The anti-inflammatory effects of fluoxetine on activated microglia  

A systematic review

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Summary

Introduction: This article reviews current literature about the anti-inflammatory effects of Fluoxetine on activated microglia in the brain. Evidence of this effect would strengthen the belief that inflammation plays a role in the development of depression and explain the therapeutic effect of SSRI’s.

Methods: A search in PubMed for the Medical Subject Headings (Mesh) term Microglia in combination with the Mesh term Serotonin Uptake Inhibitors or Fluoxetine.

Results: We found 7 eligible articles. Fluoxetine treated microglia showed significant reduction of microglia activation in 2 articles and 4 articles showed significant reduction in nitric oxide (NO) and inducible nitric oxide synthase (iNOS). Inflammatory-markers were also significantly reduced in 5 articles and 4 articles showed significant suppression of NF-κB activity. Reactive oxygen species (ROS) were significantly reduced in 3 articles. 6 studies found a reduction of micoglial activity and 4 found significant reductions.

Conclusion: In all studies we found at least 2 significant reductions in microglial inflammatory parameters due to Fluoxetine. All articles showed reduction in inflammatory parameters. This supports the idea that SRRI’s have an anti-inflammatory effect in the brain.

Introduction

Selective Serotonin Re-uptake inhibitors (SSRI’s) are a group of anti-depressants, often prescribed for depression because they are safe and well tolerated.[1-3] The effect of SSRI’s used to be explained by the monoamine theory which stated that depression was caused by a deficiency of serotonin. However, as proven with tryptophan depletions experiments, this could not explain the entire effect of the SSRI’s.[4,5]

In recent years there is the increasing amount of evidence for the role of the immune system in the pathogenesis of depression. Studies have proven that the activity of the innate immune system is increased in depression, usually with elevated plasma levels of tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6) and C-reactive protein (CRP).[6-10] Because of this speculation arose that SSRI’s may partially work through an anti-inflammatory effect.[11-13] It was for example found that Fluoxetine, a SSRI, suppressed the number of inflammatory cells and the TNF-α release from monocytes in animal models.[14]

The question is however, if this anti-inflammatory effect translates to the brain. This finding would strengthen the belief that inflammation plays a role in the development of depression and explain the therapeutic effect of SSRI’s. This article investigates if current literature supports the anti-inflammatory effect of SSRI’s in the brain.

The central component in the inflammatory reaction in the brain is the microglia, a macrophage specific for the central nervous system.[15] In activated state these microglia produce various pro-inflammatory cytokines, such as TNF-α, and no longer function in a growth stimulating way as they do in steady state.[16]

This article reviews current literature about the effects of Fluoxetine, a commonly prescribed SSRI, on activated microglia to examine its anti-inflammatory effect in the brain.[3] Microglial activation was achieved by treatment with lipopolysaccharide (LPS), kainic acid (KA), 1-methyl-4-phenyl-pyridinium (MPP+) or by causing cerebral ischemia in several animal models.[17-23]

Methods

We conducted a search in PubMed at January 26th 2013. We searched PubMed for the Medical Subject Headings (Mesh) term Microglia in combination with the Mesh term Serotonin Uptake Inhibitors or Fluoxetine. Our exact search was: “Microglia”[Mesh] AND (“Serotonin Uptake Inhibitors”[Mesh] OR “Fluoxetine”[Mesh]). Based on title and abstract we included all articles which studied the effects of Fluoxetine on activated Microglia. We only looked at the response of activated microglia to Fluoxetine to prevent confounding factors. No other in- or exclusion criteria were necessary. All studies compared Fluoxetine treatment with either a placebo or no treatment in activated microglia.
We looked at the effects of Fluoxetine on microglial activation in general and more specifically several parameters which show inflammation, namely production of nitric oxide (NO), inducible nitric oxide synthase (iNOS), pro-inflammatory of nitric oxide (NO), inducible nitric oxide synthase (iNOS), pro-inflammatory markers, reactive oxygen species (ROS) and transcription factor NF-κB.

**Results**

Our PubMed search produced 14 articles. After applying the inclusion criteria, 7 articles remained [17–23], which investigated the effect of fluoxetine on activated microglia. All articles were published within the last 4 years, as seen in Table 1. Microglia were cultured from rat brain [18,20,23], mouse brain [19,21] and BV2 microglial cell lines [17,22]. The microglia were activated using LPS, MPP+, KA or cerebral ischemia. Six studies investigated the microglia separately [17,18,20–23], one study measured microglial parameters within hippocampus regions. All articles defined statistical significance as $p < 0.05$.

**Dosage**

Out of the 7 studies, 5 used different dosages of Fluoxetine. [17,19,20,22,23] In these 5 studies, we noticed in at least one microglial parameter, that a lower dose yielded no significant reduction whereas a higher dose did. One study mentioned a significant pro-inflammatory effect on activated microglia which were treated with a lower dose of Fluoxetine.[17]

**Table 1 - Study Characteristics**

<table>
<thead>
<tr>
<th>Article</th>
<th>Mechanism of microglia activation</th>
<th>Microglia source</th>
<th>Parameters investigated</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tynan et al, 2012 [17]</td>
<td>LPS</td>
<td>BV2 microglial cell line</td>
<td>TNF-α, NO</td>
</tr>
<tr>
<td>Chung et al, 2010 [18]</td>
<td>LPS</td>
<td>rat brain (SN)</td>
<td>activation (ED1, OX-42), iNOS, NADPH oxidase, ROS, protein carbonyl</td>
</tr>
<tr>
<td>Jin et al, 2009 [19]</td>
<td>KA</td>
<td>CA1 and CA3 mouse hippocampus regions</td>
<td>activation (Iba-1, COX-2#, IL-1β#, TNFα#, NF-κB (IκBα))</td>
</tr>
<tr>
<td>Lim et al, 2009 [20]</td>
<td>LPS, cerebral ischemia</td>
<td>rat brain, primary microglia culture</td>
<td>activation (Iba-1, Mac2), COX-2#, IL-1β#, TNFα#, iNOS#, NO, NF-κB</td>
</tr>
<tr>
<td>Chung et al, 2011 [21]</td>
<td>MPP+</td>
<td>mouse brain (SN), microglia culture</td>
<td>activation (ED1, Mac1), IL-1β#, TNFα#, iNOS#, NO, NADPH oxidase, ROS, protein carbonyl</td>
</tr>
<tr>
<td>Liu et al, 2011 [22]</td>
<td>LPS</td>
<td>BV2 microglial cell line, primary microglial culture</td>
<td>activation (CD11b), TNF-α*, IL-6*, iNOS*, NO, NF-κB (p65, DNA binding)</td>
</tr>
<tr>
<td>Zhang et al, 2012 [23]</td>
<td>LPS, MPP+</td>
<td>rat brain cultures</td>
<td>activation (Iba-1), TNF-α*, IL-1β*, iNOS#, NO, ROS, NF-κB (p65, IκBα, IκBβ)</td>
</tr>
</tbody>
</table>

# RNA expression was studied

* RNAand protein expression were studied

Abbreviations: LPS; lipopolysaccharide, SN; substantia nigra, KA; kainic acid, MPP+; 1-methyl-4-phenylpyridinium, TNF-α; tumour necrosis factor-α, NO; nitric oxide, iNOS; inducible nitric oxide synthase, IL; interleukin, ROS; reactive oxygen species, NF-κB; nuclear factor-kappaB, IκBα; nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor alpha, IκBβ; inhibitor of nuclear factor kappa-B kinase subunit beta, COX; Cyclooxygenase, NADPH; Nicotinamide adenine dinucleotide phosphate.
We limited ourselves to the effect of Fluoxetine on activated Microglia because SSRI’s seem to have an inflammatory effect in steady state microglia. To eliminate unknown differences in effects between SSRI’s we chose to look only at Fluoxetine, one of the most studied SSRI’s and to give clear explanation about the dosage used.

Despite the limitations of our review, all research supports the anti-inflammatory effect of Fluoxetine on the brain. This could lead to new insights in the pathophysiology of depression and successful treatment of depression.

This orientating review only studied the effect of the highest dosage of SSRI’s. We also limited ourselves to Fluoxetine to prevent confounding factors because of possible differences between SSRI’s. In the orientation process these were necessary but important limitations but we encourage future research to compare all types of SSRI’s at different dosages.

At least 2 of the following parameters were significantly reduced in all studies: microglial activation, NO, iNOS, ROS, and NF-κB production, and pro-inflammatory marker expression (COX-2, TNF-α, IL-1β, IL-6). The effect seems to be dose-dependent in most studies. Six studies looked at microglial activation. All found a reduction of activity and 4 found significant reductions. This makes it likely that fluoxetine does have an anti-inflammatory effect in the brain.

The anti-inflammatory effect in the brain can also help to explain the functional pathway of SSRI’s in depression. The underlying mechanism of this anti-inflammatory effect however is still unknown. One hypothesis for the mechanism of action is the suppression of NF-κB by Fluoxetine.[14] NF-κB is an important transcription factor for the production of pro-inflammatory factors like TNF-α, NO and IL-6 via the transcription of Nos2.[17,24] However, the effect seems strongly dose and cell dependant and it is not known how Fluoxetine exactly suppresses NF-κB.[20,24] This mechanism should be further investigated. Possibly, this knowledge could lead to a better understanding of the pathogenesis and successful treatment of depression.

This orientating review only studied the effect of the highest dosage of SSRI’s. We also limited ourselves to Fluoxetine to prevent confounding factors because of possible differences between SSRI’s. In the orientation process these were necessary but important limitations but we encourage future research to compare all types of SSRI’s at different dosages.

Also, some of the studies only visualised effects of Fluoxetine without quantifying these differences or calculating if these differences were statistically significant. This makes it difficult to compare findings. We advise future researcher to quantify their research data instead of solely using visualisation, and to give clear explanation about the dosage used.

Table 2 - Effects of the highest Fluoxetine dose on activated microglia

<table>
<thead>
<tr>
<th>Article</th>
<th>Activation</th>
<th>NO</th>
<th>iNOS</th>
<th>pro-inflammatory factors*</th>
<th>NF-κB</th>
<th>ROS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tynan et al, 2012 [17]</td>
<td>↓</td>
<td></td>
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<tr>
<td>Chung et al, 2010 [18]</td>
<td>↓*</td>
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<tr>
<td>Jin et al, 2009 [19]</td>
<td>↓*</td>
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<td>Chung et al, 2011 [21]</td>
<td>↓*</td>
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<td>Zhang et al, 2012 [23]</td>
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</tbody>
</table>

Empty boxes represent parameters that were not determined in the concerning study

* Any of the following molecules: COX-2, TNF-α, IL-1β, IL-6
* Studied the effects on NO and NF-κB in the whole hippocampus
↓ significant reduction, p < 0.05
↓* Visualised reduction without p-value

**Oxidative stress**

Three studies assessed the influence of Fluoxetine on ROS produced by activated microglia.[18,21,23] In all 3 studies ROS was significantly lower in Fluoxetine treated microglia (Table 2). Furthermore, 5 studies examined NO production by activated microglia, 4 of which mentioned significant reduction in Fluoxetine treated microglia (Table 2).[17,20-23] The expression of iNOS in Fluoxetine treated microglia was also measured in 5 articles using iNOS mRNA expression, protein levels, or both.[18,20-23] Three studies found a significant reduction in the mRNA expression of iNOS.[21-23] Two studies found a significant reduction in the iNOS protein expression.[18,21] More reductions in iNOS mRNA and protein expression were found, unfortunately these results were not quantified, but only visualised.[18,20,22]

**NF-κB activity**

The NF-κB pathway activity was studied in 4 articles.[19,20,22,23] All 4 studies found that the NF-κB activity was significantly supressed in Fluoxetine treated microglia.

Discussion/Conclusions

For a long time the effect of SSRIs was explained by the inhibition of serotonin re-uptake.[1-5] However, in the last years, increasing evidence has been found that this group of anti-depressants also exerts an anti-inflammatory effect.[14] It is important to study if this effect translates to the brain because it would give a new perspective on the development and treatment of depression. It also suggests that SSRI’s could be used in the treatment of inflammation. Therefore in this review recent literature was studied to give a current view on the anti-inflammatory effect of SSRI’s in the brain.

We limited ourselves to the effect of Fluoxetine on activated Microglia because SSRI’s seem to have an inflammatory effect in steady state microglia.[24] To eliminate unknown differences in effects between SSRI’s we chose to look only at Fluoxetine, one of the most studied SSRI’s and different dosages, at its highest dosage.

We found significant and visualised reductions due to Fluoxetine in microglial activation and inflammatory factors in all studied disease models, both in vivo and in vitro.

Table 2 - Effects of the highest Fluoxetine dose on activated microglia
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References