Long-term morphological and functional evaluation of the neuroprotective effects of post-ischemic treatment with melatonin in rats

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Presented by Tidaporn Wongsuthin

Cerebral ischemia

- Reduction of blood supply
- Pathophysiological processes
- Irreversible damage of neurons

During Ischemia

Pathogenic of brain damage

- Vascular occlusion
  - Cerebral blood flow
- Failure of high-energy metabolism
  - Ion dyshomeostasis:
    - $\downarrow$Na$^+$, $\uparrow$Ca$^{2+}$, $\uparrow$Cl$^-$, $\uparrow$K$^+$
- Calcium overload
- Activate cellular enzyme:
  - Phospholipase, cyclooxygenase, nNOS
- Vitamin E, Glutathione
- Cell death
- Organelle injury
Melatonin

- A secretory product of the pineal gland
- Many biological effects of melatonin are produced through activation of melatonin receptors

Previous studies, they examined the protective effect of melatonin against oxidative stress during brain ischemia and reperfusion injury through short-term morphologic and functional evaluations.

Melatonin treatment increase survival of pyramidal CA1 neurons

Barone et al. (1999)

However, cellular mechanisms of neuronal repair leading to functional recovery may occur over periods longer than 30 days following cerebral ischemia.

Cozzone et al. (2000), Reiter et al. (2004) and Macleod et al. (2005)
On the other hand, drug effects which do not impede neuronal death but merely slow the temporal course of neuronal damage, may be associated with delayed neuronal death.

Finklestein et al. (1999)

The magnitude of neuronal damage does not always correlate with the degree of functional impairment.

Olsen et al. (1994)

**Aim of the present study**

The long-term morphological and functional assessment of the neuroprotective effect of melatonin in the four-vessel occlusion (4-VO) rat models.

**Materials and methods**

- **Behavioral test**
  - Morris water maze
  - Olton eight-arm radial maze

- **Histological assessment**
  - Cresyl violet

**Animals**

- Adult male Sprague-Dawley rats
- Weight 320-400 g
- Control light-dark cycle (12hr/12hr) and room temperature at 20 ± 2°C
- Food and water were provided ad libitum
4-VO model of global cerebral ischemia

- Bilateral vertebral arteries occlusion (permanent)
- Bilateral common carotid arteries occlusion (transient)

The vertebral arteries were permanently occluded by electrocautery.

The both common carotid arteries were occluded for 15-min using microvascular clamps.

The clamps were removed and both carotid arteries were inspected for recovery of carotid blood flow.

Groups

1: Intact
2: Sham
3: Ischemia + Vehicle
   - Vehicle 3.0 mL/kg/hr (10% ethanol in 0.9% saline)
4: Ischemia + Melatonin
   - Melatonin 10mg/kg/hr in vehicle

n = 8
10/07/50

- Removed clamps
- Carotid arteries clamping
- Vertebral arteries occlusion
- Injection for 6-hr
- Behavioral tests

48-hr

- 15-min
- 30-min
- 90 days

Morris water maze performance

- Invisible platform
- Probe trial
- Target quadrant

Each rat performed two trials with a 20-min interval for 7 days.
Morris water maze performance

- Escape latencies (s)
- Swimming path lengths (cm)
- Inner/outer index
- Time spent in the target quadrant

Olton eight-arm radial maze performance

Adaptation days

On day 1

• Food pellets were scattered over entire surface of all eight arms

On days 2 and 3

• A single food pellet was placed in each distal hole of the eight arms

Experimental tests

Time to find the eight pellets was recorded

Entries into the arms having food pellets were recorded as correct choices

Re-entries into arms were defined as working memory errors

Rats were given a total of 10 trials within 1 trial per day for 2 weeks
Histological assessment

- Anesthetized with pentobarbital
- Brain perfusion
- Brain removal
- Pyramidal neurons cell counts
- Stained with Cresyl violet
- Embedded in paraffin and sliced

Hippocampal pyramidal cell counts

- CA1
- CA2
- CA3
- Hilus

Prefrontal cortex cell counts

- Prefrontal cortex

Only pyramidal neuron showing normal morphology with distinct cytoplasmic and nuclear outline and a visible nucleolus were counted.
Statistical analysis

Within – group differences
- Friedman’s ANOVA followed by Wilcoxon test

Between – group differences
- Kruskall – Wallis ANOVA followed by Mann – Whitney U-test

Number of pyramidal cells
- One-way ANOVA followed by the Tukey test

Results

Morris water maze test

Between group differences

Mann Whitney U-test

P<0.05

Results

Swimming path lengths

Thigmotaxis (concentric swimming pattern)

Inner/outer index

P<0.05
Results

Percent time spent in target quadrant during the probe trial

Results

Number of working memory error in the Olton eight-arm radial maze

Results

Pyramidal cell counts

Results

Number of hippocampal pyramidal neurons
Results

Percent number of pyramidal neurons

Discussion

Neuroprotective effect of melatonin in spatial memory

Melatonin treatment during the 6-hr following a 15-min period of global cerebral ischemia results in a significant long-term preservation of pyramidal CA1 neurons.

The amount and location of long-term neuronal loss seems to be a main factor leading to permanent deficits in spatial learning and memory in water maze after a 4-VO-induce global cerebral ischemia.

It occurs when neuronal loss exceeds 60% of the normal pyramidal CA1 neurons.

Block et al. (1999)
Discussion

Percent number of CA1 neurons

Percent time spent in the target quadrant

Ischemia + Vehicle 23.4 %
Ischemia + Melatonin 78.7 %

Discussion

Neuroprotective effect of melatonin in working memory

They tested in the Olton eight-arm radial maze, that was shown by the significant delay in the reduction in the number of working errors to minimal values

Discussion

The neural substrate underlying working memory includes both hippocampal and prefrontal cortical structures

Floreso et al. (1997), Laroche et al. (2000)

Impairment of working memory in the Isch + Veh group could be associated with both neuronal loss and modification of neuronal connectivity
- Severe reduction of pyramidal CA 1 neurons
- Neuronal loss was not observed in prefrontal cortex

Discussion

The preservation of a high proportion of hippocampal pyramidal neurons in the Isch + Mel group possibly favored the coordinated functioning of hippocampal and prefrontal cortical structures
Histological observations of the pyramidal layer of CA-1 showed a reduction of neuronal loss in animals that received melatonin.

Melatonin improves brain injury

Antioxidant actions of melatonin

1. Free radical scavenging
2. Activation of antioxidant enzymes

Discussion

The timing of melatonin administration in the present study is coincident with a therapeutic window for its neuroprotective effect.

Neuroprotective effect: between 30-min before reperfusion to 6-hr after reperfusion

Reiter et al. (2000), Cuzzocrea et al. (2000)
Conclusion

The efficacy of melatonin in counteracting ischemia-induced pathophysiological mechanisms which otherwise lead to severe damage and impairment of functioning of vulnerable brain structures.

Melatonin receptors

<table>
<thead>
<tr>
<th>Receptor (Previous Name)</th>
<th>MT1 (ML1A, Mel1a)</th>
<th>MT2 (ML1B, Mel1b)</th>
<th>MT3 (ML2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Transduction Mechanism</td>
<td>Adenylyl cyclase (G)</td>
<td>Adenylyl cyclase (G)</td>
<td>PT turnover (Gαi)</td>
</tr>
<tr>
<td>Location</td>
<td>SCN, pars tuberalis, hypothalamus, kidney</td>
<td>Retina, hippocampus</td>
<td>Hamster/mouse brain, kidney, testes</td>
</tr>
<tr>
<td>Selective Agonists</td>
<td>None reported</td>
<td>-</td>
<td>None reported</td>
</tr>
<tr>
<td>Selective Antagonists</td>
<td>None reported</td>
<td>DH-497 (9118)</td>
<td>4-P-PDOT (1034)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Luzindole (0877) (19-fold selective)</td>
</tr>
<tr>
<td>Non-selective Ligands</td>
<td>6-Chloromelatonin (0443) (agonist)</td>
<td>6-Chloromelatonin (0443) (agonist)</td>
<td>6-Chloromelatonin (0443) (agonist)</td>
</tr>
<tr>
<td></td>
<td>2-Iodomelatonin (0737) (agonist)</td>
<td>2-Iodomelatonin (0737) (agonist)</td>
<td>2-Iodomelatonin (0737) (agonist)</td>
</tr>
<tr>
<td></td>
<td>Prazosin (0623) (antagonist)</td>
<td>Prazosin (0623) (antagonist)</td>
<td>Prazosin (0623) (antagonist)</td>
</tr>
</tbody>
</table>

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