UNDERGRADUATE RESEARCH IN NATURAL AND CLINICAL SCIENCE AND TECHNOLOGY (URNCST) JOURNAL
Read more URNCST Journal articles and submit your own today at: https://www.urncst.com

RESEARCH PROTOCOL OPEN ACCESS

Infusion of Lanosterol to Restore Cholesterol Metabolism in R6/2 Mice: A Research Protocol

Tony Lin, BSc Student [1]*, Abha Ranjitkar, BSc Student [1], Eden Samson, BHSc Student [1], Tracy Xie, BSc Student [1]

[1] University of Ottawa, Ottawa, Ontario, Canada K1N 6N5

*Corresponding Author: tlin109@uottawa.ca

Abstract

Huntington's disease (HD) is an autosomal-dominant, neurodegenerative disease characterized by motor dysfunction, cognitive decline, and drastic behavioural changes. The mutant huntingtin gene contains a CAG trinucleotide repeat resulting in a polyglutamine expansion in the mutant huntingtin protein (muHTT). While the mechanisms are not yet fully understood, muHTT is linked to the disruption of nerve and glial cells, particularly in their role in synthesizing cholesterol. Abnormal interactions between muHTT and sterol regulatory element-binding proteins (SREBP), which are transcription factors that control the cholesterol biosynthesis pathway, are implicated in HD. About 25% of the human body's total cholesterol is found in the brain and is involved in many vital roles, such as synaptogenesis, axonal growth, and creating efficient synaptic transmissions. Cholesterol biosynthesis is shown to be diminished in HD models, along with decreased levels of cholesterol precursor molecules, thus lowered cholesterol levels may be linked to the symptoms and progression of HD. This protocol aims to increase cholesterol biosynthesis by infusion of lanosterol, a cholesterol precursor, in R6/2 mice via osmotic mini-pumps. Three increasing doses of lanosterol will be administered to three groups of R6/2 mice, while one R6/2 and one wild type group will receive saline. These doses will be administered continuously over 7 weeks, starting from age 6-weeks when mice begin displaying progressive R6/2 symptoms until age 12-weeks when they show end-stage disease. Seven weekly rounds of tests will be conducted to assess motor and cognitive functioning, consisting of a rotarod performance test, Morris Water Maze test, and Novel Object Recognition test. At age 12-weeks, the mice will be sacrificed for immunohistochemistry analysis, gas chromatography-mass spectrometry, and flow cytometry to compare muHTT aggregate numbers, cholesterol levels, and striatal neuron count between the treatment and control mice. As a result of restored cholesterol homeostasis, amelioration in motor defects, cognitive performance, and striatal neuron survival rate in lanosterol-receiving mice compared to controls are expected. These anticipated results would suggest that lanosterol infusion via osmotic mini-pumps holds therapeutic potential and could be used to improve the prognosis of HD patients.

Keywords: Huntington's disease; cholesterol; SREBP2; astrocytes; lanosterol; striatum; R6/2; huntingtin

Introduction

Huntington's disease (HD) is an autosomal-dominant neurodegenerative disease characterized by a CAG trinucleotide repeat in the huntingtin (*HTT*) gene. The CAG repeats result in the abnormal expansion of polyglutamine (polyQ) region of the mutant huntingtin protein (muHTT). Normally, the CAG segment is repeated 10-35 times, but recurs more in HD patients. CAG repeats of 36-39 are associated with incomplete penetrance of the disease, by which only some develop the HD phenotype [1]. The age of the onset of symptoms is inversely related to the number of CAG repeats: individuals with 40-55 CAG repeats typically develop symptoms around 35-45 years of age, while CAG repeats of 70 or more can lead to a juvenile onset of the disease [2]. Aberrant muHTT is fragmented and polymerizes to form insoluble fibrils that prevent proteolytic digestion, causing accumulation of the fibrils along with other proteins that cannot undergo degradation [3]. This phenomenon alters crucial functions of neurons, such as synaptic transmission, through the accumulation of the toxic fragmented muHTT which eventually leads to neuronal loss [4]. Neuronal loss primarily occurs in the striatal part of the basal ganglia, as well as in cortical pyramidal neurons that project to the striatum [5]. The striatum is a structure crucial for voluntary movement and enlargement of the lateral ventricles. Damage to the striatum leads to loss of motor control, one of the hallmark symptoms of HD [6], as well as cognitive deficits and significant behavioural and emotional changes [7,8]. In addition, the polyQ expansions in muHTT make it prone to misfolding and self-aggregation, a biomarker of polyQ diseases, which interfere with important cellular processes and ultimately lead to cell death [9,10].

MuHTT also affects the differentiation of glial cells, the support cells that maintain optimal environment and myelination for efficient synaptic transmissions [11]. Astrocytes are specialized glial cells that produce cholesterol, an essential molecule to the central nervous system (CNS) that plays many key roles during both development and adulthood such as synaptogenesis and axonal growth. In addition, up to 70% of the cholesterol in the CNS is found in myelin sheaths [12]. Approximately 25% of the total cholesterol in the human body is located in the brain—while the brain volume only accounts for 2.1% of the body mass—making the brain the most cholesterol-rich organ [13,14]. Since cholesterol is unable to cross the blood-brain-barrier, it must be synthesized locally in the brain [15].

Cholesterol biosynthesis is shown to be significantly reduced in rodent HD models and in HD patients, especially in the striatum. Numerous studies found a decreased level of cholesterol precursors in HD mice and patient plasma that correlated with the progression of the disease, along with decreased expression and activity of sterol regulatory element-binding protein 2 (SREBP2)—the main transcription factor that activates cholesterol metabolism gene expression—suggesting that muHTT interferes with brain cholesterol synthesis [16]. The cholesterol biosynthesis pathway contains a sequence of enzymatic reactions beginning with acetate. After several steps, mevalonate is synthesized in the most highly-regulated reaction in the pathway and is converted into squalene [17]. The cyclization of squalene produces lanosterol, the first sterol precursor and a key intermediate of cholesterol [18–20]. A study by Boussicault et al. found that lanosterol and desmosterol, another cholesterol precursor, both significantly improved the survival of striatal neurons expressing muHTT in vitro [19]. Treatment using lanosterol was also found to dramatically reduce aggregation of expanded polyQ ataxin-3(84Q), which is a pathogenic-expanded polyQ protein like those implicated in neurodegenerative diseases such as HD. This highlights the possible role of lanosterol in the clearing of muHTT aggregates [21]. Thus, lanosterol shows therapeutic potential in reversing the neurodegenerative effects of HD as a precursor of cholesterol and as a neuroprotective molecule.

This study proposes the infusion of lanosterol in the striatum of R6/2 mice via osmotic mini-pumps to ameliorate HD symptoms and slow disease progression. The R6/2 mouse model has the most rapid onset of disease symptoms out of all the current HD mouse models [2], allowing the experiment to be run in 3 months or less. This intervention is predicted to promote the endogenous biosynthesis of cholesterol, increase the survival rate of striatal neurons expressing muHTT, and clear muHTT aggregates in the striatum. Expected results include reversal of disease-related motor defects, improved cognitive performance, and reduced muHTT aggregates in the treated mice compared to controls.

Methods

R6/2 Mice and Colony Management

R6/2 are transgenic mice that display an accelerated, progressive disease phenotype which models the key features of HD. These mice express *exon* 1 of the human *HD* gene with around 150 CAG repeats. This results in transgene expression levels at around 75% of the mice's endogenous huntingtin, which is driven by the human huntingtin promoter [2]. R6/2 mice begin displaying HD symptoms at 5-weeks of age and reach end-stage disease at approximately 12- to 14-weeks of age, thus the experiment can be run in 3 months or less [22]. R6/2 mice (N = 24) and wild type (WT) mice (N = 6) will be used, with equal numbers of males and females to control for sex-based differences. The sample size was determined through the resource equation method, in which the value "E", representing the degree of freedom of analysis of variance (ANOVA), determines the significance of results in animal experiments. E is calculated by the following formula: E = Total number of animals – Total number of groups. For this experiment, 30 total mice will be used, with 15 male and 15 female, across five total groups (three treatment groups and two control groups). Therefore for each sex, E = 15 – 5 = 10. Values of E between 10 and 20 are considered adequate for any sample size. [23]. The R6/2 mice will be received at age 3-weeks and screened for HD phenotypes, which emerge at around 5-weeks. Mice will be housed at 22º ± 1ºC, by a 12 hours light/dark schedule, and with free access to food and water.

Osmotic Mini-Pump Implantation

Osmotic mini-pumps will be used to infuse lanosterol and saline into the striatum. At 6-weeks old, a mini-pump attached to a catheter will be implanted subcutaneously into the back of each mouse, targeting the striatum [24]. Mice will be anesthetized using 15 µL of Avertin 2.5% per gram of body weight. The scalps of the mice will be shaved followed by placement of the animals into a stereotaxic apparatus. A subcutaneous pocket will be made on the back of the mice in the midscapular region to insert the osmotic mini-pump (Azlet). The brain infusion microcannula, which is connected to the mini-pump via a catheter, will be stereotaxically implanted into the right stratum; the striatum is the brain region most influenced by HD and the earliest identified site of reduced cholesterol biosynthesis [14,25]. The mini-pump will infuse at a constant rate for approximately 49 days. Eighteen R6/2 mice will receive increasing doses of lanosterol: 3 mg/L (N = 6), 10 mg/L (N = 6), and 20 mg/L (N = 6). One R6/2 group (N = 6) and one WT group (N = 6) will each receive 10 mg/L of saline, totalling twelve control mice.

Behavioural Tests

At 6-weeks of age, a testing round consisting of a rotarod performance test, Morris Water Maze (MWM) test, and Novel Object Recognition (NOR) test will be

performed prior to mini-pump implantation. R6/2 mice progressively develop many motor characteristics of HD patients, while memory loss and cognitive decline are the earliest and most sensitive indicators of HD [26,27]. The rotarod will assess motor function whereas the MWM and NOR will evaluate cognitive and memory impairment in treated and control mice. Following implantation and administration of lanosterol or saline, the same tests will be carried out weekly until 12-weeks of age, totalling seven testing rounds.

Rotarod Performance Test

The rotarod is a common performance test used to assess motor coordination and balance [28]. Treatment and control mice will be tested once weekly over the testing period. Each weekly test will first include a 5 min training trial on the rotarod at 4 RPM. One hour later, the mice will be tested in three consecutive trials with the rotarod speed linearly accelerating from 4 to 45 RPM over 300 seconds, with a 30 min inter-trial interval (ITI). The riding time will be recorded and mice remaining on the rotarod for more than 300 seconds will be removed and their time scored as 300 seconds.

Morris Water Maze Test

The MWM test will be used to assess spatial learning and memory. A 122 cm diameter circular tank will be filled with 30 cm deep opaque water at 22ºC. The water will be mixed with non-toxic white paint to obscure the location of the submerged platform [29]. A 12 cm diameter circular escape platform will be submerged 1 cm below the surface of the water. Treatment and control mice will be tested once a week with four trials per testing day. Prior to the first testing week, mice will be given a cued trial where the platform will be placed at the centre of the MWM with a visible flag hanging 15 cm overhead. Four training trials will be administered from the four starting locations, North (N), South (S), East (E), and West (W), to train mice to swim to the escape platform and ensure swimming ability and visual acuity [30]. During each testing day, the location of the platform (in the centre of the NE, NW, SE, or SW quadrants) will be randomly assigned and consistent for each mouse and the four starting locations (N, S, E, and W) will be used over four trials [31]. Mice will be placed at the starting position facing the tank wall and given 60 seconds to locate the hidden platform. The mice that are unable to find the platform within 60 seconds will be placed on the platform and given an escape latency of 61 seconds. All mice will be allowed to remain on the platform for 10 seconds before being removed from the tank, dried, and given an ITI of 30 seconds. The swim speed, distance travelled, and escape latency will be tracked and recorded using Viewpoint VideoTrack. The software will record the probability of finding the escape platform (number of correct trials / total trials).

Novel Object Recognition Test

The NOR test will be administered to evaluate cognition and long-term memory in a gray-coloured, non-reflective arena (44 × 44 × 44 cm) [15]. Treatment and control mice will be tested weekly over the span of two days. On day one, each mouse will be individually habituated to the empty arena for 10 min. The habituation phase will be followed by the familiarization phase in which two identical objects (A' and A") will be presented to each mouse for 10 min. After 24 h, the testing phase will occur: the mice will be exposed to one familiar object (A') and a novel object (B) for 10 min. The times taken to explore object A' (tA) and the novel object B (tB) will be recorded. Spontaneous recognition memory will be represented by the index of discrimination [(tB – tA) / (tA + tB)] × 100. Mice exploring for less than 5 seconds during the testing phase will be excluded from the analysis due to their inability to perform the task [32].

Immunohistochemistry Analysis

At 12-weeks of age, the mice will be anesthetized via intraperitoneal injection of Avertin 2.5% and transcardially perfused with paraformaldehyde (PFA) 4%. Brains will be removed from the skull, post-fixed in PFA 4% for 4 h, then cryoprotected in 25% sucrose in phosphate-buffered saline (PBS) solution, pH 7.4, for 24 h [14]. 20 µm coronal sections will be cut using a freezing microtome and incubated in citrate buffer, pH 6, for 20 min at 98ºC [33]. Sections will then be incubated with anti-huntingtin EM48 antibody (1:300, Millipore) overnight at room temperature (22º ± 1ºC) [34]. After 24 h, the sections will be washed in PBS three times then stained with a horse anti-mouse secondary antibody (1:200, Vector Laboratories) for 2 h at room temperature. Sections will be counterstained with nuclear dye Hoechst 33258 (1:10,000, Invitrogen), then mounted under cover slips.

To count muHTT aggregates, images will be acquired using a LEICA SP5 laser scanning confocal microscope. To quantify the number of aggregates, ImageJ software will be used to measure the fluorescence.

Gas Chromatography-Mass Spectrometry (GC-MS) Analysis

Levels of neural sterols and metabolites such as 24S-hydroxycholesterol (24S-OHC) will be quantified through GC-MS. Mouse striatal sections will be used to prepare striatal cell homogenates in PBS. 50 µL of homogenate will be added to a screw-capped vial sealed with a Teflon-lined septum, along with 50 µg of D6-cholesterol (CDN Isotopes), 1 µg of D4-lathosterol (CDN Isotopes), 100 ng of D6-lanosterol (Avanti Polar Lipids), and 400 ng of D7-24S-OHC (Avanti Polar Lipids) as internal standards [14]. In each vial, 25 µl of butylated hydroxytoluene (5 g/L) and 25 µl of EDTA (10 g/L) will be added to prevent auto-oxidation, along with argon to flush out air. Alkaline hydrolysis will proceed at room temperature for 1 h in the presence of 1 M

ethanolic KOH solution under magnetic stirring. After hydrolysis, the sterols (cholesterol, lathosterol, and lanosterol) and oxysterols (24S-OHC) will be extracted twice with 5 mL of cyclohexane. The organic solvent will be evaporated under a gentle stream of argon and converted into trimethylsilyl ethers with BSTFA + TMCS 1%.

GC-MS analysis will be performed on a Clarus 600 GC/MS (Perkin Elmer) with the GC equipped with an Elite-5MS capillary column (30 m × 0.32 mm × 0.25 m, Perkin Elmer). The injection will be performed in the spitless mode using helium as a carrier gas at a flow rate of 1 mL/min. The initial temperature of 180ºC will be held for 1 min, linearly increased by 20ºC/min to 270ºC, then increased by 5ºC/min to 290ºC, which will be maintained for 10 min. The MS will acquire data in selected ion monitoring mode. Peak integration will be performed manually and the sterols will be quantified from the selected ion monitoring analyses by comparison against internal standards using standard curves [35].

Flow Cytometry

To measure striatal neuronal survival rate, flow cytometry with neuronal nuclei (NeuN) staining will be used. After sacrificing the mice and sectioning of the brain, striatal sections will be immersed in 10 times volume of zinc-fixative solution (0.1 M Tris-HCl pH 6.5, 0.5% $ZnCl_2$, 0.5% Zn acetate, 0.05% $CaCl_2$, final pH 6.3) at 4ºC overnight [36]. The striatal tissues will be washed in ice-cold PBS before being finely minced to approximately 1 mm^3 pieces [33]. The tissue sample will be transferred with a 1 mL syringe with no needle into a low-protein binding tube. Four rounds of trituration will be completed in 600 µL of cold PBS, starting with 12 passes of the tissue through a 21 G needle, avoiding bubbles, followed by 5 min of settling. Afterwards, the cloudy supernatant will be removed and separated to another low-protein binding tube, with 500 µL of additional cold PBS for another trituration round, which will use a 23 G needle. The final two rounds will use a 25 G needle. All supernatants will be combined and filtered through a 70 µm mesh. Filtered striatal neurons are centrifuged at 250 g for 3 min in a swinging bucket centrifuge at 4ºC. The supernatant will be removed and the cells will be resuspended in 200 µL PBS. The filtered cells, maintained at 4ºC, will be stained with anti-NeuN-phycoerythrin (PE) (1:200; Millipore) and 4',6-diamidino-2-phenylindole (1.43 µM) in 1 mL of PBS for 2 h on a rotating platform at 200 RPM. Flow cytometric data will be acquired using a FACSAria flow cytometer (Becton-Dickinson) [37] and analyzed using FlowJo software [38].

Statistical Analysis

The data obtained from the behavioural tests and quantitative analyses will be statistically analysed using the Statistical Package for the Social Sciences software with a p-level of 0.05. To analyze the rotarod performance test, repeated measures analysis of variance (RM-ANOVA) will measure changes between groups across the 7-week testing period. Post-hoc comparisons, such as Fisher's least significant difference test, will determine if there are significant differences in riding time between the treatment and control groups, with increased riding times expected in the former [39].

In the MWM test, an RM-ANOVA would analyze differences between the probability of locating the escape platform between each group. Similarly, a 2-way analysis of variance test will be performed to analyze the NOR test results, to compare the index of discrimination between the treatment and control groups over the 7-week long testing. Multiple t-tests will be employed to compare the results of each lanosterol-treated group with the controls.

Quantitative analyses following the testing period (immunohistochemistry, GC-MS, and flow cytometry) can all be conducted using multiple 1-way RM-ANOVAs, where the mean values of the lanosterol-infused R6/2 mice will be compared with the mean values of the two control groups in each respective method.

Results

As this is a research protocol, the results are merely hypotheticals and may be different if the experiments are performed. The treated mice are expected to perform better in the physical, memory, and cognitive function tests. In the rotarod performance test, treated mice will hypothetically have higher riding times in all trials compared to controls, indicating stronger motor functions and balance. Treatment groups in the MWM should yield lower times compared to control as weeks proceed, demonstrating amelioration in spatial memory. The index of discrimination in the NOR test should be greater in lanosterol-infused mice compared to controls, indicating rescued recognition memory and cognitive function. In addition, dose-dependent improvements are expected in motor defects, memory, and cognition between the three treatment groups.

Following the last round of behavioural tests at 12-weeks of age, quantitative analysis of mice samples should favour the lanosterol-infused mice versus the controls. Immunohistochemical analysis conducted with EM48 antibodies, commonly used to stain muHTT, will allow for the comparison of the numbers of muHTT aggregates in treated mice compared to those in the controls [34]. Increased rescuing of aggregates in the lanosterol-infused mice versus controls is expected. GC-MC measures levels of neural sterols and metabolites, allowing comparison of cholesterol biosynthesis between the treatment and control groups [35]. Higher detected sterol and metabolite levels are predicted in the treated mice. Lastly, in the flow cytometry performed on striatal tissue with NeuN, the treatment groups should show a statistically significant difference with a larger NeuN-positive population compared to controls, indicating enhanced striatal neuron survival rate resulting from treatment [36].

Discussion

The major objectives of the presented research protocol were to slow down the progression of HD and examine the in-depth effects of lanosterol infusion in HD mice. By targeting the striatum, this protocol aims to restore cholesterol homeostasis—subsequently reducing motor dysfunction, cognitive deficits, neuronal death rate in the striatum, and muHTT aggregates in R6/2 treatment mice compared to controls. Increasing doses of lanosterol will be delivered to the striatum via surgically implanted osmotic mini-pumps, followed by rounds of behavioural tests and quantitative analyses to assess the effects of the treatment on HD symptoms.

The experimental results may be used to guide future research in developing a therapeutic intervention for HD. Next steps would include optimizing the therapeutic dose of lanosterol in R6/2 mice that would generate the maximal reversal of HD symptoms; this would subsequently be used in future studies examining lanosterol infusion in R6/2 mice versus other variables, such as other neuronal targets, other cholesterol precursor molecules, or age of implantation. This protocol may also help illuminate how the cholesterol biosynthesis pathway is implicated both in HD pathology and in broader research about neurodegenerative diseases. This research can help lay the foundation of potential treatment for HD patients, slowing the actual onset and progression of the disease rather than simply treating the symptoms.

Conclusions

This research protocol defines an approach of infusing lanosterol to the striatum of R6/2 mice, which is predicted to ameliorate hallmark HD symptoms through the restoration of cholesterol homeostasis. Given the hypothetical benefits of lanosterol, the next directions include optimizing a proper therapeutic dose in R6/2 to be used in further experiments. It may also help elucidate the dysregulation of cholesterol biosynthesis in HD literature, as well as neurodegeneration in general. In the future, the striatal infusion of lanosterol in mouse models may help pave the way for therapeutic interventions that improve the prognosis and quality of life of HD patients.

List of Abbreviations Used

24S-OHC: 24S-hydroxycholesterol
ANOVA: analysis of variance
CNS: central nervous system
GC: gas chromatography
GC-MS: gas chromatography-mass spectrometry
HD: Huntington's disease
HTT: huntingtin gene
HTT: huntingtin protein
ITI: inter-trial interval
MS: mass spectrometry
muHTT: mutant huntingtin protein
MWM: Morris water maze
NeuN: neuronal nuclei
NOR: novel object recognition
PE: phycoerythrin
PFA: paraformaldehyde
PI: propidium iodide
polyQ: polyglutamine
RM-ANOVA: repeated measures analysis of variance
SREBP2: sterol regulatory element binding protein-2
WT: wild type

Conflicts of Interest

The authors declare that they have no conflict of interests.

Ethics Approval and/or Participant Consent

This study did not require ethics approval and/or participant consent as the experiment was performed in mice hypothetically.

Authors' Contributions

TL: Substantial contribution to the study design and methodology, drafted & revised the manuscript critically, and approved the version to be published.
AR: Substantial contribution to the study design, interpretation, analysis of hypothetical data, and drafted the manuscript, and approved the version to be published.
ES: Substantial contribution to the study design, interpretation, analysis of hypothetical data, and drafted the manuscript and approved the version to be published.
TX: Substantial contribution to the study design and methodology, drafted & revised the manuscript critically, and approved the version to be published.

Acknowledgements

The authors acknowledge SciNapse for hosting their case competition, their judges for providing helpful feedback, and editors, peer reviewers, and staff working at URNCST for providing an opportunity to submit the manuscript.

Funding

This study was not funded.

References

[1] Myers RH. Huntington's disease genetics. NeuroRX. 2004;1(2):255–62. https://doi.org/10.1602/neurorx.1.2.255
[2] Li JY, Popovic N, Brundin P. The use of the R6 transgenic mouse models of Huntington's disease in attempts to develop novel therapeutic strategies. NeuroRX. 2005;2(3):447–64. https://doi.org/10.1602/neurorx.2.3.447
[3] Waelter S, Boeddrich A, Lurz R, Scherzinger E, Lueder G, Lehrach H, et al. Accumulation of mutant Huntingtin fragments in aggresome-like inclusion bodies as a result of insufficient protein degradation. Molecular Biology of the Cell. 2001;12(5):1393–407. https://doi.org/10.1091/mbc.12.5.1393

[4] Huntington disease [Internet]. MedlinePlus Genetics. 2020 [cited 2023 Aug 14]. Available from: https://medlineplus.gov/genetics/condition/huntington-disease/
[5] Reiner A, Dragatsis I, Dietrich P. Genetics and neuropathology of Huntington's disease. International Review of Neurobiology. 2011;325–72. https://doi.org/10.1016/b978-0-12-381328-2.00014-6
[6] Trafton A. How Huntington's disease affects different neurons [Internet]. MIT News. 2023 [cited 2023 Aug 14]. Available from: https://news.mit.edu/2023/huntingtons-disease-affects-different-neurons-striosomes-0120
[7] Gusella JF, Wexler NS, Conneally PM, Naylor SL, Anderson MA, Tanzi RE, et al. A polymorphic DNA marker genetically linked to Huntington's disease. Nature. 1983 Nov 17;306(5940):234–8. https://doi.org/10.1038/306234a0
[8] Reilmann R, Leavitt BR, Ross CA. Diagnostic criteria for Huntington's disease based on natural history. Movement Disorders. 2014 Sept 15;29(11):1335–41. https://doi.org/10.1002/mds.26011
[9] Arrasate M, Finkbeiner S. Protein aggregates in Huntington's disease. Experimental Neurology. 2021 Nov 12;238(1):1–11. https://doi.org/10.1016/j.expneurol.2011.12.013
[10] Jarosińska OD, Rüdiger SG. Molecular strategies to target protein aggregation in Huntington's disease. Frontiers in Molecular Biosciences. 2021 Nov 12;8. https://doi.org/10.3389/fmolb.2021.769184
[11] Parpura V, Heneka MT, Montana V, Oliet SH, Schousboe A, Haydon PG, et al. Glial cells in (patho)physiology. Journal of Neurochemistry. 2012 Apr 2;121(1):4–27. https://doi.org/10.1111/j.1471-4159.2012.07664.x
[12] Kacher R, Lamazière A, Heck N, Kappes V, Mounier C, Despres G, et al. CYP46A1 gene therapy deciphers the role of brain cholesterol metabolism in Huntington's disease. Brain. 2019 Aug 1;142(8):2432–50. https://doi.org/10.1093/brain/awz174
[13] Kacher R, Mounier C, Caboche J, Betuing S. Altered cholesterol homeostasis in Huntington's disease. Frontiers in Aging Neuroscience. 2022 Apr 19;14. https://doi.org/10.3389/fnagi.2022.797220
[14] Birolini G, Valenza M, Di Paolo E, Vezzoli E, Talpo F, Maniezzi C, et al. Striatal infusion of cholesterol promotes dose-dependent behavioral benefits and exerts disease-modifying effects in Huntington's disease mice. EMBO Molecular Medicine. 2020 Oct 7;12(10). https://doi.org/10.15252/emmm.202012519
[15] Birolini G, Verlengia G, Talpo F, Maniezzi C, Zentilin L, Giacca M, et al. SREBP2 gene therapy targeting striatal astrocytes ameliorates Huntington's disease phenotypes. Brain. 2021 Nov 29;144(10):3175–90. https://doi.org/10.1093/brain/awab186
[16] González-Guevara E, Cárdenas G, Pérez-Severiano F, Martínez-Lazcano JC. Dysregulated brain cholesterol metabolism is linked to neuroinflammation in Huntington's disease. Movement Disorders. 2020 Jul;35(7):1113–27. https://doi.org/10.1002/mds.28089
[17] Valenza M, Leoni V, Tarditi A, Mariotti C, Björkhem I, Di Donato S, et al. Progressive dysfunction of the cholesterol biosynthesis pathway in the R6/2 mouse model of Huntington's disease. Neurobiology of Disease. 2007;28(1):133–42. https://doi.org/10.1016/j.nbd.2007.07.004
[18] Risley JM. Cholesterol biosynthesis: Lanosterol to cholesterol. Journal of Chemical Education. 2002 Mar 1;79(3):377. https://doi.org/10.1021/ed079p377
[19] Boussicault L, Alves S, Lamazière A, Planques A, Heck N, Moumné L, et al. CYP46A1, the rate-limiting enzyme for cholesterol degradation, is neuroprotective in Huntington's disease. Brain. 2016 Mar;139(3):953–70. https://doi.org/10.1093/brain/awv384
[20] Russell DW. Cholesterol biosynthesis and metabolism. Cardiovascular Drugs and Therapy. 1992;6(2):103–10. https://doi.org/10.1007/bf00054556
[21] Upadhyay A, Amanullah A, Mishra R, Kumar A, Mishra A. Lanosterol suppresses the aggregation and cytotoxicity of misfolded proteins linked with neurodegenerative diseases. Molecular Neurobiology. 2018 Feb;55(2):1169–82. https://doi.org/10.1007/s12035-016-0377-2
[22] Klapstein GJ, Fisher RS, Zanjani H, Cepeda C, Jokel ES, Chesselet M-F, et al. Electrophysiological and morphological changes in striatal spiny neurons in R6/2 Huntington's disease transgenic mice. Journal of Neurophysiology. 2001 Dec;86(6):2667–77. https://doi.org/10.1152/jn.2001.86.6.2667
[23] Charan J, Kantharia ND. How to calculate sample size in animal studies? Journal of Pharmacology and Pharmacotherapeutics. 2013;4(4):303–6. https://doi.org/10.4103/0976-500x.119726
[24] Sanchez-Mendoza EH, Carballo J, Longart M, Hermann DM, Doeppner TR. Implantation of miniosmotic pumps and delivery of tract tracers to study brain reorganization in pathophysiological conditions. Journal of Visualized Experiments. 2016 Jan 18;(107). https://doi.org/10.3791/52932
[25] Shankaran M, Di Paolo E, Leoni V, Caccia C, Ferrari Bardile C, Mohammed H, et al. Early and brain region-specific decrease of de novo cholesterol biosynthesis in Huntington's disease: A cross-validation study in Q175 knock-in mice. Neurobiology of Disease. 2017 Feb;98:66–76. https://doi.org/10.1016/j.nbd.2016.11.013
[26] Carter RJ, Lione LA, Humby T, Mangiarini L, Mahal A, Bates GP, et al. Characterization of progressive motor deficits in mice transgenic for the Human Huntington's Disease Mutation. The Journal of Neuroscience. 1999;19(8):3248–57. https://doi.org/10.1523/jneurosci.19-08-03248.1999

[27] Paulsen JS. Cognitive impairment in Huntington disease: Diagnosis and treatment. Current Neurology and Neuroscience Reports. 2011;11(5):474–83. https://doi.org/10.1007/s11910-011-0215-x
[28] Menalled L, El-Khodor BF, Patry M, Suárez-Fariñas M, Orenstein SJ, Zahasky B, et al. Systematic behavioral evaluation of Huntington's disease transgenic and knock-in mouse models. Neurobiology of Disease. 2009 May 21;35(3):319–36. https://doi.org/10.1016/j.nbd.2009.05.007
[29] Vorhees CV, Williams MT. Morris water maze: Procedures for assessing spatial and related forms of learning and memory. Nature Protocols. 2006 Jul 27;1(2):848–58. https://doi.org/10.1038/nprot.2006.116
[30] Fink KD, Rossignol J, Crane AT, Davis KK, Bombard MC, Bavar AM, et al. Transplantation of umbilical cord-derived mesenchymal stem cells into the striata of R6/2 mice: Behavioral and neuropathological analysis. Stem Cell Research & Therapy. 2013 Oct 24;4(5). https://doi.org/10.1186/scrt341
[31] Hebb ALO, Robertson HA, Denovan-Wright EM. Phosphodiesterase 10A inhibition is associated with locomotor and cognitive deficits and increased anxiety in mice. European Neuropsychopharmacology. 2008 May;18(5):339–63. https://doi.org/10.1016/j.euroneuro.2007.08.002
[32] López-Hurtado A, Burgos DF, González P, Dopazo XM, González V, Rábano A, et al. Inhibition of DREAM-ATF6 interaction delays onset of cognition deficit in a mouse model of Huntington's disease. Molecular Brain. 2018 Mar 9;11(1). https://doi.org/10.1186/s13041-018-0359-6
[33] Lee M, Im W, Kim M. Exosomes as a potential messenger unit during heterochronic parabiosis for amelioration of Huntington's disease. Neurobiology of Disease. 2021 Jul;155:105374. https://doi.org/10.1016/j.nbd.2021.105374
[34] Bayram-Weston Z, Jones L, Dunnett SB, Brooks SP. Comparison of mHTT antibodies in Huntington's disease mouse models reveal specific binding profiles and steady-state ubiquitin levels with disease development. PLOS ONE. 2016 May 19;11(5). https://doi.org/10.1371/journal.pone.0155834
[35] Marullo M, Valenza M, Leoni V, Caccia C, Scarlatti C, De Mario A, et al. Pitfalls in the detection of cholesterol in Huntington's disease models. PLoS Currents. 2012 Oct 11; 4: e505886e9a1968. https://pubmed.ncbi.nlm.nih.gov/23145355/
[36] Martin D, Xu J, Porretta C, Nichols CD. Neurocytometry: Flow cytometric sorting of specific neuronal populations from human and rodent brain. ACS Chemical Neuroscience. 2017 Feb 1;8(2):356–67. https://doi.org/10.1021/acschemneuro.6b00374
[37] Okada S, Saiwai H, Kumamaru H, Kubota K, Harada A, Yamaguchi M, et al. Flow cytometric sorting of neuronal and glial nuclei from central nervous system tissue. Journal of Cellular Physiology. 2010 Aug 17;226(2):552–8. https://doi.org/10.1002/jcp.22365
[38] Park HJ, Lee SW, Im W, Kim M, Van Kaer L, Hong S. INKT cell activation exacerbates the development of Huntington's disease in R6/2 transgenic mice. Mediators of Inflammation. 2019 Jan 15;2019:1–10. https://doi.org/10.1155/2019/3540974
[39] Curzon P, Zhang M, Radek RJ, Fox GB. The behavioral assessment of sensorimotor processes in the mouse: Acoustic startle, sensory gating, locomotor activity, rotarod, and beam walking. In: Methods of Behavior Analysis in Neuroscience. 2nd ed. Boca Raton (FL): CRC Press/Taylor & Francis; 2009. Chapter 8. https://pubmed.ncbi.nlm.nih.gov/21204341/

Article Information
Managing Editor: Jeremy Y. Ng
Peer Reviewers: Clara Schott, Miriam Basta
Article Dates: Received May 23 23; Accepted Aug 04 23; Published Aug 28 23

Citation
Please cite this article as follows:
Lin T, Ranjitkar A, Samson E, Xie T. Infusion of lanosterol to restore cholesterol metabolism in R6/2 mice: A research protocol. URNCST Journal. 2023 Aug 28: 7(8). https://urncst.com/index.php/urncst/article/view/503
DOI Link: https://doi.org/10.26685/urncst.503

Copyright
© Tony Lin, Abha Ranjitkar, Eden Samson, Tracy Xie. (2023). Published first in the Undergraduate Research in Natural and Clinical Science and Technology (URNCST) Journal. This is an open access article distributed under the terms of the Creative Commons Attribution License (https://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work, first published in the Undergraduate Research in Natural and Clinical Science and Technology (URNCST) Journal, is properly cited. The complete bibliographic information, a link to the original publication on http://www.urncst.com, as well as this copyright and license information must be included.

URNCST Journal
"Research in Earnest"

Funded by the Government of Canada | Canada

Do you research in earnest? Submit your next undergraduate research article to the URNCST Journal!
| Open Access | Peer-Reviewed | Rapid Turnaround Time | International |
| Broad and Multidisciplinary | Indexed | Innovative | Social Media Promoted |
Pre-submission inquiries? Send us an email at info@urncst.com | Facebook, Twitter and LinkedIn: @URNCST
Submit YOUR manuscript today at https://www.urncst.com!