CHAPTER 8

Studies On The Antioxidant Role Of Pyrimidines In Their Cerebroprotective Actions Against Ischemia-Reperfusion Induced Cerebral Infarction In Rats
Studies On The Antioxidant Role Of Pyrimidines In Their Cerebroprotective Actions Against Ischemia-Reperfusion Induced Cerebral Infarction

Introduction

Oxygen-derived free radicals produced on reperfusion of ischemic brain could constitute the main cause of reperfusion injury. Brain is the most susceptible organ to the damage due to oxidative stress because neurons are rich in polyunsaturated fatty acids, and levels of endogenous antioxidant enzymes (superoxide dismutase (SOD), catalase, glutathione peroxidases) and non enzymes (vitamins C and E) in neuronal tissue are low [1-3].

Mitochondria are considered to be the main source of ROS production, but another ROS source are activated inflammatory cells, such as neutrophils and microglial cells. The superoxide anion radicals (O$_2^−$) and hydrogen peroxide (H$_2$O$_2$) are the main ROS. These constantly produced reactive oxygen species (ROS) are scavenged by superoxide dismutase (SOD), glutathione peroxidase (GPx), and catalase. Other small molecular antioxidants, including glutathione (GSH), ascorbic acid, and α-tocopherol, are also involved in the detoxification of free radicals. But during reperfusion these endogenous antioxidative defenses are likely to be disturbed and results in overproduction of free radicals causing oxidative damage to biomolecules like lipids, proteins and DNA. Hydroxyl radical (OH$^\cdot$) formed from H$_2$O$_2$ (in itself not highly reactive) in the presence of
divalent metal ions, especially Fe$^{2+}$ and Cu$^{2+}$, via the Fenton reaction and peroxynitrite (ONOO$^-$) formed by interaction of super oxide (O$_2^•$) with nitric oxide (NO$^•$) a strong oxidant initiates lipid peroxidation. Lipid peroxidation causes damage to mitochondria further and generated ROS. In addition, lipid peroxides degrade to reactive aldehyde products, including malondialdehyde (MDA), 4-hydroxy nonenal (HNE), and acrolein [4-10].

Several experimental studies indicate that this cascade of reactions induced by ischemia followed by recirculation causes membrane disintegration and irreversible energy failure, leading to the aggravation of brain edema and loss of neuronal functions. Since reperfusion injury is associated with an imbalance of oxidative stress and antioxidant defense system, theoretically it would be possible to limit oxidative damage and ameliorate disease progression by supplementing antioxidants. Indeed, numerous natural antioxidants have shown cerebroprotective effect in I/R induced cerebral injury [11-19].

Therefore the present study was undertaken to determine if the antioxidant mechanism of pyrimidines is attributed to their cerebroprotective activity in I/R induced cerebral infarction in rats. Antioxidant role of pyrimidines was determined by estimation of end product of lipid peroxidation, MDA levels and antioxidant enzymes SOD and CAT in brain tissue.
Experimental Protocol

Sprague Dawley rats of either sex weighing between 250 - 300 g were used in the study. Experimental protocol is as follows

<table>
<thead>
<tr>
<th>S.No</th>
<th>Group</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Group 1</td>
<td>Served as sham control (without I/R)</td>
</tr>
<tr>
<td>2.</td>
<td>Group 2</td>
<td>Rats received 0.2 mL of saline and served as Ischemia-reperfusion control (I/R)</td>
</tr>
<tr>
<td>3.</td>
<td>Group 3</td>
<td>Rats received 0.2 mL of 50% DMSO 10 min before reperfusion and served as vehicle control</td>
</tr>
<tr>
<td>4.</td>
<td>Group 4</td>
<td>Rats received <strong>pyrimidine 1</strong> (20 mg/kg) 10 min before reperfusion</td>
</tr>
<tr>
<td>5.</td>
<td>Group 5</td>
<td>Rats received <strong>pyrimidine 2</strong> (20 mg/kg) 10 min before reperfusion</td>
</tr>
</tbody>
</table>

Animals

Rats were randomly divided into five groups. Each group consists of 6 animals. Animals in all groups were anesthetized with thiopental sodium (30 mg/kg, intraperitoneally) and were subjected to bilateral common carotid artery occlusion for 30 min and followed by 4 h reperfusion. Surgical procedure is described in the chapter 6.

Pyrimidines were dissolved in 50% DMSO and administered intraperitoneally 10 min before reperfusion. At the end of the experiment the brain was removed and the homogenate was prepared as described in the previous chapter 6 for estimation of oxidative stress markers.
Results

Results of tissue MDA levels are presented in Table 8.1 & Fig. 8.1. Results of superoxide dismutase (SOD) levels are presented in Table 8.2 & Fig. 8.2. Results of catalase (CAT) levels are presented in Table 8.3 & Fig. 8.3.

Results shown in the above mentioned tables indicate that the cerebral ischemia and reperfusion significantly increased the level of lipid peroxidation (MDA) and decreased antioxidant enzyme levels (SOD and CAT) in the injured brain tissue of rats as compared with the sham control group.

However, the treatment of rats with pyrimidine 1 and pyrimidine 2 increased SOD and CAT activity and decreased MDA content in the brain tissue of rats in comparison with the I/R control group.

Table 8.1: Effect Of Pyrimidines On MDA (nmol/g Wet Tissue) Levels In Infarcted Tissue In Cerebral Ischemia Reperfusion Injury In Rats

<table>
<thead>
<tr>
<th>Normal control</th>
<th>Sham Control</th>
<th>I/R Control</th>
<th>Vehicle control</th>
<th>Pyrimidine 1 (20 mg/kg)</th>
<th>Pyrimidine 2 (20 mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>157.28</td>
<td>160.25</td>
<td>513.45</td>
<td>510.12</td>
<td>448.36</td>
<td>374.89</td>
</tr>
<tr>
<td>156.73</td>
<td>158.69</td>
<td>518.12</td>
<td>510.65</td>
<td>462.06</td>
<td>349.34</td>
</tr>
<tr>
<td>155.21</td>
<td>158.36</td>
<td>520.652</td>
<td>522.36</td>
<td>460.00</td>
<td>384.89</td>
</tr>
<tr>
<td>156.12</td>
<td>160.69</td>
<td>519.65</td>
<td>510.63</td>
<td>452.25</td>
<td>359.45</td>
</tr>
<tr>
<td>157.56</td>
<td>160.35</td>
<td>518.16</td>
<td>511.58</td>
<td>435.56</td>
<td>368.68</td>
</tr>
<tr>
<td>156.25</td>
<td>159.61</td>
<td>519.32</td>
<td>512.96</td>
<td>449.65</td>
<td>358.15</td>
</tr>
<tr>
<td>156.52±0.34</td>
<td>159.65±0.38</td>
<td>518.22±1.03</td>
<td>513.05±1.90</td>
<td>451.31±3.87</td>
<td>365.90±5.24</td>
</tr>
</tbody>
</table>

All values are expressed as Mean±SEM (n=5), p<0.05
**Fig. 8.1:** Effect Of Pyrimidines On MDA (nmol/g Wet Tissue) Levels In Infarcted Tissue In Cerebral Ischemia Reperfusion Injury In Rats

All values are expressed as Mean±SEM (n=5), p<0.05

**Table 8.2:** Effect Of Pyrimidines On SOD (U/mg Protein) Levels In Infarcted Tissue In Cerebral Ischemia Reperfusion Injury In Rats

<table>
<thead>
<tr>
<th>Normal Control</th>
<th>Sham Control</th>
<th>I/R Control</th>
<th>Vehicle control</th>
<th>Pyrimidine 1 (20 mg/kg)</th>
<th>Pyrimidine 2 (20 mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>9.31</td>
<td>8.86</td>
<td>4.53</td>
<td>3.62</td>
<td>7.12</td>
<td>7.68</td>
</tr>
<tr>
<td>9.41</td>
<td>8.23</td>
<td>4.61</td>
<td>5.92</td>
<td>6.12</td>
<td>7.96</td>
</tr>
<tr>
<td>8.36</td>
<td>9.56</td>
<td>5.82</td>
<td>5.61</td>
<td>6.91</td>
<td>8.23</td>
</tr>
<tr>
<td>9.58</td>
<td>8.65</td>
<td>4.92</td>
<td>3.97</td>
<td>6.87</td>
<td>9.52</td>
</tr>
<tr>
<td>9.58</td>
<td>8.96</td>
<td>5.23</td>
<td>5.32</td>
<td>6.98</td>
<td>8.45</td>
</tr>
<tr>
<td><strong>9.31±0.19</strong></td>
<td><strong>8.94±0.20</strong></td>
<td><strong>4.99±0.19</strong></td>
<td><strong>4.89±0.37</strong></td>
<td><strong>6.74±0.15</strong></td>
<td><strong>8.18±0.31</strong></td>
</tr>
</tbody>
</table>

All values are expressed as Mean±SEM (n=5), p<0.05
Table 8.2: Effect Of Pyrimidines On SOD (U/mg Protein) Levels In Infarcted Tissue In Cerebral Ischemia Reperfusion Injury In Rats

All values are expressed as Mean±SEM (n=5), p<0.05

Table 8.3: Effect Of Pyrimidines On Catalase (μmoles/min/ mg Protein) Levels In Infarcted Tissue In Cerebral Ischemia Reperfusion Injury In Rats

<table>
<thead>
<tr>
<th>Normal Control</th>
<th>Sham Control</th>
<th>I/R Control</th>
<th>Vehicle control</th>
<th>Pyrimidine 1 (20 mg/kg)</th>
<th>Pyrimidine 2 (20 mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>109.93</td>
<td>105.96</td>
<td>34.12</td>
<td>37.69</td>
<td>65.02</td>
<td>80.12</td>
</tr>
<tr>
<td>110.92</td>
<td>102.63</td>
<td>36.23</td>
<td>38.12</td>
<td>67.12</td>
<td>90.65</td>
</tr>
<tr>
<td>115.92</td>
<td>106.18</td>
<td>40.12</td>
<td>36.35</td>
<td>68.12</td>
<td>88.53</td>
</tr>
<tr>
<td>114.05</td>
<td>104.58</td>
<td>35.68</td>
<td>35.98</td>
<td>69.69</td>
<td>86.58</td>
</tr>
<tr>
<td>113.91</td>
<td>105.25</td>
<td>39.84</td>
<td>38.25</td>
<td>68.36</td>
<td>89.89</td>
</tr>
<tr>
<td>116.32</td>
<td>109.25</td>
<td>46.32</td>
<td>39.54</td>
<td>63.46</td>
<td>92.36</td>
</tr>
<tr>
<td><strong>113.50±1.05</strong></td>
<td><strong>105.64±0.88</strong></td>
<td><strong>38.71±1.80</strong></td>
<td><strong>37.65±0.53</strong></td>
<td><strong>66.96±0.94</strong></td>
<td><strong>88.02±1.77</strong></td>
</tr>
</tbody>
</table>

All values are expressed as Mean±SEM (n=5), p<0.05
**Fig. 8.3:** Effect Of Pyrimidines On Catalase (μmoles/min/ mg Protein) Levels In Infarcted Tissue In Cerebral Ischemia Reperfusion Injury In Rats

All values are expressed as Mean±SEM (n=5), p<0.05

**Discussion**

Ischemia and reperfusion cause brain injury via multiple pathways. Previous studies demonstrate that reactive oxygen species are elevated during cerebral ischemia and reperfusion, which plays a major role in the pathophysiology of ischemic stroke or cerebral I/R related injury. In order to investigate the mechanism of protection induced by pyrimidines against the ischemic cerebral injury, lipid peroxidation and antioxidant defenses including SOD and CAT in the injured brain tissue of rats were measured [20].

The results demonstrate an increase in tissue MDA levels in parallel to significant increase in infarct size in the I/R control group when compared to sham control group.
Since MDA is end product of lipid peroxidation, the results clearly indicates the cytotoxic effect of free radical by peroxidation on brain tissue, because it contains large amount of phospholipids that are rich in polyunsaturated fatty acids leading to neuronal death.

Pyrimidines treatment significantly reduced the elevated tissue MDA levels contributing to partial protection. This may be because of involvement of multiple pathways in global cerebral ischemia-reperfusion injury. The beneficial effects of these pyrimidines are attributed to their antioxidant and anti-inflammatory properties. The evidence from our study clearly indicates that besides the peripheral organs, these pyrimidines may also help to prevent tissue damage from oxidative stress in the brain [21].

Increased nitric oxide concentrations associated with ischemia and reperfusion may have dual effects on lipid peroxidation. Nitric oxide reacts with free radicals, thereby producing the highly damaging peroxynitrite. Nitric oxide injury takes place for the most part through the peroxynitrite route because peroxynitrite can directly oxidize low density lipoproteins, resulting in irreversible damage to the cell membrane. When these pyrimidines are used free radicals are scavenged and therefore can no longer react with nitric oxide, resulting in less damage. In contrast, nitric oxide itself may directly inhibit lipid peroxidation by intercepting alkoxyl and peroxyl radical intermediates thereby terminating chain propagation reactions.[22]

Present study demonstrated that SOD and CAT levels were significantly reduced in ischemia-reperfusion (I/R) control group when compared to sham
To prevent oxidative damage, mammalian cells have developed a complex antioxidant defense system that include enzymatic activities (SOD, CAT, glutathione peroxidase) and free radical scavengers such as glutathione, vitamin C and E. SOD catalyzes the dismutation of superoxide radicals forming hydrogen peroxide. GPx and CAT are the unique enzymes scavenging hydroperoxides and therefore act in concert with SOD. Decrease in the antioxidant enzyme activities during ischemia and reperfusion is due to the attack of sulphydryl (-SH) groups of enzymes by oxygen free radicals and interaction of enzymes with peroxidation products, which can affect the active site of the enzyme. Another reason for reduction of enzyme activities can be attributed to the reduction in pH, i.e. acidosis. Ischemia renders the cells to undergo anaerobic metabolism thereby producing lactic acid and acidosis. Enzymes that are pH-sensitive will therefore be easily affected. Thus significant alteration in the antioxidant enzyme activities during cerebral ischemia and reperfusion may be responsible for more neurodegeneration than ischemia.[23]

Pyrimidines treatment in our present investigation increased the endogenous antioxidant enzymes SOD and CAT indicating enhanced biochemical defenses to scavenge the overproduced free radicals. The observed increase in antioxidant enzymes and decrease in MDA levels was more with pyrimidine 2 when compared to pyrimidine 1.

However, pyrimidines could not completely antagonize the lipid peroxidation observed in I/R control animals and at the same time, pyrimidines
could not completely restore the antioxidant reserves depleted in I/R control animals. Therefore, results indicate that antioxidant effects of these drugs are partially responsible for cerebroprotective activity.

Conclusion

The cerebroprotective action of pyrimidines is partially attributed to their antioxidant effect against cerebral ischemia reperfusion injury.

Further studies are needed to explore other possible mechanisms involved in cerebroprotective activity of pyrimidines.
REFERENCES


[15] Reddy MK, Labhasetwar V. Nanoparticle-mediated delivery of


