Neuroprotective Effects of Phenylbutyrate in the N171-82Q Transgenic Mouse Model of Huntington’s Disease*

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Huntington’s disease (HD) is caused by an expansion of exonic CAG triplet repeats in the gene encoding the huntingtin protein (Htt), however, the means by which neurodegeneration occurs remains obscure. There is evidence that mutant Htt interacts with transcription factors leading to reduced histone acetylation. We report that administration of the histone deacetylase inhibitor phenylbutyrate after onset of symptoms in a transgenic mouse model of HD significantly extends survival and attenuates both gross brain and neuronal atrophy. Administration of phenylbutyrate increased brain histone acetylation and decreased histone methylation levels as assessed by both immunocytochemistry and Western blots. Phenylbutyrate increased mRNA for components of the ubiquitin-proteosomal pathway and down-regulated caspases implicated in apoptotic cell death, and active caspase 3 immunoreactivity in the striatum. These results show that administration of phenylbutyrate, at doses that are well tolerated in man, exerts significant neuroprotective effects in a transgenic mouse model of HD, and therefore represents a very promising therapeutic approach for HD.

Huntington’s disease (HD)¹ is caused by a mutation that leads to an expansion of a stretch of polyglutamines in the Htt protein. The expanded polyglutamine domains can interact with other polyglutamine containing proteins, including several transcription factors (1). Alterations in gene transcription occur as an early feature of transgenic mouse models of HD and other polyglutamine diseases (2–4). One example is recruitment of CREB-binding protein (CBP) into aggregates of polyglutamine containing proteins in cultured cell lines (5, 6). CBP is depleted from its normal nuclear localization and sequestered into Htt containing aggregates in cell culture, transgenic mice, and human HD postmortem brain tissue (7). CBP functions as a histone acetyltransferase enzyme. Mutant huntingtin directly binds to the acetyltransferase domains of CBP and another protein, P300/CBP associated factor, which results in a reduction of the acetyltransferase activity in cell-free assays as well as a decrease in the level of histone acetylation in mammalian cells (8). There is, however, evidence of increased CRE-mediated transcription in a transgenic mouse model of HD (9).

Histone acetylation helps transcription factors gain access to specific regions of DNA when it is tightly packed in chromatin, and thereby increases gene transcription. In a Drosophila model of polyglutamine-dependent neurodegeneration, histone deacetylase (HDAC) inhibitors arrest the ongoing progressive neuronal degeneration (8). The toxic effects of polyglutamines in yeast were mitigated by the HDAC inhibitor trichostatin A (10). Furthermore, histone acetylation is reduced in cell lines that express a mutant androgen receptor with an expanded polyglutamine repeat, and this is reversed by overexpression of CBP or by treatment with HDAC inhibitors, with a concomitant reduction in cell loss (11). The HDAC inhibitor suberoylanilide hydroxamic acid (SAHA) ameliorates motor deficits in the R6/2 transgenic mouse model of HD, although its effects on survival were not assessed. (12). The HDAC inhibitor sodium butyrate improves survival and attenuates striatal atrophy in the R6/2 transgenic mouse model of HD when administered presymptomatically starting at 21 days of age (13). A critical issue, however, is whether HDAC inhibitors exert neuroprotective effects when administered after the onset of symptoms, analogous to the situation in HD patients. We found that the HDAC inhibitor phenylbutyrate exerts significant effects on survival and ameliorates histopathologic degeneration in the N171-82Q transgenic mouse model of HD when administered after the onset of symptoms. Furthermore, we show for the first time that histone methylation is markedly increased in the N171-82Q transgenic mouse model of HD, and that this is ameliorated by phenylbutyrate treatment.

MATERIALS AND METHODS

Animals—Transgenic N171-82Q mice were originally obtained from Drs. Ross and Borchelt (The Johns Hopkins University, Baltimore, MD), and maintained on a B6C3F1 background (Jackson Laboratories, Bar Harbor, ME). The offspring were genotyped using a PCR assay on tail DNA. Mice were housed 4–5 per cage with free access to food and water, under standard conditions with a 12-h light/dark cycle. All

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1 The abbreviations used are: HD, Huntington’s disease; Htt, huntingtin; HDAC, histone deacetylase; CBP, CREB-binding protein; CRE, cAMP-response element-binding protein; PBS, phosphate-buffered saline; RT, reverse transcriptase; SAHA, suberoylanilide hydroxamic acid.
animal experiments were performed in accordance with the NIH Guide for the Care and Use of Laboratory Animals and were approved by the Weill Medical College of Cornell University Institutional Animal Care and Use Committee. Male and female N171-82Q mice, and littermates, were equally distributed between experimental groups.

Treatment—Mice received intraperitoneal injections of 4-phenylbutyric acid sodium salt (100 mg/kg/day, volume 3.33 ml/kg; Triple Crown USA, Inc., Perkasie, PA) or vehicle (PBS, 3.33 ml/kg), 6 days per week from 75 days of age.

Survival—Thirty-six mice (24 in the drug and 12 in the vehicle group) were used in survival, weight, and behavior studies. Mice were observed daily, and deemed to have reached end-stage of the disorder when they could no longer initiate movement after being gently agitated for 2 min. Death was recorded when animals reached this stage and were euthanized by Na-pentobarbital overdose, or at the time of natural death if this occurred between daily inspections.

Behavior and Weight Assessment—Motor performance was assessed using an accelerating rotarod apparatus (Columbus Instruments, Columbus, OH). The rotarod was accelerated from 0 rpm at a rate of 5.3 rpm/min over 180 s, then maintained at 16 rpm for a further 120 s (300 s total). The time elapsed on the rotarod measures the motor competency of the mouse in the task. Mice were given two rotarod training sessions to acclimate them to the apparatus, and then tested twice weekly from 75 days of age. Each mouse undertook three 50-s trials per test session, with the best result recorded. Mice were weighed twice a week.

Histone Acetylation and Methylation Levels in Brain Tissue—Fifteen N171-82Q and littermate wild-type mice (aged 4–5 months) received single intraperitoneal injections of 100 mg/kg Na-phenylbutyrate or PBS (vehicle). Mice were sacrificed 0, 1, 2, 3, and 4 h post-injection (n = 3 per group), brain and spleen were harvested and immediately frozen in liquid nitrogen. Nuclear fractionation was carried out according to Ref. 15. Levels of acetylated H3 histones were measured by Western blot analysis according to published methods (anti-acetylated H3 dilution 1:3000; anti-acetylated H4 dilution 1:2000) (16). Levels were compared with butyrate-treated and untreated HeLa cell extracts. Histone methylation in both vehicle and Na-phenylbutyrate-treated wild-type and N171-82Q mice at 0, 1, 2, 3, and 4 h was measured by Western blot analysis using an anti-dimethylated H3 (Lys-9) antibody (Upstate Biotechnology, Lake Placid, NY). Anti-acetylated H3 and H4 antibodies as well as butyrate-treated and control HeLa cell extracts were obtained from Upstate Biotechnology (Lake Placid, NY) and anti-rabbit IgG horseradish peroxidase from Amersham Biosciences. The Western blots were quantitated using computer-assisted densitometry.

Histone Quantitation and Methylhistone Localization of Acetylated and Methylated Histones—Mice were treated with 100 mg/kg Na-phenylbutyrate or PBS from 75 days of age. At 100 or 120 days of age, mice were deeply anesthetized and transcardially perfused with 4% paraformaldehyde (n = 5 per group). Brains were removed, post-fixed with the perfusant for 2 h, and then cryoprotected in 20% glycerol, 2% Me2SO. Brains were then cut into frozen 50-μm thick sections, and serial sections stained for Nissl substance (creosyl violet) or immunostained with an antibody recognizing the first 256 amino acids of human huntingtin (EM48, dilution, 1:1,000). An antibody to ubiquitin (dilution, 1:200, Dako Corp., Carpintena, CA) was also used to confirm the presence of protein aggregates. Immunocytochemical localization of acetylated histone H3 and acetylated histone H4 was performed in adjacent sections, as previously described (17). Immunocytochemical detection of histone methylation was done using an antibody to anti-dimethyl-histone H3 (Lys-9) (Upstate Biotechnology, Lake Placid, NY). Caspase activity was examined using an affinity purified anti-active caspase 3 antibody (dilution, 1:1,000, BD Pharmingen).

Stereology/Quantitation—Serial-cut coronal tissue sections beginning from the most rostral segment of the neostriatum to the level of the substantia nigra were sampled. The optical dissector method was employed estimating the number of huntingtin-positive aggregates. Striatal neuron areas were analyzed by microscopic videocapture using a Windows-based image analysis system for area measurement (Optimas, Biospec Inc., Edmonds, WA). The software automatically identifies and measures profiles. All computer identified cell profiles were manually verified as neurons and exported to Microsoft Excel. Cross-sectional areas were analyzed using Statview.

RNA Isolation and Microarray Analysis—Striata were dissected from mouse brains and immediately frozen in liquid nitrogen. Total RNA was isolated by extraction with TRIzol (Invitrogen). Labeled cRNA probes were generated from total RNA samples using the MessageAmpTM cRNA kit (Ambion Inc., Austin, TX). Briefly, the procedure consists of reverse transcription of 2 μg of total RNA with a T7 oligo(dT) primer bearing a T7 promoter sequence followed by in vitro transcription of the cRNA. Gel-purified cRNA was used as a substrate for microarray hybridization. The microarray chip was sealed to 250 target intensity, using the Microarray Suite software (Affymetrix, Santa Clara, CA). Drug-induced alterations in gene expression were analyzed using the GeneSpring software (Silicon Genetics, Redwood, CA). Data analysis was carried out on 10 microarrays (5 PBS-treated and 5 phenylbutyrate-treated mice). Normalization was carried out using default normalization parameters by GeneSpring software as follows: per sample, by dividing the raw data by the 50th percentile of all measurement, and per gene, by dividing the raw data by the median of the expression level to specific samples. Data from probe sets representing genes that failed the detection criteria (labeled “absent” or “marginal” in all 10 microarrays) were eliminated, and all further analyses were carried out in the remaining 7,116 probe sets. The Welch’s t test statistical method was used to find differentially expressed genes by two-group comparisons, and genes were assumed to be significantly up-regulated or down-regulated if the calculated p value was <0.05.

Real-time RT-PCR—One-step quantitative real-time RT-PCR using a LightCycler thermal cycler system (Roche Diagnostics) was performed to confirm microarray results. PCR was carried out with the SYBR Green quantitative RT-PCR system (Sigma) and gene-specific primers for 40 cycles according to the manufacturer’s protocols. After amplification, a melting curve analysis and length verification by gel electrophoresis were carried out to confirm the specificity of PCR products. As a negative control, template RNA was replaced with PCR-grade water. Calculations of threshold cycle and difference were analyzed with LiCycler analysis software (Roche). Gene-specific primers were designed using LightCycler probe design software (Roche). PCR primer pairs used for each gene were as follows: Gfer, 5'-GGCTGCAACAT-GAGGT-3' and 5'-GGCTCAGATGCACCTTATAT-3'; Gstm3, 5'-CTCTGCT-CTCATAGAGAG-3' and 5'-GGAGAGAGAGCCGGGA-3'; Pmsa3, 5'-ACGGAGACAGCAG-3' and 5'-ATACGGCAAGTATCTT-3'; Casp9, 5'-AGAACACCTGACTG-3' and 5'-CTCCCGTGAAGATAT-TAC-3'; Cflar, 5'-TGGATACCGTGCAGCG-3' and 5'-CTTCGAT-
RESULTS

Systemic administration of phenylbutyrate at a dose of 100 mg/kg intraperitoneal 6 days per week starting at 75 days of age produced a significant increase in survival of 23% in the N171-82Q transgenic mouse model of HD (Fig. 1) (p < 0.0001). Life span was 153.2 ± 4.8 days in the phenylbutyrate-treated mice and 124.5 ± 5.4 days in mice that received vehicle.

Surprisingly, phenylbutyrate treatment had no significant effects on weight loss or on motor performance as assessed by rotarod performance in the N171-82Q mice (data not shown). This contrasts with findings using other therapeutic interventions including creatine and minocycline, in which improved survival is accompanied by improved motor performance and delayed weight loss (18). Treatment with another HDAC inhibitor, SAHA, before onset of symptoms ameliorated rotarod deficits, but had no effect on grip strength (12). Treatment with sodium butyrate starting at 21 days of age improved both rotarod performance and weight loss (13). The lack of beneficial effects on motor symptoms in the present study may reflect the fact that therapy was initiated after symptom onset.

Histopathologic studies in brains of treated and untreated mice were performed at both 100 and 120 days of age. These studies showed a significant attenuation of gross brain atrophy and ventricular enlargement, as well as neuronal atrophy after phenylbutyrate treatment, which was significant at 120 days (Fig. 2). At 120 days the total brain volume in the phenylbutyrate-treated mice was 21.6% greater than in PBS (p < 0.001) and the ventricular volume was 82.4% less (p < 0.001). Whereas marked striatal neuron atrophy was present in untreated N171-82Q mice at 120 days, phenylbutyrate treatment significantly reduced striatal neuron atrophy in N171-82Q mice (wild-type littermate control: 143.9 ± 11.9 μm²; phenylbutyrate-treated N171-82Q mice: 116.5 ± 17.5 μm²; PBS-treated N171-82Q mice: 54.2 ± 25.3 μm²; F5,15 = 12.85; phenylbutyrate versus PBS, p < 0.01). The numbers of Htt and...
ubiquitin-stained aggregates were unaltered by phenylbutyrate administration (Fig. 3) (Huntingtin aggregates in phenylbutyrate-treated N171-82Q mice: 3.25 × 10^6 ± 1.11; PBS-treated N171–82Q mice: 3.11 × 10^6 ± 1.02, F_{3,10} = 1.09; p < 0.38). These results are similar to observations with sodium butyrate in the R6/2 transgenic mouse model of HD (13). Treatment with SAHA had no effect on numbers of aggregates and gross morphology, but showed a tendency to reduce neuronal atrophy (12). To verify that the effects we observed were because of an increase in histone acetylation, we performed both immunocytochemistry and Western blots. Administration of phenylbutyrate for 2 weeks increased immunostaining for both acetylated histone H3 and histone H4 in striatal neurons (Fig. 4). Administration of phenylbutyrate at 100 mg/kg increased histone acetylation in the spleen (not shown) and brain at 2 h post-administration (Fig. 5). Another histone modification that can repress gene transcription is methylation of lysine 9 on histone 3. Using immunocytochemistry we found a marked increase in methylation of histone 3 in the striatum at 120 days of age, which was markedly attenuated by phenylbutyrate treatment (Fig. 6). This finding was confirmed by Western blots (Fig. 7). We verified that phenylbutyrate dose dependently inhibited histone deacetylase activity in vitro with an IC_{50} of ~10 mM.

We also examined the effects of phenylbutyrate on gene expression levels using Affymetrix gene arrays. Phenylbutyrate was administered for 2 weeks starting at 75 days of age to 5 N171-82Q mice, whereas 5 mice received vehicle. Transcription products in striatum of the two groups were compared (Table 1). To validate the alterations of gene expression at the mRNA level, which appeared on the microarray, real-time RT-PCR was performed using a LightCycler thermal cycler system (Fig. 8). Expression of selected genes (Gfer, Gstm3, and Pama3) were significantly up-regulated after phenylbutyrate treatment compared with controls (p < 0.05), whereas the other genes (Casp9, Cflar, and Prkce) showed significantly lower expression after phenylbutyrate treatment than in controls (p < 0.05). Because caspase 9 is involved in activation of caspase 3, and is increased in HD patients, we examined caspase 3 immunoactivity at 120 days of age (19) (Fig. 9). It was markedly increased in the striatum of the N171-82Q mice as compared with controls, similar to the observations in the R6/2 mice. The increase was markedly attenuated by phenylbutyrate treatment.

**DISCUSSION**

There is substantial evidence that impaired gene transcription plays a role in the pathogenesis of HD. Prior studies showed that treatment with HDAC inhibitors is effective in attenuating polyglutamine toxicity *in vitro* (10, 11). Treatment with HDAC inhibitors arrests ongoing progressive neuronal degeneration in a *Drosophila* model of polyglutamine neurotoxicity (8). The HDAC inhibitor SAHA was recently shown to improve motor function in the R6/2 transgenic mouse model of HD (12), and sodium butyrate improves motor function and exerts neuroprotective effects in this model when administered presymptomatically (13).

In the present study we administered the HDAC inhibitor phenylbutyrate to the N171-82Q transgenic mouse model of HD. N171-82Q mice express a cDNA encoding a 171-amino acid N-terminal fragment of huntingtin exon 1 containing 82 CAG repeats (20). The mice develop tremors, progressive weight loss, incoordination, and abnormal hind limb clasping by 3 to 4 months of age, and die prematurely at 4–6 months of age (18, 20). Mice appear normal for the first 2 months of life, but the initial phenotypic abnormality is failure to gain weight from 2 months onwards. Striatal N-acetylaspartate concentration is reduced as early as 50 days of age (indicating neuronal dysfunction), and abnormal systemic glucose tolerance occurs by 75 days of age (18, 21). Significant reductions in gene expression include reductions in dopamine D2 receptors, adenosine A2a receptors, and adenylyl cyclase (4). Diffuse nuclear accumulation of Htt and rare nuclear aggregates as well as cytoplasmic aggregates occur as early as 4 weeks of age (20). There

**FIG. 3.** Huntingtin and ubiquitin immunoreactivity in phenylbutyrate-treated N171-82Q mice. Huntingtin-immunostained tissue sections from the neostriatum of PBS-treated (A) and phenylbutyrate-treated N171-82Q mice at 120 days (B) show no difference in the number and size of huntingtin aggregates and inclusions between treated and untreated mice. Similarly, there were no differences in ubiquitin-positive inclusions between PBS-treated and phenylbutyrate-treated N171-82Q mice (C and D, respectively). Scale bar in A equals 50 μm and corresponds to all photomicrographs.

**FIG. 4.** Striatal tissue immunohistochemistry of acetylated histone 3 and 4 in phenylbutyrate-treated N171-82Q mice. Robust acetylated histone 3 and 4 immunohistochemistry was present in wild-type littermate control striatal tissue specimens (A and D, respectively), with hypoacetylation in the N171-82Q mice (B and E, respectively). Phenylbutyrate treatment increased acetylation of histone 3 and 4 in N171-82Q mice (C and F, respectively). Bar in A equals 100 μm and applies to all figures.
are degenerating neurons as assessed by caspase staining, terminal deoxynucleotidyl transferase-mediated UTP nick end labeling, and electron microscopy (22).

We found that administration of phenylbutyrate starting at 75 days of age produced a significant 23% improvement in survival. This is particularly impressive because we started therapy at 75 days, which is a time point after which the N171-82Q mice have shown initial symptoms (20, 23). Administration of other therapies is much less effective when initiated after onset of symptoms. Administration of a combination of coenzyme Q10 and remacemide to N171-82Q mice from 21 days of age extended survival by 17% and improved motor performance (24), however, when this dosing regimen was initiated at 56 days of age motor performance deterioration was delayed, but there was no beneficial effect on survival (25). Creatine administration in the N171-82Q mice improved survival by 19% when administered from 28 days of age (18), however, it is much less effective when treatment is initiated after the onset of symptoms in the R6/2 transgenic mouse model of HD (26).

**FIG. 5.** Western blots showing the effects of phenylbutyrate treatment (100 mg/kg intraperitoneal) on histone acetylation in the brain of N171-82Q at 0, 1, 2, 3, and 4 h after administration. There were significant increases in acetylated histones 3 (AcH3) and 4 (AcH4) at 2 h (p < 0.05, analysis of variance followed by Newman-Keuls). Control histones from untreated (−) or sodium butyrate-treated (+) HeLa cells were used as a positive control for histone acetylation detection (lanes − and +, respectively).

**FIG. 6.** Striatal tissue immunohistochemistry for methylation of lysine 9 in histone 3 at 120 days of age. The immunocytochemical staining for methylation is markedly increased in the N171-82Q mice (B) as compared with wild-type controls (A). Phenylbutyrate markedly attenuated the increase in methylation (C). Bar in A equals 100 μm and applies to all figures.

**FIG. 7.** Western blots showing the effects of phenylbutyrate treatment on histone methylation in the brain of N171-82Q mice and age-matched wild-type (Wt) mice at 0, 1, 2, and 3 h after administration, or 3 h after vehicle (PBS). There was a significant decrease in histone methylation at 2 and 3 h after phenylbutyrate (PBA) administration. *, p < 0.05; **, p < 0.01, significant difference between phenylbutyrate. PBA-treated mice and control mice were sacrificed immediately after PBA injection (PBA + 0 h); † p < 0.05, significant difference relevant to vehicle (Veh, PBS)-injected mice. n = 4/group.
The finding that phenylbutyrate is effective in symptomatic transgenic HD mice is consistent with observations in a *Drosophila* model of polyglutamine toxicity, which showed that HDAC inhibitors were neuroprotective even when administered to animals already exhibiting neurodegeneration (8). In addition, phenylbutyrate extends the lifespan in wild-type *Drosophila* when administered late in life (27). These observations suggest that phenylbutyrate administration may be beneficial to HD patients after onset of symptoms. Surprisingly, there were no significant effects on weight loss or motor deficits, which may reflect the fact that therapy was initiated after symptom onset. Similarly, SAHA did not effect weight loss, although it improved both rotarod performance and grip strength when administered presymptomatically (12).

Histopathologic studies of phenylbutyrate in the N171-82Q mice showed a trend toward reduced atrophy at 100 days of age. At 120 days of age there was a significant reduction in gross brain atrophy, ventricular enlargement, and striatal neuron atrophy. Presymptomatic treatment with sodium butyrate also attenuates brain atrophy, ventricular enlargement, and striatal neuron atrophy (13). Interestingly, phenylbutyrate treatment had no effect on huntingtin and ubiquitin-stained aggregates, which is consistent with observations made with SAHA and sodium butyrate (12, 13). The effects of HDAC

![Fig. 8. Quantitative real-time RT-PCR analysis of genes identified by microarray analysis as differentially expressed.](Image)

**Table 1**

| Gene | GenBankTM accession number | Expression pattern | p value | Description                                                                 | Chromosome location | Affymetrix probe set ID |
|------|---------------------------|-------------------|---------|------------------------------------------------------------------------------|---------------------|------------------------|
| Ccl27| AW124975                  | Induced           | 0.0088  | Chemokine (C-C motif) ligand 27                                             | 412.7 cM            | 100972_s_at            |
| Syb1 | X96737                    | Induced           | 0.0157  | Synaptobrevin like 1                                                         | X 0.5 cM            | 102885_at              |
| Lypla1| A1875934                  | Induced           | 0.0318  | Lyso phospholipase 1                                                         | 1_A1                | 97207_f_at             |
| Gfer | U40494                    | Induced           | 0.0342  | Growth factor, erv1 (augmenter of liver regeneration)                        | 1710.0 cM           | 160269_at              |
| Gstm3| J03953                    | Induced           | 0.0435  | Glutathione S-transferase                                                    | 3                   | 97682_r_at             |
| Strn3| AW124985                  | Induced           | 0.0667  | sTriatin, calmodulin-binding protein 3                                      | 12 C1               | 100878_at              |
| Cd3  | A1837005                  | Induced           | 0.0669  | Carnitine deficiency associated gene expressed in ventricle 3                | 9                   | 160326_at              |
| Usp29| AA673236                  | Induced           | 0.0191  | Ubiquitin-specific protease 29                                               | 76.5 cm             | 96433_at               |
| Psma3| AF055983                  | Induced           | 0.0121  | Proteasome (prosome, macropain) subunit, α type 3                            | 3                   | 92544_f_at             |
| Tceg1| AB203485                  | Induced           | 0.0039  | Transcription elongation regulator 1 (CA150)                                 | 18 B3               | 101008_at              |
| Psme3| AA409481                  | Induced           | 0.0129  | Proteasome (prosome, macropain) 26 S subunit, ATFase 3                      | 247.0 cm            | 100225_f_at            |
| Casp9| AB019600                  | Repressed         | 0.0494  | Caspase 9                                                                   | 4 E1                | 100367_g_at            |
| Ephx1| U89491                    | Repressed         | 0.0004  | Epoxide hydrolase 1, microsomal                                             | 198.5 cm            | 101587_at              |
| Prkce| AP028009                   | Repressed         | 0.0409  | Protein kinase C                                                             | 17 E4               | 94161_at               |
| Cflar| Y14041                    | Repressed         | 0.0190  | CASP8 and FADD-like apoptosis regulator                                       | 5.1 cm              | 100883_at              |
| Prsmd10| AB022022                  | Repressed         | 0.0126  | Proteasome (prosome, macropain) 26 S subunit, non-ATFase, 10                | X F1                | 103319_at              |
| Stk10| D89728                    | Repressed         | 0.0453  | Serine/threonine kinase 10                                                   | 1116.0 cm           | 93680_at               |
inhibitors contrast with the therapeutic effects of agents such as creatine, coenzyme Q10 with remacemide, and cystamine, which significantly reduce numbers of Htt immunoreactive aggregates (18, 24, 28). Minoicline and dichloroacetate also increase survival in transgenic HD mice, without altering aggregate load (29, 30). Our findings therefore provide further evidence that therapeutic effects in transgenic mouse models of HD can occur independently of effects on aggregate deposition.

We verified that phenylbutyrate increases acetylation of both histone H3 and histone H4 in the brains of treated mice using both immunocytochemistry and Western blots. We also examined histone methylation. Whereas histone acetylation is increased in symptomatic N171-82Q mice relative to wild-type levels, which was markedly attenuated by treatment with phenylbutyrate, the finding suggests that phenylbutyrate mediates its effects by both increasing histone acetylation as well as reducing histone methylation, thereby increasing expression of genes critical to cell survival. We also examined the effects of phenylbutyrate on gene expression levels. Genes that showed significant increases in expression following phenylbutyrate administration included glutathione S-transferase, striatin calmodulin-binding protein 3, ubiquitin-specific protease 29, proteasome subunit alpha type 3 and the proteasome 26 S subunit (ATPase 3), whereas caspase 9, caspase 8/FADD-like apoptosis regulator, and proteasome 26 S subunit (non-ATPase 10) were significantly decreased. Consistent with the up-regulation of caspase 9, active caspase 3 was also increased in the striatum, and this increase was attenuated by phenylbutyrate treatment. Because aberrant protein degradation and apoptosis are implicated in HD pathogenesis, these alterations may contribute to the therapeutic effects of phenylbutyrate (36, 37).

Our findings show that administration of the HDAC inhibitor phenylbutyrate exerts neuroprotective effects and increases survival in symptomatic HD mice. Supporting studies in which HDAC inhibitors were administered presymptomatically (12, 13). The findings are robust and the 23% increase in survival is the best overall therapeutic effect yet reported in the N171-82Q HD model. The effects of this HDAC inhibitor are consistent with findings both in cell culture models and in Drosophila models of polyglutamine toxicity (8, 10, 38), and Sp1 can abrogate cell death produced by proteins containing polyglutamine expansions in vitro (7, 39, 40). In addition, a recent study showed that HDAC inhibitors can prevent oxidative neuronal death via an Sp1 dependent pathway (41), which is of particular interest with regard to HD mouse models because we and others found evidence of increased oxidative damage in HD transgenic mice (42–44).

Phenylbutyrate is a particularly promising agent for therapeutic trials in man because of extensive experience with its use in patients for treatment of urea cycle disorders, sickle cell anemia, thalassemia minor, and cystic fibrosis (45–47). Patients with ornithine transcarbamylase deficiency have been treated long-term with doses of phenylbutyrate between 350 and 600 mg/kg/day without significant side effects (48, 49). Phenylbutyrate effects in cancer patients showed good tolerability at doses of 400–500 mg/kg/day (50, 51). Our effective dose of 100 mg/kg/day in mice is therefore well within the tolerable range. These findings suggest that phenylbutyrate is an extremely promising agent for treatment of HD.

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