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Low Dose Bexarotene Treatment Rescues Dopamine Neurons and Restores Behavioral Function in Models of Parkinson's Disease

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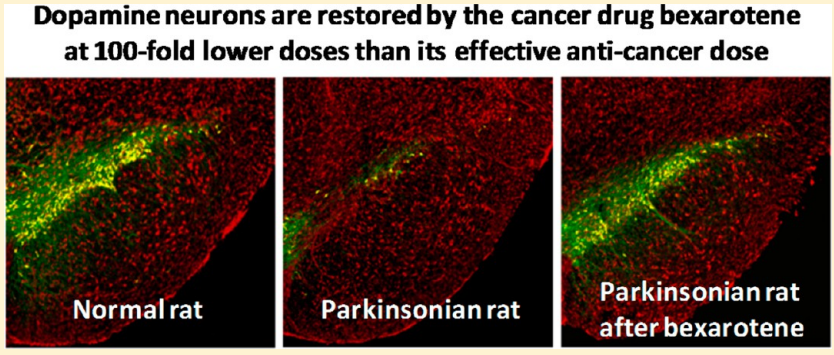
Supporting Information



ABSTRACT: Nurr1 is a nuclear hormone receptor (NucHR) strongly implicated in the growth, maintenance, and survival of dopaminergic neurons. Nurr1 may be unable to bind ligands directly, but it forms heterodimers with other NucHRs that do. Using bioluminescence resonance energy transfer (BRET) assays to directly monitor interactions of Nurr1 with other NucHRs, we found the cancer drug bexarotene (Targretin, also LGD1069) displayed biased interactions with Nurr1-RXR heterodimers compared with RXR-RXR homodimers. Remarkably, at doses up to 100-fold lower than those effective in rodent cancer models, bexarotene rescued dopamine neurons and reversed behavioral deficits in 6-hydroxydopamine (6-OHDA) lesioned rats. Compared to the high doses used in cancer therapy, low doses of bexarotene have significantly milder side effects including a reduced increase in plasma triglycerides and less suppression of thyroid function. On the basis of extrapolations from rat to human doses, we hypothesize that low oral doses of bexarotene may provide an effective and tolerated therapy for Parkinson's disease (PD).

KEYWORDS: *Bexarotene, Nurr1, retinoid X receptor, dopamine neuron, behavior, Parkinson's disease*

Nurr1 has essential roles in both development and maintenance of dopamine neurons.[1,2] It regulates expression of many genes involved in dopamine turnover and trophic support of dopamine neurons,[3−7] limits inflammatory responses in the CNS, and protects dopamine neurons from neurotoxicity.[8] Mutations that reduce Nurr1 expression are associated with PD in humans.[9−12] These observations suggest that Nurr1 might play a pathophysiological role in aspects of PD ranging from modulating inflammatory responses to promoting dopaminergic neuron function and survival.

For these reasons, Nurr1 agonists would be expected to have potential as PD disease modifying drugs. However, Nurr1 is an "orphan NucHR", with no known endogenous agonist. Moreover, the crystal structure of Nurr1 suggests its ligand binding cavity may be inaccessible to small molecules.[13] A number of compounds have been proposed to act as Nurr1 agonists;[14−16] however, their mechanism of activation of Nurr1 is unclear. Presently, no drugs targeting Nurr1 are available.

Nurr1 can form dimers with other NucHRs.[17,18] Unlike Nurr1, several of these other NucHRs are ligand activated transcriptions factors. Therefore, Nurr1 activity may be regulated indirectly through agonist activation of its druggable binding partners. Consistent with this idea, it was previously shown that RXR agonists could transactivate Nurr1.[19]

We used BRET assays to directly monitor interactions of small molecules with Nurr1 alone or coexpressed with other NucHRs and screened a small molecule library in BRET assays for Nurr1-RXR heterodimers. Surprisingly, we identified compounds that displayed biased interactions with Nurr1-RXR heterodimers compared to RXR-RXR homodimers. Among the compounds showing this bias was bexarotene,[20,21] an FDA approved drug used to treat certain types of cancer.[22] This finding motivated us to examine bexarotene using both in vitro and in vivo models of PD.

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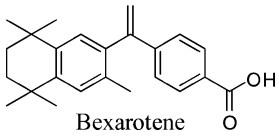

Bexarotene

RESULTS AND DISCUSSION

We used BRET assays to directly monitor interactions of small molecules with Nurr1 alone or coexpressed with other NucHRs. We found that the FDA approved drug bexarotene[20,21] interacted more potently with Nurr1-RXR heterodimers than RXR-RXR homodimers, as did a structural analogue of bexarotene called LG100268, whereas other compounds had equal or greater potency at RXR-RXR, and no compounds were identified that directly interacted with Nurr1 (Figure 1, Supporting Information Tables 1 and 2).

LG100268 (also LG268) was previously shown to prevent toxin-induced degeneration of dopamine neurons in a prophylactic manner.[26] Using a more therapeutically relevant protocol whereby treatment was started 1 day after application of MPP+, bexarotene fully restored both the number and morphology of cultured tyrosine hydroxylase (TH) positive neurons with equal efficacy to BDNF, and similar potency to its activity in the BRET assays (Figure 1e–j).

Next we determined if bexarotene had efficacy in 6-OHDA lesioned rats using intracerebroventricular (icv) administration to avoid limitations of drug delivery and exposure. Bexarotene (1 mM, approximately 6 μg/day, Table 1) was infused continuously icv into rats for 28 days beginning 3 days following bilateral 6-OHDA lesions of the substantia nigra pars compacta (SNc), a time point when significant loss (~50%) of TH-positive neurons has occurred (Supporting Information Figure 1).

As shown in Figure 2a–c, bexarotene treatment prevented the development of motor deficits caused by 6-OHDA administration and significantly protected the dopamine neurons in the SNc, as assessed by quantification of TH and the neuronal marker Neurotrace (Figure 2d–g). Dopamine transporter (DAT) and vesicular monoamine transporter 2 (VMAT2) immunohistochemistry (IHC) on striatal slices demonstrated the protective effects of bexarotene extended to the striatum where highly significant improvements in DAT (Figure 2h, j–l) and VMAT2 (Figure 2i, m–o) immunoreactivity were seen.

Having established that bexarotene has protective effects on dopamine neurons previously damaged by either 6-OHDA or MPP+, we conducted a series of pharmacokinetic (PK) evaluations to establish the brain exposure of bexarotene in the icv experiments described above, and determine the subcutaneous (sc) and oral (po) doses of bexarotene that produce equivalent or higher brain exposure. The icv infusions produced stable bexarotene brain concentrations in rats of 34 nM (Table 1). This level of brain exposure was achieved with continuous sc infusion of 4 and 16 mM bexarotene (approximately 0.25 and 1 mg/kg/day, see Table 1). Once-daily oral doses of 1 mg/kg/day surpassed 34 nM for at least 8 h per day (Figure 3), whereas oral doses of 0.3 mg/kg/day only briefly reached this level (not shown).

Next, we conducted a series of experiments with 6-OHDA lesioned rats to assess the dose–effect–exposure relationships for bexarotene administered sc or po. Consistent with the icv results, continuous sc infusion of the two concentrations that delivered brain exposures equal or greater than the effective brain exposure in the icv experiments fully preserved motor function and restored TH staining to nearly the levels in the sham control group, whereas two lower doses had no significant effect on behavior or TH (Supporting Information Figure 2).

The results with oral dosing were also consistent with the pharmacokinetic experiments described above. Oral doses of 1 and 3 mg/kg/day fully blocked the development of motoric deficits produced by 6-OHDA lesion, whereas 0.3 mg/kg/day had little or no effect (Figure 4a, b). Similarly, oral doses of 1 and 3 mg/kg/day preserved TH expression in the SNc neurons (Figure 4e–i). In addition, striatal TH was also preserved by 1 and 3 mg/kg/day of bexarotene (Supporting Information Figure 3).

The bilateral 6-OHDA lesion model was also used to investigate nonmotoric effects of dopamine cell loss.[27] 6-OHDA lesioned rats experienced a loss of cognitive function, as assessed in a novel object recognition test, which was effectively blocked by oral bexarotene doses of 1 and 3 mg/kg/day (Figure 4C). 6-OHDA lesioned rats also displayed increases in spontaneous head twitches which were evident at the start of drug treatment (PreTx). Head twitches were significantly reduced within 10 days after starting bexarotene treatment at 1 and 3 mg/kg/day and partially reduced at 0.3 mg/kg/day (Figure 4D).

To more accurately assess the level of neuroprotection afforded by bexarotene in this lesion model, we repeated the experiment described in Figure 4 using oral doses of 1 mg/kg/day of bexarotene, and analyzed the number of TH positive cells in the SNc using unbiased stereology (see Methods). We observed a large increase in the number of TH positive cells in lesioned animals treated with bexarotene compared to vehicle with an average of 19 636 ± 829 (mean ± SEM) cells in sham animals, 3412 ± 819 cells in vehicle-treated lesioned animals and 9456 ± 1039 cells in bexarotene-treated lesioned animals. These results were highly significant ($F(2,23)= 82.75$, $p < 0.0001$). Newman-Keuls multiple comparison posttest identified significant differences in all groups as compared to each other ($p < 0.0001$). Head twitch and performance in the challenging beam motor task were very similar both in terms of the deficit produced by the lesion and the restoration produced by bexarotene treatment in this repeat experiment (Supporting Information Figure 4) compared to the experiment shown in Figure 4.

Bexarotene is an FDA approved drug used to treat cutaneous T-cell lymphoma[21,22] and is currently being developed to treat other cancers.[28] The recommended starting clinical dose of bexarotene for treatment in humans is 300 mg/m2/day (equivalent to 8.1 mg/kg/day or ~650 mg/day for an 80 kg person), and this dose may be increased if there is insufficient response.[21,22] The effective anticancer dose of bexarotene in rats is 100 mg/kg/day po.[21] In contrast, we have found that 1 mg/kg/day po is effective in blocking the development of behavioral deficits and dopamine neuron degeneration in a rat model of PD. Interestingly, oral doses that exceeded effective brain exposures in the icv and sc experiments for only ~8 out of 24 h per day (see Figure 3) were fully effective showing that continuous receptor occupancy may not be necessary for efficacy. This is consistent with the idea that even transient activation of NucHRs may lead to persistent downstream physiological effects. Because the effective doses in the rat PD model were so much lower than the effective doses in rat cancer models, we estimated what doses of bexarotene might be used to treat PD in humans compared to the doses that are currently

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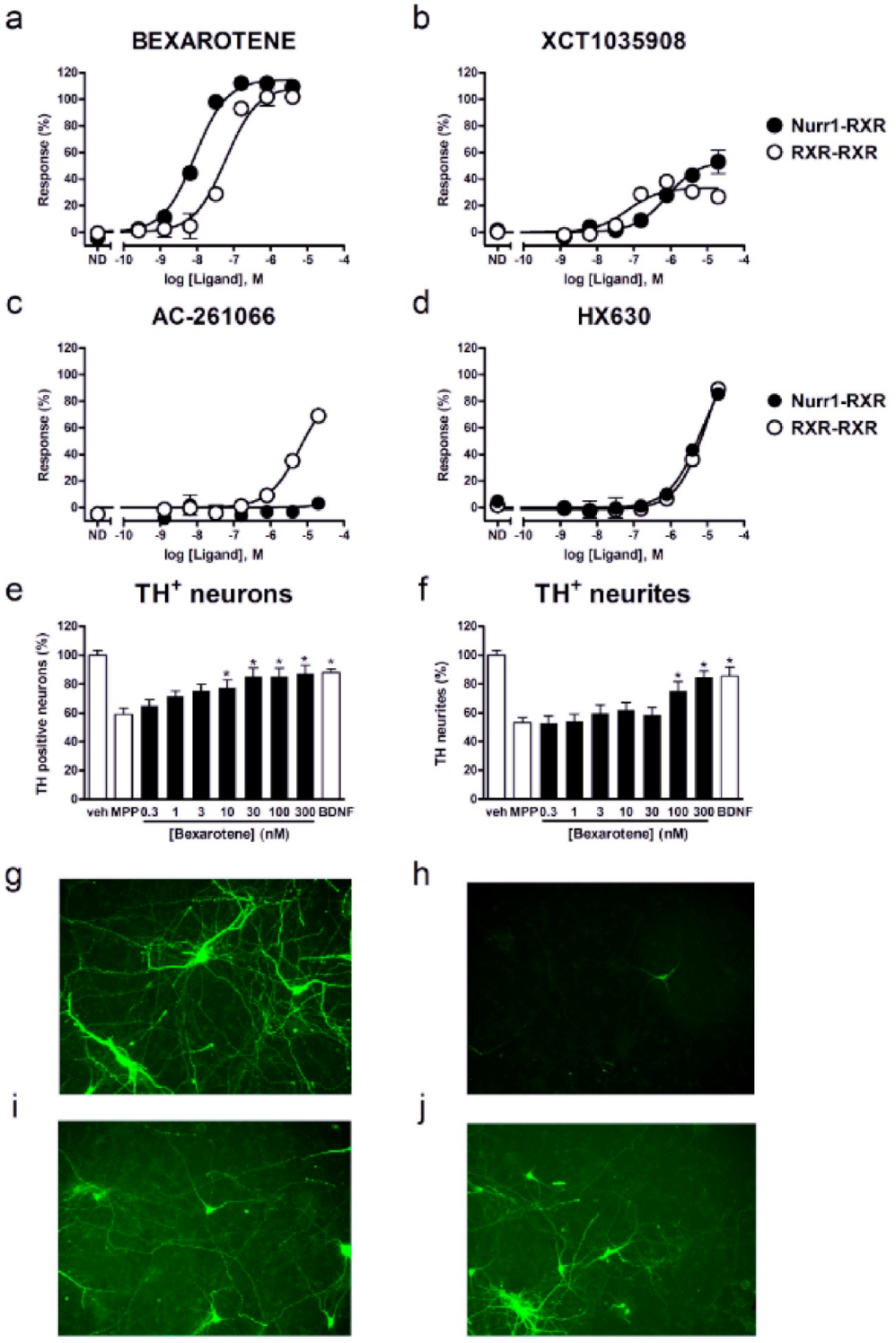

Figure 1. In vitro activity of bexarotene. BRET assays were performed as described in the Methods using the indicated pairs of receptors and concentrations of ligands. Responses were normalized to 9-*cis*-retinoic acid (see Supporting Information Table 2). (a) Bexarotene; (b) XCT1035908; (c) AC-261066; (d) HX630 (see Supporting Information Table 1 for references). (e–j) Trophic effects of bexarotene applied to primary cultures of dopaminergic neurons previously exposed to MPP+. The cultures were stained for TH. Dose effect curve of bexarotene and BDNF (50 ng/mL) on (e) TH positive neurons and on (f) total TH neurite length after a 24h MPP+ injury (4 μM) expressed as percent of control. Representative images of (g) control cells, (h) MPP+ treated cells receiving vehicle, (i) BDNF (50 ng/mL), or (j) bexarotene (300 nM). Data for the restoration of dopamine neurons were analyzed using ANOVA test following by Dunnett's test. *$p < 0.05$ groups vs MPP+.

used in cancer therapy. We estimate that oral doses of 10–60 mg/day (approximately 5–30 mg/m^2/day) may be sufficient to treat PD in humans (see Supporting Information Figure s5 and Supporting Information Table 3).

Bexarotene cancer therapy is often accompanied by significant side effects, including increased plasma triglycerides and decreased thyroid function.[23−25] We therefore compared the effect of 5 consecutive days of oral administration of 100 to

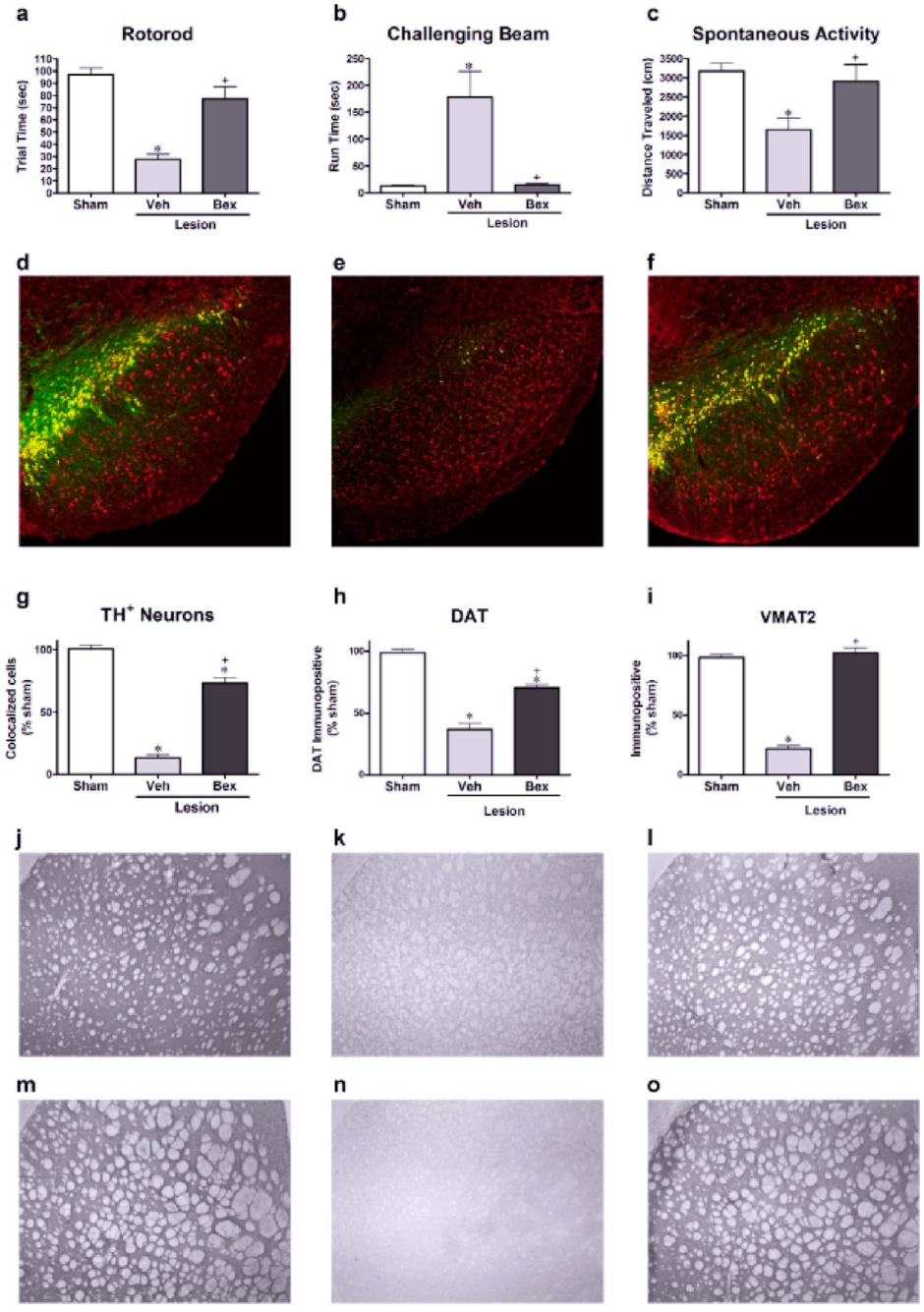

Figure 2. Efficacy of bexarotene in restoring dopamine cells and behavior in 6-OHDA lesioned rats. Animals were treated for 28 days with continuous icv infusion of either vehicle (Veh) or 6 μg/kg/day bexarotene (Bex) beginning 72 h following 6OHDA infusion. Panels (a−c) show the trial time on the rotorod, time required to traverse the challenging beam, and the distance traveled during a 15 min spontaneous locomotor activity session, respectively, for sham (all treatments combined) and 6-OHDA (lesion) animals treated with vehicle or bexarotene. Panels (d−f) show representative images of TH (green), Neurotrace (red) and colocolized (yellow) immunohistochemistry in SNc sections from sham, vehicle treated, and bexarotene treated lesioned rats, respectively. Panel (g) shows the quantification of cells positively stained for TH and Neurotrace (denoted colocalized cells), normalized to sham controls. Panels (h, i) show the percentage of the image that was immunopositive for DAT and VMAT2 staining in the striatum respectively, normalized to sham controls. Panels (j−l) show representative images of DAT staining in striatal sections from sham, vehicle-treated, and bexarotene-treated lesioned rats, respectively. Panels (m−o) show representative images of VMAT2 staining in striatal sections from sham, vehicle-treated, and bexarotene-treated lesioned rats, respectively. Data were analyzed with one-way ANOVAs followed by Bonferroni's post hoc comparisons. Data are presented as mean ± SEM. Asterisk (∗) indicates a significant difference from Sham, $p < 0.05$; plus sign (+) indicates a significant difference from vehicle/6OHDA, $p < 0.05$. $N = 9-15$ animals per group.

1 mg/kg/day on serum levels of triglycerides and thyroxine (T4) in rats. Administration of 100 mg/kg/day caused a dramatic increase in serum triglycerides and a sharp drop in serum T4. In contrast, 1 mg/kg/day produced significantly reduced changes in both of these serum markers. (Figure 5a,b).

Several clinical studies also suggest that low doses of bexarotene will be significantly better tolerated than the high doses used in cancer therapy.[24,25,29,34] For example, there is a clear dose dependence of hyperlipidemia in patients,[24,25] and based on this relationship, doses within the range we estimate

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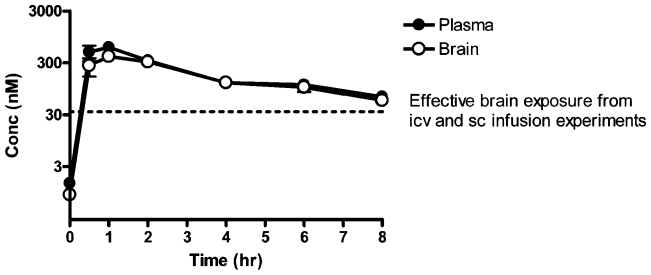

Figure 3. Pharmacokinetics of bexarotene. Male Sprague−Dawley rats received once daily oral doses of 1 mg/kg/day bexarotene. Prior to the fifth dose, plasma and brain samples were analyzed to get $t = 0$ values. After the fifth dose, plasma and brain samples were obtained at the indicated time intervals and analyzed for bexarotene concentrations. Dashed line denotes the effective brain concentration determined at steady state in the continuous infusion icv dosing experiments.

may benefit PD patients would be expected to cause much smaller increases in serum triglycerides and cholesterol (Figure 5c,d). Presumably, other side effects would be reduced in severity and frequency as well.

Our interest in testing bexarotene in models of PD originated first from our interest in Nurr1, and subsequently from our BRET experiments identifying compounds including bexarotene with a bias toward Nurr1-RXR heterodimers over RXR-RXR homodimers. In our experiments, we observed a number of Nurr1-regulated targets that were upregulated by bexarotene including DAT and VMAT2 (see Figure 2) suggesting Nurr1 activation is probably involved in the protective effects we observed. However, it is not possible from these experiments to say whether Nurr1 activation alone is sufficient to account for the neuroprotective effects we observed or whether other targets may also contribute to the protective effects of bexarotene. We also found that bexarotene increases expression of the liver X receptor (LXR)-dependent genes ApoE and the ABCA1 transporter at these same low doses (A. Björklund, personal communication). A protective role for LXRβ receptors in MPTP-induced dopaminergic cell death has been proposed,[30] and it was recently shown that high-dose bexarotene (100 mg/kg) cleared β-amyloid in a mouse model of Alzheimer's disease, in part, through upregulation of ApoE and ABCA1.[31] Thus, the mechanism of neuroprotection afforded by bexarotene is likely to be complex.

Since we are relying on experimental models, caution must be used in drawing inferences about the potential clinical benefits of bexarotene. Nevertheless, because bexarotene is already an FDA approved drug, a great deal of preclinical and clinical data is available and the pathway to developing bexarotene for use in treating PD patients might be considerably shorter than it would be for a new chemical entity. Based on the results presented above, we hypothesize that low oral doses of bexarotene may provide an effective and tolerated therapy for PD.

METHODS

Subjects. The subjects were male Sprague−Dawley rats purchased from Charles River Laboratories (Hollister, CA) weighing 225−250 g upon arrival. Rats were housed in pairs in polypropylene cages within a temperature controlled vivarium maintained on a 12 h light/dark cycle (lights on 7 a.m.). For the duration of the experiments, animals received free access to food and water. All procedures were conducted in accordance with the NIH *Guidelines for the Care and Use of Laboratory Animals* and were approved by the Institutional Animal Care and Use Committee (IACUC) at ACADIA Pharmaceuticals. Animals were acclimated to vivarium conditions and handling for a minimum of one week prior to surgery.

Lesion Surgery. In order to protect norepinephrine terminals, each animal received an injection of desipramine (10 mg/kg) about 15 min prior to being anesthetized using isofluorane. Animals were placed into a stereotaxic apparatus and bilateral infusions of 6OHDA (8 μg/4 μL/side) or 0.2% ascorbic acid vehicle were aimed at the SNc (A/P −5.0 mm, M/L ± 1.6 mm, D/V −8.2 mm relative to bregma).

Drug Delivery. For icv administration of bexarotene (LC Laboratories, Woburn, MA), an Alzet osmotic pump (Braintree Scientific, Braintree, MA) was attached to an intracranial guide cannula and implanted subcutaneously between the shoulder blades of each animal. The guide was placed intracerebroventricularly (icv, A/P −0.8 mm, M/L −1.4 mm, D/V −4.5 mm relative to bregma) and was attached to the skull with jeweler's screws and dental acrylic and the incision was closed with staples. For sc infusions of bexarotene, 3 days following 6-OHDA infusion, Alzet osmotic pumps were implanted subcutaneously between the shoulder blades of each animal. Animals received supportive care following surgery, including administration of subcutaneous (sc) fluids (10 mL/day) and soft food mashes, until they surpassed their surgical weights. The osmotic pumps (Alzet, model 2004 for icv use; model 2ML4 for sc use) were weighed and then filled with bexarotene (1 mM for icv experiments; 4 mM or 16 mM for sc experiments) or vehicle (1% DMSO in saline) 48 h prior to surgery. They were then incubated in 0.9% physiological saline at 37C until surgically implanted. The pumps infused at a rate of 0.25 μL/h (icv) or 2.5 μL/h (sc) for 28 days after implantation. Infusion pumps were connected to the icv or sc cannula with vinyl tubing and bexarotene infusion started 72 h after infusion of 6OHDA occurred. For oral dosing, rats were gavaged with vehicle (6% DMSO in safflower oil, 1 mL/kg) or bexarotene using a plastic feeding tube (model FTP-15-100, Solomon Scientific, San Antonio, TX) once daily for 28 days, starting 72 h after infusion of 6OHDA.

BRET Assays. BRET assays were performed as described[32] using HEK293T cells transiently transfected with plasmid DNAs expressing a receptor tagged with Renilla luciferase (1 μg plasmid DNA) and a receptor tagged with GFP2 (20 μg plasmid DNA). Nurr1 (NR4A2) and RXRα receptors were subcloned in frame to vectors expressing humanized Renilla luciferase (pRluc(h)) and green fluorescent protein 2 (pGFP2) from PerkinElmer Life and Analytical Sciences (Waltham, MA). All clones were sequence verified before use and generated the correct R. luciferase and GFP2 emission spectra when expressed. BRET signals were calculated as the ratio between the Renilla luciferase emission and the GFP2 emission corrected by the background emissions of nontransfected cells.

Spontaneous Locomotion. Rats were placed in automated activity chambers (42 × 42 × 30 cm^3) equipped with 16 infrared photobeams along each horizontal axis (front to back and side to side, from Accuscan Instruments, Inc., (Columbus, OH). Activity was recorded during a 15-in test session, and distance traveled (cm) was analyzed.

Head Twitch, Rotorod, and Challenging Beam Tests. These tests were performed as previously described.[27]

Novel Object Recognition (NOR). NOR was conducted in a novel environment in two phases: sample and test. For sample, rats were placed into the NOR chamber, where two identical objects were placed. Each rat was allowed to explore for 3 min, and the time spent exploring at each position recorded. After 3 min, each rat was removed from the arena and placed back into its cage. There were no statistical differences among groups in exploration times during the sample phase (not shown). The test phase was conducted 4 h after the sample phase. During test, one familiar object (seen during sample) and one novel object were placed into the chamber, and each rat was allowed 3 min to explore. The test sessions were recorded on video and scored by an observer blind to each rats treatment condition. For test data, percent of exploration time spent at the novel object was determined.

Immunohistochemistry. Brains fixed in 4% paraformaldehyde were sectioned (50 μm) through the SNc (−5.2 mm relative to bregma) and striatum (1.2 mm relative to bregma) according to the

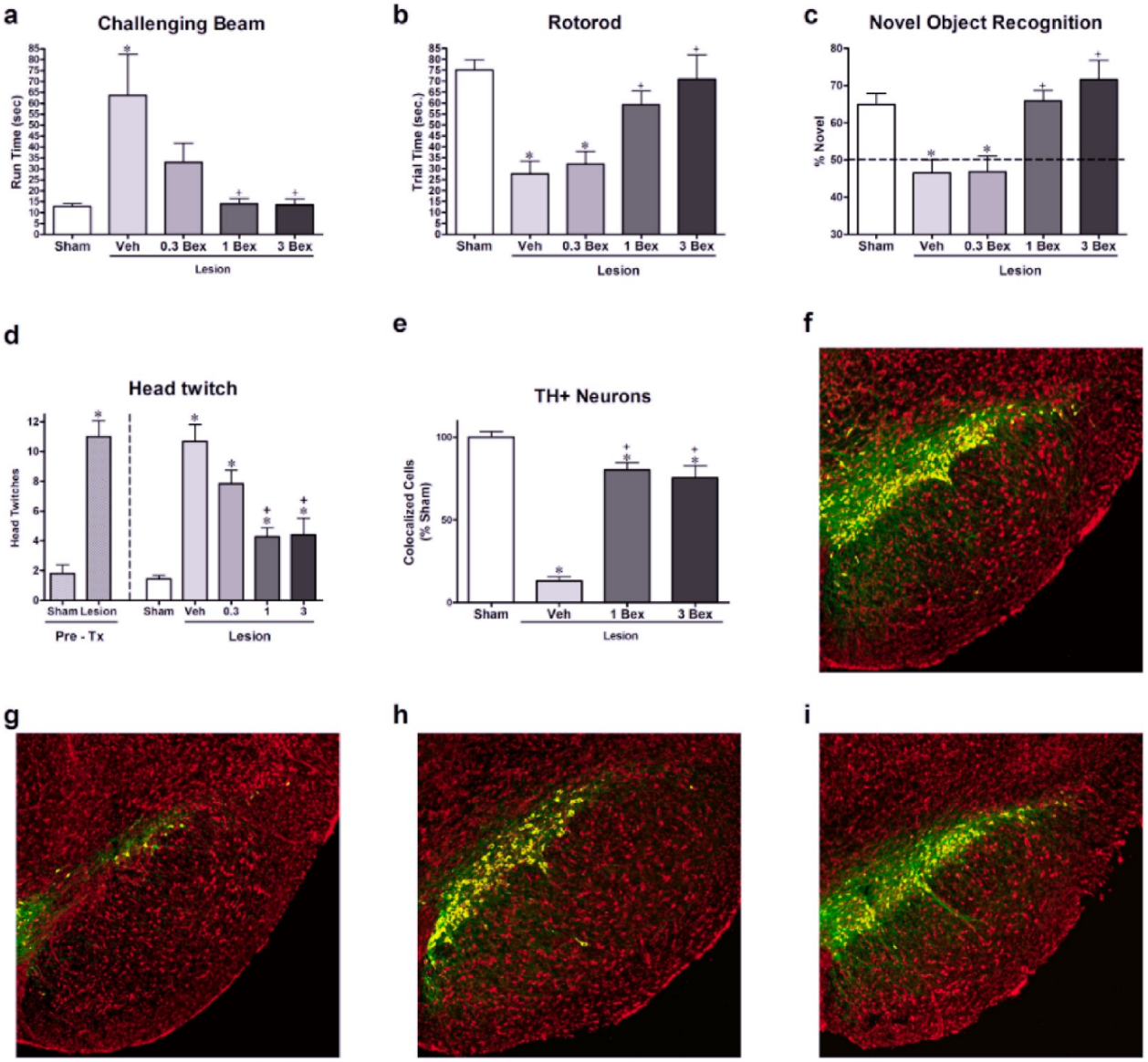

Figure 4. Efficacy of orally administered bexarotene. Animals were treated for 28 days with once daily oral gavage of vehicle (Veh) or bexarotene (0.3, 1, or 3 mg/kg/day) beginning 72 h following 6OHDA infusion. Panels (a–d) show time to traverse the challenging beam, trial time on the rotorod, time spent exploring a novel object, and spontaneous head twitches, respectively, for sham (all treatments combined) and 6-OHDA (lesion) animals treated with vehicle or bexarotene. Head twitch data were obtained 10 days after beginning treatment, nearly identical results were observed 20 days after treatment (not shown). All other behavioral data were obtained after 28 days of treatment were completed. Panel (e) shows quantification of cells positively stained for TH and Neurotrace (denoted colocalized cells), normalized to sham controls. Data were analyzed with one-way ANOVAs with Bonferroni's post hoc comparisons for (a–e). *Significantly different from Sham, $p < 0.05$. $^{+}$Significantly different from vehicle/6OHDA, $p < 0.05$. $N = 9–15$ per group. Panels (f–i) show representative images of TH (green) and Neurotrace (red) in SNc sections from sham, lesioned/vehicle, 6-OHDA/bexarotene (1 mg/kg/day), and 6-OHDA/bexarotene (3 mg/kg/day), respectively.

atlas of Paxinos and Watson, 1997. The SNc sections were immunolabeled for TH using rabbit anti-tyrosine hydroxylase polyclonal antibody (AB152, Chemicon) and Neurotrace (N21482, Invitrogen). Striatal tissue was labeled for TH, DAT, and VMAT2 using rabbit anti-tyrosine hydroxylase polyclonal antibody (AB152, Chemicon), rat anti-DAT monoclonal antibody (MAB369, Millipore), and rabbit anti-VMAT2 polyclonal antibody (NB100-68123, Novus Biologicals), respectively. Single optical plane images were obtained using an Olympus BX51 fluorescent/light microscope (Olympus America Inc.) equipped with a digital camera (Retina 2000R, Qimaging). Images were acquired using a 4× air objective with 2× digital magnification. Images were analyzed by an observer blind to each subject's treatment condition using ImageJ software (available at http://rsb.info.nih.gov/nih-imageJ). Controls (omission of the primary antibody) revealed no nonspecific staining (not shown).

Unbiased Stereology. Coronal sections of each brain were prepared by FD Neurotechnologies (Ellicott City, MD). Briefly, rat brains were cryoprotected with 0.1 M phosphate buffer (pH 7.4) containing 20% sucrose for 72 h, and then rapidly frozen in isopentane precooled to −70 °C with dry ice. Serial cryostat sections (30 μm) were cut coronally through the brain regions containing the substantia nigra (approximately from Bregma −4.36 mm to −6.72 mm), and every fourth section (interval: 120 μm) was processed for this study. The sections were processed for TH-immunofluorescence with a primary antibody directed against the tyrosine hydroxylase protein (AB152, Millipore, Billerica, MA). Following washes in 0.01 M phosphate-buffered saline (PBS), sections were incubated free-floating in PBS containing 1.0% Triton X-100, blocking serum, and the specific antibody for 2 days at 4 °C, followed by incubation with a secondary antibody conjugated with Alexa Fluor 488. After thorough washes, all

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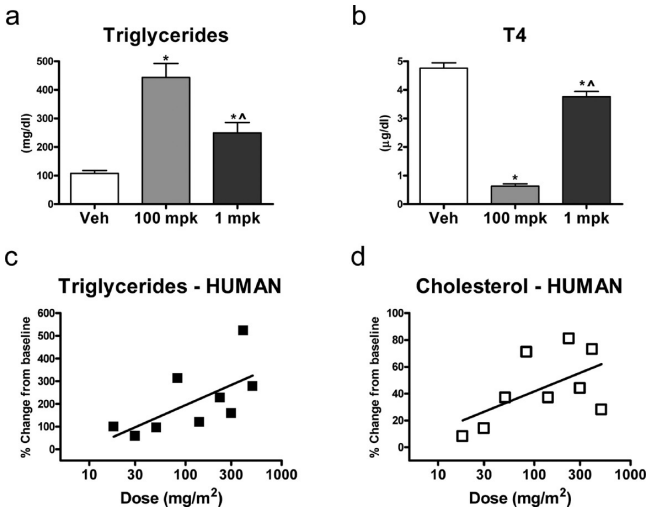

Figure 5. Dose dependence of bexarotene-induced side effects. Bexarotene or vehicle was administered orally for 5 consecutive days at the indicated doses. Rats were sacrificed, and plasma analyzed for (a) triglycerides and (b) T4. Asterisk (*) indicates a significant difference from vehicle $p < 0.05$. Up arrowhead ^ indicates a significant difference from 100 mpk, $p < 0.05$. Dose dependence of (c) serum triglycerides and (d) serum cholesterol in human cancer patients. Data represent the % change from individual baseline values plotted against the logarithm of the dose in mg/m^2. Data adapted from ref 24. Extrapolations from the rat data (see Supporting Information Table 3) predict oral doses of 10−60 mg/day (approximately 5−30 mg/m^2/day) may be sufficient to treat PD in humans.

sections were incubated with DAPI, mounted on gelatin-coated slides, and coverslipped with Vectashield (Vector Laboratories). The stained sections were shipped to MBF Laboratories (Williston, VT) for stereologic analysis. Quantitative assessment was performed with a modified light microscope (Zeiss AxioImager Z1; Germany) with Zeiss objectives; EC Plan-Neofluar 5× (NA = 0.16), Plan-Apochromat 20× (NA = 0.60), EC Plan-Neofluar 40× (N.A. = 0.75), Plan-Apochromat oil 63× (NA = 1.40), and Plan-Apochromat oil 100× (NA = 1.4), motorized specimen stage for automated sampling (Ludl Electronics; Hawthorne, NY), CCD color video camera (mRm; Zeiss, Germany), Lumencore Sola fluorescent light source, and stereology software (Stereo Investigator, v11.00; MBF Bioscience, Williston, VT). Cells were marked following the counting rules for stereology when the tyrosine hydroxylase labeled cell with a DAPI stained nucleus comes into focus within the dissector. Both MBF Labs and FD Neurotechnologies were blinded to the group assignments of the brains throughout the study.

Analysis of Plasma and Brain Drug Concentrations. Sprague−Dawley rats were administered bexarotene either by continuous icv or sc infusion, or by oral gavage as described above. Animals were sacrificed, and plasma and brain samples were harvested at the indicated times, and analyzed for bexarotene levels using LC-MS/MS according to the vendor's (Agilux Laboratories, Worcester, MA) procedures.

MPP+ Exposure to Primary Cultures of Dopaminergic Neurons. These experiments were performed at Neuron Experts SAS (Marseille, France). Rat dopaminergic neurons derived from fetal (15 day gestation) midbrains were cultured as described.[33] On day 6 of culture, medium was removed and fresh medium added, without or with 4 μM MPP+. On day 7, the culture was washed with fresh medium without (containing vehicle) or with test drugs for 48 h. After 48 h, cells were fixed (all conditions) by paraformaldehyde 4% solution, permeabilized with 0.1% saponin (Sigma), and labeled for TH. For each condition (6 culture wells), 2 × 10 pictures per well were taken in the same condition using an InCell AnalyzerTM 1000 instrument (GE Healthcare) with 10× magnification. The analyses were automatically done using developer software (GE Healthcare) to measure the total number of TH positive neurons and neurite length. Two means of 10 pictures were automatically performed by well. Data were expressed in percentage of control condition. Statistical analyses (using Graph Pad Prism's package) were done on the different conditions using ANOVA test following by Dunnett's test (when allowed), and significance was set for $p \leq 0.05$.

■ ASSOCIATED CONTENT

Supporting Information

Additional experimental details as described in the text. This material is available free of charge via the Internet at http://pubs.acs.org.

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Table 1. Dose/Exposure/Effect Summary for Bexarotene in Rats

dosing route	dose (mg/kg/day)	Plasma cone (nM)	brain cone (nM)	plasma AUC (μM·hr)	brain AUC (μM·hr)	brain/plasma ratio	effective in ratPD model?	effective in rat cancer model?
icv	0.006	<5	34	<0.1	0.8	>6	yes	–
sc	0.06	10	–	0.2	–	–	no	–
sc	0.25	34	40	0.8	1.0	1.2	yes	–
sc	1	100	136	2.4	3.3	1.4	yes	–
po	1	597	402	2.3	1.9	0.8	yes	–
po	3[a]	714	–	4.4	–	–	yes	–
po	10	3931	1265	13.2	7.0	0.5	–	–
po	10[a]	1552	–	14.1	–	–	–	no
po	30[a]	3334	–	24.3	–	–	–	yes/no
po	100[a]	5416	–	42.1	–	–	–	yes

[a]Data from bexarotene NDA #21055. AUC for sc dosing calculated using the trapezoidal rule. AUC for po dosing calculated using prizm software. The rat PD model was bilateral 6-OHDA lesioning of the substantia nigra and the cancer model was the NMU (*N*-nitroso-*N*-methylurea) induced mammary tumor carcinoma model (see Targretin NDA #21055). Plasma and brain concentrations from icv and sc dosing are steady state levels after 4−8 days of continuous infusion. Plasma and brain concentrations from oral dosing experiments are peak concentrations obtained after 5 days of dosing, except data from Targretin NDA #21055 was after 15−50 days of dosing. Brain-plasma ratio = AUC brain/AUC plasma. Yes/no indicates partial efficacy. (−) denotes not evaluated.

Author Contributions

K.M., T.A.S., and E.S.B. designed experiments. K.M., T.A.S., D.H., and J.M. conducted experiments. K.M., T.A.S., D.H., J.M., and E.S.B. performed data analysis. K.M., T.A.S., R.O., and E.S.B. helped conceive the project. E.S.B. wrote the manuscript.

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Notes

The authors declare the following competing financial interest(s): All authors are either past or present employees of ACADIA Pharmaceuticals Inc. and hold stock in the company.

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■ ABBREVIATIONS

NucHR, nuclear hormone receptor; BRET, bioluminescence resonance energy transfer; RXR, retinoid X receptor; BDNF, brain-derived neurotrophic factor; 6-OHDA, 6-hydroxydopamine; MPP+, 1-methyl-4-phenylpyridinium; DAT, dopamine transporter; VMAT2, vesicular monoamine transporter 2; TH, tyrosine hydroxylase; PD, Parkinson's disease

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