A small molecule therapy for MS patients appears to override inhibitors of oligodendrogenesis to induce remyelination

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Abstract

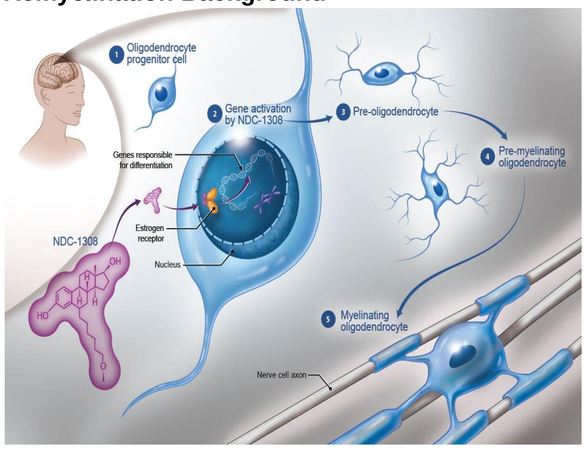
There is an unmet need for remyelinating therapies to treat multiple sclerosis (MS) patients. NDC-1308 is an analog of estradiol (E2) that harnesses the body's natural remyelinating system to drive oligodendrogenesis, a process resulting in mature, myelinating oligodendrocytes (OLs) that can repair damaged myelin sheaths.

NDC-1308 was previously shown in oligodendrocyte progenitor cell (OPC) cultures to induce a 3-fold increase in OLs compared to vehicle. Structurally related estrogens, E2 and estriol, do not possess this activity. Side-by-side comparison of NDC-1308 and E2 activity, following chronic treatment in the cuprizone mouse model of demyelination, showed only NDC-1308 could significantly repair the myelin sheath (a 44% increase in hippocampus). NDC-1308 can apparently accomplish this by overriding inhibitors of oligodendrogenesis, such as Lingo-1.

Here, we investigated how NDC-1308 has gained the biological activity to repair demyelinated axons, but lost the deleterious side-effects commonly associated with estrogens. While NDC-1308 and E2 are both ER agonists, we found the remyelinating activity of NDC-1308 can be traced back to its unique ability to significantly up-regulate key genes (OLIG2, DNER, MOG and MBP) for oligodendrogenesis. Real-time qPCR analysis showed these same genes are up-regulated 2-3 fold in human PBMCs treated for 12 hours with NDC-1308, suggesting they could serve as potential therapeutic biomarkers. Potential safety concerns for NDC-1308 were addressed. 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. Unlike E2, NDC-1308 was not found to be estrogenic. Further testing revealed that NDC-1308 is not mutagenic (Ames assay) and not genotoxic (micronucleus assay). The OPC pool remained intact after six weeks of chronic NDC-1308 treatment, demonstrating that it can serve as a renewable source for sustaining oligodendrogenesis.

In conclusion, NDC-1308 is a potential first-in-class remyelinating therapy that possesses many key qualities needed to effectively treat secondary progress (SPMS) and relapsing-remitting (RRMS) MS patients.

Remyelination Background

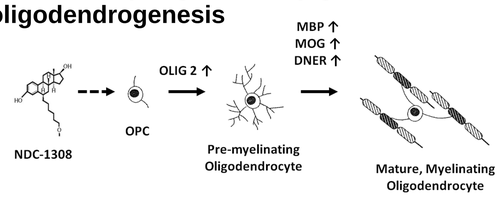


Normal myelin production
Myelin sheaths are derived from OPCs (1,2), which differentiate (3,4) into mature, myelinating oligodendrocytes (5).

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.

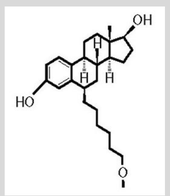
How NDC-1308 works
Induces OPCs to become mature, myelinating oligodendrocytes leading to a new myelin sheath.

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 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).

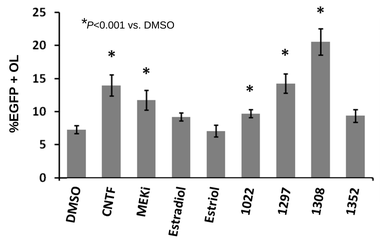
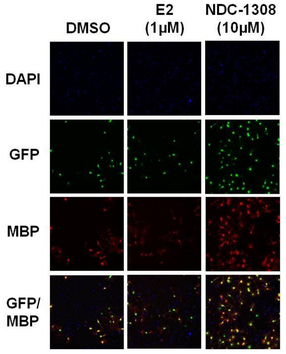


Figure A. NDC-1308 (EC_{50} = 1.0 μM) is the most potent of ENDECE' Neural's analogs for inducing oligodendrogenesis.

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

B. Image Analysis



C. ER-specific Differentiation

Figure C. 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.

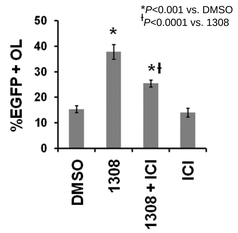


Figure B. cells that are co-expressing GFP and MBP appear yellow and are predominantly detected in NDC-1308 treated cells (lower right). Figure C, the ER antagonist ICI 182,780 inhibits about 50% of NDC-1308's activity to induce OPC differentiation, demonstrating that NDC-1308 is functioning through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

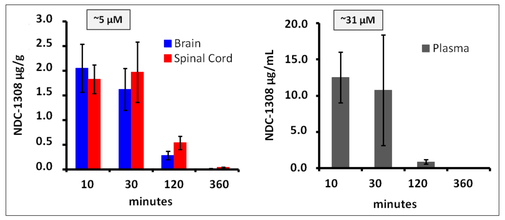
D. NDC-1308 regulates oligodendrogenesis genes distinctly from estradiol (E2) in both OPCs and PBMCs.

Gene Name	Gene Expression (fold change)			
	mouse OPCs		human PBMCs	
	NDC-1308	E2	NDC-1308	E2
OLIG2	10.42	2.11	1.74	0.98
MBP	8.46	1.79	2.83	2.10
MOG	4.86	1.00	1.84	0.95
DNER	7.33	4.16	2.32	1.06

Figure D. NDC-1308 (10 μM), E2 (10 nM) or vehicle was used to treat either mouse OPCs (5 days) or human PBMCs (12 hours). For OPCs, %EGFP analysis of replicate wells verified that the cells differentiated only with the NDC-1308 treatment. Cells were harvested with Trizol and RNA purified by Qiagen RNeasy for qPCR analysis. Comparisons were made for NDC-1308 and E2 relative to vehicle.

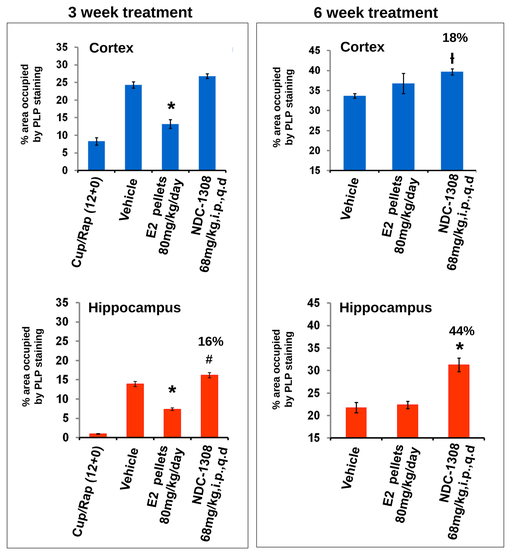
NDC-1308 is rapidly absorbed into CNS tissues

- Female C57BL/6 mice (N=5) each with single i.p. 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.***



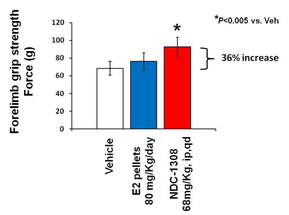
NDC-1308 induces remyelination in a cuprizone mouse model of demyelination

- 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.

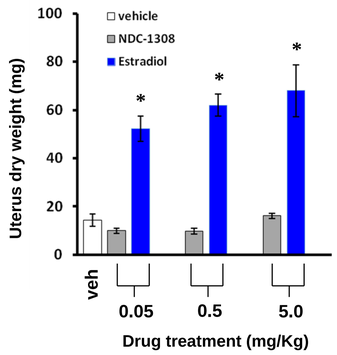
This test could potentially be used to assess improvement of function in Secondary Progressive MS patients during clinical trials.

Favorable kinetics for NDC-1308 in dog

Species	NDC-1308 injected (mg/Kg)	NDC-1308 Detected			NDC-1308 Plasma:Brain
		Brain (mg)	Brain (μM)	Plasma (mg)	
Mouse (i.p.,10 min)	75.00	‡0.0008	§5.3	*0.012	15.0
Dog (i.v., 5 min)	2.81	#0.432	^19.0	¦1.422	3.3

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
¦450 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. **Although structurally related, the biological 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.**
2. **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.**
3. **In the mouse cuprizone model, the remyelinating activity of NDC-1308 is associated with a functional improvement in forelimb grip strength.**
4. ***In vitro*, NDC-1308 can induce OPCs to differentiate into mature, myelinating oligodendrocytes.**
5. **Mechanistically, NDC-1308 is an estrogen receptor agonist that up-regulates several key genes which drive oligodendrogenesis.**
6. **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.**

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