Potential of protease inhibitor in 3-nitropropionic acid induced Huntington's disease like symptoms: Mitochondrial dysfunction and neurodegeneration

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ABSTRACT

Huntington’s disease (HD) is a genetic, neurodegenerative disorder mainly characterized by motor dysfunction, cognitive decline and psychiatric disturbances. 3-Nitropropionic acid (3-NP) is an inhibitor of succinate dehydrogenase (Complex II) of the mitochondrial respiratory chain, which thereby reduces production of ATP. It induces neurotoxicity by causing striatal degeneration, energy deficit and oxidative stress. Angiotensin converting enzyme (ACE) is an important protease in the renin angiotensin system (RAS) responsible for the conversion of Angiotensin I to Angiotensin II. Angiotensin-II stimulates mitochondrial oxidant release leading to depression of energy metabolism. ACE inhibitors have shown promise in disorders like stress, anxiety, and depression in addition to showing beneficial effects in cognitive disorders like Alzheimer’s. Angiotensin-II inhibition enhances energy production by lowering mitochondrial oxidant production, and hence protects mitochondrial structure. Trandolapril is a centrally active ACE inhibitor. 3-NP administered systemically (20 mg/kg, i.p) for 4 days consecutively induced HD like symptoms – loss of body weight, neurobehavioral alterations like memory dysfunction (elevated plus maze, Morris water maze performance), Hind-limb impairment (Narrow beam test), motor incoordination (locomotor activity). Biochemical studies on brain tissue showed increased lipid peroxidation, nitrite levels and acetylcholinesterase activity along with decreased levels of reduced glutathione, catalase activity. Mitochondrial enzyme complex activities (I, II, IV and MTT assay) were found to be significantly lowered in brain mitochondria. Administration of Trandolapril (4 and 6 mg/kg, p.o) daily for 12 days showed significant improvement in body weight, neurobehavioral parameters, oxidative stress and mitochondrial enzyme activities in rat brain. These findings were further confirmed by histopathological studies which showed improvement in 3-NP induced brain lesions. This study indicates that Trandolapril could be an effective treatment option for the management of HD.

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1. Introduction

Neurodegeneration is a condition that describes progressive loss of structure and/or functions of neurons (Uttara and Singh, 2009). Diseases like Alzheimer’s disease and Huntington’s disease can be classified as neurodegenerative proteinopathies. This is so because they are mainly characterized by the accumulation of misfolded proteins, resulting in the progressive and selective loss of anatomically or physiologically related neuronal systems (Ocampo, 2012). Huntington’s disease (HD) is autosomally dominant, an archetypical polyglutamine disorder, in which the CAG nucleotide triplet repeat is translated into an expanded polyglutamine (polyQ) tract, resulting in the formation of protein aggregations within the cell (Everett and Wood, 2004). The HD gene is localized to chromosome 4p16, and encodes a widely expressed, 348 kDa protein named Huntingtin (htt) which has highest levels in neurons (Cattaneo et al., 2001). Some of the mechanisms by which degeneration develops in HD are Excitotoxicity, energy deficit, oxidative stress, inflammatory processes, and protein aggregation (Bordelon, 2012).

HD is mainly characterized by motor dysfunction, cognitive decline and psychiatric disturbances. Chorea is the main feature of this disease, and it is characterized by brief, semi-directed,
irregular movements that appear to flow from one muscle to the next, but are not repetitive or rhythmic (Chittnis and Karunanupuzha, 2009). The mutant Htt causes cell death by a variety of mechanisms. It causes mitochondrial dysfunction by interacting with the organelle and producing defects in the dynamics of the mitochondria which, in turn, may result in bio-energetic failure (Ferreira et al., 2011). Studies have demonstrated reduced activity of the complex II enzyme succinate dehydrogenase (SDH), a 55–60% decrease in complex II–III activity in the basal ganglia of patients with Huntington’s disease decrease along with a decrease in complex IV activity in HD striatum (Leegwater-Kim and Cha, 2004). Disruption of mitochondrial activity is associated with the abnormal formation of reactive oxidative species (ROS) like the superoxide radical (O2•−), hydrogen peroxide (H2O2), and the hydroxyl radical (OH•−), thereby generating oxidative stress (Ramaswamy et al., 2007).

For a model of HD to be considered reliable, it should be capable of reproducing the neuropathology and symptomology of the disease. 3-NP inhibits SDH which is involved in both the tricarboxylic acid cycle (TCA) and the electron transport chain (ETC) and thus, causes mitochondrial damage (Tabrizi et al., 1999). This leads to an increase in electron leakage from the mitochondria, production of reactive oxygen and nitrogen species, accompanied by depletion of antioxidant defences (Browne et al., 1997). 3-NP can mimic as well as reproduce the hyperkinetic and hypokinetic movement symptoms of HD (Túnez et al., 2010). In addition to the above, striatal lesions caused by 3-NP have been reported to closely mimic the anatomical, histological and neurochemical pathology seen in HD (Ramaswamy et al., 2007). Hence, it is an efficient model for studying the features of HD.

Proteases belong to a family of enzymes that catalyze the cleavage of peptide bonds hydrolytically. Currently there are 570 human proteases and based on the nature of their catalytic site, they are divided into 5 major classes: serine, threonine, cysteine, aspartic and metalloproteases (Meredith, 2009). Angiotensin converting enzyme (ACE) is a dipeptidase that belongs to the class of metalloproteases and catalyses the conversion of angiotensin I to angiotensin II. Expression of truncated forms of mutant huntingtin protein (Htt) induces cell death by apoptosis. Therefore, it has been hypothesized that toxic protein fragments derived from full-length mutant Htt are required for the initiation of disease. Abnormal proteolytic processing of mutant Htt has been implicated as a critical step in the initiation of HD (Gafni and Ellerby, 2002). Hence, proteases may be involved in the progression of this disease. Protease inhibitors avert apoptosis in part by preventing mitochondrial outer membrane permeabilization (MOMP), but the precise mechanism by which they work is not known (Hisatomi et al., 2008).

ACE belongs to the renin angiotensin system (RAS). The brain has been reported to have an intrinsic RAS which is independent of peripheral RAS. Drugs which act on RAS include angiotensin receptor (AT) blockers and angiotensin converting enzyme (ACE) inhibitors (Awasthi et al., 2012). There has been increasing evidence that brain RAS is involved in multiple neurological phenomena like Alzheimer’s disease, stroke, memory and learning etc., in addition to its involvement in neurogenic hypertension (Phillips and de Oliveira, 2008). It is believed that the RAS of the brain is involved not only in the regulation of blood pressure, but also in the modulation of various other functions in the brain, including processes of sensory information, learning and memory, and the regulation of emotional responses (Petrescu et al., 1999). ACE inhibitors and Angiotensin II antagonists have been postulated to be effective in preventing cognitive decline and even improving cognitive function in hypertensive patients (Mugellini et al., 2003). It has also been reported that chronic administration of ACE inhibitor reduced cholinergic dysfunction and oxidative stress (Awasthi et al., 2012). Trandolapril is a centrally active, ethyl ester prodrug of a nonsulfhydryl ACE inhibitor, trandolaprilat. It is approximately eight times more active as an inhibitor of ACE activity as compared to trandolaprilat. It also possesses a long elimination half-life, strong lipophilicity, high ACE inhibitor potency, high affinity for the ACE and a long biological half-life (Ciobica et al., 2011).

Keeping the above findings in mind, the present study was designed to evaluate the effects of Trandolapril against the neurobehavioral, oxidative and mitochondrial impairment induced by 3-NP in a rat model of HD.

2. Materials and methods

2.1. Chemicals

All the chemicals used in the study were of analytical grade and obtained from Sigma Chemicals Co., and SD Fine Chemicals. 3-Nitropropionic acid was procured from Sigma Chemicals Co. Trandolapril was obtained as a gift sample.

2.2. Animals and treatment schedule

Female Wistar rats weighing 200–250 g were used for the study. The animals were maintained on 12-h light/dark cycle and given free access to food and water. Animals were acclimatized to laboratory conditions before the start of the experimental study. They were kept under standard laboratory conditions. Experiments were performed between 9.00 and 17.00 h. The experimental protocol was approved by the Institutional Animal Ethics Committee (IAEC).

3-nitropropionic acid (3-NP) was diluted in saline (pH 7.4) and administered intraperitoneally. Trandolapril was suspended in 0.5% sodium carboxymethyl cellulose and administered orally to the animals in accordance with their body weights.

The animals were randomly selected and grouped as follows: (n = 6)

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>Vehicle control (sodium CMC, p.o), Saline (pH 7.4, i.p)</td>
</tr>
<tr>
<td>Group II</td>
<td>3-Nitropropionic acid (20 mg/kg, i.p)</td>
</tr>
<tr>
<td>Group III</td>
<td>Trandolapril (4 mg/kg, p.o) + 3-NP (20 mg/kg, i.p)</td>
</tr>
<tr>
<td>Group IV</td>
<td>Trandolapril (6 mg/kg, p.o) + 3-NP (20 mg/kg, i.p)</td>
</tr>
<tr>
<td>Group V</td>
<td>Trandolapril (6 mg/kg, p.o)</td>
</tr>
</tbody>
</table>

Trandolapril, at doses of 4 mg/kg and 6 mg/kg were given as a suspension in 0.5% sodium CMC. It was administered orally by a gavage, every day for 12 days. 3-nitropropionic acid (3-NP) was administered for induction of HD symptoms like chorea, mitochondrial dysfunction and oxidative stress. It was administered intraperitoneally on the last four days (days 9–12). It was given 1 h after the administration of Trandolapril.

2.3. Measurement of body weight (Kumar and Kumar, 2010)

Body weight was recorded on day zero and the last day (day 12) of the experiment. Percent change in body weight was calculated as:

\[
\text{Body weight} \left( \frac{1st \ day - 15th \ day}{1st \ day \ body \ weight} \right) \times 100
\]