Review

Pridopidine: Overview of Pharmacology and Rationale for its Use in Huntington's Disease

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Abstract. Despite advances in understanding the pathophysiology of Huntington's disease (HD), there are currently no effective pharmacological agents available to treat core symptoms or to stop or prevent the progression of this hereditary neurodegenerative disorder. Pridopidine, a novel small molecule compound, has demonstrated potential for both symptomatic treatment and disease modifying effects in HD. While pridopidine failed to achieve its primary efficacy outcomes (Modified motor score) in two trials (MermaiHD and HART) there were consistent effects on secondary outcomes (TMS). In the most recent study (PrideHD) pridiopidine did not differ from placebo on TMS, possibly due to a large enduring placebo effect.

This review describes the process, based on *in vivo* systems response profiling, by which pridopidine was discovered and discusses its pharmacological profile, aiming to provide a model for the system-level effects, and a rationale for the use of pridopidine in patients affected by HD. Considering the effects on brain neurochemistry, gene expression and behaviour *in vivo*, pridopidine displays a unique effect profile. A hallmark feature in the behavioural pharmacology of pridopidine is its state-dependent inhibition or activation of dopamine-dependent psychomotor functions. Such effects are paralleled by strengthening of synaptic connectivity in cortico-striatal pathways suggesting pridopidine has potential to modify phenotypic expression as well as progression of HD. The preclinical pharmacological profile is discussed with respect to the clinical results for pridopidine, and proposals are made for further investigation, including preclinical and clinical studies addressing disease progression and effects at different stages of HD.

Keywords: Huntington's disease, pridopidine, prefrontal cortex, striatum, motor control, dopamine, stabilizer, indirect pathway, dopamine D2 receptors

INTRODUCTION

Huntington's disease (HD) is a rare neurodegenerative disorder of the central nervous system (CNS) characterized by progressive deterioration of motor and cognitive functions, as well as behavioral and psychiatric disturbances [1]. The disease has an autosomal dominant inheritance and is caused by an expanded CAG repeat in the *huntingtin* (*HTT*) gene on chromosome 4, encoding the mutant protein huntingtin [2, 3]. The hallmark neuropathological feature of HD is degeneration of medium spiny neurons (MSNs) in the striatum [4, 5] and such atrophy is

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evident some years before a formal clinical diagnosis can be made [6, 7]. The onset of clinical symptoms is usually in the fourth or fifth decade of life, but may occur at any time from childhood until old age. A diagnosis of HD is made following unequivocal signs of motor impairment, and may also be confirmed by genetic testing. Following disease onset, motor and cognitive functions steadily decline, ultimately leading to a state of immobility and severe dementia, and to premature death [8]. Pridopidine is a small molecule in clinical development for the treatment of motor symptoms in HD. This article provides an overview of the published pre-clinical pharmacology of pridopidine. The pharmacology is discussed in terms of major neuronal pathways disrupted in HD, aiming to provide a mechanistic rationale for the use of pridopidine in HD.

MOTOR SYMPTOMS IN HUNTINGTON'S DISEASE

The motor phenotype in HD consists of a number of symptoms, including involuntary choreatic movements and a loss of voluntary motor functions such as a progressive decline in fine and gross motor skills, motor impersistence, speech and swallowing difficulties, gait disorder and postural dysfunction. While chorea is considered a hallmark symptom of HD, the severity of disease and disability is more precisely defined by the progressive impairment in voluntary motor function [9, 10]. Also, health economic investigations suggest that voluntary motor impairment is a major determinant of burden of disease in HD [11]. The neuronal mechanisms underlying many of these symptoms are hypothesized to be linked to dysfunctions in cortico-striatal circuits [12–15] and a recent study observed strong correlations between motor symptoms and levels of degeneration in the motor cortico-striatal pathway [16]. Post-mortem neuroanatomical studies have shown that the motor impairment is strongly correlated to the degree of atrophy and cell loss in the striatum [17–19].

The dopamine system in Huntington's disease

Dopamine is a monoamine neurotransmitter modulating several aspects of brain function, including motor control [20], and disrupted dopaminergic signaling has been implicated in a number of neurological and psychiatric conditions [21–24]. Motor control is exerted by dopamine released from the nigrostriatal pathway, modulating the activity of MSNs involved in the facilitation of movement, and inhibition of unwanted movement [25]. MSNs are GABAergic neurons, expressing high densities of dopamine receptors, and a progressive decline in striatal dopamine receptor density is one of the earliest findings in patients with HD [26, 27]. Such changes have been well described in *post mortem* studies, and corroborated *in vivo* by positron emission tomographic (PET) studies [28–31]. In comparison to these post-synaptic changes, the integrity of the pre-synaptic dopaminergic system in HD has been less extensively studied. While the dopamine neuron population in the substantia nigra appears preserved [32], a loss of dopamine terminals has been reported [33]. The latter finding has been confirmed in a PET study including a smaller number of patients with HD [28]. Studies in transgenic animal models suggest that a change in dopaminergic function, such as compromised dopamine release, is an early sign of neuropathology in HD [34–36]. Clinical studies have demonstrated that HD is characterized by pre-synaptic as well as post-synaptic dopamine-related dysfunctions with reduction in striatal dopamine synthesis, dopamine storage, dopamine transporter binding, and both dopamine D1 and D2 receptor binding [37]. Loss of both pre- and post-synaptic markers of dopamine neurotransmission is positively correlated with cognitive performance in both asymptomatic and symptomatic HD patients [38], but the integrity of extrastriatal dopamine D2 receptors has been reported to appear relatively well preserved in patients with HD [39]. A recent review on dopamine changes in Huntington's disease outlined an integrated view, in essence suggesting a continuous decline in dopamine receptors over the patient's life-span, and biphasic changes in striatal dopamine levels, initially increased but thereafter decreasing, in parallel with the transition from hyper- to hypokinetic motor disturbances [40].

It is well established that pharmacological treatments that modify dopaminergic function impact on the motor symptoms of HD. Levodopa challenges were used to provoke chorea as a clinical diagnostic test more than a decade before genetic testing was available [41]. Conversely, tetrabenazine, a monoamine-depleting drug, and dopamine D2 receptor antagonist drugs (antipsychotics) are used to alleviate chorea [42, 43]. On the other hand, Parkinsonian symptoms such as bradykinesia and hypokinesia in HD are hypothesized to be linked to dopaminergic impairment as these symptoms are

aggravated by the use of antipsychotic medication [44, 45]. Furthermore, HD patients treated with antidopaminergic drugs have been reported to display a more rapid disease progression [46] and a more severe phenotype [47]. Thus, motor symptoms in HD are sensitive to drugs that alter dopaminergic transmission, where enhancement of dopaminergic activity is associated with increased chorea, and attenuation is conceivably associated with worsening of negative motor symptoms such as bradykinesia.

THE DIRECT AND INDIRECT PATHWAY IN HUNTINGTON'S DISEASE

Two major components of the cortico-striatal network are the so-called indirect and direct pathways, each forming part of one closed, cortico-basal ganglia-thalamo-cortical feedback circuit [12, 48–50]. The indirect pathway projects from MSNs co-expressing dopamine D2 receptors and enkephalin, and involves relays in the external segment of the external globus pallidus and the sub-thalamic nucleus, before reaching the internal globus pallidus (GPi) and the substantia nigra pars reticulata (SNr). Output from GPi/SNr targets the thalamus, projecting further to glutamatergic neurons of the cortex which project back onto the striatum. The indirect pathway forms part of a negative feedback loop, involved in suppression of movement. Striatal neurons that co-express dopamine D1 receptors, substance P and dynorphin give rise to the direct pathway. In essence, the direct pathway connects GABAergic MSNs expressing excitatory dopamine D1 receptors, substance P and dynorphin, with the GPi/SNr via a single neuron pathway. Further projections from GPi via the thalamus reach the cortex, closing the circuit by a glutamatergic corticostriatal connection back to the MSNs. This circuit, functioning as a positive feedback loop, is involved in the selection and facilitation of voluntary movements. In healthy conditions, the direct and indirect pathways act in balance leading to adequate control of voluntary movement and suppression of involuntary movements [12, 48]. In HD a number of changes that affect striato-thalamic output arise as a consequence of the pathologic process occurring in the disease. A progressive degeneration of striatal MSNs leads to weakened output in both pathways (Fig. 2). Cortico-striatal and nigrostriatal inputs are progressively weakened, leading to decreased striatal glutamate and dopamine release. In manifest HD, a progressive metabolic

decline is seen in the thalamus, an observation likely to reflect a net loss of pallidothalamic output [51].

A decreased output from the MSNs in the indirect pathway results in reduced inhibition of unwanted movements. In patients with HD this is hypothesized to underlie the presence of involuntary movements, such as chorea and dystonia [52]. This would explain why dopamine D2 antagonists, or dopamine depleters, could suppress chorea, as blockade of the inhibitory influence of dopamine on the indirect pathway would strengthen the GABAergic output from the MSNs expressing dopamine D2 receptors, thereby facilitating the suppression of involuntary movements [12]. Decreased activity in the direct pathway, due to cellular degeneration and loss of connectivity in D1-receptor-expressing MSNs, is hypothesized to lead to impaired ability to perform voluntary motor functions in patients with HD [12, 53, 54]. In addition to the striatal degeneration, deterioration of cortical function and cortico-striatal connectivity is observed in HD [53, 55]. Animal studies exploiting regionally specific expression of mutant huntingtin suggest that cortical expression of *htt* is required for the complete HD phenotype to develop. Furthermore, abnormalities specifically affecting neuronal activity and morphology in the cortex, are being increasingly recognized as determinants contributing to the HD phenotype [56]. Such aberrations are likely to contribute to the impaired motor control as well as psychiatric disturbances and cognitive impairments in patients with HD [52, 57, 58].

PHARMACOLOGICAL TREATMENT OF MOTOR SYMPTOMS IN HUNTINGTON'S DISEASE

Numerous medications from different classes are prescribed off-label to ameliorate the motor symptoms associated with HD. These medications include, e.g., antidopaminergic drugs, energy metabolites, benzodiazepines, and glutamate-modifying drugs (riluzole and amantadine) [43, 59]. However, the evidence for the effectiveness of such treatments is poor [60]. Among the motor symptoms of HD, chorea is the most frequently treated symptom, and a vast majority of medications prescribed for this indication are based on the principle of reducing dopaminergic tone. Thus, antipsychotics drugs, i.e., dopamine D2 receptor antagonists, and tetrabenazine, a vesicular monoamine transporter (VMAT) inhibitor, are regarded as "first line" treatment of chorea [43]. In

the US, tetrabenazine is approved for the treatment of chorea in HD [42], but no beneficial effects on the more functionally determining voluntary motor function have been demonstrated [60]. Thus, there are no approved or established treatments for general improvement of the multifaceted motor symptoms. Hence, there is a significant unmet medical need to ameliorate both positive and negative motor symptoms of HD [42, 60] and to slow or halt the progression [61].

PRIDOPIDINE IN HUNTINGTON'S DISEASE

Pridopidine (Teva Pharmaceutical Industries Ltd, Israel) belongs to a novel class of compounds characterized by their psychomotor stabilizing properties, i.e., the ability to restore normal levels of psychomotor activity in both hypo- and hyperactive states, in pre-clinical *in vivo* behavioral models. The efficacy of pridopidine in HD was first investigated in a small-scale study, which suggested that pridopidine could improve voluntary motor functions in patients with HD [62]. Subsequently, two large clinical trials in patients with manifest HD indicated that pridopidine can improve motor function [63, 64]. The Unified Huntington's Disease Rating Scale (UHDRS) total motor score (TMS) was improved by around 3 points versus placebo in both these trials. One point on this scale represents an approximately 10% reduced likelihood of being able to work, manage finances, drive a car or supervise children [65]. Further analysis of subscales of the TMS indicated that pridopidine significantly improved voluntary movements (balance and gait), including hand movements, in both trials. There were also signals of effects on eye movements and involuntary movements. Furthermore, both studies concluded that pridopidine displayed a good safety and tolerability profile [63, 64], consistent with available reports from the recently concluded PRIDE-HD study, a placebo-controlled, dose-ranging study with 52 weeks treatment duration [66]. Long-term safety and tolerability over 36 months was assessed in the recently published OPEN-HART study, showing similar results [67]. 29.7% of the safety population ($n = 118$) had adverse events (AEs) considered to be related to study drug, the most common of which were falls, decreased weight, anxiety, insomnia, and depression. Two subjects were reported to have seizures, one of which was considered possibly treatment related.

A meta-analysis of pooled data sets from these studies supports the conclusions drawn from each individual trial, indicating significant effects on overall motor function (measured by TMS) as well as on voluntary movements (hand movements, and gait and balance), eye movements and involuntary movements (dystonia) [68]. Pridopidine is, therefore, the first compound showing promise in large-scale clinical trials in treating the core motor symptoms of HD without worsening other symptom domains.

Pridopidine – discovery and overview of pharmacology

Pridopidine was discovered using an *in vivo* systems pharmacology approach, focusing on measuring biomarkers of neurotransmission in neuronal circuits known for their involvement in the control of psychomotor functions. The theory is that systematic *in vivo* profiling of CNS compounds, using a comprehensive range of neurochemical, gene expression and behavioral biomarkers collected in both healthy and diseased states, would give a reliable handle on preclinical properties and allow for predictions of clinical features of the novel compounds tested in the system. Data on a wide variety of known therapeutic agents including antipsychotics, antidepressants, anxiolytics and psychostimulants, as well as experimental reference compounds, are collected, and then used to define a multidimensional compound "map" serving as a guide towards the sought after *in vivo* profile [69]. This methodology was developed to enable a rational and predictive drug discovery process in a field where target-based discovery approaches have hitherto shown to be largely unsatisfactory in terms of determining clinically promising molecules and molecular action mechanisms [70]. In the specific program that led to the discovery and subsequent development of pridopidine in HD, the vital impact of central monoaminergic and specifically dopaminergic pathways in the cortico-subcortical circuitry regulating motor function was considered. The complex, progressive symptomatology in HD, combining hypo- and hyperkinetic motor disturbances, cognitive and psychiatric symptoms, suggest any pharmacological intervention should be delicately balanced not to disturb remaining functions or worsen aspects of the heterogeneous symptoms. The combined basic research efforts and considerations described above led to the formulation of a preclinical target profile. In short, a compound of interest should: 1) show no interference with spontaneous locomotor patterns

over a wide dose range; 2) have the ability to normalize states of hypoactivity; 3) have the ability to normalize states of hyperactivity; and 4) act primarily through the dopamine system. The initial screening was performed *in vivo* as described in the following (see section *Overall in vivo phenotypic response profile*).

From the medicinal chemistry perspective, the fourth criterion above suggested the conceptual starting point of the program to focus on design and synthesis of compounds with dopamine D2 receptor agonist-like chemical motifs. The key chemical strategy in the program was to modify the agonist motif so that the pharmacophore elements essential for intrinsic activity were lost, to obtain compounds that primarily interact with the dopamine D2 receptor in a similar way as agonists, but without the ability to stabilize the active and catalytic conformation(s) of the receptor G-protein complex. The working hypothesis was that this should result in a portfolio of compounds with agonist-like receptor interactions but with antagonist-like pharmacological features. Further, the hydrophilic characteristics of the agonist motif were to be retained to ensure similar binding mode and adherence to druggability features important for favorable drug metabolism and pharmacokinetics, and reduced safety and toxicological risks.

Starting from a series of compounds with partial dopamine D2 agonist properties reported by Mewshaw et al. [71] including 3-(1-benzylpiperidin-4-yl)phenol, the key elements for its intrinsic activity were identified to be the phenol group, the anilinic nitrogen and the large *N*-alkyl group. A series of modifications of these elements led to a range of phenylpiperidines, including 4-[3-(methylsulfonyl) phenyl]-1-propylpiperidine (ACR16, pridopidine) [72], displaying the desired profile.

Overall in vivo phenotypic response profile

The initial *in vivo* pharmacological assessment of pridopidine was performed by acute dose response studies in intact rats, collecting behavioral data and *ex vivo* monoaminergic neurochemical indices according to a systematic, standardized model, followed by multivariate analysis of the response profile, including comparisons to a wide range of CNS compounds and a tentative therapeutic classification [69, 73]. This "screening" suggested that three major criteria were met; i.e., no behavioral inhibition, subtle behavioral activation in the

hypoactive phase, and significant effects on dopaminergic indices reflecting dopamine D2 receptor modulation. Furthermore, the multivariate comparison to reference compounds indicated clear similarities with the cluster of anti-psychotic compounds tested, as well as some effects in cortical brain areas overlapping with effects of pro-cognitive compounds. This prompted further preclinical investigations in specific models of dopaminergic hyperactivity as well as glutamatergic hypoactivity, and assays of cortical neurotransmission.

Behavioral pharmacology

Pridopidine reduces both hyperactivity and the behavioral abnormalities pharmacologically induced in animal models of elevated dopamine or decreased glutamate neurotransmission, while the locomotor activity of intact animals is unaffected over the same dose range. Hence, pridopidine counteracts hyperactivity induced by psychotomimetics including d-amphetamine and *N*-methyl-d-aspartate (NMDA) antagonist MK-801 [72, 74]. In addition, pridopidine enhances locomotor activity in animals with a low baseline psychomotor activity, as seen in animals habituated to their environment [74], as well as in rats co-treated with the VMAT inhibitor tetrabenazine [75]. Pridopidine is unable to induce profound hypoactivity or catalepsy, indicating that it has a low likelihood of displaying the adverse neurological effects associated with classical dopamine D2 receptor antagonist antipsychotics [74, 76]. Pridopidine does not induce catalepsy, even at doses producing D2 receptor occupancy reaching 80% or above [76]. Considering qualitative aspects of the behavioral effects of pridopidine, it has been shown to restore social interactions in rats treated with MK-801 [77], and ameliorate the behavioral primitivization induced by MK-801 in mice [78]. Both findings are proposed to imply beneficial effects on cognitive symptoms. Furthermore, pridopidine is efficacious in the conditioned avoidance response model of antipsychotic activity [76]. Pridopidine has also been shown to display potent and efficacious antidepressant activity in the mouse tail suspension test [74]. A chronic study in a rodent model of L-DOPA induced motor complications (sensitization to repeated L-DOPA upon unilateral 6-OH-dopamine lesion) demonstrated that pridopidine reduced L-DOPA induced rotational behavior while not impairing forward locomotion [79]. In behavioral studies using the R6/2 mouse model of HD, Squitieri

et al., recently reported data indicating that pridopidine reduces and/or prevents the expression of the HD motor phenotype on the horizontal ladder task and in open-field locomotor measurements during 6 weeks' treatment, starting in the pre-symptomatic stage. It also prolonged the life-span in R6/2 mice. Wild-type behaviors in the same assays were not affected by pridopidine [80].

Neurochemical effects

Pridopidine increases synthesis, release and metabolism of dopamine in sub-cortical areas [74], mimicking the effects of dopamine D2 antagonists in general [22]. The increases in dopamine turnover and transmission biomarkers produced by dopamine D2 antagonists in sub-cortical areas are due to inhibition of dopamine-D2-receptor-dependent negative feedback [22, 81]. These results indicate that pridopidine acts as an antagonist at dopamine D2 receptors *in vivo*. Furthermore, in an *in vivo* study designed to detect agonist properties, Natesan et al., tested pridopidine's ability to reverse DOPA accumulation induced by reserpine (an inhibitor of the vesicular monoamine transporter) [76]. They found that pridopidine had no effect in this sensitive agonist assay. Pridopidine increases plasma levels of prolactin in rats, suggesting antagonistic effects at hypothalamic dopamine D2 receptors *in vivo* [82]. The interaction of pridopidine at dopamine D2 receptors is further demonstrated by *in vivo* binding experiments showing that pridopidine dose-dependently displaces the dopamine D2 antagonist raclopride from dopamine D2 receptors [76]. Taken together, the *in vivo* dopaminergic neurochemistry and *in vivo* binding data on pridopidine consistently indicate that pridopidine acts as a full dopamine D2 antagonist. *In vivo* microdialysis studies in conscious rats have demonstrated that pridopidine dose-dependently increases the efflux of dopamine and noradrenaline in the cortical and subcortical projection areas of the ascending dopamine projections [74]. At doses efficacious with respect to the key behavioral effects, prefrontal cortex and striatal levels of both dopamine and noradrenaline are increased.

Analysis of gene expression profiles has demonstrated that pridopidine increases the expression of activity-regulated cytoskeleton-associated protein [83] (*Arc*) mRNA in the frontal cortex and the striatum [84]. Furthermore, pridopidine has been shown to reverse the reduction in striatal Arc levels induced by MK-801 [85]. The increase of Arc mRNA expression suggests an increase in synaptic activity [86], possibly related to facilitation of NMDA receptor activation [87, 88]. In addition to the *ex vivo* biomarker studies demonstrating cortical effects of pridopidine, a recent study shows that pridopidine increases firing in prefrontal pyramidal cells [79]. The pridopidine-driven increase in pyramidal cell firing was antagonized by the dopamine D1 antagonist SCH23390, suggesting that the pyramidal cells are indirectly activated by pridopidine through increased levels of dopamine binding with D1 receptors. The cortical effects of pridopidine, as reflected, by increased dopamine release in the frontal cortex, increased Arc gene expression, and increased cortical neuronal activity, may contribute to its potency in behavioral assays. This is reported to be higher than the potency in increasing striatal 3,4-dihydroxyphenylacetic acid, essentially reflecting D2 antagonism *in vivo* [74]. It has been shown that addition of a compound that increases cortical dopamine release improves the potency of D2 antagonists in behavioral models of antipsychotic activity [89, 90]. Also, dopamine D1 agonists *per se* have been shown to exert anti-psychotic like behavioral effects [91].

In vitro pharmacology

In vitro binding studies demonstrate affinity in the micromolar range at dopamine D2 receptors. [72]. Assessments on functional responses in different settings *in vitro* show that pridopidine displays competitive antagonism with a fast dissociation rate from the dopamine D2 receptor [92], and that pridopidine, just as in the *in vivo* assays, lacks intrinsic activity at dopamine D2 receptors [92–94].

The affinity of pridopidine measured at dopamine D2 receptors is slightly higher using agonist vs antagonist counter ligands, $k_{i\,antagonist}/k_{i\,agonist} = 2.3$ [72]. Dopamine D2 receptors exists in two states (i) the resting and low-affinity state ($D2R^{Low}$) and (ii) the active, catalytic, high-affinity state ($D2R^{High}$). Dopamine D2 receptors agonists bind with preference to receptors in $D2R^{High}$ and induce a full catalytic reaction, i.e. they have affinity and intrinsic activity. Pridopidine has been proposed to preferentially bind to the high-affinity state, but without intrinsic activity [72, 95, 96]. This would differentiate pridopidine from classical D2 receptor antagonists, which, in contrast, stabilize the $D2R^{Low}$ state and do not show preference for either receptor state. In summary, *in vitro* as well as *in vivo* studies

addressing dopamine receptor interactions consistently indicate that pridopidine acts as a competitive, low-affinity dopamine D2 antagonist with fast-off receptor-dissociation kinetics and with a slight preference for the agonist binding site.

Pridopidine has also been reported to display *in vitro* affinity in the micromolar range at adrenergic alpha 2A/C receptors (k_i 20/2.3 μM), serotonergic 5HT1A (k_i 3.1 μM) and 5HT2A (k_i 15 μM) receptors, and histamine H3 (k_i 7.2 μM) receptors [79]. Interactions at these receptors, each of which has been shown to modulate extracellular levels of monoamines [97–100], as well as glutamatergic transmission in the cortex [101–104], may contribute to the *in vivo* effects of pridopidine. Apart from the monoaminergic receptors, pridopidine has been reported to display moderate affinity at the sigma-1 receptor *in vitro* [105]. The significance of this is unclear, as sigma-receptor binding is displayed by a wide range of CNS-active compounds from different therapeutic classes, but it has been proposed to contribute to neuroprotective properties of pridopidine [80]. In support of this, a recent *ex vivo* study on striatal gene expression in rats showed upregulation of several neuroprotective pathways by pridopidine, including brain derived neurotrophic factor (BDNF), glucocorticoid receptor, and AKT/phosphoinositide 3-kinase (PI3K), and dopamine D1 pathways, and further suggested that sigma1-receptor activation is necessary for the effect of pridopidine on the effect on the BDNF pathway, based on assessment of *in vitro* BDNF secretion in neuroblastoma cells, which was antagonized by co-incubation with a sigma-1 antagonist [106]. Recently published additional gene expression analyses from the same study demonstrated upregulation of calcium regulating proteins including calbindin and homer1a upon chronic treatment with pridopidine. Furthermore, it was shown that pridopidine normalized calcium homeostasis and prevented spine loss in aging corticostriatal co-cultures from YAC128 mice. Spine rescue and normalized Ca2 + levels were prevented by deleting sigma-1 receptors, suggesting pridopidine exerts synapto-protective effects, through sigma-1 receptors and effects on calcium signaling [107].

Summary of preclinical pharmacology

The *in vivo* pharmacological profile of pridopidine can be summarized as *state-dependent* inhibition or activation of dopamine-dependent psychomotor functions (Fig. 1). This is achieved through
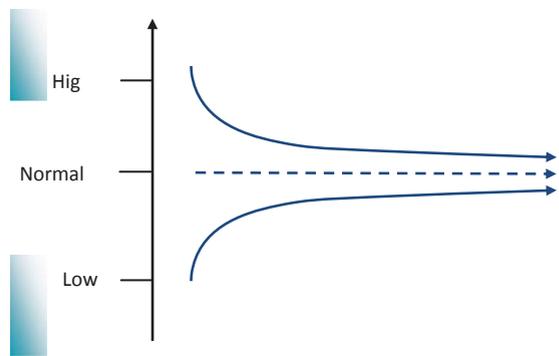

Fig. 1. Pridopidine is able to enhance or inhibit dopamine-dependent functions. Graphic illustration of psycho-motor stabilization: *In vivo* pharmacological studies have consistently demonstrated state dependent behavioral effects of pridopidine; reducing psychomotor activity in hyperactive states, and enhancing activity in hypoactive states. This is proposed to translate to stabilization of both hyper- and hypokinetic motor disturbances in HD; to some extent overlapping with observations of disturbed dopamine transmission over the course of the disease [40].

low-affinity/fast-off antagonism of dopamine D2 receptors, combined with an increased cortical and sub-cortical monoamine release resulting in enhanced activity of prefrontal cortex neurons. It thus appears as if pridopidine exerts its behavioral stabilizing effects by acting on several key nodes of the cortico-striatal network regulating psychomotor functions. In addition to these acute *in vivo* effects as described in terms of major neuronal pathways, emerging data suggest sigma-1 receptor mediated effects, putatively involving regulation of intraneuronal calcium signaling, which may be of relevance in particular with regard to neuroprotective effects of pridopidine.

PRIDOPIDINE: MODE OF ACTION AT THE INTEGRATED CIRCUITRY LEVEL

The pharmacological effects of pridopidine as outlined above, at the levels of receptor interactions, neurochemistry, gene expression firing activity and behavior, can be brought together in a tentative, integrated and testable model of the system-level mode of action of pridopidine based on three main core features (Fig. 2). The discussion is focused on the relief of motor symptoms in manifest HD, as the primary clinical indication for pridopidine at present. First, pridopidine is a low-affinity/fast-off dopamine D2 receptor antagonist, thus modifying
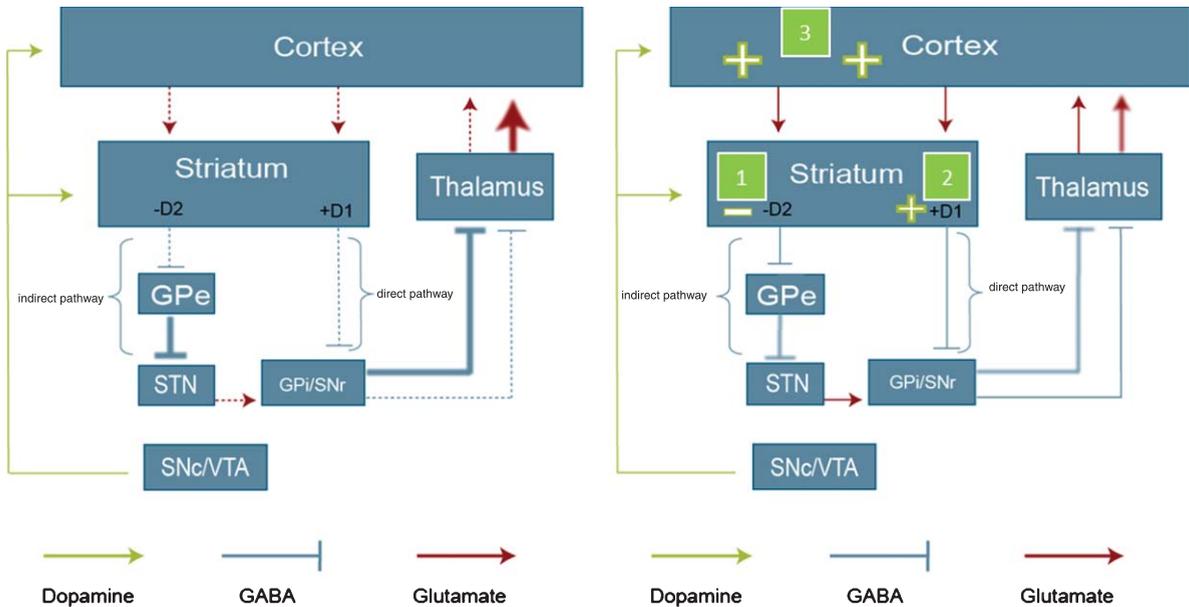
Fig. 2. Proposed *in vivo* mode of action of pridopidine in manifest HD. A schematic overview of the organization of the basal ganglia, involving the direct and indirect pathway, and the proposed *in vivo* effects of pridopidine. The left panel shows the direct and indirect pathway in the state of manifest HD. Dashed lines represent reduced transmission, thick lines increased transmission. In manifest HD, output in both striatal pathways is attenuated, and cortico-striatal connectivity is impaired [53, 55]. The right panel illustrates the suggested mode of action for pridopidine: (1) Pridopidine normalizes the aberrant function in the indirect pathway, by blocking DA D2 receptors, which results in attenuation of involuntary movements. (2) Pridopidine improves voluntary movements by stimulating the direct pathway via activation of the DA D1 receptor. (3) Pridopidine strengthens the prefrontal cortex, which indirectly stimulates both the direct and indirect pathways. The schemes shown represent the changes in late stage, symptomatic HD, and the tentative impact of pridopidine. At earlier stages of HD an increase in cortico-striatal glutamate transmission has been suggested (not shown). D1, dopamine D1 receptors; D2, dopamine D2 receptors; GPe, globus pallidus pars externa; SNc, substantia nigra pars compacta; VTA, ventral tegmental area; GPi, globus pallidus pars interna.

output in the indirect striato-thalamic output pathway, tentatively leading to improved control of involuntary movements in HD. Secondly, pridopidine induces dopamine release in the basal ganglia and the frontal cortex. This, in combination with the D2 inhibiting properties, leads to a shift in balance towards D1 receptor signaling, strengthening the direct striato-thalamic output pathway, which would enhance voluntary motor functions. Thirdly, pridopidine enhances dopamine transmission and neuronal activity in the frontal cortex, leading to strengthened cortico-striatal signaling. These three core features of pridopidine are proposed to act in synergy to reduce the complex mixture of negative and positive motor symptoms associated with cortical and striatal degeneration in HD.

Pridopidine strengthens the indirect pathway via antagonism of dopamine D2 receptors

The mechanism of action for pridopidine involves dopamine D2 receptor antagonism [72, 92, 95, 96]. MSNs projecting to the indirect pathway are negatively modulated by dopamine through dopamine D2 receptors. Hence, dopamine attenuates the GABAergic output from these neurons. Diminished activity in this pathway results in a reduced capacity to suppress involuntary movements. By blocking the dopamine D2 receptors on the MSNs of the indirect pathway, the inhibitory influence of dopamine is reduced, and the output via the indirect pathway is strengthened. Therefore, by antagonizing dopamine D2 receptors in the striatum, pridopidine normalizes the aberrant function in the indirect pathway, which results in attenuation of involuntary movements (Fig. 2, right panel). This is supported by clinical results suggesting reduced involuntary motor symptoms in HD patients treated with pridopidine, reported from large randomized clinical trials [63, 64].

Pridopidine strengthens the direct pathway by stimulating dopamine D1 receptors

MSNs projecting to the direct pathway are positively modulated by dopamine through dopamine D1

receptors. Hence, dopamine enhances the GABAergic output from these neurons. Administration of pridopidine increases the release of striatal dopamine [74]. This implies that pridopidine, by increasing synaptic availability of dopamine, thus indirectly stimulating the dopamine D1 receptors, could strengthen the striatal output in the direct pathway (Fig. 2). This would result in improvements of voluntary movement control in HD. In our view, this hypothesis seems to be supported by clinical outcomes of the MermaiHD (Multinational European Multicentre ACR16 study in Huntington's Disease) and HART (Huntington's disease ACR16 Randomized Trial) trials indicating improvement in voluntary motor control such as oculomotor and postural function, and hand movements [63, 64].

Consistent with the notion that pridopidine activates striatal D1 receptors, and antagonizes D2 receptors, pridopidine has been demonstrated to dose-dependently increase expression of striatal *Arc* mRNA [84]. To the best of our knowledge, pridopidine displays no affinity or direct activity at any glutamate receptors investigated. Therefore, such NMDA receptor activation is not likely to occur as a direct effect. Rather, given the functional association between D1 and NMDA receptors in MSNs [108] the increase in striatal *Arc* mRNA levels could arise indirectly as a consequence of synaptic NMDA receptor modulation, related to activation of dopamine D1 receptors. This is in line with the findings that dopamine D1 receptor agonists increase, and dopamine D1 antagonists decrease striatal *Arc* expression [109, 110]. Activation of DA D1 receptor mediated pathways by pridopidine was corroborated in a recent gene profiling study indicating upregulation of D1 mediated gene expression upon repeated administration of pridopidine [106]. The effect of pridopidine on striatal Arc levels may also be associated with the direct antagonism of striatal D2 receptors, leading to reduced inhibitory tone on cortico-striatal glutamate release, and therefore to increased glutamate transmission. Induction of striatal Arc gene expression has been reported for several D2 receptor antagonists [111, 112]. Further studies, *e.g.* investigating the localization of *Arc* induced in D1 vs D2 receptor expressing MSNs, would be needed to determine more precisely how MSNs of the direct and indirect pathways are affected by pridopidine. Furthermore, animal studies have suggested a biphasic pattern of changes in cortico-striatal communication in HD, with increased signaling in early stages, before reaching the late stage represented in Fig. 2 [53, 55].

Thus, it would be of interest to assess the impact of pridopidine on cortico-striatal communication at different stages of the disease.

Pridopidine strengthens cortical activity

In manifest HD, progressive thinning of the cortex is observed [113], and preclinical studies suggest decreased communication in the cortico-striatal glutamatergic projections, in addition to the degeneration of striatal MSNs [53]. These alterations are associated with cognitive impairments [114] and are proposed to result in reduced activation of the direct and indirect pathway [53] (Fig. 2, left panel), hampering motor control [53]. The pathogenetic impact of disrupted cortico-striatal connectivity for the HD phenotype has further been demonstrated in pre-clinical HD models, showing that expression of mutant *htt* in both the cortex and the striatum is required to develop the full pathological phenotype [115].

Pridopidine has been demonstrated to dose-dependently increase dopamine in the prefrontal cortex [74]. The strengthening of frontal cortex dopamine transmission is further hypothesized to drive down-stream effects in the cortico-striatal circuitry, regulating motor functions.

As a more direct read-out of neuronal activity in the frontal cortex, administration of pridopidine has been demonstrated to dose-dependently increase Arc gene expression in rat frontal cortex, interpreted as increased activation of synaptic NMDA receptors [84]. Preliminary observations that the extra-synaptically acting NMDA receptor antagonists memantine and ifenprodil display similar effects as pridopidine, with respect to frontal cortex *Arc* gene expression, provide indirect support for the notion that pridopidine shifts the balance towards synaptic NMDA receptor signaling [85]. Given the synergistic interaction between dopamine D1 and NMDA receptor signaling in cortical pyramidal cells, it is proposed that such enhancement of synaptic NMDA receptor signaling by pridopidine arises indirectly due to increased cortical dopamine transmission followed by activation of dopamine D1 receptors. This is supported by recently recorded observations that pridopidine increases the firing frequency of rat pyramidal neurons in the frontal cortex, and that this effect could be blocked by administration of the D1 antagonist SCH23390 [79]. Increased activity of dopamine D1-expressing glutamatergic cells in the cortex would promote

cortico-striatal communication, and indirectly drive the indirect and direct pathways (Fig. 2). The effects of pridopidine to reduce hypoactivity in partially monoamine-depleted rats, concurring with increased frontal cortex Arc gene expression, provide some support for a cortically driven improvement of voluntary motor function by pridopidine.

Pridopidine displays clear-cut effects on cortical dopamine transmission, which are likely to contribute to effects such as those on Arc gene expression and pyramidal cell firing activity, and to the overall behavioral profile. However, pridopidine may also influence cortical neurons by other mechanisms. *In vivo* microdialysis studies have demonstrated increased levels of not only dopamine, but also noradrenaline, which modulates cortical activity through alpha 1 and alpha 2 receptors [74, 79]. α2-adrenoceptor blockade has been shown to induce cortical *Arc* gene expression, likely by increased NA release [116]. Furthermore, the affinity of pridopidine at adrenergic alpha2c, 5HT1a and histamine H3 receptors [79], as well as sigma-1 receptors [105], may be of relevance. Regardless of the exact mechanism for the Arc upregulation *in vivo*, it provides evidence of unique cortical effects of the dopidines, as compared to other DA modulating compounds in general, and antipsychotics in particular [84]. Such effects could contribute to the characteristic behavioral pharmacology of the dopidines, and further support the notion of potential cognitive enhancing properties.

The proposed *in vivo* mode of action of pridopidine is based on the state of manifest HD, a symptomatic stage in which impairment of both the indirect and direct pathways has developed (Fig. 2). This would suggest the use of pridopidine for motor symptom alleviation in advanced HD, targeting both voluntary and nonvoluntary motor impairment. However, emerging evidence suggesting potential beneficial effects on disease progression could motivate earlier interventions.

THE CLINICAL POTENTIAL OF PRIDOPIDINE

Results from multicenter trials MermaiHD and HART were recently published [63, 64]. While the primary endpoint, defined as reduction in a modified motor score, was not met, a 3-point reduction in the UHDRS total motor score, compared to placebo, was observed in both studies. Exploratory analysis

of these data indicated that negative motor symptoms such as gait and balance, hand movements and oculomotor function improved. There were also improvements on involuntary motor features. Of note, the clinical results indicate that the dopamine-enhancing properties of the compound are not translated into an increase in involuntary movements, such as those seen after as example L-dopa treatment in patients with HD. Furthermore, in contrast to classical D2 receptor blocking antipsychotics or dopamine depleters like tetrabenazine, pridopidine does not give rise to the bradykinesia and rigidity associated with such treatments. Rather, the data reported so far suggest that pridopidine reduces negative motor symptoms.

There is also a possibility that pridopidine, via the aforementioned pharmacological profile, including modulation of dopaminergic transmission as well as through sigma-1 receptor interactions, may modify disease progression itself in HD. Neurodegeneration in HD is strikingly selective where striatal MSNs are most vulnerable to the pathological process. The underlying causes for this selectivity are not completely known. Striatal MSNs receive massive glutamatergic input from the cortex and a longstanding hypothesis is that changes in NMDA-receptor-dependent plasticity and transmission are a major factor contributing to this selective vulnerability. It was more recently proposed that the balance between synaptic and extrasynaptic NMDA receptors determines whether resulting signaling is beneficial or detrimental. Synaptic activation promotes a number of pro-survival pathways whereas extrasynaptic signaling opposes these and triggers pro-death pathways [117]. Pridopidine increases Arc mRNA expression, and increases pyramidal cell firing in the frontal cortex, both effects likely driven by dopamine release and D1 receptor stimulation leading to enhanced NMDA receptor activity. Thus, pridopidine may indirectly enhance synaptic NMDA receptor signaling in the frontal cortex. In support of this interpretation, memantine, which has been shown to preferentially antagonize extrasynaptic NMDA receptors, and hence shifts the balance in NMDA-receptor-mediated transmission from extrasynaptic to synaptic sites, displays similar effects as pridopidine on cortical Arc mRNA expression [85]. Memantine has been shown to display neuroprotective effects *in vivo* [118, 119]. Recently, pridopidine was reported to promote brain cell survival, activate pro-survival pathways and improve motor phenotype in R6/2 mice, providing support for a protective

Table 1
Progression rates in terms of TFC decline in randomized and observational studies. Shown are mean values of annual TFC change estimated for each study listed; negative values indicate worsening on this scale. For Pride-HD, exact baseline data were not available; however, the study population was similar to, e.g., the HART study population, i.e. ambulatory patients, a large proportion in TFC stage I or II. The delay of TFC decline observed in pridopidine treated subjects was particularly evident in the early-stage population. CoQ: Coenzyme Q. HSG: Huntington study Group

Study	Baseline TFC	Treatment	Annual TFC progression	Follow-up time
HSG [121]	7.5	Observational	−0.72	18 (median)
HSG [121]	Stage 2 (tfc 7–10)	Observational	−0.84	18 mo
REGISTRY [122]	8 (M), 7.4 (F)	Observational	−0.74(M), 0.94 (F)	21 mo
CARE-HD [123]	10.3	Placebo	−1.096	30 mo
CARE-HD [123]	10.1	CoQ	−0.96	30 mo
CARE-HD [123]	10.0	Remacemide	−1.08	30 mo
2CARE [124]	11.0	Placebo	−0.952	60 mo
2CARE [124]	10.8	CoQ	−0.906	60 mo
CREST-E [125]	10.2	Placebo	−0.7	18–48 mo
CREST-E [125]	10.2	Creatine	−0.82	18–48 mo
Tetra-HD continuation [126]	7.6	Tetrabenazine	−1.3	18 mo
Open-HART [67]	9.1 (cohort completing 36 mo)	Pridopidine	−0.57	36 mo
Open-HART [67]	8.4 (cohort completing 12 mo)	Pridopidine	−0.6	12 mo
Pride-HD [66]	–	Pridopidine	−0.04	12 mo
Pride-HD [66]	–	Placebo	−0.83	12 mo

potential in HD [80]. A tentative mechanism for such an effect could be indirect facilitation of synaptic NMDA receptor transmission. There are also studies suggesting neuroprotective effects *in vitro* and *in vivo* mediated by sigma-1 receptor interactions [105–107, 120]. Mechanisms directly related to altered dopaminergic neurotransmission could also be involved. Of note, given the proposed biphasic changes in cortico-striatal glutamate transmission, it could be argued that indirect enhancement of DA D1, as well as NMDA receptor mediated transmission, in particular in the striatum, could be unfavorable in early HD, potentially aggravating chorea, and accelerating disease progression, e.g., by triggering apoptotic pathways. The studies in R6/2 mice speaks against such concerns; rather supporting the notion of neuroprotective effects, tentatively arising as a net system level effect of dopamine modulation at multiple sites as outlined above, combined with sigma-1 receptor interactions. Also, the phenotype improved. Furthermore, there have been no indications of worsened chorea in pridopidine treated patients, to the contrary, involuntary motor features tend to improve.

No clinical studies primarily assessing disease-modifying aspects of pridopidine have been performed. In a recently concluded phase 2 study, Pride-HD, disease progression as represented by total functional capacity (tfc) score was measured as an exploratory end-point. The primary endpoint, the change in total motor score at 26 weeks, was not met in this study which was complicated by a large, enduring placebo response [66]. Considering tfc, pridopidine 45 mg b.i.d. treated subjects displayed a slower decline at 52 weeks, compared to placebo. Recently published data from Open-HART, an extension of the HART study reporting outcomes upon up to 36 months of treatment with pridopidine, suggested a numerical reduction in clinical progression in terms of tfc as compared to the expected natural course of progression, however not statistically significant based on a *post-hoc* analysis comparing a limited sample of matched placebo control subjects from two other studies in HD (2CARE and CARE-HD) with pridopidine treated subjects [67], when adjusting for multiple comparisons. In Table 1, rates of progression in terms of tfc decline over 1 year or more for observational and randomized studies in HD are listed. Rate of progression is related to baseline tfc, with faster rates observed in early stages of HD (Marder). The rate of functional decline observed in observational cohorts, or with placebo or other treatments are consistently faster than the rates observed upon long term treatment with pridopidine.

CONCLUSION

HD is a devastating hereditary neurodegenerative disorder causing brain atrophy and rapidly progressive disability. Clinical and preclinical data in HD patients and transgenic rodent models have provided evidence for multiple neuronal and neurotransmitter dysfunctions in the cortico-basal ganglia systems. The neuronal degeneration and dysfunction in HD explains the motor symptoms seen in this disorder, and the involvement of both the direct and indirect

output pathways from the striatum, underlies the complex mixed negative and positive motor symptoms presented. Preclinical pharmacology data on pridopidine demonstrate balancing effects on motor function through the dopamine system, and indirect enhancement of cortico-striatal synaptic activity. Hence, negative motor features such as impairment of fine motor skills, bradykinesia and gross motor coordination difficulties, may be improved by pridopidine through the activation of cortical dopamine transmission and downstream cortico-striatal synaptic activation, strengthening both the direct and the indirect pathways. The involuntary motor symptoms, dystonia and chorea, may be further alleviated by antagonism of striatal dopamine D2 receptors. In addition to effects directly and indirectly related to modulation of dopamine signaling, emerging evidence points towards a role for sigma-1 receptor interactions and regulation of calcium signaling contributing to potential disease modifying effects of pridopidine. Mechanistic studies specifically testing these hypotheses could be performed in animal models of HD. In addition, further human studies are warranted to investigate whether pridopidine may modify HD progression rate or prolong the time to phenoconversion in HD gene carriers.

CONFLICT OF INTEREST

CS, NW, SW, and JT are co-inventors on a composition of matter patent covering pridopidine, held by Teva Pharmaceutical Industries Ltd, Israel.

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