Oligodendrogenesis via a small molecule therapy for treating multiple sclerosis patients

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ENDECE neural
neurological drug development

Renovo neural

MS

Abstract

Background: There is an unmet need for remyelinating therapies for diseases like multiple sclerosis (MS). Remyelination depends upon the ability of endogenous oligodendrocyte progenitor cells (OPCs) to differentiate into mature oligodendrocytes that can then repair the damaged myelin sheath. NDC-1308 is an analog of estradiol (E2) that was previously shown in culture to cause 3-fold more mouse OPCs to differentiate into mature myelinating oligodendrocytes compared to vehicle. E2 and estriol do not possess this myelinating activity. Side-by-side comparison of NDC-1308 and E2 in the cuprizone mouse model of demyelination showed that only NDC-1308 dramatically increases the level of remyelination (up to 44% in the hippocampus).

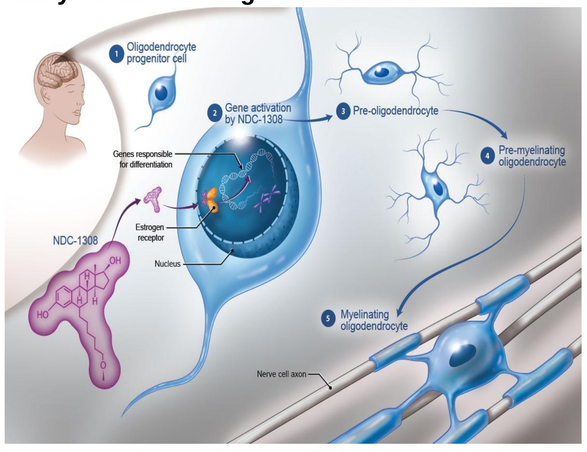
Objectives: We investigated how NDC-1308 has gained the function to remyelinate axons, but lost the deleterious side-effects commonly associated with estrogens.

Methods: *In silico* modeling was used to compare the orientation of NDC-1308 in the estrogen receptor (ER) ligand binding domain to E2. The affinity of NDC-1308 for different ER subtypes was characterized using a fluorescence polarization-based assay. Intracellular pathway activation of NDC-1308 was compared to E2 by real-time qPCR in several human cell lines. We determined whether NDC-1308 is estrogenic, a potential safety concern for treating MS patients. Estrogenicity was directly measured in a mouse uterotrophic assay since E2 treatment is known to cause a rapid and dramatic increase in uterine weight in this assay.

Results: *In silico* modeling studies suggest that NDC-1308 interacts with different moieties in the ER ligand binding domain compared to E2, thereby eliciting a distinct pattern of gene expression that is beneficial for myelin repair. Indeed, while NDC-1308 and E2 are both ER agonists, the unique remyelinating activity of NDC-1308 can be traced back to its ability to significantly up-regulate key genes (OLIG2, DNER, MOG and MBP) for oligodendrogenesis and remyelination. Real-time qPCR analysis showed these same genes are up-regulated in human PBMCs treated with NDC-1308, suggesting they could serve as potential therapeutic biomarkers. Unlike E2, NDC-1308 was not found to be estrogenic in the uterotrophic assay. Further testing revealed that NDC-1308 is not mutagenic and not genotoxic.

Conclusions: Because of its unique mechanism of action, its potent remyelinating activity and its demonstrated lack of harmful side-effects, NDC-1308 possesses many desirable attributes of an effective MS therapy.

Remyelination Background

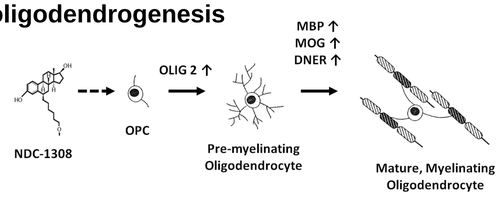


Normal myelin production
Myelin sheaths are derived from OPCs (1,2), which differentiate (3,4) into mature, myelinating oligodendrocytes (5).

Myelin block in SPMS patients
OPCs are present but quiescent in SPMS patients; no drugs exist that induce OPC differentiation to repair the damaged myelin sheath.

How NDC-1308 works
Induces OPCs to become mature, myelinating oligodendrocytes leading to a new myelin sheath.

NDC-1308 up-regulates key genes involved in oligodendrogenesis



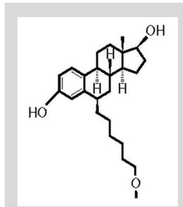
Adapted from Baumann and Pham-Dinh, Physiological Reviews, Vol 81, No. 2, April 2001

NDC-1308 regulates oligodendrogenesis genes distinctly from estradiol

- Human cell lines Panc-1 or SK-OV-3 cells were treated with NDC-1308 (10 μM); NDC-1308 (10 μM) + estrogen receptor (ER) antagonist ICI 182,780 (10 μM); estradiol (E2, 10 nM) or vehicle for 72 hrs.
- Real-time PCR of key genes for OPC differentiation and myelin synthesis were significantly up-regulated (3 biological replicates, duplicate RT-PCR)

Gene	Gene Expression (Fold Increase)					
	Panc-1 ER α (+), ER-β (+)			SK-OV-3 ER-α (-), ER-β (+)		
	NDC-1308	NDC-1308 + ICI	E2	NDC-1308	NDC-1308 + ICI	E2
OLIG2	2.33	1.21	3.99	7.99	2.66	1.02
MBP	1.54	0.95	3.93	10.76	3.87	2.34
MOG	0.90	0.34	1.67	15.66	5.12	1.79
DNER	2.27	0.96	3.56	0.64	0.40	0.57

NDC-1308 Characterization



NDC-1308

- **Induces oligodendrogenesis to repair the myelin sheath of demyelinated axons *in vivo*.**
- **Has lost the harmful side-effects commonly associated with estradiol**
- **Estrogen Receptor agonist.**
- **Injectable (stable modified beta-cyclodextrin formulation).**
- **GMP-manufacturing process for estradiol-free API is in place.**

NDC-1308 induces OPC differentiation *in vitro*

A. Oligodendrogeneis

- OPCs were isolated from PLP-EGFP transgenic mice.
- OPCs were treated with 10μM test agents for 5 days.
- Mature oligodendrocytes (EGFP expressing) were detected by luminescence
- % EGFP + OL was calculated with imaging software from 20 fields (+/- S.D).

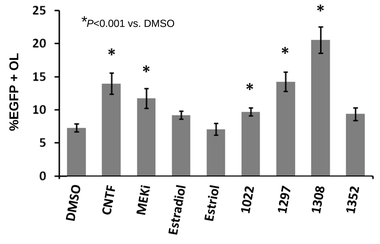
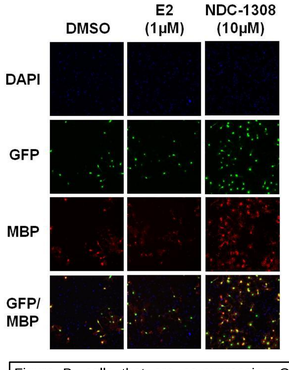


Figure A, NDC-1308 (EC_{50} = 1.0 μM) is the most potent of ENDECE' Neural's analogs for inducing oligodendrogenesis.

Estradiol and estriol *do not* induce OPCs to differentiate into mature oligodendrocytes.

B. Image Analysis



C. ER-specific Differentiation

OPCs were treated with NDC-1308 (10 μM) in the absence or presence of the ER antagonist ICI 182,780 (10 μM) for 5 days.

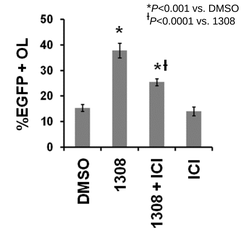
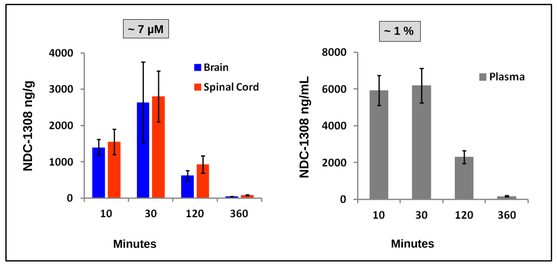


Figure B, cells that are co-expressing GFP and MBP appear yellow and are predominantly detected in NDC-1308 treated cells (lower right). Figure C, the ER antagonist ICI 182,780 inhibits about 50% of NDC-1308's activity to induce OPC differentiation, demonstrating that NDC-1308 is functioning through the ERs.

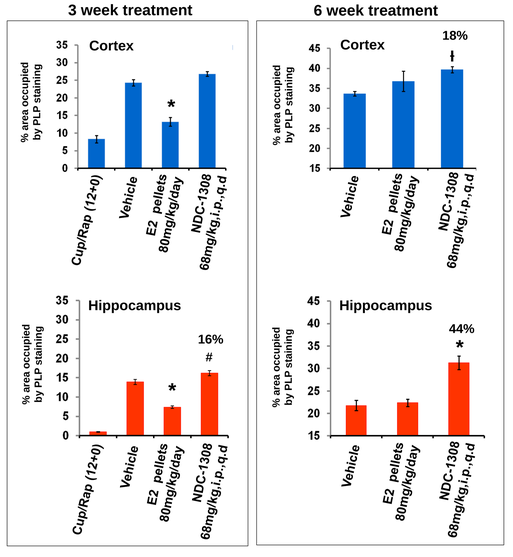
NDC-1308 is rapidly absorbed into CNS tissues

- Female C57BL/6 mice (N=5) each with single s.c. injection
- 75 mg/Kg of NDC-1308 formulated in SBE-β-cyclodextrin.
- Brain, spinal cord and plasma were collected at termination.
- NDC-1308 (+/-S.D.) measured by mass spectrometry
- ***CNS absorption: i.v. >> s.c. ≥ i.p. >> p.o.***



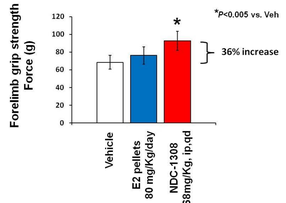
NDC-1308 induces remyelination in a cuprizone mouse model of demyelination

- Mice were demyelinated with a cuprizone/rapamycin treatment for 12 weeks.
- NDC-1308, estradiol (E2) or vehicle was administered for 3 or 6 weeks.
- Myelin synthesis in hippocampus and cortex was detected by PLP staining.
- N=7-12 per group; Average %PLP +/- SEM.
- #*P* < 0.05 vs. veh; †*P* < 0.01 vs. veh; **P* < 0.0001 vs. veh.



Functional improvement following NDC-1308 treatment of demyelinated mice

- 3-week NDC-1308 (68 mg/Kg, i.p., q.d.) treatment of cuprizone demyelinated mice.
- Forelimb grip strength assessment with 10 trials per animal.
- N=12 per treatment group.
- Force (g) +/- S.D.



NDC-1308 treated animals have significantly higher forelimb grip strength compared to vehicle.

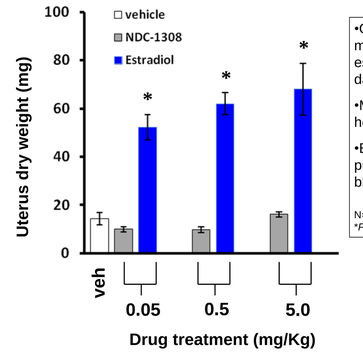
This test could potentially be used to assess improvement of function in Secondary Progressive MS patients during clinical trials.

Favorable kinetics for NDC-1308 in dog

Species	NDC-1308 injected (mg/Kg)	NDC-1308 Detected			NDC-1308 Brain:Plasma
		Brain (mg)	Brain (μM)	Plasma (mg)	
Mouse (i.p.,10 min)	75.00	‡0.0008	§5.3	*0.012	0.066
Dog (i.v., 5 min)	2.81	#0.432	^19.0	ǂ1.422	0.304

Assumptions for a 20 g mouse: ‡Ave brain wt = 0.4 g
§Ave brain volume = 0.4 mL
*1 mL plasma
Assumptions for a 10 Kg dog: #Ave brain wt = 72 g
^Ave brain volume = 59 mL
ǂ450 mL plasma

NDC-1308 is not estrogenic



- Ovariectomized C57BL/6 mice were dosed with either estradiol or NDC-1308 once daily for 4 days.
- Mice were euthanized 6 hours after last dose.
- Each uterus was removed, punctured and dried by blotting.

N=6
**P* < .0001 vs. veh

NDC-1308 is not mutagenic or genotoxic

Bacterial reverse mutation assay

Tester Strain	Treatment	Average Revertants ±SD	Fold Induction Response
TA100	DMSO	19 ± 1	
	NDC-1308 (30 μg)	23 ± 4	1.2
	Sodium Azide (0.2μg)	113 ± 4	6.1
TA1535	DMSO	1 ± 1	
	NDC-1308 (30 μg)	3 ± 2	nd
	Sodium Azide (0.2μg)	170 ± 1	170

In vitro TK6 micronucleus assay

Treatment	Population Doubling	Cytotoxicity %	%MN
DMSO	1.9	0	0.5
NDC-1308, 5.36 μg/mL	1.7	8	0.4
NDC-1308, 10.9 μg/mL	1.5	19	0.4
NDC-1308, 22.3 μg/mL	0.8	55	0.6
Vinblastin, 12 ng/mL	1.2	36	1.4*

*P≤0.05 vs. DMSO

Conclusions

1. **NDC-1308 is a small, lipophilic molecule that is systemically administered and absorbed into CNS tissues in amounts sufficient for inducing remyelination of cortical and hippocampal brain regions.**
2. **In the mouse cuprizone model, the remyelinating activity of NDC-1308 is associated with a functional improvement in forelimb grip strength.**
3. ***In vitro*, NDC-1308 can induce OPCs to differentiate into mature, myelinating oligodendrocytes.**
4. **NDC-1308 appears to override inhibitors of OPC differentiation leading to formation of mature, myelinating oligodendrocytes that express MBP, a key component of the myelin sheath.**
5. **Mechanistically, NDC-1308 is an estrogen receptor agonist that up-regulates several key genes which drive oligodendrogenesis.**
6. **The activity of NDC-1308 is in strong contrast to that of estradiol. NDC-1308 has gained the function of remyelination compared to estradiol, but lost commonly associated side-effects, such as estrogenicity.**

***Conflicts:** SHN and JGY are shareholders of ENDECE Neural. **Funding:** this work was funded in part by a Fast Forward grant from the National MS Society*