Neuroprotective Effect of Nilotinib on Cortical Pyramidal Cells, Histological, Morphometric and Biochemical Study

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Author’s contribution
The sole author designed, analyzed, interpreted and prepared the manuscript.

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Introduction: Death of neuronal cell and gliosis are the two main pathological hallmarks induced by nervous tissue stress like conditions such as status epilepticus. Previous studies have mentioned that neuronal cell death occur as a result of different mechanisms, namely, necrosis and apoptosis. Although more recent studies have explained the cell death on the basis of autophagy. Many antiepileptic drugs are marketed, taking into consideration the antioxidant role of nilotinib and support its use as a favorable antiepileptic drug. The aim of the present study is to assess the neuroprotective effect of antiepileptic drug nilotinib on cortical tissue in rats.

Materials and Methods: Sixty adult male rats were divided into three groups: (1) Control group, (2) pentylenetetrazol group (injected with pentylenetetrazol 60 mg/kg, subcutaneously), (3) nilotinib and pentylenetetrazol group (pretreated with nilotinib, 25 mg/kg daily for seven days prior to pentylenetetrazol administration). Latency of seizure and level of either oxidant or antioxidant enzymes in the cortical tissue was assessed. The histopathological changes in the cerebral cortex were studied also using hematoxylin and eosin stain.

Results: Nilotinib increased the latency period of convulsions, increased the antioxidant enzymes levels with regain of the normal histological features.

Conclusions: Nilotinib proved to promote the antioxidant, antiapoptotic pathways, anti-inflammatory and inhibiting autophagy which favor its use as an anti-epileptic drug.
Keywords: Antiepileptic; cerebral cortex; epilepsy; nilotinib; rats.

1. INTRODUCTION

Epilepsy is a chronic disease affecting different human age groups [1]. It is characterized by recurrent status epilepticus (SE) attacks [2]. As per many medical resources, epilepsy is considered the fourth commonest neurological disorder affecting humans [3]. Recent evidence suggests that, oxidative stress marked with reactive oxygen species (ROS) and nitric oxide (NO) production, is considered the main mechanism that causing SE [4-8]. Oxidative stress biochemical pathways are evoked by various stress conditions such as seizures and the underlying mitochondrial metabolic processes generate highly reactive free radical molecules [9,10].

Cerebral cortex neurons are most sensitive to SE. They show cellular sclerosis and loss [11-14]. Apoptosis, necrosis and autophagic changes are the main three causes of neuronal such loss [15,16]. Ablation off these cellular changes has been a major issue to research projects.

In apoptosis (programmed cell death), cascade of events includes cellular blebbing & cell shrinkage [17-19], nuclear fragmentation, chromatin condensation & fragmentation, and mRNA decay [20-23]. These events lead to morphological cellular death [24]. Necrosis is a form of cellular injury which leads to premature cellular death, characterized by nuclear karyolysis, Karyorrhexis and pyknosis [25-27]. Recent evidence suggests that autophagy pathways (naturally, regulated mechanisms helps in the removal and recycling of unnecessary and dysfunctional cellular components [28,29]) are present in a variety of neurological disorders, which range from chronic neurodegenerative diseases to acute neurological insults such as status epilepticus [30,31].

Nilotinib (NIL) (Tasigna), which was approved for the treatment of chronic myelogenous leukemia, has a favorable action in reducing oxidative stress and fibrosis in the rat model for renal disorders and liver injuries [32-34].

1.1 Aim of the Study

The aim of the present study is to assess the neuroprotective effect of nilotinib on cortical tissue, in rats.

2. MATERIALS AND METHODS

This research study was approved by Research and Ethics Committee, Quality Assurance Unit, Faculty of Medicine, Tanta University, Egypt.

2.1 Drugs

Pentylenetetrazol (PTZ) was purchased from LOPAC, Sigma-Aldrich Co. LLC, USA, dissolved in 0.9% sterile normal saline. Nilotinib (NIL) was purchased from Selleck Chemicals, Canada, dissolved in 0.5% carboxymethyl cellulose.

2.2 Animals

Sixty adult male Wister rats were used with average weight 250–300 g. They were observed for one week before the start of experimental process under normal condition (12/12 light–dark cycle, standard temperature 25 degree Celsius and humidity 55%).

2.3 Experimental Design

The sixty rats were divided into 3 groups: (1)The control group (n = 20), (2)PTZ group (n = 20) received a subcutaneous injection with PTZ dissolved in saline (60 mg/kg b.w.), [35], (3) NIL-PTZ group (n = 20) received NIL (25 mg/kg/b.w.); orally by a nasogastric tube daily for one-week [36] then received single subcutaneous injection of PTZ dissolved in saline( 60 mg/kg b.w.) After PTZ injection, seizure and its latency time were recorded over 45 min [37]. The seizure behavior was classified as follows: no response, stage 0; facial twitching, stage 1; myoclonic convulsions, stage 2; forelimb clonus, stage 3; limb clonic convulsions, stage 4; generalized clonic convulsions, stage 5 [38].

2.4 Cerebral Cortex Tissue Collections

One hour after PTZ injection, cortical samples were collected. Right hemispheres were stained with hematoxylin and eosin stain. While the left hemispheres were rapidly frozen for the biochemical assay [39].

2.5 Biochemical Assays

Left Cortical hemisphere sample was rapidly frozen, kept in 0.05 M phosphate buffer (pH 7.4) then centrifuged for 10 min. The centrifuged sample was used to measure different antioxidant markers including superoxide...
dismutase (SOD), catalase (CAT), glutathione peroxidase (GP-x) and glutathione (GSH) together with oxidative state markers including malondialdehyde (MDA), nitrate/nitrite (NOx) [40].

2.6 Morphometric Study

Cerebral cortex pyramidal cell layer thickness (µm) was measured microscopically at 1000× magnification.

2.7 Statistical Study

SPSS software was used for the analysis. All data were expressed as mean ± SEM. Statistical analysis was performed. P > 0.05, P ≤ 0.05 and P ≤ 0.001 were considered nonsignificant, significant and highly significant, respectively.

3. RESULTS

3.1 H&E Sections

3.1.1 Control group

H&E sections of the control group showed normal thickness of external pyramidal layer (lamina pyramidalis externa). The common cells inside this layer are the pyramidal (medium sized) in addition to glial cells (small sized). The pink stained background was interconnected cell processes. Pyramid cell bodies have round vesicular nuclei and prominent nucleoli with dendritic spines, whereas glial cells were small with small round dark nuclei. No neuronal death was detected (Fig. 1a).

3.1.2 PTZ group

Showing moderate pyramidal cells degenerative changes evidenced by vacuolization. Multiple neurons are deformed, other neurons exhibit dark nuclei and acidophilic cytoplasm. Reduction of pyramidal nerve cell layer thickness was found (Fig. 1b).

3.1.3 NIL-PTZ group

In NIL-PTZ sections, the shape for the majority of nerve cells and thickness of the pyramidal nerve cell layer were similar to those seen in the control group (Fig. 1c).

3.2 Biochemical Assays

On comparison PTZ group with control group, there was significant elevation in cortical MDA with a significant decrease in levels of SOD, CAT, GP-x and GSH. While NIL-PTZ group, there was reduction in MDA level (if compared to PTZ group) but still significantly higher than the control value. On the other hand, a highly significant improvement in antioxidants levels of SOD, CAT, GP-x and GSH; were observed in NIL-PTZ group, as compared to the PTZ group. Regarding to the level of NOx, it was observed that PTZ group presented high level when compared to control rats. This elevated NOx level was significantly decreased in NIL-PTZ group as compared to the PTZ group (Table 1).

3.3 Latencies (Seizures threshold)/min

PTZ group showed that onset of the first myoclonic seizures was after 1.40 ±0.7 min and the onset of the first generalized tonic-clonic seizure was after 2.50 ±0.07 min. It was observed that PTZ -NIL group showed delay the of onset of seizures as compared to PTZ group, which indicates the protective effect of NIL against PTZ-induced seizures (Table 2).

Table 1. Mean concentration of MDA, SOD, GSH and GP-x, in control, PTZ, and NIL-PTZ groups. N = 10/group

<table>
<thead>
<tr>
<th>Groups</th>
<th>MDA (nmol/gm tissue)</th>
<th>GSH (uM/gm tissue)</th>
<th>SOD (U/gm tissue)</th>
<th>GP-x (uM/gm tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>113.70 ±3.24</td>
<td>154.78 ±2.53</td>
<td>12.20 ±0.55</td>
<td>178.30 ±3.39</td>
</tr>
<tr>
<td>PTZ</td>
<td>260 ±3.88</td>
<td>74.50 ±1.95</td>
<td>3.04 ±0.32</td>
<td>35.30 ±1.02</td>
</tr>
<tr>
<td>NIL-PTZ</td>
<td>142 ±2.24</td>
<td>138.50 ±5.00</td>
<td>10.70 ±0.48</td>
<td>152.70 ±4.25</td>
</tr>
<tr>
<td>P1 PTZ</td>
<td>≤ 0.0001**</td>
<td>≤ 0.0001***</td>
<td>≤ 0.0001***</td>
<td>≤ 0.0001***</td>
</tr>
<tr>
<td>P2 NIL-PTZ</td>
<td>≤ 0.0001***</td>
<td>≤ 0.0001***</td>
<td>≤ 0.0001***</td>
<td>≤ 0.0001***</td>
</tr>
</tbody>
</table>

Data are represented as mean ±SEM

P – probability *significant, **highly significant, ***very highly significant

P1 – significance between Control and PTZ groups

P2 – significance between NIL-PTZ and PTZ groups
3.4 Morphometric Study

The mean thickness of the pyramidal nerve cell layer showed a very highly significant decrease in the PTZ only treated group (22.33 ±0.61, P1 ≤ 0.0001) as compared to the control group (46.10 ±0.97). NIL-PTZ groups showed a marked increase in the thickness of the pyramidal layer (32.63 ±1.03, P2 ≤ 0.0001 and 38.27 ±0.98, P3 ≤ 0.0001, respectively) as compared to the PTZ group.

4. DISCUSSION

Pentylenetetrazol is a central nervous system stimulant and so it is used to study experimentally induced seizures and to compare newly marketed antiepileptic drugs [41].
Fig. 1. H&E sections of the cerebral cortex of control group showed the common cells inside this layer which are the pyramidal (P), stellate (S) in addition to microglial cells (M). The pink stained background was a mat of neuronal and glial cell processes (arrowhead). Pyramid cell bodies have round vesicular nuclei and prominent nucleoli with dendritic spines, whereas glial cells were small with small round dark nuclei 1A. No neuronal death was detected. Sections of the PTZ group showed moderate pyramidal cells (P) degenerative changes with vacuolization. Multiple deformed stellate (S) neurons and multiple neurons exhibiting dark nuclei and acidophilic cytoplasm (arrow) 2A. Reduction of pyramidal nerve cell layer thickness was found. H&E sections of the NIL-PTZ group showed the shape for the majority of nerve cells and thickness of the pyramidal nerve cell layer were similar to those seen in the control group 3A.

Table 2. Mean latencies (Seizures threshold)/min to the onset of myoclonic and generalized tonic-clonic in control, PTZ and NIL-PTZ groups. N = 10/group

<table>
<thead>
<tr>
<th>Groups</th>
<th>Latency to the 1st myoclonic seizures/min</th>
<th>Latency to the 1st generalized tonic-clonic seizures/min</th>
</tr>
</thead>
<tbody>
<tr>
<td>PTZ</td>
<td>1.39 ±0.6</td>
<td>2.40±0.05</td>
</tr>
<tr>
<td>NIL-PTZ</td>
<td>22 ±0.76</td>
<td>32 ±0.79</td>
</tr>
<tr>
<td>P1 PTZ</td>
<td>≤ 0.0001**</td>
<td>≤ 0.0001***</td>
</tr>
<tr>
<td>P2 NIL-PTZ</td>
<td>≤ 0.0001***</td>
<td>≤ 0.0001***</td>
</tr>
</tbody>
</table>

Data are represented as mean ±SEM

P – probability *significant, **highly significant, ***very highly significant
P1 – significance between Control and PTZ groups
P2 – significance between NIL-PTZ and PTZ groups

It blocks GABA^A receptor function. The sequence of induced seizures is myoclonic followed by clonic and by tonic-clonic phase [42]. These seizures are accompanied by nervous tissue oxidative stress state with production of a flow of free radicals [43-45]. Nilotinib was approved as a treatment for chronic myelogenous leukemia and proved its antioxidant effect on either kidney or liver tissues [46-48]. It was investigated in the present research to study its neuroprotective role against PTZ induced seizures. Present study showed a significant delay of the onset of...
seizures in the NIL-PTZ group if compared to PTZ rats, indicating that NIL has a neuroprotective effect against PTZ. The decreased latency of seizures was used as an indicator of protective antiepileptic action of NIL [49,50].

Oxidative stress arises from overproduction of reactive oxygen species (ROS) by metabolic reactions that use oxygen and disturb the balance between oxygen containing molecules (oxidants) and antioxidants in favor of the oxidants [51-53]. Production of ROS and reduction of antioxidant activities are the main pathological changes noticed in nervous tissue after seizures [54,55]. The complex interconnected event including lipid deposition, glucose, mitochondrial calcium, glutamate, neuromitter and ribonucleic acid oxidation; explains the sensitive of the brain to oxidative stress [56-58]. The cerebral cortex is mainly sensitive to hypoxic states evoked by severe brain activity as occurs in status epilepticus (SE) [59,60].

Markers for antioxidant state include nitric oxide synthases (NOS), Superoxide dismutase (SOD) an enzyme that enhance the transformation of the superoxide (O2−) radical into either ordinary molecular oxygen (O2) [61]. Catalase (CAT) a common enzyme that provoke the degradation of hydrogen peroxide into water and oxygen [5,23], Glutathione peroxidase (GPx) which reduce both lipid hydroperoxides and free hydrogen peroxide [62,63], in addition to Glutathione (GSH) preventing damage of important cellular components [64,65]. Marker for oxidative state include Malondialdehyde (MDA) which is one of the final products of lipid oxidation in the nerve cell [66].

Oxidative stress in SE can cause reduction in the activity of certain antioxidant enzymes such as NOS, CAT, SOD, GPx in addition to GSH levels [67,68], which comes in line with our results. In our study brain cells oxidant state was also determined by measuring MDA. The present study showed a significant improvement in the antioxidant levels in the NIL-PTZ group when compared to PTZ. Nilotinib has been previously reported to show significant beneficial antioxidant, anti-inflammatory and anti-fibrotic activity in other research paper [69].

Studies emphasized that imbalance between oxidants and antioxidants results in a state of oxidative stress which favors the hypothesis that NOx has a main role in nervous tissue pathological state [70]. The present study showed a marked elevation in NOx in PTZ treated rats. On the other hand, NIL treated group was found to have improved antioxidant state demonstrated the elevated NO level to an almost normal control level. Such findings suggest that NIL plays its role via NO-mediated mechanisms [71].

During normal neural activity, glutamate concentration is kept constant. whether it is elevated to an uncontrolled level, the cell intentionally destroy itself via a process of apoptosis. Cell death during SE is explained by many researchers by the fact that glutamate causes swelling of the cellular organelles and damages to the intercellular membranes secondary due to the increased Ca++ overload [72]. This may provide an explanation to our findings on the histopathological level. Corrupted nerve cells evidenced by the shrunken nuclei, shape distortion and the diminish in the mean thickness of the pyramidal nerve cell layer, which were patent in the PTZ group, proved neuronal death. Gliosis, and vacuolation which were also reported in other research papers correlate the fact that neuronal cell death following SE was mainly located in cerebral cortex [73-75].

NIL treated group showed more pyramidal cell layer preservation when compared to PTZ treated one. Neuronal injury is accompanied by gliosis, which is characterized by remarkable molecular alterations in glial cells [76]. Recent studies have demonstrated the importance of astrogliosis and its essential roles in nervous tissue maintenance [77-80].

5. CONCLUSIONS

The present study showed that Nilotinib plays a role in inhibiting cellular oxidative stress. It provides a crucial neuro-protective effect through its antifibrotic, anti-inflammatory and antiapoptotic role. Further experimental and clinical studies should be done to confirm this beneficial effect at a molecular level.

ETHICAL APPROVAL

This research study was approved by Research and Ethics Committee, Quality Assurance Unit, Faculty of Medicine, Tanta University, Egypt.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our
area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

COMPETING INTERESTS

Author has declared that no competing interests exist.

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