Dexamethasone induces heat shock response and slows down disease progression in mouse and fly models of Huntington’s disease

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INTRODUCTION

Huntington’s disease (HD) is an inherited neurodegenerative disorder caused by abnormal expansion of glutamine repeats in the protein huntingtin. In HD brain, mutant huntingtin undergoes proteolytic processing, and its N-terminal fragment containing poly-glutamine repeats accumulate as insoluble aggregates leading to the defect in cellular protein quality control system and heat shock response (HSR). Here we demonstrate that the defective HSR in the brain is due to the down-regulation of heat shock factor 1 (HSF1) in both mice and fly models of HD. Interestingly, treatment of dexamethasone (a synthetic glucocorticoid) to HD mice or flies significantly increased the expression and transactivation of HSF1 and induction of HSR and these effects are mediated through the down-regulation of HSP90. Dexamethasone treatment also significantly decreased the aggregate load and transient recovery of HD-related behavioural phenotypes in both disease models. These results suggest that dexamethasone could be a potential therapeutic molecule for the treatment of HD and related poly-glutamine disorders.
In HD, as well as other poly-glutamine diseases, components of HSR are found to be sequestered into inclusion bodies (16–18). This results in reduced functional levels of chaperones and further disruption of protein homeostasis. Various reports have shown that up-regulations of chaperones such as HSP70, HSP40 and HSP27 are able to ameliorate disease pathogenesis only to a certain extent (19–26). However, it is believed that inducing multiple chaperones together in disease condition may prove to be more effective (24,27–30). Because HSF1 transcribes multiple chaperones, its activation has recently been a popular target for therapeutic intervention. HSP90 is known to have a major role in maintaining HSF1 in an inert state (31). Thus, compounds that inhibit HSP90 present one way to activate HSF1 and induce downstream chaperones (32–34). HSP90-independent up-regulation of HSF1 activity has also been shown to suppress poly-glutamine toxicity (35–37). However, the status of endogenous HSF1 in HD brain is poorly understood. In this study, we report that HSF1 is down-regulated in murine and fly models of HD and that administration of dexamethasone, a synthetic glucocorticoid, significantly increased the expression of HSF1 and stimulated HSR. We also report the alleviation of disease pathology in these models upon treatment of dexamethasone.

RESULTS

HSF1 is reduced in HD mice brain

HSR is known to be severely compromised in HD brain. Its components such as chaperones co-localize with huntingtin aggregates, and their soluble levels are significantly reduced (30,38). Because HSF1 plays a crucial role in HSR, we first assessed its level in R6/2 HD mouse brain. Cortex, hippocampus and cerebellum of 12-week-old wild-type and R6/2 mice brains were immunohistochemically stained for HSF1. Unlike HSP70, there was no co-localization of HSF1 with insoluble huntingtin aggregates, but its soluble levels were significantly reduced in HD brain compared with wild-type control (Fig. 1A). Notably, up to 12 weeks of age, no cell death was seen in HD mouse brain. We further monitored protein level of HSF1 by immunoblot analysis and found to be significantly reduced in different regions of HD mouse brain in comparison with their age-matched wild-type controls (Fig. 1B and C). HSF1 protein levels across all regions of HD mice brain were reduced to ~60–70% at 12 weeks of age. Immunoblot analysis also indicated a reduced level of posttranscriptionally modified HSF1 in HD mouse brain. Levels of HSP70, which are already known to be decreased in various models of HD, were reconfirmed across different brain regions at 12 weeks of age (Fig. 1B). Surprisingly, reduced protein levels of HSF1 and HSP70 were also observed at 4- to 5-week-old HD mice brain (Supplementary Material, Fig. S1). Next we analyzed the mRNA level of HSF1. Striatum and cortex of wild-type and HD mice brain of two different ages (5 and 12 weeks) were compared for HSF1 mRNA levels in real-time reverse transcription-polymerase chain reaction (RT-PCR) and were also found to be significantly reduced in an age-dependent manner (Fig. 1D and E).

HSF1 is induced upon dexamethasone treatment

Drugs or molecules that can activate HSF1 are known to elicit HSR. Dexamethasone has been shown to activate HSF1 in rat cardiomyocytes (39,40). In order to explore the ability of dexamethasone to induce HSR, 5-week-old wild-type and R6/2 mice were injected subcutaneously with either saline (vehicle) or dexamethasone at a dose of 4 mg/kg/day for 20 days. By 5 weeks of age, poly-glutamine aggregates were observed throughout the R6/2 mouse brain and by 8 weeks of age, behavioural deficits were well manifested. We have also noticed a significant drop in HSF1 at 5 weeks of age in these mice. Hence, we chose this window for chronic dexamethasone treatment. Chronic administration of dexamethasone showed significant induction of HSF1 in different brain regions of wild-type and HD mice in comparison with that of respective vehicle-treated group, as evident from both immunohistochemical staining and immunoblot analysis (Fig. 2A–C). At transcriptional level, HSF1 mRNA level was assessed in cerebellum using real-time RT-PCR and were found to be significantly induced in dexamethasone-treated wild-type and HD mice brain (Fig. 2D). Interestingly, the levels of HSF1 in brain samples of HD mice were restored back to the level of wild-type mice upon chronic dexamethasone treatment.

Dexamethasone treatment induces transactivation of HSF1 and up-regulation of HSP70

The activation of HSF1 is essential for the induction of HSR. The activation is achieved through a dynamic multi-step process of trimerization of HSF1 monomers into a competent DNA-binding form followed by several posttranslational modifications. Hyperphosphorylation at several serine residues has been reported to promote trans-activating capacity of HSF1. To examine whether dexamethasone-induced HSF1 is transactive, Serine-230 phosphorylation level of HSF1 was checked and found to be up-regulated in different brain regions of dexamethasone-treated mice in comparison with vehicle-treated group (Fig. 3A). In HD mice brain, phosphorylated HSF1 level was low compared with that in wild-type mice, which was clearly increased upon dexamethasone treatment. Unfortunately, this phospho-specific HSF1 antibody did not work in immunoblot. The level of HSP70 was significantly increased in dexamethasone-treated mice brain. In HD mice, HSP70 levels were almost restored back to wild-type control levels upon dexamethasone treatment (Fig. 3B–D). As expected, HSP70 mRNA levels were also significantly increased in the cerebellum of dexamethasone-treated mice (Fig. 3E and F). These findings suggest that dexamethasone induce not only the expression of HSF1 but also its transactivation in both wild-type and HD mice brain.

Dexamethasone treatment reduces aggregate load and improves motor performance of HD mice

The activation of HSF1 and the consequent up-regulation of HSP70 are known to decrease aggregate load and improve the behavioural phenotype in HD mice and fly models. Since dexamethasone induces the expression and transactivation of HSF1, we further evaluated its effect on aggregate load of mutant huntingtin in HD mouse brain. Chronic treatment of dexamethasone significantly reduced the aggregate load in cortex, striatum and cerebellum of HD mice brain in comparison with saline-treated control group (Fig. 4A and B). Throughout the duration of dexamethasone administration, body weights were measured and various behavioural tests...
were conducted to assess phenotypic recovery. Body weight of HD mice began to decrease from 6 weeks, and at 8 weeks there was a significant drop. Dexamethasone treatment did not significantly recover the body weight (Fig. 4C). Aspects of locomotion were analysed by gait analysis and motor coordination in rotarod test. Both dexamethasone- and vehicle-treated wild-type mice behaved equally well, showing no adverse effect of chronic drug treatment. Up to 5 weeks of age, no deficits of locomotion were found in HD mice compared with wild-type control. However, at 6, 7 and 8 weeks of age, HD mice developed gait abnormalities with reduced stride length, which was significantly improved upon dexamethasone treatment (Fig. 4D). Dexamethasone-administered HD mice stayed for longer time on rotating rod than vehicle-treated HD mice at 6, 7 and 8 weeks of age (Fig. 4E). Similarly, the proportion of HD mice that clasped or showed abnormal limb extension was always less when treated with dexamethasone as compared with saline (Fig. 4F). Striatal volume was unaffected in 8-week-old HD mice compared with wild-type control, a result that is consistent with the observations of others (41), and dexamethasone

Figure 1. Down-regulation of HSF1 in R6/2 HD mice brain. (A) Immunohistochemical staining for HSF1 in different brain regions of wild-type and HD mice. Brain sections of 20 μm thickness collected from 12-week-old mice (wild type and HD) were placed on the same slide and processed for staining. Scale bar = 20 μm. (B) Immunoblot analysis of the levels of HSF1 and HSP70 in cortex, hippocampus and cerebellum of 12-week-old wild-type and HD mice brain. (C) The band intensity of HSF1 normalized to β-tubulin was quantified from five different mice brain samples in each group using NIH ImageJ software. *P < 0.001 with respect to wild-type mice (Student’s t-test). (D and E) Quantitative real-time reverse transcription-polymerase chain reaction (RT-PCR) analysis of HSF1 mRNA in striatum (D) and cortex (E) of 5- and 12-week-old wild-type and HD mice brain. Values are means ± SEM; n = 3 in each group. *P < 0.001 in comparison with wild-type mice (Student’s t-test).
Figure 2. Chronic treatment of dexamethasone induces expression of HSF1 in brain. Treatment was started at 5 weeks of age for 21 days, and all mice were 8 weeks old at the time of sacrifice. (A) Immunohistochemical staining of HSF1 in cortex, hippocampus and cerebellum of vehicle- or dexamethasone-treated wild-type and HD mice. Sections were of 20 μm thickness. Brain sections obtained from wild-type, HD and dexamethasone-treated mice were kept on the same slide and then processed for staining. Scale bar = 20 μm. (B) Immunoblot analysis of HSF1 in different regions of brain. (C) Band intensities of HSF1 normalized to β-tubulin were plotted. Values are mean ± SEM of five different animals in each group. *P < 0.05 calculated using two-way ANOVA followed by Holm-Sidak post hoc test. (D) Semi-quantitative RT-PCR analysis of HSF1 mRNA in cerebells collected from vehicle and dexamethasone-treated wild-type and HD mice. (E) Quantitative real-time RT-PCR analysis of HSF1 mRNA of the samples shown in D. Data are means ± SEM; n = 5 in each group. *P < 0.05 calculated using two-way ANOVA followed by Holm-Sidak post hoc test.
Figure 3. Dexamethasone treatment transactivates HSF1. (A) Immunohistochemical staining of phospho-HSF1 (Ser230) in different brain regions of wild-type and HD mice treated with dexamethasone or vehicle as described in Figure 2. Scale bar = 20 μm. Up-regulation of HSP70 protein in cortex (B), hippocampus (C) and cerebellum (D) after dexamethasone treatment. (E, F) Induced HSP70 mRNA (semi-quantitative and quantitative real-time RT-PCR) after dexamethasone treatment. Values are mean ± SEM; n = 5 in each group. * P < 0.05 calculated by two-way ANOVA followed by Holm-Sidak post hoc test.
Figure 4. Reduced aggregate load and improved motor performance in HD mice upon dexamethasone administration. (A) Immunohistochemical staining of ubiquitin-positive aggregates in vehicle- or dexamethasone-treated HD mice brain sections. Mice were treated with dexamethasone as described in Figure 2 and sacrificed at 8 weeks of age. Scale bar = 20 μm. (B) The approximate number of ubiquitin-positive nuclear aggregates in different brain regions of vehicle and dexamethasone-treated HD mice. Ubiquitin-positive nuclear aggregates were counted in ×40 images (0.200 × 0.150 mm area) obtained from 3 to 5 different fields in each region and plotted. Values are mean ± SEM of five different mice in each group. *P < 0.01 compared with vehicle-treated HD mice (Student’s t-test). (C) Comparison of body weight. Significant improvement of motor performance and coordination as evaluated from stride length of foot print gait analysis (D), rotarod test (E) and clasping (F). All these test were conducted in blinded manner. Data are represented as mean ± SEM; n = 5 in each group. *P < 0.05 calculated by two-way ANOVA followed by Holm-Sidak post hoc test.
treatment had no effect on it (Fig. 4G). Although dexamethasone treatment decreased aggregate load and improved behavioural abnormalities, it did not significantly increase the survival rate of HD mice (Supplementary Material, Fig. S2).

Effect of dexamethasone in a cellular HD model

A mouse Neuro2a cell line stably and inducibly expressing truncated N-terminal huntingtin containing 150Q fused with GFP named HD150Q was used to analyse effects of dexamethasone on HSF1 induction and mutant huntingtin aggregation. Cells were treated with 1 μM ponasterone A to induce the expression of mutant huntingtin and at the same time exposed with different doses of dexamethasone for 24 h. The treatment of dexamethasone increased the level of HSF1 in HD150Q cells as evident from immunofluorescence staining as well as immunoblot analysis (Fig. 5A and B). Dexamethasone-treated HD150Q cells exhibited strong nuclear staining of HSF1 in comparison with control, indicating that it is involved in the up-regulation as well as transactivation of HSF1. Immunoblot analysis also revealed up-regulation of HSF1 and HSP70 in the dexamethasone (1 μM dose)-treated HD 150Q cells. As a positive control of HSF1 activation, 17AAG was used. The frequency of mutant huntingtin aggregation was also significantly reduced upon exposure of dexamethasone (Fig. 5A and C).

Effects of dexamethasone in HD are recapitulated in a fly model

To model HD in Drosophila, human Huntingtin exon 1 containing expanded poly-glutamine repeats is overexpressed (42). When driven in the eyes using GMR-GAL4, UAS-HTTEX1-PQ93 results in the formation of aggregates in larval eye imaginal discs and a subsequent age-dependent retinal degeneration accompanied by impaired vision. In order to investigate whether dexamethasone is able to induce HSF1 and HSR in a manner similar to that in mice described above, the drug was administered to HD flies by adding it to the food in two concentrations: 1 and 100 μM. Total RNA was extracted from the heads of wild-type and HD untreated controls along with dexamethasone-fed HD flies. HSF1 mRNA was found to be reduced in 15-day-old HD flies compared with age-matched wild-type controls. Treatment

Figure 5. Treatment of dexamethasone up-regulates HSF1 and reduces aggregate numbers in a cellular model of HD. HD150Q cells were simultaneously induced (with 1 μM of ponasterone A) and treated with dexamethasone for 24 h and then subjected to immunofluorescence staining (A) and immunoblot analysis (B) of HSF1 and aggregate counting (C). In (B), 17AAG was used as the positive control, which is known to activate HSF1. In (C), data represented as mean ± SEM of three independent experiments (*P < 0.05 calculated by one-way ANOVA followed by Holm-Sidak post hoc test).
with 100 μM dexamethasone was able to significantly induce HSF1 mRNA (Fig. 6A). As indicated above, this additionally transcribed HSF1 is probably active as its downstream target, HSP70, which was also down-regulated in HD flies, was induced to control levels by 100 μM dexamethasone (Fig. 6B). In both the cellular and murine model, it has been shown that dexamethasone treatment, and the subsequent induction of HSR, results in a reduction of mutant huntingtin aggregate load. Thus, mutant huntingtin aggregates were stained in the eye imaginal discs of third instar larvae of HD flies developed on dexamethasone. A drastic reduction of aggregates was visible upon 100 μM dexamethasone treatment compared with vehicle-fed HD controls (Fig. 6C). To confirm that the effect of dexamethasone occurs through the induction of HSF1, HSF1 mRNA was down-regulated in HD flies using RNAi. To confirm that HSF1 is down-regulated in UAS-HSF1-RNAi, these flies were crossed to GMR-GAL4. Levels of mRNA for both HSF1 and HSP70 were found to be greatly reduced when compared with controls (Fig. 6D). These HSF1-RNAi flies were then used to down-regulate HSF1 in HD (hereby called HD HSF-RNAi). This resulted in an increase in aggregate load in HD HSF-RNAi in comparison with HD control and though dexamethasone treatment does reduce these aggregates to some extent, the aggregate load is still greater than that of dexamethasone-fed HD flies (Fig. 6C).

Since dexamethasone treatment resulted in a reduction of aggregate load in larvae, we investigated whether this correlates with a rescue of retinal degeneration. In HD flies, on the day of eclosion (Day 1), slight retinal degeneration was visible, which accelerates as the fly ages, with maximum degeneration at 15 days of age. No such degeneration is seen in age-matched wild-type flies (Fig. 7A). Upon administration of dexamethasone, on the day of eclosion itself, a marked rescue of retinal degeneration was visible, and this effect persisted across the ages of both 7 and 15 days as seen in the photographs showing the external eye.

![Figure 6](image-url)
morpology (Fig. 7A). Eyes of 15-day-old HSF-RNAi, HD and HD HSF1-RNAi flies treated with vehicle or dexamethasone are shown in Supplementary Material, Fig. S3.

Wild-type flies are phototropic in nature and when subjected to a choice between light and dark chambers, they move towards the light chamber. In HD flies, on the other hand, retinal degeneration is accompanied by a loss of vision and flies distribute randomly across the two chambers. While older HD flies showed reduced phototropism (40% at 15 days), flies treated with dexamethasone preferentially chose the light chamber, but GMR-GAL4;UAS-HTTEX1-PQ93 flies showed a drastic reduction in phototaxis, which worsened with age. This progressive decline in phototaxis did not occur upon treatment of 1 and 100 μM dexamethasone. Instead, vision appeared to improve marginally. (C) Locomotion was studied using climbing assay. HD flies (ELAV-GAL4;UAS-HTTEX1-PQ93) did not show any deficits immediately after eclosion, but their locomotion declines by 7–8 days of age. Treatment of 100 μM dexamethasone was able to significantly rescue these locomotor deficits (*P < 0.01 by two-way ANOVA and Holm-Sidak post hoc test). Data are represented as mean ± SEM.

Figure 7. Rescue of retinal degeneration, vision and locomotor deficits upon dexamethasone treatment in HD fly. (A) Photographs of the eyes of GMR-GAL4;UAS-HTTEX1-PQ93 (HD-Vehicle) flies showed an age-dependent degeneration, which was absent in wild-type flies. Treatment of 1 and 100 μM dexamethasone resulted in reduced retinal degeneration across the three ages. (B) Vision was analyzed using phototactic assay. Vehicle-treated wild-type flies preferentially chose the light chamber, but GMR-GAL4;UAS-HTTEX1-PQ93 flies showed a drastic reduction in phototaxis, which worsened with age. This progressive decline in phototaxis did not occur upon treatment of 1 and 100 μM dexamethasone. Instead, vision appeared to improve marginally. (C) Locomotion was studied using climbing assay. HD flies (ELAV-GAL4;UAS-HTTEX1-PQ93) did not show any deficits immediately after eclosion, but their locomotion declines by 7–8 days of age. Treatment of 100 μM dexamethasone was able to significantly rescue these locomotor deficits (*P < 0.01 by two-way ANOVA and Holm-Sidak post hoc test). Data are represented as mean ± SEM.

It is reported that locomotion in these flies is impaired when compared with controls in a climbing assay and lifespan is drastically reduced to ~12 days. No increase in lifespan was observed in dexamethasone-fed HD flies when compared with untreated controls (data not shown). However, a rescue was observed in locomotion of these flies. On the first day after eclosion, the percentage of HD flies that were able to climb up to 8 cm in 10 s was similar to that of wild-type controls. However, at 7–8 days of age, untreated HD flies were compromised in locomotion, and this defect was rescued upon the treatment of 100 μM dexamethasone (Fig. 7C). Thus, in the fly model too, dexamethasone is able to reduce aggregate load and rescue neurodegeneration as well as behavioural phenotype in HD without increasing lifespan.
Dexamethasone treatment down-regulates HSP90

HSP90 inhibitors are well known to activate HSF1, and recently they also have been shown to induce the expression of multiple genes including HSF1 (31,43). In order to investigate the possible mechanism of dexamethasone-induced up-regulation of HSF1, we checked HSP90 levels in the dexamethasone-treated cells, mice, and fly. Interestingly, we have noticed significant decrease in the level of HSP90 in the dexamethasone-treated HD 15Q cells and HD mice cortex as well as in the dexamethasone-fed HD fly head (Fig. 8A, B and C). Overexpression of HSP90 significantly prevented the dexamethasone-induced decrease in mutant huntingtin aggregates in HD 15Q cell line (Fig. 8D). These data indicate that dexamethasone-induced expression and activation of HSF1 is probably mediated via down-regulation of HSP90.

DISCUSSION

In this work, we have shown a progressive global reduction in HSF1 mRNA level in mice model of HD, and similar finding has been reproduced in HD fly model. Because transcriptional repression of various genes is a hallmark of HD, it is possible that HSF1 too is directly or indirectly repressed by mutant huntingtin (44). Subsequent to mRNA reduction, endogenous HSF1 protein was also found to be reduced in HD mice brain at a very early stage of disease progression. A similar reduction in HSF1 protein has been demonstrated recently in the striatum and cerebellum of HD knockin mice (45). Apart from reduced levels of the protein, there are indications that the activity of HSF1 could also be compromised in HD. For instance, in HD mice, Sirt1, which deacetylates HSF1, interacts with mutant

Figure 8. Dexamethasone treatment down-regulates HSP90. (A) HD15Q cells were left untreated or treated with 1 μM of dexamethasone for 10 h and then the cell lysates were made and subjected to immunoblot analysis using antibodies against HSP90 and β-actin. Values are means ± SEM of three independent experiments. (B) Cortex samples obtained from HD and dexamethasone-treated HD mice (as described in Fig. 2) were processed for immunoblot analysis using HSP90 and β-actin antibodies. Values are means ± SEM; n = 3 in each group. (C) Head lysates of HD and dexamethasone-fed HD flies (described in Fig. 6) were subjected to immunoblot analysis using HSP90 and β-actin antibodies. Data are represented as mean ± SEM; n = 5. *P < 0.05 in comparison with respective control (Student’s t-test; HD fly data were analyzed by one-way ANOVA and Holm-Sidak post hoc test). (D) Overexpression of HSP90 prevented dexamethasone-induced decrease in mutant huntingtin aggregation in HD 15Q cells. Cells were transiently transfected with either empty pcDNA3.1 or HSP90 plasmid, and 24 h later, cells were induced with 1 μM of ponasterone A in the absence or presence of dexamethasone for 24 h. Aggregate counting was done at 24 h. Values are mean ± SEM; n = 3 (*P < 0.05 by one-way ANOVA and Holm-Sidak post hoc test).
HSP70, one of the most important chaperones, is known to be ery, their deregulation results in increased burden on the cell pathogenesis. Notably, the dysfunction of HSF1 correlates well with the onset of aggregates in these mice at ~4–5 weeks of age, suggesting a prominent role of HSF1 in early stage of disease pathogenesis.

Since chaperones are integral to the protein refolding machinery, their deregulation results in increased burden on the cell and disruption of its healthy protein milieu. In particular, HSP70, one of the most important chaperones, is known to be down-regulated in cellular and mice models of HD (30,38). We have also demonstrated such a reduction of HSP70 in R6/2 mice and additionally, in HD flies as well. Although reduced levels of HSP70 have not been previously reported in HD flies, it has been shown that up-regulating its level improved disease phenotype in both flies and mice (23,47). However, apart from HSPs, HSF1 has multiple other targets including various chaperones, transcription factors, scaffold proteins and cytokines (48). In cells, it has been shown that overexpression of some of these novel HSF1 targets is able to reduce poly-glutamine aggregate load indicating that they are also protective in nature. Therefore, down-regulation of HSF1 not only affects normal cellular HSR and protein quality control but also could lead to other consequences.

In an attempt to rescue reduced levels of HSF1 in HD, we administered dexamethasone to murine and fly HD models. We found that mRNA, and subsequent protein levels, of HSF1 was rescued to about control levels in all regions of HD mice brains. The fact that HSF1 mRNA is increased upon dexamethasone treatment in both flies and mice implies that it is able to transcribe HSF1 directly or indirectly. Additionally, the ability of dexamethasone to activate HSF1 has been shown in rat cardiomyocytes (39,40). The dexamethasone-induced transcriptional activation of HSF1 was evident from increased phosphorylation of HSF1 at Ser230 in control and HD mice brain. HSF1 has multiple phosphorylation sites that can be either inhibitory or stimulatory to its activity (12). Phosphorylation at Ser230 is one of the crucial sites known to enhance HSF1 activity (49). Additionally, HSP70 mRNA and protein levels were also found to be increased upon dexamethasone treatment, further indicating that induced HSF1 is transcriptionally active. Thus, it is possible that other targets transcribed by HSF1 would also be enhanced in a similar manner, which can collectively act to slow down disease progression. Our findings also indicate that dexamethasone-induced expression and activation of HSF1 could be mediated through the down-regulation of HSP90. Inhibitors of HSP90 are known to activate HSF1 (31), and recently inhibition of HSP90 has been shown to increase the transcription of HSF1 (43).

The up-regulation of HSF1 and its downstream targets could be responsible for the reduced aggregate load seen in all the three dexamethasone-treated HD models used in this work. Genetically modifying HSF1 by both overexpression and knockout of the gene has been shown to reduce and increase poly-glutamine aggregate load, respectively (36,50). Here, we report that pharmacologically inducing HSF1, using dexamethasone, also reduces aggregate load in vivo. Recently, activation of HSF1 using geldanamycin, 17AAG, radicicol, celastrol, HSF1A and HSP90 has been shown to be able to ameliorate pathology of poly-glutamine diseases (32–35,37).

Aged HSF1 knockout mice have been shown to have motor defects, while pharmacological induction of HSF1 in HD has shown improvement in motor performance (34,50). In agreement with this, we too show that treatment of dexamethasone is able to rescue behavioural phenotype of motor deficits in both HD mice and flies. However, we did not observe any decreased mortality rate in either of the HD models, which could be the result of either the treatment protocol or the aggressive model systems used. It is also important to note that dexamethasone has a potent anti-inflammatory activity and inflammation was reported in human HD brain (51). However, R6/2 mice model of HD rarely shows signs of inflammation, even in the advance stage of disease progression (52–54). Therefore, HSF1-inducing property of dexamethasone is probably playing a major role in rescuing the behavioural abnormalities in the early stage of disease progression.

Altogether, our study provides evidence that the reduced HSR in HD could be primarily because of the down-regulation of HSF1. Our study also identified dexamethasone as an inducer of HSF1 and HSR that has the ability to slow down the disease progression in both murine and fly models of HD. Thus, an anti-inflammatory and HSR-inducing property of dexamethasone offers immense potential for its therapeutic use in HD and related other poly-glutamine disorders.

**MATERIALS AND METHODS**

**Materials**

Dexamethasone, 17AAG (HSP90 inhibitor), anti-rabbit HSF1 and all cell culture reagents were obtained from Sigma. Ponasterone A, zeocin and G418 were purchased from Invitrogen. Rabbit polyclonal anti-phospho (Ser230)-HSF1, anti-GAPDH, anti-β-tubulin, mouse monoclonal anti-HSP70 and goat polyclonal anti-huntingtin were purchased from Santa Cruz Biotechnology. Rabbit polyclonal anti-ubiquitin was from DAKO, anti-HSP90 and anti-HSF1 were obtained from Cell Signalling. Goat anti-rabbit IgG-Rhodamine, AP- and HRP-conjugated anti-mouse, anti-rabbit IgG and ABC kit were purchased from Vector Laboratories. The truncated N-terminal huntingtin (tNht) expression constructs fused with EGFP, pLND-tNht-150Q and the generation of the stable cell line of this construct (HD 150Q) have been described previously. HSP90 plasmid was obtained from Addgene.

**HD transgenic mice, drug administration and tissue preparation**

Ovarian transplanted hemizygous females carrying Htt exon1 with ~150 CAG repeats (strain name: B6CBA-Tg(HD exon1)62Gph/J) were obtained from Jackson Laboratory and maintained by crossing with B6CBAF1/J males. Genotyping was carried out using a previously described method (55). All animal experiments were conducted in accordance with the approval of the Institutional Animal Ethics Committee of National Brain Research Centre, Manesar. Animals had free access to a
pelleted diet and water ad libitum. Dexamethasone solution was prepared in 0.9% sodium chloride and was administered subcutaneously for 20 days at a dose of 4 mg/kg body weight/day. Control mice were injected with equal volume of vehicle (saline). Twenty-four hours after the last dose of injection, mice were either sacrificed by cervical dislocation and brain parts were stored at −80°C or anesthetised and transcardially perfused with PBS containing 4% PFA (w/v).

**Behavioural studies in mice**

**Gait analysis**
For footprint gait analysis, the fore and hind paws of the mice were dipped in blue and red nontoxic paints, respectively. Mice were placed at an open end of a wooden tunnel (40 × 5 cm) lined with paper. The mice were trained for 3 days to walk through the tunnel and then tested for three trials per week. Two to four steps from the middle portion of each run were measured for hind-stride length and hind-base width.

**Rotarod test**
Mice were placed on the rotarod (Scientific Instruments, New Delhi) and were trained to stay on the rod, which was rotating with a constant speed of 5 rpm. Mice that would fall were repeatedly placed back on the rod until they were able to stay on the rotarod for at least 30 s. Mice were trained for 3 days and then tested for five trials per week (each trial comprises three sessions of 60 s) at a constant rod speed of 10 rpm. Sessions of all five trials in each week were averaged to generate the overall time for each mouse, with a maximum possible score of 60 s. Data were then expressed as per cent of total time in rotarod.

**Clasping behaviour**
Mice were suspended by their tails for 60 s and the proportion of mice that either clasped by firmly bringing their limbs together or just began to clasp was recorded. All the behavioural tests were performed in a blinded fashion.

**Measurement of striatal volume**
Mice were perfused with 4% paraformaldehyde and brains were dissected out. Coronal cryosections (20 μm) were cut and subjected to Nissl staining. The stereological volume of striatum was measured in random sections of a systematic set (e.g. every fifth section, with the first section selected randomly) was measured in random sections of a systematic set (e.g. every fifth section, with the first section selected randomly) and was projected to obtain 20 μm thick sections. The sections were then processed for immunohistochemistry using reagents from Vector Laboratories as described earlier (58). Briefly, after antigen retrieval of 45 min at 70°C, sections were blocked and probed using various primary antibodies. HSF1, phospho-HSF1 (Ser230) and huntingtin antibodies were used at 1:500, 1:200 and 1:1000 dilutions, respectively. Biotinylated secondary antibodies were used at a dilution of 1:500, and signal was enhanced using ABC kit and developed using ImmPACT Novared Peroxidase Substrate. Stained sections were imaged using bright-field microscopy on a Leica DM RXA2 microscope and all images were taken at ×40 magnification.

**Cell culture, immunofluorescence staining and counting of aggregates**
A stable mouse Neuro2a cell line, containing edcsyne-inducible a plND-tNht-150Q-GFP construct, has already been described (59). Cells were maintained on DMEM supplemented with heat-inactivated 10% fetal bovine serum and antibiotics (0.4 mg/ml Zeocin and 0.4 mg/ml G418). Expression of GFP-tagged tNHtt-150Q protein was induced with 1 μM ponasterone A and simultaneously treated with different doses of dexamethasone for 24 h, followed by immunofluorescence staining of HSF1, immunoblot analysis and aggregate counting. In some cases, cells were transiently transfected with HSP90 plasmid using lipofectamine 2000 and then induced with ponasterone A in the presence or absence of dexamethasone. Cells were fixed in 4% PFA for 30 min at room temperature, permabilized with Triton X-100 and then processed for immunofluorescence using a previously described protocol (60). HSF1 antibody was used at 1:500 dilutions, and Alexa-fluor 594-conjugated secondary antibody (rabbit specific) was used at 1:1000 dilutions. To count GFP positive aggregates, random images were taken across regions of the chambers. Three independent experiments were done to calculate percentage of cells with aggregates.

**Fly stocks and drug administration**
Fly stocks were maintained at 25°C in BOD incubators on standard sugar yeast food containing nipagin and propionic acid to prevent bacterial and fungal contaminations. Fly stocks used in this study were w; GMR-GAL4;+, w; w; UASHTT-EX1-PQ93/(CyO);+, w; ELAV-GAL4; UASGFP;+;+ and w;+; UAS-HSF1-RNAi (stock number 48692, VDRC, Vienna). Dexamethasone was dissolved in 100% ethanol and mixed in lukewarm food to allow the ethanol to evaporate. The concentration of dexamethasone in the food was set at either 1 or 100 μM. Flies that were fed the food supplemented only with ethanol were used as controls. Appropriate crosses to generate HD flies were set on dexamethasone-supplemented food and the progeny was examined.

**Examination of fly eye structure and eye imaginal disc immunostaining**
The external gross morphology of the eye was imaged on a Leica DM RXA2 microscope using a × 5 objective. Eye imaginal discs of third instar larvae were dissected in PBS and fixed in 4% PFA for 45 min. They were then processed for immunostaining using standard protocols. Goat anti-huntingtin was used at 1:100 dilutions, and anti-goat secondary antibody conjugated with FITC was used at a dilution of 1:500. The tissue was mounted in DAPI-mounting medium. Z-series images (1 μm per section) were taken on a confocal microscope (Carl Zeiss) using a 40 × oil objective and processed using Adobe Photoshop and ImageJ. When...
comparing aggregate load between genotypes, all microscope settings were kept constant.

**Fly behavioural assays**

**Vision**
Flies were subjected to phototropic T-maze to assay defects in vision. Briefly, groups of ten flies were introduced to the T-maze, one arm of which was covered with foil to maintain darkness and the other arm was brightly illuminated. At the end of 1 min, flies in the light chamber were counted as being positively phototropic. Each set of flies was given three trials, and three sets of flies were used per condition.

**Climbing assay**
To assay locomotion, groups of 10 flies were placed in graduated empty plastic vials. They were gently tapped to the bottom and the number of flies able to cover a distance of 8 cm was counted at the end of 10 s. Each set of flies was given five trials, and 5–10 sets of flies were used per condition.

**Immunoblotting**
HD150Q cells were lysed in RIPA lysis buffer (50 mM Tris, pH 8.0, 150 mM NaCl, 1% NP-40, 0.5% sodium deoxycholate, 0.1% SDS and complete protease inhibitor cocktail) and briefly sonicated. Mice brains parts were carefully dissected and homogenized in RIPA lysis buffer. All lysed protein samples were centrifuged at 15 000 g for 10 min, and the supernatant was used for immunoblotting as described elsewhere (61). Primary antibodies used were rabbit HSF1 (1:1000 dilutions), mouse HSP70 (1:1000 dilutions), mouse β-tubulin (1:5000 dilutions) and rabbit GAPDH (1:1000 dilutions).

**Semi-quantitative and quantitative real-time RT-PCR**
RNA was extracted from mouse brains and fly heads using Trizol reagent (Sigma) and following manufacturer’s instruction. Total RNA was quantified and 75 ng (flies) or 100 ng (mice) was used in each RT-PCR reaction. RT-PCR was done using one-step Superscript III RT-PCR kit (Invitrogen). Quantitative real-time RT-PCR for HSF1 was carried out using iQ SYBR Green Super Mix (Bio-Rad) after cDNA synthesis from total RNA. Real-time RT-PCR products were normalized in RIPA lysis buffer. All lysed protein samples were centrifuged at 15 000 g for 10 min, and the supernatant was used for immunoblotting as described elsewhere (61). Statistical analysis was performed using SigmaStat software. Values were expressed as mean ± SEM. Data were analyzed by using one- or two-way ANOVA, followed by Holm-Sidak post hoc test. In some experiments, two-tailed Student’s t-test was used for inter group comparison. P < 0.05 was considered statistically significant.

**SUPPLEMENTARY MATERIAL**
Supplementary Material is available at HMG online.

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**REFERENCES**


