Studies on the Anti-inflammatory role of Resveratrol

in Its Cerebroprotective Potential against Cerebral

Ischemia-reperfusion Injury

11.1 Introduction

Resveratrol was reported to possess anti-inflammatory activity in vivo and in vitro (Issuree et al. 2009). Though its in vivo mechanisms of anti-inflammatory effects are not fully understood. In the inflammation, endogenous mediators (cytokine and chemokines) and recruitment of circulating leukocytes are involved (Gouwy et al. 2005). Cytokines play a key role in the attraction of leukocytes as potent inducers of Chemokines. Therefore cytokines act as primary mediators and chemokines act as secondary mediators to attract leucocytes in the inflammation. There is increasing evidence which suggests that reactive astrocytes are involved in ischemia-reperfusion injury to cause inflammation. They produce nitric oxide via cytokine-mediated induction iNOS, likely contributing to an increase in free radical formation during inflammation (Vaughan and Delanty 1999). The free radicals and inflammation come together which cause tissue apoptosis. Therefore, treatment regimes were aimed at modulation of neuroinflammation which may act to diminish devastating injury caused by ischemia-reperfusion.

Initially leucocytes accumulate in vasculature by adhering to endothelial tissues. This leads to excess ROS production and cause endothelial cell damage, vascular smooth muscle contraction, activated platelet factor. In addition, activated astrocytes and microglia are produced by cytokines and chemokines. These molecules appear to be responsible for accumulation of inflammatory cells in injured brain tissue. TNF-α, IL-1β and IL-6 are the important cytokines which initiate inflammatory mediators and inflammatory reactions and induce expression
of other cytokines after ischemic reperfusion injury. The ischemic brain was observed with increased levels of TNF-α, IL-6 and IL-1β. They are considered as a part of damaging response. IL-10 is a main cytokine which inhibits expression of TNF-α and IL-1β activity in the injured brain tissues. Several reports suggested IL-10 act as anti inflammatory cytokine in cerebral ischemia (Zhang et al. 2011; Spera et al. 1998).

The protective role of resveratrol in an acute model of ischemia-reperfusion, just at the point of reperfusion was evaluated in the present study to assess the anti-inflammatory role of resveratrol against cerebral reperfusion injury by quantifying MPO, TNF-α, IL-6, ICAM-1 and IL-10.

11.2 Experimental protocols

Wistar rats of either sex weighing between 250 to 300 g were used in the study. Experimental protocol was as follows

Group-1 Normal
Group-2 Sham control
Group-3 I/R (Rats recived 30 min BCCA occlusion and 4 hr reperfusion)
Group-4 Vehicle treated (0.1ml 10% of DMSO)
Group-5 Resveratrol treated (20 mg/kg)

Vehicle and Resveratrol (in 10% DMSO) were administered i.p. 5 min before reperfusion. Each group consisted of 6 animals.

Animals in all groups were anesthetized with thiopental sodium (30 mg/kg i.p) and were subjected to BCCA occlusion for 30min and 4 hr reperfusion as described in Chapter 8. At the end of experiment, the brains were removed quickly and the homogenate was prepared as described in the Chapter 8 for estimation of inflammatory markers.
11.3 Results

In this study it was found that MPO, TNF-α, IL-6 and ICAM-1 levels were increased significantly and IL-10 levels were decreased significantly in I/R group of rats as compared to Sham control group. In resveratrol treated groups, MPO, TNF-α, IL-6 and ICAM-1 levels were reduced significantly and IL-10 levels were increased significantly.

The results were shown in Table and Figures: 1&2

Table: Effect of resveratrol on inflammatory markers

<table>
<thead>
<tr>
<th>Assessments</th>
<th>Normal</th>
<th>Sham Control</th>
<th>I/R</th>
<th>Vehicle Treated I/R</th>
<th>Resveratrol (20 mg/kg i.p.) Treated</th>
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</thead>
<tbody>
<tr>
<td>MPO (Units/gm of wet tissue)</td>
<td>3.520 ± 0.191</td>
<td>4.603 ± 0.205</td>
<td>64.490 ± 0.316</td>
<td>64.220 ± 0.642</td>
<td>4.232 ± 0.229</td>
</tr>
<tr>
<td>TNF-α (ng/mg of tissue)</td>
<td>0.110 ± 0.004</td>
<td>0.095 ± 0.004</td>
<td>0.283 ± 0.016</td>
<td>0.277 ± 0.016</td>
<td>0.103 ± 0.014</td>
</tr>
<tr>
<td>IL-6 (ng/mg of tissue)</td>
<td>0.163 ± 0.019</td>
<td>0.188 ± 0.020</td>
<td>0.490 ± 0.037</td>
<td>0.442 ± 0.023</td>
<td>0.235 ± 0.021</td>
</tr>
<tr>
<td>ICAM-I (pg/mg of tissue)</td>
<td>243.80 ± 14.580</td>
<td>253.30 ± 16.230</td>
<td>517.80 ± 46.020</td>
<td>515.70 ± 29.300</td>
<td>246.50 ± 18.420</td>
</tr>
<tr>
<td>IL-10 (ng/mg of tissue)</td>
<td>1.665 ± 0.085</td>
<td>1.485 ± 0.095</td>
<td>0.680 ± 0.137</td>
<td>0.678 ± 0.095</td>
<td>1.422 ± 0.083</td>
</tr>
</tbody>
</table>
Fig 1 and 2: Effect of Resveratrol on Inflammatory Markers

P≤ 0.05, I/R indicates ischemia and reperfusion, MPO indicates Myeloperoxidase, TNF-α indicates Tumor necrosis factor-alpha, IL-6 indicates Interleukin-6, ICAM-1 indicates Intracellular adhesive molecule-1, IL-10 indicates Interleukin-10.
11.4 Discussion

In cerebral ischemia-reperfusion injured tissue, ROS and pro-inflammatory mediators (cytokines and chemokines) will be released. Furthermore, these mediators induce release of intercellular adhesive molecules on leukocytes and thus promote the adhesion and transendothelial migration of circulatory leucocytes (Frank et al. 2008). Thus adhesion molecules are need to excavate neutrophils. Vascular adhesion molecules have been shown to play a prominent role in cerebral ischemia-reperfusion injury. TNF-α has been shown to be involved in generation of oxidant and activation of proteinkinase C (PKC) (Rahman et al. 1999). TNF-α is mainly responsible for activation of NF-κB (nuclear factor kappa-light-chain-enhancer of activated B cells) signaling pathway and for the expression of adhesion molecules such as ICAM-1 and VCAM-1 in the endothelium (Rahman et al. 1999, 2011). PKC activation and oxidant generation were both necessary for ICAM-1 expression and NF-kB activation (Rahman et al. 1999, 2011). These adhesion molecules allow the leukocytes to the endothelium and may allow their subsequent transmigration into peripheral tissue (Frank et al. 2008). IL-6 is an endogenous and hematopoietic cytokine that plays an important role after traumatic injuries. It is involved in induction of B cell differentiation and helps to attract T-lymphocytes into the brain, contributing to exacerbation of the inflammatory response. IL-6 induces neutrophilia and reduces CD162 expression on neutrophils in inflammation (Hashizume et al. 2011).

During progressive conditions of inflammation leukocytes will interact with the endothelium that will permit these cells to cross the barrier created by endothelial cells. In this phase, infiltrating leucocytes release cytokines and chemokines; particularly they exacerbate ROS production and stress signaling
pathways, which amplifies the brain inflammation, destruction of blood brain barrier (BBB), brain edema and neuronal death (Frank et al. 2008). Cerebral ischemia and reperfusion injury is followed by appearance of inflammatory cytokines such as MPO, IL-1, IL-6, TNF-α and ICAM-1 in the circulation (Bruning et al. 2012; Wong et al. 2008), which further worsens the ischemia-reperfusion damage. IL-10 is a regulatory cytokine, which regulates the expression of TNF-α, IL-6, IL-1β and other cytokines. Several evidences also suggest that IL-10 act as an anti-inflammatory cytokine in cerebral ischemia (Spera et al. 1998).

In normal brain microglial cells are defense against pathogens, activated microglia cells can protect neurons through the engulfment of invading polymorphonuclear neutrophils or through the release of neurotrophic and anti-inflammatory factors (Block et al. 2007; Neumann et al. 2008). In ischemia-reperfusion injury, microglia become over activated and induces highly detrimental neurotoxic consequences through the excess production of many cytotoxic factors such as superoxide, nitric oxide, TNF-α, adhesion molecules, free radicals and cytokines (Kato et al. 1996; Colton and Gilbert 1987; Liu et al. 1996; Moss and Bates 2001; Liu et al. 2002 and Sawada et al. 1989).

In the present study, MPO, TNF-α, IL-6, ICAM-1 and IL-10 in brain tissue were estimated by using ELISA kits. A significant increase in the levels of MPO, TNF-α, IL-6, ICAM-1 and a significant decrease in the levels of IL-10 was observed in I/R group rats when compared to Sham control group. A significant reduction in the levels of MPO, TNF-α, IL-6, ICAM-1 and a significant increase in the levels of IL-10 was observed in resveratrol treated group. TNF-α, IL-6, ICAM-1 are pro-inflammatory cytokines (Saito 1996; Park et al. 2009) and IL-10
is an anti-inflammatory cytokine (Liu et al. 2009). These results indicate that infiltration of neutrophil and release of cytokines are involved in I/R injury. These results are in accordance with the earlier reports (Liu et al. 2011; Park et al. 2009; Bruning et al. 2012). Anti inflammatory mechanism of resveratrol was confirmed by reduction of infiltration of neutrophils, reduction of pro inflammatory mediators (TNF-alpha, IL-6, ICAM-1) and increasing of anti-inflammatory mediator (IL-10). From these findings we suggest that resveratrol might be having cerebroprotection against cerebral ischemia-reperfusion injury by anti-inflammatory effect.

11.5 Conclusion

Resveratrol treated rats showed significant diminishing of the pro-inflammation response (TNF-α, IL-6 and ICAM-1) and significantly improved anti inflammatory response (IL-10). Hence the anti inflammatory effect of resveratrol is attributed to its cerebroprotective potential against cerebral ischemia and reperfusion injury. However, further studies are needed to investigate other possible mechanisms involved in cerebroprotective potential of resveratrol.
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