Short Communication

Imatinib methanesulfonate reduces hippocampal amyloid-beta and restores cognitive function following repeated endotoxin exposure

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ABSTRACT

Alzheimer’s disease (AD) is characterized, in part, by atrophy of the adult brain and increased presence of extracellular amyloid-beta (Aβ) plaques. Previous studies in our lab have shown that peripheral inflammation can lead to increased central Aβ and deficits in learning and memory. In order to determine whether Aβ accumulation in the brain is responsible for the learning deficits, we attempted to decrease peripheral production of Aβ in order to reduce central Aβ accumulation. It has previously been shown that Aβ is produced in large quantities in the liver, and is transferred across the blood–brain barrier (BBB). Recent research has shown that peripheral treatment with imatinib methanesulfonate salt (IM), known to interfere with the interaction between gamma (γ)-secretase and the γ-secretase activating protein (GASAP), decreases the cleavage of peripheral amyloid precursor protein into Aβ. Because IM poorly penetrates the BBB, we hypothesized that co-administration of IM with LPS would decrease peripheral production of Aβ in the presence of LPS-induced inflammation, leading to a decrease in Aβ accumulation in the hippocampus. We show that peripheral IM treatment eliminates hippocampal Aβ elevation that occurs during peripheral inflammation, leading to a decrease in Aβ accumulation and thus rescue hippocampus-dependent cognitive deficits.

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1. Introduction

The Alzheimer’s Association estimates that 5.4 million Americans are currently living with Alzheimer’s disease (AD), resulting in over $200 billion in medical bills and lost wages (Alzheimer Fact Sheet, 2012). AD symptoms usually begin with mild cognitive deficits, typically affecting short-term memory, followed by a decline in cognitive abilities (Hennek and O’Banion, 2007). These behavioral symptoms correspond with a reduction in synapses and neurons (Mattson, 2004), as well as increases in neurofibrillary tangles and senile plaques (Cras et al., 1995; Murphy and LeVine, 2010). Further, several gene mutations are associated with the early-onset form of AD, including mutations in the amyloid precursor protein (APP) and presenilin-1 and -2 (PS-1, -2) genes. These genes are involved in the processing and production of the amyloid-beta (Aβ) peptides, which aggregate to form plaques in an age-dependent manner (Terry, 2006). Thus, decreasing Aβ production or deposition has become an important therapeutic goal for the treatment of AD (Hardy and Selkoe, 2002).

A common feature in the emerging research on neurodegenerative diseases, such as AD, is the impact of inflammatory processes on progression of the disease (Minghetti, 2005). For example, when animals with pre-existing neurodegenerative diseases were administered the bacterial mimetic lipopolysaccharide (LPS), an exacerbated inflammatory response occurred that accelerated the pre-existing neurodegeneration (Cunningham et al., 2009; Kitazawa et al., 2005; McAlpine et al., 2009). Such results indicate that immune challenges could possibly induce early onset and/or more rapid progression of an underlying neurodegenerative disease. However, these findings do not implicate peripheral inflammation as a causative factor in the onset of neurodegenerative diseases in animals that are not already predisposed to such an outcome. Recent studies have shown that repeated peripheral injections of LPS in non-transgenic animals can lead to an increase in central Aβ (Kahn et al., 2012; Lee et al., 2008), accompanied by hippocampus-dependent cognitive deficits (Kahn et al., 2012). Both laboratories hypothesized that the cognitive disruption was due to the elevation in hippocampal-Aβ. Here, we extend these findings by proposing that γ-secretase inhibition can attenuate the accumulation of hippocampal-Aβ that occurs during peripheral inflammation and thus rescue hippocampus-dependent cognitive deficits.
Imatinib (IM), the active component of the FDA approved anti-
cancer drug Gleevec™, is an ABL-specific tyrosine kinase inhibitor
with known secondary effects, including the ability to interfere
with the interaction of γ-secretase activating protein (GSAP) and
γ-secretase (He et al., 2010). The disruption of this interaction re-
sults in a reduction of γ-secretase activity, and concomitantly re-
duced cleavage of amyloid precursor protein into the toxic Aβ
peptide. Recently, IM was shown to decrease both plasma and
brain levels of Aβ in wild-type mice (Sutcliffe et al., 2011). These
studies reinforce the idea that γ-secretase inhibition is a plausible
therapeutic mechanism in the fight against AD.

The present study tested the hypothesis that IM could decrease
the central accumulation of Aβ in our LPS-induced inflammatory
model (Kahn et al., 2012; Lee et al., 2008). Further, we propose that
because IM poorly crosses the blood–brain barrier (BBB) (Takay-
ama et al., 2002; Williams et al., 2007; Wolff et al., 2003), Aβ must
be produced in the periphery and is transported across the BBB. We
demonstrate here that IM treatment blocks accumulation of Aβ in
the hippocampus and subsequently restores normal cognitive
function following peripheral inflammation.

2. Methods

2.1. Subjects

Male C57BL/6j mice (4–6 month old), bred in the TCU vivarium
from a breeding stock from The Jackson Laboratory (Bar Harbor,
ME), were utilized in all experiments. All animals were housed and
cared for in accordance with the Guide for the Care and Use
of Laboratory Animals (National Research Council, 2010), and
in accordance with protocols approved by the Institutional Animal
Care and Use Committee (IACUC) of TCU.

2.2. Treatment conditions

Intraperitoneal (i.p.) injections of 20 mg/kg IM or volume-
equivalent saline were administered twice a day for 14 days (days
1–14). During days 7–14, an additional single injection of 250 μg/
kg LPS (Escherichia coli serotype: 055:B5; Sigma–Aldrich, St. Louis,
MO) or saline was also administered (Fig. 1A). This procedure re-
sulted in four treatment groups: IM-saline, IM-LPS, saline-saline,
and saline-LPS. The dose of IM was chosen based on previously
published work (Sutcliffe et al., 2011) and should have no toxic af-
ects at that dose (Williams et al., 2007).

2.3. Tissue preparation

After completion of all treatments, mice were euthanized by
CO₂ inhalation and hippocampal tissue was immediately extracted
and prepared for protein assay and ELISA procedures. For the ELISA
procedure, tissues were homogenized with protein extraction solu-
tion (PRO-PREP, Boca Scientific, Boca Raton, FL) containing prote-
ase inhibitors and left on ice for an additional 30 min, followed
by overnight storage at −80 °C. This crude lysate was next centrifu-
ged at 16,000g for 30 min, and the purified lysate removed for
DC Protein Assay (Bio-Rad Laboratories, Hercules, CA) and subse-
quent Aβ analysis ELISA (Covance Research Products, Dedham, MA).

2.4. Aβ ELISA procedure

The BetaMark Aβ ELISA (Covance Research Products, Ded-
ham, MA) was performed in accordance with manufacturer
instructions and read on a multi-well spectrophotometer at
620 nm (BMG LabTech FLUOstar Omega, Cary, NC).

2.5. Blood assays

Blood was collected from the tail vein of mice 4 h after the first
LPS injection. Serum was isolated and used to measure peripheral
pro-inflammatory cytokines IL-6, TNF-α, as well as chemokines,
MCP-1, and MIP-1α, by cytometric bead array (CBA; BD Biosci-
ences, San Jose, CA), in accordance with kit instructions. Data were
collected on a FACSCalibur flow cytometer (BD Biosciences, San
Jose, CA) using CellQuest Pro software (BD Biosciences), and ana-
lyzed using FCAP Array software (Soft Flow, Inc., New Brighton,
MN). From other animals, plasma was isolated and used for Aβ
ELISA.

2.6. Contextual fear conditioning

During contextual fear conditioning (CFC), freezing behavior
was measured in automated fear conditioning chambers (Coulbo-
urn Instruments, Whitehall, PA, 7Wx7Dx12H), and monitored
using FreezeFrame™ software (ActiMetrics Software, Wilmette,
IL). The chambers use an electrified grid floor through which the
aversive stimulus was delivered. Dotted wall patterns and an off-
tory cue are also included, similar to work conducted previously
in our lab (Kranjac et al., 2012). Animals were trained on day 15, 24 h
after the last injection, and tested 24 h later, on day 16. The train-
ing session began with a 120-s acclimation period, followed by
a single 2-s 0.7 mA shock. Animals then remained in the apparatus
for 60 s. On the testing day, no shocks were delivered, though the
system recorded the movement of the animal for 90 s. Freezing
behavior, an innate response to fear in rodents, was monitored,
and total freezing time (sec) was collected as the dependent vari-
able indicating learning of the context/aversive stimulus pairing.

2.7. Statistical analyses

Data obtained from biological assays and behavioral paradigms
were analyzed using Statview 5.0.1 software (SAS Institute Inc.,
Cary, NC). For the plasma ELISA, a one-way Analysis of Variance
(ANOVA) was utilized. For all other biological assays, as well
as CFC, a 2 (IV: LPS, saline) × 2 (IV: IM, saline) ANOVA was used to
determine any significant main effects and interactions. All data
in figures are shown as mean ± SEM. The alpha level used for all
statistical analyses was 0.05, followed by Fisher’s PLSD post hoc
tests for significant omnibus effects.

3. Results

3.1. LPS-induced inflammation is observed despite IM co-
administration

In order to show that co-administration of IM for 7 days prior to
endotoxin exposure would not alter the inflammatory process trig-
gered by LPS administration, it was necessary to detect pro-inflam-
mmatory cytokine levels 4 h after the first injection of LPS. As
hypothesized, there was no significant main effect of treatment
(IM or saline) on the levels of pro-inflammatory cytokines (IL-6 and
TNF-α) or chemokines (MCP-1 and Mip-1α), indicating that
cytokine and chemokine levels in animals injected with IM-saline
did not differ from those injected with saline-saline (Fig. 1). Also
as hypothesized, analysis revealed a main effect of condition (LPS
or saline) for IL-6 (*F(1,16) = 33.984, p < 0.0001), TNF-α
(*F(1,16) = 16.304, p < 0.01), MCP-1 (*F(1,16) = 51.152, p < 0.0001),
and Mip-1α (*F(1,16) = 6.524, p < 0.05), indicating significantly in-
creased levels of pro-inflammatory cytokines and chemokines in
both groups of animals receiving LPS. Fisher’s PLSD post hoc analy-
yses revealed that there was no difference between LPS treatment
groups that received either saline or IM in any of the cytokines or chemokines measured \( (p > 0.05) \). Our results indicate that 7 days of IM treatment did not interfere with the production of these pro-inflammatory cytokines and chemokines, indicating that potential explanations, based upon the reduction in Aβ, could not be confounded by a possible reduction in peripheral inflammation.

### 3.2. IM reduces LPS-induced plasma and hippocampal Aβ levels

To assess whether i.p. LPS administration leads to peripheral Aβ production, and whether IM pre-treatment could alter this Aβ production, an Aβ ELISA was conducted on plasma that was isolated 4 h after the first LPS injection. As a control, we used animals that received 7 days of Sal, followed by a single injection of Sal. We found a main effect of treatment \( (F(2,10) = 9.045, p < 0.05) \). Fisher’s PLSD analysis revealed that animals that received Sal for 7 days, followed by LPS displayed a significant elevation of plasma Aβ \( (p < 0.01) \) when compared to animals that were instead pre-treated for 7 days with IM, or over the control group.

In order to determine whether co-administration of IM with repeated LPS exposure could lead to a decrease in the accumulation of Aβ in the hippocampus, tissue was analyzed using an Aβ ELISA. As expected, there was a main effect of condition (LPS or saline; \( F(1,36) = 15.143, p < 0.001 \)), a main effect of treatment (IM or saline; \( F(1,36) = 5.435, p < 0.05 \)), and a significant interaction of condition \( \times \) treatment \( (F(1,36) = 6.577, p < 0.05) \) on Aβ production. As we hypothesized, there were no significant increases in Aβ when animals were injected with either saline-saline or IM-saline. Fish-
er’s PLSD post hoc analyses revealed that i.p. administration of saline-LPS led to a significant increase in hippocampal Aβ, as compared to animals that were co-administered IM and LPS (p < 0.05). Our results indicate that IM does in fact decrease hippocampal Aβ, most likely via its effects in the periphery.

3.3. IM administration restores cognitive function following LPS exposure

In order to assess whether co-administration of IM could restore cognitive function following LPS exposure, freezing behavior in the hippocampus-dependent CFC task was analyzed. As anticipated, there were no significant main effects of condition (LPS or saline; F(1,36) = 0.134, NS), treatment (IM or saline; F(1,36) = 3.314, NS), or interaction of condition x treatment (F(1,36) = 0.839, NS) on behavior during the training day. This indicated that there were no significant behavioral differences in our four experimental groups prior to treatment. On the testing day, as hypothesized, there was a main effect of condition (F(1,36) = 5.399, p < 0.05), a main effect of treatment (F(1,36) = 9.173, p < 0.01), and a significant interaction of condition x treatment (F(1,36) = 4.251, p < 0.05) on freezing behavior (see Fig. 2). Fisher’s PLSD post hoc analyses revealed that animals treated for 7 days with LPS (our saline-LPS group) displayed significant cognitive deficits as compared to all other groups (p < 0.05). Interestingly, we found that when animals were co-administered IM and LPS, cognitive function was rescued, and was not significantly different from animals administered saline-saline or IM-saline, suggesting that LPS-induced cognitive deficits may be due to the accumulation of significant levels of Aβ peptide in the hippocampus.

4. Discussion

Our lab has shown that repeated peripheral LPS administration in the mouse leads to increased hippocampal-Aβ and cognitive deficits found using the hippocampus-dependent Morris water maze and contextual fear conditioning paradigms, but not hippocampus-independent auditory-cued conditioning (Kahn et al., 2012). In addition, we demonstrated that the cognitive deficits were not confounded by sickness, as animals became tolerant of the LPS. By the seventh day of LPS treatment, animals no longer produced elevations in pro-inflammatory cytokines (Kahn et al., 2012). Findings from Sutcliffe et al. (2011) suggested that a major source of rodent Aβ is the liver, and that Aβ is transported into the brain. In order for us to determine whether the cognitive deficits seen in the current and previous experiments were due to peripherally produced Aβ, IM was administered for 14 consecutive days, along with LPS during the final 7 days. Our results indicate that IM blocks the elevation in Aβ found in the blood and in the hippocampus following LPS-induced inflammation. Because IM poorly penetrates the BBB, and because IM blocks the acute elevation of plasma Aβ, these results suggest that the LPS-induced peripheral inflammation likely leads to peripherally produced Aβ that is transported into the brain.

Fig. 2. A. LPS-induced Aβ production is blocked by IM co-administration. Following seven consecutive days of LPS treatment, Aβ is significantly elevated in the mouse hippocampus (p < 0.05; n = 10). Prior (7 days) and LPS-concomitant (7 days) administration of IM blocks the elevation in Aβ in the hippocampus. (B) LPS-induced cognitive deficit is rescued by IM co-administration. Following seven consecutive days of LPS treatment, contextual fear conditioning is significantly impaired (p < 0.05; n = 10). Prior (7 days) and LPS-concomitant (7 days) administration of IM rescues cognitive function. (C) Administration of IM for one week prior to a single i.p. injection of LPS blocks the peripheral production of Aβ measured in the serum. Animals receiving Sal prior to LPS demonstrated significant elevation in serum Aβ (p < 0.05; n = 3–5) Bars represent mean ± SEM. Saline (Sal) or IM administered for 14 days; Sal or LPS administered for the final 7 days. Control represents animals treated for 7 days with Sal and receiving a single control Sal injection.
Brain. While we do not attempt to measure changes in BBB permeability, it has been shown that LPS enhances Aβ influx into the brain and decreases Aβ efflux from the brain, independent of changes in BBB permeability (Jaeger et al., 2009). Even if BBB disruption leads to an increased influx of IM, the result would still be the reduction of Aβ. An alternative explanation in which IM blocks the inflammatory response leading to peripheral Aβ production seems unlikely, given the significant elevation of pro-inflammatory cytokines and chemokines even when IM is co-administered with the LPS. In addition, daily animal weights revealed that all animals receiving LPS lost weight during the first 4 days, irrespective of IM treatment, but recovered weight to near starting values by the end of the study (data not shown), matching what we have previously reported (Kahn et al., 2012). This suggests that LPS triggers inflammation, regardless of IM administration, but that IM reduces the production of Aβ. Finally, and most importantly, blocking peripheral Aβ production during an extended inflammatory response rescues cognitive function that is normally disrupted following this type of inflammation. In light of numerous studies, beautifully reviewed by Mucke and Selkoe (2012) that link Aβ to synaptic dysfunction, our findings posit a potential link between peripheral inflammation, peripheral production of Aβ, and cognitive deficits caused by Aβ transported into the brain.

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References
