Tegaserod mimics the neurostimulatory glycan polysialic acid and promotes nervous system repair

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ABSTRACT

Glycans attached to the cell surface via proteins or lipids or exposed in the extracellular matrix affect many cellular processes, including neuritogenesis, cell survival and migration, as well as synaptic activity and plasticity. These functions make glycans attractive molecules for stimulating repair of the injured nervous system. Yet, glycans are often difficult to synthesize or isolate and have the disadvantage to be unstable in a complex tissue environment. To circumvent these issues, we have screened a library of small organic compounds to search for structural and functional mimetics of the neurostimulatory glycan polysialic acid (PSA) and identified the 5-HT4 receptor agonist tegaserod as a PSA mimetic. The PSA mimicking activity of tegaserod was shown in cultures of central and peripheral nervous system cells of the mouse and found to be independent of its described function as a serotonin (5-HT4) receptor agonist. In an in vivo model for peripheral nerve regeneration, mice receiving tegaserod at the site of injury showed enhanced recovery compared to control mice receiving vehicle control as evidenced by functional measurements and histology. These data indicate that tegaserod could be repurposed for treatment of nervous system injuries and underscores the potential of using small molecules as mimetics of neurostimulatory glycans.

1. Introduction

Polysialic acid (PSA) is a homopolymer of ω-(2,8)-linked sialic acid residues numbering up to 200 in length, and is attached predominantly to the neural cell adhesion molecule (NCAM) (Finne et al., 1983; Muhlenhoff et al., 1996). PSA is expressed in the developing and adult nervous system of vertebrates, with expression localized to migrating cells, processes of neurons and glial cells, synapses and stem cells (Angata and Fukuda, 2010; Durbec and Cremer, 2001). PSA has been suggested to promote cell motility in the nervous system by primarily expanding the extracellular space due to its large hydration volume (Yang et al., 1994) and decreasing homophilic interactions of NCAM (Durbec and Cremer, 2001). PSA has also been suggested to promote cell motility in the nervous system by primarily expanding the extracellular space due to its large hydration volume (Yang et al., 1994) and decreasing homophilic interactions of NCAM (Durbec and Cremer, 2001). PSA also mediates interactions of NCAM with heparan sulfate proteoglycans (Storms and Rutishauser, 1998), brain derived neurotrophic factor (Muller et al., 2000), ω-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors (Vaithianathan et al., 2004), N-methyl-o-aspartate receptors...
(Hammond et al., 2006; Senkov et al., 2006), histone H1 (Mishra et al., 2010) and myristoylated alanine-rich C kinase substrate (Theis et al., 2013).

The cell motility promoting effects of PSA are of interest for the treatment of nervous system injuries and disorders. Viral-induced expression of PSA enhances regeneration after spinal cord injury, promotes sensory neuron integration into the injured spinal cord, and increases Purkinje cell dendrite formation following injury (Zhang et al., 2007a, 2007b, 2007c). Overexpression of PSA on astrocytes improves axonal extension across spinal cord injuries (El Maarouf et al., 2006), and transplanted Schwann cells over-expressing PSA augment repair in both spinal cord (Luo et al., 2011; Papastefanaki et al., 2007) and peripheral nerve (Jungnickel et al., 2012). However, continuously expressed PSA slows the rate of myelination in vivo (El Maarouf et al., 2006; Franceschini et al., 2004), and high PSA expression correlates with invasiveness and malignancy of cancers (Petridis et al., 2009; Tanaka et al., 2001), suggesting that a dose- and time-constrained approach must be considered.

As PSA is rapidly degraded by sialidases in the complex tissue environment (Franz et al., 2005; Martini et al., 1994; Nagai et al., 1989), peptide mimetics of PSA were identified and shown to act as false neurotransmitter counterparts of PSA (Torregrossa et al., 2004). Linear and cyclic PSA mimetic peptides have improved functional recovery following peripheral nerve and spinal cord injuries in mice (Marino et al., 2009; Mehanna et al., 2010, 2009). Small organic molecule mimetics of PSA offer further advantages for the development and regulatory approval for therapies. To build upon previous advances in identifying PSA mimetics and to increase the translational potential, we have screened a library of small compounds for mimetics of PSA and identified tegaserod. Tegaserod is a drug that received clinical approval for treatment of irritablebowel syndrome and constipation (Muller-Lissner et al., 2001) by stimu-

2.2. Surface plasmon resonance (SPR)

Binding affinities of organic compounds to the antibody 735 were evaluated in a competition experiment by SPR measurements carried out on a BIAcore 3000 in-

2.3. Molecular modeling of tegaserod with the PSA-specific antibody 735

A model of PSA bound to the binding pocket of the antibody 735 was con-

2.4. In vitro analysis of neurite/process outgrowth

Primary cultures of cerebellar granule neurons (cGNs), dorsal root ganglion (DRG) neurons or Schwann cells were prepared from cerebellar or dorsal root ganglia of 7-day-old (P7) C57BL/6J wild type mice as described (Kleene et al., 2001; Loers et al., 2005; Mehanna et al., 2009) and motorneurons were prepared from C57BL/6J wild type or NCAM−/− 14-day-old (E14) mouse embryos as described (Sirova et al., 2006). In brief, 48-well plates were coated with 0.01% poly-γ-lysine (PLL) or poly-γ-ornithine (PLO) overnight at 4 °C. Schwann cells, cerebellar neurons, DRG neurons (PLL) or motorneurons (PLO) were seeded at a density of 1.25 × 10^4 (Schwann cells, DRG neurons and motorneurons) or 2.5 × 10^5 cells (cGNs) per well in 250 μl of corresponding serum-free culture medium and compounds were added 1 h after seeding. After maintenance for 24 h at 37 °C, cells were fixed with 2.5% glutaraldehyde and stained with 1% methylene blue/toluidine blue in 1% borax. Morphological quantification of neurite or process lengths was performed as described (Mehanna et al., 2009). Schwann cell processes and neurites of cGNs, DRG neurons and motorneurons with a length of at least one cell body diameter were counted and total neurite or process length per cell was determined by counting 50 cells in each of two wells per experiment using an Axiovision system 4.6 (Carl Zeiss). At least three independent experiments were performed for each culture condition.

2.5. Effects of tegaserod on femoral nerve regeneration

2.5.1. Mice

All experiments were conducted in accordance with the Rutgers Animal Care and Facilities Committee and the Institutional Animal Care and Use Committee (IACUC) and every effort was made to minimize animal suffering and the number of animals used in experiments. C57BL/6J wild type and NCAM−/− mice were used for all experiments and kept under standard laboratory conditions with food and water supply ad libitum and with an artificial 12 h light/dark cycle. Twelve-week-old C57BL/6J mice were subjected to femoral nerve injury as described (Mehanna et al., 2009), but with tegaserod substituting for the PSA peptide mimetic. Briefly, Panumatrix hydrogel (BD Biosciences, Franklin Lakes, NJ) contained within a poly-

3-(dimethylaminopropyl) carbodiimide and 50 mM N-hydroxysuccinimide. The PSA mimetic peptide solution (10 nM in 10 mM sodium acetate, pH 5.2) was then injected for 5–10 min. Ethanolamine (1 M, pH 8.5) was used to block remaining activated carboxyl groups (1 h). Ligand densities of 100–150 fmol/mm² were reached. Immobilized control peptide (10 nM PSA scrambled peptide mimetic coupled to catalase) was used as a reference surface. Regeneration of the sensor chip was achieved by injection of 10 nM glycine, pH 2.5, at 10 ml/min (two 30 s pulses). The data were analyzed using the BIAevaluation 3.0 software. All sensorgrams were corrected for background and bulk refractive index subtraction of the reference.

To confirm that tegaserod binds specifically to antibody 735, a competition experiment was performed. First, the antibody (10 nM) was pre-incubated for 1 h at room temperature with different molar concentrations (1, 2, 5, 8, 15 and 30 μM) of tegaserod or the negative control compound nitrendipine. Then, the antibody/ organic compound solutions were injected (1 ml/min) to the PSA peptide mimetic coupled chip and binding was determined over 30 min. The surface of the sensor chip was subsequently regenerated with 10 mM glycine, pH 2.5, at 10 ml/min (three 30 s pulses).
2.5.2. Determination of locomotor parameters

The foot base angle (FBA) was measured before surgery and every week following surgery before the sacrifice of animals as described (Mehanna et al., 2009; Simova et al., 2006). Single frame video motion analysis (Simi Reality Motion Systems, Simi Sportsplayer) was used to quantify the angle between the beam and the foot when the toes from the left leg are fully extended.

The protraction limb ratio (PLR) was similarly measured using single frame motion analysis (Irintchev et al., 2005) and is taken as an indicator of voluntary movement. Mice are suspended by the tail above a pencil and allowed to grasp the pencil. The PLR is measured by dividing the relative length that the uninjured hind leg extends to the pencil by the length that the injured hind limb extends.

![Image of graphs](image-url)
To account for variabilities between animals, an index is calculated that takes into account the initial FBA and PLRs of animals before surgery. The recovery index is calculated in percent by the equation: $RI = \frac{(X_{\text{post}} - X_{\text{pre}})}{(X_{\text{pre}} - X_{\text{rm}})} \times 100$, where $X_{\text{pre}}$, $X_{\text{post}}$, and $X_{\text{rm}}$ are values prior to operation, during the state of denervation (7 days after injury) and at the end-point of the study (here 42 days after injury), respectively. An RI value of 100 indicates complete recovery of locomotor function.

2.5.3. Determination of quadriceps muscle mass

At the end of the locomotor measurements, the quadriceps muscles were removed from the injured and uninjured hind limbs. The muscle was bluntly dissected starting distally at the level of the patellar tendon, working proximally, and cut free from its attachments from the patellar tendon distally and from the pelvis and femur proximally. Muscles were blotted dry using a paper towel and the mass was weighed using a Mettler Toledo XS105 Dual Range analytical balance.

2.5.4. Histology

Animals were perfused with 4% paraformaldehyde and explanted nerves were post-fixed in osmium tetroxide and embedded in resin as described (Masand et al., 2012). One and 2 μm thick cross sections were cut midway into the region of the regenerated nerve within the conduits, and sections were stained with 1% toluidine blue and 1% borax. Sections were imaged with a Zeiss Axiocam, using 20 x, 40 x, and 100 x objectives. ImageJ software was used to calculate the number of axons as described (Mehanna et al., 2009).

3. Results

3.1. Identification of tegaserod as potential mimetic of PSA

NIH Clinical Collection 1 Library was screened via competition ELISA for compounds that inhibit binding of the PSA receptor site of antibody 735 to a previously identified peptide mimic of PSA (Mehanna et al., 2009). This screen resulted in the identification of six drugs, one of which was tegaserod. To confirm the results of the initial screen, a second competition ELISA was performed with a range of tegaserod concentrations. Tegaserod inhibited binding of antibody 735 to the PSA peptide mimetic in a dose dependent manner (Fig. 1A), with maximal inhibition at approximately 100 nM. The ability of tegaserod to inhibit binding of the PSA peptide mimetic to antibody 735 was compared against the control compound nitrendipine, which did not impede antibody binding at any concentration.

The capacity of tegaserod to interact with the antibody 735 antigen binding site was also evaluated by surface plasmon resonance (SPR), which provides a more sensitive and kinetic platform to probe molecular interactions. Tegaserod disrupted binding of antibody 735 to the PSA peptide mimetic that was bound to the chip surface by 80% compared to antibody in the absence of tegaserod (Fig. 1B). Nitrendipine showed markedly less inhibition than tegaserod. In addition, a concentration dependent inhibition of binding between antibody 735 and PSA peptide mimetic could be shown by SPR in the presence of tegaserod, but not in the presence of the control compound nitrendipine (Fig. 1C).

3.2. Molecular modeling of tegaserod in the PSA receptor pocket

To identify how a small molecule such as tegaserod could act as a mimic for the larger and negatively charged PSA, molecular modeling of PSA and tegaserod was performed within the published structure for the PSA binding pocket of antibody 735 (Evans et al., 1995). The broad complementary determining region (CDR) of antibody 735 contains two biochemically distinct regions: a hydrophobic region rich in aromatic residues and several polar residues, and a positively charged region consisting of multiple lysine residues. An eight-residue PSA fragment forms a half-helical turn that binds within a broad cleft in the antibody CDR region (Fig. 2A). PSA residues 1–4, which expose N-acetyl groups to the antibody surface bind to the hydrophobic region of the CDR and form hydrogen bonds with aspartic acid (D)105 — heavy chain (H) and arginine (R)55 ! light chain (L). These interactions are thought to be critical to the specificity of antibody 735 for N-acetyl over N-propyl PSA. By contrast, PSA residues 5–8 present largely negatively charged carboxyl groups to the positively charged region of the CDR, forming salt-bridges with lysine (K)65-H and K101-H. Overall, the model confirms previous observations (Evans et al., 1995), in that the aromatic/polar region of the CDR is responsible for N-acetyl PSA specificity, while the positively charged region forms more promiscuous interactions with multiple negatively charged groups in PSA.

Using the ligand docking software Glide (Friesner et al., 2004), tegaserod was docked into the CDR region of antibody 735 without further modifications to the receptor structure. The top-ranked pose (Fig. 2B) shows tegaserod binding to the...
hydrophobic region of the antibody CDR, forming hydrogen bonds with two key residues, D105-L and R55-H, that also mediate PSA binding. The pose suggests that tegaserod may be further stabilized by cation-π interactions between R55-H and the tegaserod ring as well as aromatic interactions with numerous residues in the binding site such as tyrosine (Y)37-L, Y54-L, Y32-H, Y33-H, and phenylalanine (F)102-H. These docking results suggest that tegaserod competes with PSA by binding to a hydrophobic groove within the PSA-binding site of antibody 735 and reproducing a number of intermolecular hydrogen bonds found in the antibody 735 complex model.

3.3. In vitro activity of tegaserod

Functional activity of tegaserod was assessed in vitro using PSA-responsive murine cGNs, DRG neurons, Schwann cells, and motoneurons. Schwann cells, DRG neurons and motoneurons are key cell types involved in peripheral nerve regeneration and muscle reinnervation and cGNs were chosen because they are, like motoneurons and Schwann cells, responsive to PSA, but are known not to be responsive to serotonin (5-hydroxytryptamine; 5-HT) for neurite extension (Zilkha-Falb et al., 1997). A dose–response curve of tegaserod for motoneurons, Schwann cells and cGNs, and revealed that tegaserod affects neurite and process extension of neurons and Schwann cells, respectively, at 1 nM and reached an optimal effect at 100 nM for all cell types (Fig. 3A). At doses exceeding 100 nM, the effects of tegaserod on increasing neurite and process extension began to decline and reached control levels between 1 µM and 5 µM.

Experiments were next conducted to compare the activity of tegaserod with the PSA peptide mimic and the bacterial PSA analog colominic acid on motoneuron neurite extension. Colominic acid, the PSA peptide mimic, and tegaserod all stimulated neurite extension of murine motoneurons, while cisapride (another 5-HT4 agonist (Quigley, 2011)) and serotonin did not enhance motoneuron neurite extension (Fig. 3B). Furthermore, the 5-HT4 receptor antagonist GR113808 (Gale et al., 1994) did not inhibit motoneuron extension and co-treatment of neurons with tegaserod and GR113808 did not decrease the stimulatory effect of tegaserod. Importantly, motoneurons isolated from NCAM+/− mice were not enhanced in neurite extension by colominic acid, the PSA peptide mimic, or tegaserod, suggesting that tegaserod affects motoneurons via NCAM. Similarly, the effects of tegaserod were comparable to those of colominic acid and the PSA peptide mimetic on cGNs, DRG neurons and Schwann cell neurite/process extension (Fig. 3C, D, E). Neurite/process extension of these cell types could not be duplicated with serotonin or cisapride, and was not lowered by co-administration of GR113808 with tegaserod. Finally, exposure to tegaserod also increased survival of motoneurons and cGNs (Fig. 3F).

3.4. Effects of tegaserod on regeneration following femoral nerve injury

3.4.1. Metrics of functional recovery

The in vivo efficacy of tegaserod was tested in the mouse femoral nerve injury model, with 250 nM or 2500 nM of tegaserod being encapsulated in the BD Puramatrix hydrogel within an inert conduit that was sutured between two nerve stumps with a gap of 2 mm length. The vehicle control group received the same hydrogel contained within the conduit but without tegaserod. The 2 mm gap length is sub-critical and limited recovery is expected in the control group. Locomotor recovery was quantified as described (Mehanna et al., 2009) through assessment of the foot base angle (FBA) and protraction limb ratio (PLR) as shown in Fig. 4. Studies utilizing this method have found maximal recovery of the FBAs between 75 and 85° (Irinchchev et al., 2005; Masand et al., 2012; Mehanna et al., 2009).

3.4.2. Functional recovery

Analysis of the FBA showed that tegaserod improved this functional metric of regeneration. A sustained improvement of the FBA in mice receiving 250 nM tegaserod-containing conduits first became apparent 6 weeks after the injury and became statistically significantly different from the vehicle control group at 10 weeks (Fig. 5A). The level of recovery of the FBA from 250 nM tegaserod exceeds or is equivalent to the maximal recovery found in other studies using this model (Irinchchev, 2011; Masand et al., 2012; Mehanna et al., 2009; Simova et al., 2006). Animals that received the higher dose of tegaserod, 2500 nM, tended toward improved FBA recovery but did not achieve a statistically significant difference from the vehicle control.

Given the variation between individual animals, a recovery index (RI) for the FBA was calculated which normalizes the recovery at the end of the 15-week assessments to the initial FBA and the FBA measured at week 1, which is within 5–7 days of the surgery. The RI is expressed as a percentage, with the vehicle control group showing an average RI of 37%, with 5 of the 6 animals clustered between 36% and 42% (Fig. 5B). Animals that had received 250 nM tegaserod recovered to an average of 62%, while animals that had received 2500 nM tegaserod showed an RI of 56%, with considerable variation for animals having received 2500 nM tegaserod.

In addition to the FBA measurements, the PLR was evaluated. Following injury, the injured leg does not extend to the same extent as the uninjured leg, but will do so as nerve function is restored (Fig. 4). Analysis of the PLR with and without tegaserod suggested a positive influence at a 250 nM tegaserod concentration, but not at 2500 nM (Fig. 6A). Vehicle control mice without tegaserod returned to pre-surgery PLR by 8 weeks, compared with 6 weeks for mice having received 250 nM tegaserod. In contrast, mice that had received 2500 nM tegaserod exhibited PLRs that remained higher than the other two groups. Calculation of the RI for each mouse revealed that, although the majority of mice with 2500 nM eventually did recover to an RI of 100%, one mouse within this group did not recover to pre-surgical PLR functional performance (Fig. 6B).

3.4.3. Effect of tegaserod treatment on muscle mass

Muscles rapidly atrophy if nerve conduction is lost and will regain muscle mass as reinnervation occurs. The quadriceps muscle, which is solely innervated by the femoral nerve, was weighed at the end of the 15-week recovery period as an additional metric of recovery. The quadriceps muscle from the injured limbs of mice that had received 250 nM tegaserod showed a 50% increase in muscle mass compared to the quadriceps muscle of the control group (Fig. 7). Quadriceps muscles from mice having received 2500 nM tegaserod were slightly heavier when compared to the control group.

3.4.4. Histological assessment of nerves

At the end of the recovery period, femoral nerves were fixed and histology was performed to assess the histology of the nerve 1 mm into the 2 mm gap (Fig. 8A–C). Regenerated nerves treated with vehicle control contained an average of 432 axons, compared to 589 and 434 for nerves treated with 250 nM and 2500 nM tegaserod, respectively (Fig. 8D). When the degree of myelination (in percent) and g-ratios of the myelinated axons were quantified from images obtained from light microscopy, no significant differences between groups were detectable (data not shown).
Fig. 3. In vitro activity of tegaserod on extension of neurites/processes from and survival of motoneurons, cerebellar granule neurons (cGNs), dorsal root ganglion (DRG) neurons and Schwann cells. (A) Neurite/process extension following varying doses of tegaserod after 24 h. (B) Comparison of the neurite-extending capabilities of tegaserod and related compounds on murine motoneurons from wild type and NCAM deficient (NCAM/−/−) mice. Tegaserod elicits neurite extension comparable to the PSA analog colominic acid and the PSA peptide mimetic, which is not observed in motoneurons from NCAM/−/− mice. 5-HT4 agonists serotonin and cisapride as well as the 5-HT4 antagonist GR113808 do not increase neurite extension from motoneurons, nor did GR113808 attenuate the effects of tegaserod. (C–E) Effects of tegaserod and related compounds on cGNs, DRG neurons and Schwann cells. (F) Relative survival of motoneurons and cGNs treated with tegaserod and related molecules. Concentrations of compounds, glycans and peptides: tegaserod, serotonin, cisapride and GR113808 (100 nM), colominic acid (3 mM), PSA peptide mimetic (30 μM). All treatments were performed in duplicates and at least 100 cells were counted for each treatment. Results are from two or more experiments. Mean values ± SEM are shown. (C–E) *p < 0.05, **p < 0.005, ***p < 0.0005 via Student’s t-test.
approved drugs have known toxicological and pharmacokinetic profiles and thus have the potential to avoid a repetition of these time consuming and costly studies prior to gaining approval for a new application, provided that the therapeutic dose is equal to or less than the original application. In the majority of cases, a drug is repurposed for a different indication based on its known mechanism of action. For example, celecoxib was repurposed from treating osteoarthritis to familial adenomatous polyposis based on the activity of inhibiting cyclooxygenase-2 (Phillips et al., 2002).

Other compounds, such as β-lactam antibiotics and minocycline, have been found to act via thus far undefined molecular mechanisms to upregulate expression of proteins that confer neuroprotection (Plane et al., 2010; Rothstein et al., 2005). We have, in contrast to these approaches, used an in vitro approach to investigate new functional mechanisms for a drug targeted to a different disease paradigm. We have thus been able to present what appears to be one of the first reports on a small molecule mimetic of a nervous system glycan.

The small organic compound tegaserod was identified as a PSA mimetic by a competition ELISA screen and in vitro assays confirmed its PSA mimicking effect on central and peripheral nervous system cells. Lack of activity on NCAM−/− motoneurons and the inability of serotonin or other 5-HT4 agonists to replicate the activity of tegaserod in vitro indicate a structural mimicking activity consistent with the screening method for PSA mimetics and a functional activity independent of serotonin and/or serotonin receptors. While the molecular modeling studies and cell culture assays indicate that tegaserod may act via NCAM and its co-receptors, it is also conceivable that tegaserod binds to and acts via additional PSA receptors, such as heparin sulfate proteoglycans (Storms and Rutishauser, 1998), brain derived neurotrophic factor (Muller et al., 2000), excitatory amino acid receptors (Hammond et al., 2006; Kochlamazashvili et al., 2010, 2012; Vaithianathan et al., 2004), histone H1 (Mishra et al., 2010) and/or myristoylated alanine-rich kinase C substrate (Theis et al., 2013). Although the ability of tegaserod to interact with these molecules is yet unknown, it can plausibly be expected that, given the structural and functional similarity of tegaserod with oligomers within PSA, tegaserod will subserve many interactions of PSA.

It is noteworthy that tegaserod leads to maximal neurite extension in vitro at 1/10th and 1/100th of the doses required for the peptide mimetic and colominic acid, respectively. Other in vitro experiments with modified PSA derivatives used μM to mM PSA concentrations and PSA of different chain lengths (Berski et al., 2008; Haile et al., 2008). Although these results are not strictly...
comparable to the ones of the present study, they indicate that concentrations of native PSA in the in the higher μM range are needed for optimal effects.

In a previous study, the extent of regeneration induced by μM concentrations of the PSA peptide mimetic in the same injury model (Mehanna et al., 2009) was similar to that promoted by 250 nM tegaserod in the present study which showed that 250 nM of tegaserod beneficially acted in regeneration as indicated by several parameters measured in vivo. The level of recovery was superior or equivalent to that promoted by PSA and the PSA peptide mimetic in this and other peripheral nervous system regeneration paradigms (Gravvanis et al., 2007; Haastert-Talini et al., 2010; Jungnickel et al., 2009, 2012; Marino et al., 2009; Masand et al., 2012; Mehanna et al., 2009). While the proof-of-concept experiments of the present study are encouraging, it will be important to assess the ability of tegaserod to promote nerve repair in larger peripheral nerve gaps or other paradigms of regeneration in acutely and chronically injured mammalian nervous systems.

The effects of PSA, the peptide mimetic and colominic acid on neurite extension plateau at concentrations in the μM range while higher doses of tegaserod in vitro show a reduced level of stimulation and the highest doses were comparable to unstimulated control values. It is not uncommon for drugs to have an optimal dose range and exert undesirable effects if this dose range is exceeded. It is interesting in this context that overall cell viability was not reduced at higher doses; the reason for the function reducing effects on neurite extension at higher concentrations is presently unknown. It should be noted that the reduced average values of locomotor recovery observed in vivo at 2500 nM concentrations could be due to a single animal in this group where no axons were detected by histological analysis, thus raising the possibility that this dose mirrors the less beneficial effects seen at higher doses in vitro.

Like most agonists, tegaserod is not entirely specific for its cognate receptor, the 5-HT₄ receptor and stimulates, although less efficiently, other serotonin receptors (Sanger, 2009; Smith et al., 2008). Of these receptors, 5-HT₂B has been reported in rat peripheral nerves and tegaserod’s pharmacological effect on human 5-HT₂B is only 10% of that achieved via 5-HT₄ receptor (Beattie et al., 2004; Smith et al., 2008). The 5-HT₂B receptor in rat peripheral nerves is expressed by Schwann cells (Gaietta et al., 2003; Yoder et al., 1997) and triggering this receptor leads to Ca²⁺ release from Schwann cells in vitro (Yoder et al., 1996). How serotonin affects Schwann cell process extension or peripheral nerve regeneration has not been investigated.

Expression of 5-HT₄ receptor mRNA in rat DRG neurons has been reported (Nicholson et al., 2003), but the functional implications of 5-HT₄ actions in the PNS outside of the enteric nervous system remain to be determined. Our data indicate that the 5-HT₄ receptor agonists serotonin and cisapride fail to promote neurite extension from DRG neurons, while tegaserod is effective and its action is not reduced by the 5-HT₄ receptor antagonist GR113808. Despite these effects, it is unlikely that the improvements in functional motor control and muscle mass noted in our studies would be due to an effect of tegaserod on sensory axons. Sensory axons lack the intrinsic capability of stimulating muscles and those sensory axons that mistakenly reinervate into the motor branch following nerve injury are removed in a process called pruning (Brushart, 1993; Redett et al., 2005). However, while tegaserod has been found to stimulate neurite extension in vitro as PSA does via NCAM, we cannot rule out the possibility that tegaserod may be acting through other PSA receptors or additional indirect pathways in vivo to promote peripheral nerve regeneration.
Many studies over the last 40 years have investigated the potential incorporation of biological cues to stimulate nerve regeneration. Increasing the success of off-the-shelf conduits will depend upon directional support, but are not biologically inductive. Thus, regeneration is often suboptimal for these smaller gap sizes (Kehoe et al., 2012). Nerves from animals treated with 2,500 nM tegaserod. (et al., 2012). These in-market conduits provide mechanical and nerves are reported to only be effective for gaps of ≤3 cm, and regeneration is often suboptimal for these smaller gap sizes (Kehoe et al., 2012). These in-market conduits provide mechanical and directional support, but are not biologically inductive. Thus, increasing the success of off-the-shelf conduits will depend upon incorporation of biological cues to stimulate nerve regeneration. Many studies over the last 40 years have investigated the potential of a broad range of molecules and cells to improve peripheral nerve regeneration in animal models with promising results. However, only conduits that are biologically inert have entered the market. Likely reasons for this are (a) the failure of the enhancing agent to prove sufficient benefit in longer nerve gaps, (b) the expense needed for clinical trials and establishing good manufacturing processes (GMP) for the enhancers, and (c) the time consuming and costly regulatory approval pathway.

Tegaserod would be advantageous in these respects. The concentrations needed to promote nerve regeneration are orders of magnitude lower than those administered for irritable bowel syndrome in humans: 2–12 mg/day or 0.025–0.15 mg/kg for an 80 kg patient for a 4–6 week application time as opposed to 46 pg in a single on-site dose in a 20 g mouse, comparable to $2 \times 10^{-6}$ mg/kg, for administration to an injured peripheral nerve. Application of tegaserod at lower than clinically used concentrations could avoid the need for replication of toxicological and pharmacological profiles. In the context of nerve regeneration, tegaserod would be delivered in a single on-site dose, either encapsulated within a scaffold as used in this report or preferably slowly released from a biodegradable drug-eluting conduit. On-site delivery via the conduit should also greatly minimize the likelihood of the very rare adverse coronary effects reported for daily oral administration of high doses of tegaserod (Busti et al., 2004; Schiller and Johnson, 2008). As a previously FDA approved drug with established GMP, commercial translation may likewise be facilitated. Thus, tegaserod has the potential to be repurposed for the application not only of peripheral nerve repair, but hopefully also for amelioration of central nervous system disabilities where PSA is likely to play an important therapeutic role.

5. Conclusions

Glycans, such as PSA, play an important role in nervous system development, synaptic plasticity and regeneration following injury. The application of small molecule compounds that mimic the beneficial activity of glycans, such as also the HNK-1 carbohydrate, for nervous system repair may prove to be valuable additions to treatments that rely on other classes of compounds. Also, evaluating approved drugs for alternate mechanisms of action may open novel approaches to pharmacological actions.

Conflict of interest statement

The authors declare no conflicts of interest regarding this work. The opinions and assertions contained herein are the private views of the authors and are not to be construed as of the views of the U.S. Army or the U.S. Department of Defense. This paper has been approved for public release with unlimited distribution.

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