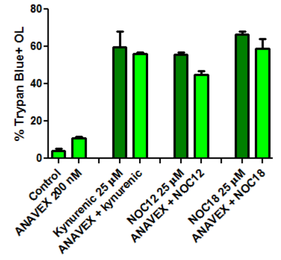


Figure 7. Inhibition of ANAVEX2-73 protection by the sigma-1 receptor antagonist BD1047

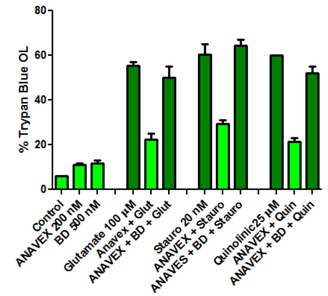
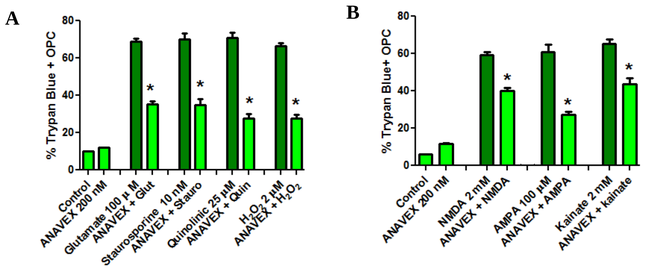


Figure 8. ANAVEX2-73 protection of OPC from toxic agents



CONCLUSIONS and DISCUSSION

ANAVEX2-73 inhibits OL cell death induced by staurosporine (apoptosis), excitotoxicity (glutamate via ionotropic receptors), ROS (induced by H_2O_2) and a mediator of inflammation (QA) but not nitric oxide or KA. For OPCs, of the toxic agents assessed so far, ANAVEX2-73 also protects from the same agents as found for OL. This pattern of *in vitro* protection for OL and OPC is the same as we reported for DM (Lisak 2014 et al.), chemically unrelated, but sharing both s-1R agonist and NMDA antagonist functions with ANAVEX 2-73. DM is a non-competitive agonist and ANAVEX2-73 a competitive agonist for s-1R. ANAVEX2-73 is also an agonist for muscarinic receptors and affects Na+ channel site 2. The relative roles of these molecules at s-1 and NMDA receptors and the role of muscarinic receptors and Na+ channel site 2 activity for ANAVEX2-73 in relation to OL and OPC protection is not as yet clear and will require further study. DM, ANAVEX2-73 and future s-1R agonists might have a protective role in treatment of patients with MS.