CHAPTER 9

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

Introduction

A number of pyrimidines are reported to possess anti-inflammatory activity *in vitro* and *in vivo*. Although not fully understood, several mechanisms of action are proposed to explain *in vivo* anti-inflammatory action. The important mechanism for anti-inflammatory activity is inhibition of eicosanoid generating enzymes including phospholipase A2, cyclooxygenases and lipoxygenases, thereby reducing the concentrations of prostanoids and leukotrienes. Other mechanisms include inhibition of histamine release, phosphodiesterase, protein kinases and activation of transcriptase [1-3].

There is increasing evidence to suggest that inflammatory processes contribute to the gush of events that lead to the progressive neuronal damage observed after cerebral ischemia followed by reperfusion. Therefore, treatment regimes aimed at modulating neuro-inflammatory processes may act to slow the progression of this devastating brain disorder. The fact that pyrimidines are able to modulate the activities of various mediators of inflammation has made us to explore the involvement of myeloperoxidase (MPO), tumor necrosis factor-α (TNF-α) and interleukin-10 (IL-10) in cerebroprotection offered by pyrimidines in I/R induced cerebral infarction in rats [4-6].
In response to inflammatory signals from ischemic and reperfused tissue, leukocytes initially accumulate in the vasculature by adhering to the vascular endothelium and plugging capillaries. This leads to excessive production of ROS, such as superoxide which increases vascular permeability and damage endothelial and/or brain directly, and produce endothelial-dependent vascular smooth muscle contraction, platelet activating factor that increases vascular permeability, neurotoxicity, and granular, enzymatic constituents including several cytotoxic lysosomal proteases, one being MPO.[7-10]

Enzymatically active MPO, together with hydrogen peroxide and chloride, produces the powerful oxidant hypochlorous acid, a key contributor to the oxygen-dependent microbicidal activity of phagocytes and radicalized oxygen species (O$_2^-$,ONOO$^-$) which induces apoptosis and nitrotyrosination of proteins. There is clear indication that the increase in brain MPO activity after cerebral ischemia and reperfusion reflects neutrophil infiltration into the brain. Thus myeloperoxidase (MPO) was used as a marker in our present study to quantify neutrophil accumulation [11-13].

In addition, inherent cells such as astrocytes, microglia, or endothelia have been found to be activated by cerebral injuries including ischemic stroke. These cells then become immunologically reactive and interact with each other by producing substances including cytokines and adhesion molecules [14-20].

These molecules appear to be responsible for the accumulation of inflammatory cells in the injured brain, and the resulting immunologic-inflammatory cascade produces an environment that may affect the survival of
j neurons subjected to ischemic injury constitutively express very low levels of MHC antigens, and are thus considered as 'immunologically silent', these cells, once stimulated, can express various cytokines and adhesion molecules. Three major cytokines namely tumor necrosis factor-α and interleukin-10 (IL-10) [21-28].

Our objective is to explore the anti-inflammatory effect of pyrimidines against I/R injury by estimating myeloperoxidase (MPO), tumor necrosis factor-α (TNF-α) and interleukin-10 (IL-10) activities in brain tissue.

**Experimental Protocol**

Wistar 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 Pyrimidine 1 (20 mg/kg) 10 min before reperfusion</td>
</tr>
<tr>
<td>5.</td>
<td>Group 5</td>
<td>Rats received Pyrimidine 2 (20 mg/kg) 10 min before reperfusion</td>
</tr>
</tbody>
</table>
**Animals**

Rats were randomly divided into five groups. Each group consists of 5 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 (1 and 2) 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 earlier chapter 6 for estimation of myeloperoxidase (MPO), tumor necrosis factor-α (TNF-α) and interleukin-10 (IL-10).

**Results**

Results of MPO levels are presented in Table 9.1 & Fig. 9.1. Results of TNF-α levels are presented in Table 9.2 & Fig. 9.2. Results of IL-10 levels are presented in Table 9.3 & Fig. 9.3. In comparison with I/R control group pyrimidines treatment significantly reduced the myeloperoxidase and TNF-α levels and thereby contributed to its anti-inflammatory activity. When compared to pyrimidine 1, pyrimidine 2 exhibited more degree of cerebroprotection in both the cases.

In comparison with I/R control group pyrimidines treatment significantly enhanced IL-10 levels and thereby initiated its anti-inflammatory activity. When compared to pyrimidine 1, pyrimidine 2 showed better increment in IL-10 levels thereby initiated its endogenous anti-inflammatory activity.
Table 9.1: Effect Of Pyrimidines On Myeloperoxidase (U/g Tissue) Levels 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>3.51</td>
<td>4.64</td>
<td>63.53</td>
<td>64.12</td>
<td>10.4</td>
<td>7.11</td>
</tr>
<tr>
<td>3.12</td>
<td>4.91</td>
<td>64.27</td>
<td>62.84</td>
<td>11.01</td>
<td>9.32</td>
</tr>
<tr>
<td>3.81</td>
<td>4.12</td>
<td>63.84</td>
<td>66.47</td>
<td>10.89</td>
<td>8.61</td>
</tr>
<tr>
<td>3.64</td>
<td>3.99</td>
<td>65.26</td>
<td>63.91</td>
<td>12.52</td>
<td>8.98</td>
</tr>
<tr>
<td>4.26</td>
<td>4.99</td>
<td>65.36</td>
<td>65.85</td>
<td>11.93</td>
<td>9.54</td>
</tr>
<tr>
<td>2.93</td>
<td>5.27</td>
<td>64.87</td>
<td>64.22</td>
<td>10.85</td>
<td>8.69</td>
</tr>
<tr>
<td>3.54±0.19</td>
<td>4.653±0.20</td>
<td>64.52±0.30</td>
<td>64.57±0.54</td>
<td>11.27±0.32</td>
<td>8.708±0.35</td>
</tr>
</tbody>
</table>

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

Fig. 9.1: Effect Of Pyrimidines On Myeloperoxidase (U/g Tissue) Levels In Cerebral Ischemia Reperfusion Injury In Rats

![Graph showing the effect of pyrimidines on myeloperoxidase levels in cerebral ischemia reperfusion injury in rats.](image)

All values are expressed as Mean±SEM (n=5), p<0.05
Table 9.2: Effect Of Pyrimidines On TNF-α (ng/mg Of Tissue) Levels 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>0.11</td>
<td>0.09</td>
<td>0.26</td>
<td>0.28</td>
<td>0.07</td>
<td>0.08</td>
</tr>
<tr>
<td>0.11</td>
<td>0.08</td>
<td>0.24</td>
<td>0.36</td>
<td>0.06</td>
<td>0.04</td>
</tr>
<tr>
<td>0.12</td>
<td>0.11</td>
<td>0.34</td>
<td>0.21</td>
<td>0.09</td>
<td>0.06</td>
</tr>
<tr>
<td>0.12</td>
<td>0.12</td>
<td>0.32</td>
<td>0.26</td>
<td>0.11</td>
<td>0.09</td>
</tr>
<tr>
<td>0.11</td>
<td>0.09</td>
<td>0.25</td>
<td>0.32</td>
<td>0.07</td>
<td>0.07</td>
</tr>
<tr>
<td>0.12</td>
<td>0.11</td>
<td>0.29</td>
<td>0.33</td>
<td>0.14</td>
<td>0.11</td>
</tr>
<tr>
<td>0.11±0.002</td>
<td>0.10±0.006</td>
<td>0.28±0.01</td>
<td>0.29±0.02</td>
<td>0.09±0.01</td>
<td>0.07±0.009</td>
</tr>
</tbody>
</table>

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

Table 9.2: Effect Of Pyrimidines On TNF-α (ng/mg Of Tissue) Levels In Cerebral Ischemia Reperfusion Injury In Rats

All values are expressed as Mean±SEM (n=5), p<0.05
Table 9.3: Effect Of Pyrimidines On IL-10 (ng/mg Of Tissue) Levels 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>1.35</td>
<td>1.45</td>
<td>0.73</td>
<td>0.72</td>
<td>1.55</td>
<td>1.55</td>
</tr>
<tr>
<td>1.51</td>
<td>1.53</td>
<td>0.93</td>
<td>0.89</td>
<td>1.26</td>
<td>1.08</td>
</tr>
<tr>
<td>1.76</td>
<td>1.54</td>
<td>0.96</td>
<td>0.76</td>
<td>1.32</td>
<td>1.32</td>
</tr>
<tr>
<td>1.81</td>
<td>1.57</td>
<td>0.96</td>
<td>0.53</td>
<td>1.46</td>
<td>1.53</td>
</tr>
<tr>
<td>1.92</td>
<td>1.65</td>
<td>0.99</td>
<td>0.79</td>
<td>1.54</td>
<td>1.72</td>
</tr>
<tr>
<td>1.64</td>
<td>1.74</td>
<td>0.89</td>
<td>0.64</td>
<td>1.26</td>
<td>1.46</td>
</tr>
<tr>
<td>1.66±0.08</td>
<td>1.58±0.04</td>
<td>0.91±0.03</td>
<td>0.72±0.05</td>
<td>1.39±0.05</td>
<td>1.44±0.08</td>
</tr>
</tbody>
</table>

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

Fig. 9.3: Effect Of Pyrimidines On IL-10 (ng/mg Of Tissue) Levels In Cerebral Ischemia Reperfusion Injury In Rats

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

Myeloperoxidase activity was significantly lower in animals treated with pyrimidines as compared with the nontreated group. These results suggest the anti-inflammatory role of pyrimidines by inhibiting neutrophil adherence to the endothelium during cerebral I/R period. Transient ischemia of the cerebral vasculature followed by reperfusion leads to a secondary cascade of pathophysiologic events, characterized by a complex inflammatory response. Inflammation in stroke has been traditionally identified on histopathology as neutrophil infiltration, which correlates positively with ischemic damage [29-35].

Myeloperoxidase (MPO) is the most abundant component in azurophilic granules in neutrophils and has often been used as a histopathological marker for neutrophils. It is also expressed in the myeloid line, especially in monocytes and macrophages/microglia. MPO interacts with hydrogen peroxide to generate highly reactive species including hypochlorite (OC1⁻) and radicalized oxygen species (O₂⁻, ONOO⁻). MPO-mediated radicalization of molecules induces apoptosis and nitrotyrosination of proteins. Thus, MPO is a key component of inflammation and has been shown to play a major role in animal models of stroke in the post hypoxic inflammatory response. MPO genotypes are associated with increased brain infarct size and poorer functional outcome. Therefore, inflammation is a complex cascade of events involving different types of cells and molecules, MPO could be used as an excellent biomarker for inflammation to access the extent of neutrophil infiltration during cerebral I/R
Pyrimidines treatment significantly reduced the inflammation characterized by decrease in myeloperoxidase activity in animal subjected to cerebral I/R injury. The evidence relating to neuroinflammatory modulation by pyrimidines supports the following mechanisms [41-48].

1) An inhibitory role on the release of cytokines, such as IL-1β and TNF-α, from activated glia.

2) An inhibitory action against iNOS induction and subsequent NO’ production in response to glial activation.

3) An ability to inhibit the activation of NADPH oxidase and subsequent ROS generation in activated glia.

4) A capacity to down regulate the activity of pro-inflammatory transcription factors such as NF-kB.

5) The potential to modulate signaling pathways such as MAPK cascade.

Similarly, pyrimidines treatment significantly reduced the inflammation characterized by decrease in tumor necrosis factor-α (TNF-α) in animal subjected to cerebral I/R injury. The evidence supports the anti-inflammatory role of pyrimidines in cerebral I/R period.

Interleukin-10 (IL-10) activity was considerably increased in animals treated with pyrimidines as compared with nontreated group. These results suggest the anti-inflammatory role of pyrimidines in cerebral I/R period.
Conclusion

The cerebroprotective actions of pyrimidines are partially attributed to their anti-inflammatory effects against I/R injury in rats as evidenced by significant reduction in pro-inflammatory markers MPO, TNF-α and significant increase in anti-inflammatory marker IL-10. **Pyrimidine 2** has offered more degree of anti-inflammatory activity when compared to **pyrimidine 1**. However further studies are needed to explore other possible mechanisms involvement in the cerebroprotective activity of pyrimidines.
REFERENCES


[20] Broughton BRS, Reutens D, Sobey CG. Apoptotic mechanisms after


