The renin-angiotensin system (RAS) is an important peripheral system involved in homeostasis modulation, with angiotensin II (Ang II) serving as the main effector hormone. The main enzyme involved in Ang II formation is angiotensin-converting enzyme (ACE). ACE inhibitors (ACEIs) such as captopril (Cap) are predominantly used for the management of hypertension. All of the components of the RAS have also been identified in brain. Centrally located hormones such as Ang II can induce glial inflammation. Moreover, in Alzheimer’s disease (AD) models, where glial inflammation occurs and is thought to contribute to the propagation of the disease, increased levels of Ang II and ACE have been detected. Interestingly, ACE overexpression in monocytes, migrating to the brain was shown to prevent AD cognitive decline. However, the specific effects of captopril on glial inflammation and AD remain obscure. In the present study, we investigated the effect of captopril, given at a wide concentration range, on inflammatory mediators released by lipopolysaccharide (LPS)-treated glia. In the current study, both primary glial cells and the BV2 microglial cell line were used. Captopril decreased LPS-induced nitric oxide (NO) release from primary mixed glial cells as well as regulating inducible NO synthase (iNOS) expression, NO, tumor necrosis factor-α (TNF-α) and induced interleukin-10 (IL-10) production by BV2 microglia. We further obtained data regarding intranasal effects of captopril on cortical amyloid β (Aβ) and CD11b expression in 5XFAD cortex over three different time periods. Interestingly, we noted decreases in Aβ burden in captopril-treated mice over time which was paralleled by increased microglial activation. These results thus shed light on the neuroprotective role of captopril in AD which might be related to modulation of microglial activation.

Keywords: Alzheimer's disease, angiotensin II, angiotensin-converting enzyme, captopril, glial inflammation

INTRODUCTION

Alzheimer's disease (AD) is a progressive neurodegenerative disease considered as the most common type of dementia worldwide (Stansley et al., 2012; Kettenmann et al., 2013). It is well accepted that glial-mediated inflammation contributes to the progression of the disease (Griffin, 2006; Tejera and Heneka, 2016). The AD brain is characterized by activated microglia located in close vicinity to extra-cellular cerebral depositions of amyloid β (Aβ) aggregates and intra-cellular tau-associated neurofibrillary tangles (NFTs; Heneka et al., 2014). As activated microglia are responsible for brain homeostasis, they mediate the innate immune response in the central nervous system (CNS; Tejera and Heneka, 2016). Microglia assume a variety of functions, ranging from the release of inflammatory mediators to phagocytosis (Mandrekar-Colucci and Landreth, 2010; Tejera and Heneka, 2016). Thus, microglial reactions to pathological conditions may result in a
detrimental inflammatory responses leading to neurodegeneration (Griffin, 2006; Heneka et al., 2014). For example, glial cytokine production plays crucial roles in the chronic and self-sustained inflammatory cycles seen in AD, subsequently leading to neuronal dysfunction (Griffin, 2006; Glass et al., 2010). High levels of pro-inflammatory cytokines, such as interleukin-1β (IL-1β) and tumor necrosis factor-α (TNF-α), were observed in the cerebrospinal fluid (CSF) and brains of AD patients (Tarkowski et al., 1999; López González et al., 2016). Reactive oxygen species (ROS), nitric oxide (NO) and elevated levels of inducible nitric oxide synthase (iNOS) enzyme, originating from resident CNS glial cells, are also observed during AD (Heneka et al., 2014). It is well established that excessive amounts of NO in the brain can shift its role from physiological neuromodulator to neurotoxic factor (Jonnala and Buccafusco, 2001). Moreover, peroxynitrite was shown to enhance Aβ peptide aggregation, leading to amyloid plaque formation via nitration of Aβ peptide residues (Kummer et al., 2011). A direct interaction between Aβ proteins and TNF-α type 1 receptor (TNFR1) was reported to stimulate inflammatory cascades leading to neuronal apoptosis (Li et al., 2004).

Over the last 25 years, anti-inflammatory agents were suggested for blocking the complement system activation in AD, induced by Aβ peptides (Breitner et al., 1995; McGeer et al., 1996). Epidemiological studies have shown various degrees of benefit from prolonged consumption of NSAIDs on the onset of AD and symptomatic severity (McGeer et al., 1996). Other prospective randomized controlled trials in adults with normal cognition or mild cognitive impairment indicated no convincing evidence for the efficacy of pharmacologic intervention with NSAIDs in reducing the risk for dementia or improving cognition (Fink et al., 2018). Interestingly, recent studies suggest a lag time of 10 and possibly 20 years as opportunity for treating AD patients with anti-inflammatory drugs prior to clinical manifestation of AD and symptomatic severity (Oakley et al., 2006). In addition to Aβ lesions and gliosis that begin to develop at 8 weeks of age in the brains of these mice, this model is one of few AD mouse models that also display cholinergic neuronal loss in different brain regions as the mice age (Yan et al., 2018).

ACEIs are widely prescribed for the treatment of cardiovascular disorders, diabetes and metabolic syndrome, limited clinical studies investigated the anti-inflammatory effects of these agents in humans (Zanchetti and Elmfeldt, 2006; Savoia and Schiffrin, 2007; Kaur et al., 2015). Recent clinical studies conducted by de Oliveira et al. (2014) with perindopril and captopril found beneficial effects for such pharmacological treatment in terms of cognitive decline in late onset-AD patients. Moreover, treatment with captopril resulted in reduced amyloidogenic processing of the amyloid precursor protein (APP) and ROS levels in the hippocampus of Tg2576 AD mice (AbdAlla et al., 2013).

In the present study, we investigated the effects of captopril, a potent ACEI, administered across a wide range of concentrations, on inflammatory mediators released by lipopolysaccharide (LPS)-induced glia. Both primary glial cells and the BV2 microglial cell line were used in these studies. Targeting microglia with LPS is a well-known model for understanding the interplay between infection and neuroinflammation associated with microglial activation in brain neurodegenerative diseases (Banks and Robinson, 2010). It is well established that LPS-induced acute systemic inflammation, via stimulation of toll-like receptors 4 (TLR4) expressed on innate immune cells, can lead to lasting changes in neuroimmunomodulation and behavior (Saavedra, 2012). Actually, in the CNS, all cell types express TLRs, however, microglia express the whole repertoire and TLR4 selectively (Pardon, 2015). Interestingly, Ang II, LPS and Aβ peptides share a common mechanism for microglial activation which involves the activation of TLR (Buchanan et al., 2010; Pardon, 2015; Winklewski et al., 2016). Moreover, we considered the time-dependent effects of intranasally administrated captopril on AD-associated pathological features, gliosis and Aβ aggregation, in the brains of five familial AD mice (5XFAD). The 5XFAD mice co-express mutations in the APP and presenilin 1 (PS1) genes, which in time lead to early expression of AD-associated brain pathological features (Oakley et al., 2006). In addition to Aβ lesions and gliosis that begin to develop at 8 weeks of age in the brains of these mice, this model is one of few AD mouse models that also display cholinergic neuronal loss in different brain regions as the mice age (Yan et al., 2018).

MATERIALS AND METHODS

Cell Culture

BV2 Microglial Cells

BV2 murine microglial cell line was provided by Professor Rosario Donato (Dep. of Experimental Medicine and Biochemical Sciences, University of Perugia, Italy). Cells were maintained at humidified atmosphere of and 37°C and 5% CO2 in RPMI-1640 medium with 10% fetal calf serum (FCS), penicillin/streptomycin (100 U/ml and 100 μg/ml, respectively) and 4 Mm of L-glutamine. For experiments, cells were cultured in 24- and 6-well plates at a density of 3 × 10⁵ and 1 × 10⁶ cells per well, respectively. Following over-night incubation, serum
free medium (SFM) was added for 4 h and additional 24 h incubation with SFM containing 10 mM HEPES, 0.1% bovine serum albumin (BSA) and drug treatments was performed.

**Primary Rat Neonatal Mixed Glial Cells Culture**

Rat primary mixed glial cells cultures of astrocytes and microglia were obtained from the whole brain of neonatal (0–24 h age) Wistar rats, according to previous protocols (Brenner et al., 1992; Torika et al., 2016). Briefly, cells were harvested following meninges removal and mesh on a nylon sieves of 60 μm pore size and seeded in poly-l-lysine-coated 24-well plates at a concentration of 1 × 10^6 cells per well. Cells were mentioned in high glucose DMEM medium supplemented with 10% FCS, penicillin/streptomycin (100 U/ml and 100 μg/ml, respectively), 0.2 mM L-glutamine and 100 U/ml insulin. Cells were grown at humified atmosphere of and 37°C and 5% CO₂ for 21 days, medium was replaced twice a week. For experiments, SFM was added for 4 h and replace with supplemented SFM with 10 mM HEPES, 0.1% BSA and drug treatments for 24 h.

Culture treatments included LPS from *Escherichia coli*, captopril and actinomycin D, all purchased from Sigma-Aldrich (St. Louis, MO, USA).

**Cell Viability Assay (XTT)**

Cell Proliferation Kit (XTT; Biological Industries, Kibbutz Beit-Haemek, Israel) was used for determination of cells viability, according to the manufacturer’s instructions. The spectrophotometric analysis of the total formazan content was performed by using a microplate reader (model 680, Bio-Rad, Hercules, CA, USA), absorbance measured at 450 nm as previously described (Kwiecińska et al., 2012).

**Measurement of Nitrite Production (Griess Reaction)**

NO production was determined by measuring the nitrite content in the supernatant of the cell culture as described previously (Zhu et al., 2010). An equal volume (100 μl) of supernatants and gries reagent (Sigma-Aldrich, St. Louis, MO, USA) were incubated for 15 min, at room temperature and light avoided atmosphere. The spectrophotometric analysis of the total nitrite content was performed by using a microplate reader (model 680, Bio-Rad, Hercules, CA, USA), absorbance measured at 540 nm. The nitrite concentration was determined using sodium nitrite as a standard (0–50 μM). Nitrite levels were normalized to cell count.

**TNF-α and Interleukin 10 (IL-10) Proteins Assay by Enzyme-Linked Immunosorbsent Assay (ELISA)**

Supernatants TNF-α and IL-10 levels in the medium were assayed using ELISA kits (BD Biosciences, San Diego, CA, USA) according to the manufacturer’s instructions.

**Western Blot Analysis**

Whole cell lysates were obtained using lysis buffer containing protease and phosphatase cocktail (Stratech Scientific LTD., UK). Samples were separated on 7.5% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), transferred to nitrocellulose membranes and blocked using 4% BSA. Overnight incubation at 4°C with specific rabbit anti-iNOS antibody (1:1000, Cayman Chemicals, Ann Arbor, MI, USA) was performed. Then, membranes were incubated with donkey anti-rabbit antibody (1:10,000, GE Healthcare, Buckinghamshire, UK) for 90 min at room temperature. The bands were visualized using enhanced chemiluminescence (ECL) solution (according to the manufacturer’s instructions) and exposure to X-ray film (Fuji medical X-ray film, FujiFilm). Computerized image analysis system (EZ Quant-Gel 2.2, EZQuant Biology Software Solutions Ltd., Israel) was used for bands analysis. Protein load was normalized by β-actin protein level measurements using membrane exposure to mouse anti-β-actin antibody (1:4000, Sigma-Aldrich) and horseradish peroxidase-conjugated goat anti-mouse antibody (1:20,000, Jackson ImmunoReaserch Inc., West Grove, PA, USA).

**Mice**

The five familial AD mouse model (5XFAD) was used for animal experiments. 5XFAD mice express total of five familial AD (FAD) mutations, three mutations in the human APP695 gene (Swedish K670N, M671L; Florida I716V and London V717I) and two mutations in the human presenilin-1 gene (PSEN-1; M146L, L286V). C57BL/6 wild type (WT) mice (Harlan, Jerusalem, Israel) were reproduced with hemizygous 5XFAD mice. DNA tail polymerase chain reaction (PCR) for 3-weeks-old neonatal mice was used for detection of the human APP gene and dividing mice into WT or 5XFAD groups. Cages temperature and humidity conditions were set to 22 ± 2°C and 65 ± 5%, respectively. Mice were kept in 12 h light/dark cycle and available food/water supply conditions. For experiments, mice of both genders were randomly divided into three groups: (1) the control group included WT mice that were treated with 5 mg/kg/day of captopril (WT+Cap; n = 11 mice; 6 females/5 males); (2) 5XFAD mice that were
treated with 5 mg/kg/day of captopril (5XFAD+Cap; n = 11; 5 females/6 males); and (3) 5XFAD mice that were treated with the vehicle (saline; 5XFAD+saline; n = 11; 6 females/5 males). Intranasal administration (2 µl drop to each nostril) of the solutions started when mice reached 8 weeks-old and lasted for 8 weeks. Surgical and experimental procedures were approved by the Institutional Animal Care and Use Committee of Ben-Gurion University of the Negev, Beer-Sheeba, Israel (approval number: IL-30-08-2011-15, IL-55-09-2016).

**Immunohistochemistry**

Cardiac perfusion was performed in ketamine/Xylazine Hydrochloride anesthetized mice as previously described (Torika et al., 2016; Asraf et al., 2017). Brains were then removed and the two separated hemispheres were incubated in cold 4% paraformaldehyde (PFA) solution (4°C, overnight). Hemispheres were transferred into 30% sucrose solution for 48 h followed by −80°C freezing in molds filled with tissue adhesive (O.C.T compound Tissue-Tek, Torrance, CA, USA). Brain tissues were sliced into 40 µm thick sagittal sections by cryostat (Leica, Germany) and then rinsed in 0.05% PBS/Tween 20 solution followed by another rinsing in 0.5% PBS/Triton X-100 solution. Primary antibody diluting buffer (GBI Labs, Bothell, WA, USA) was used for blocking of non-specific binding. Immunostaining for Aβ and CD11b proteins was performed using 2 h incubation with rabbit anti-human Aβ antibody (1:250, gift from Prof. Alon Monsonego, the Shraga Segal Department of Microbiology and Immunology, faculty of Health Sciences and the National Institute of Biotechnology in the Negev, Ben-Gurion University of the Negev, Beer-Sheeba, Israel) and rat anti-mouse/human CD11b antibody (1:25, Biolegend) followed by incubation with the corresponding secondary antibodies, Cy3-conjugated donkey anti-rabbit IgG (1:100, Jackson ImmunoResearch Laboratories, USA) and Alexa flour 488-conjugated goat anti-rat IgG (1:250, Jackson ImmunoResearch Laboratories, USA), respectively. Mounting medium with DAPI (Vector Labs, USA) was used for cells nuclei staining. Confocal images at a 1024 × 1024-pixel resolution with ×10 objective were obtained using the Olympus FluoView FV1000 confocal microscope (Olympus, Hamburg, Germany).

**Image Analysis**

The threshold function in ImageJ software (version 1.40C, NIH, Bethesda, MD, USA) was used for quantification of the area stained for Aβ and CD11b proteins. Five cortical sections of each mouse were analyzed for the indicated proteins. The fluorescence intensity measured for the WT mice group was used as the baseline intensity.

**Statistics**

Results are presented as the mean ± SEM. The statistical differences between the experimental groups were assessed by one-way analysis of variance (ANOVA) followed by post hoc multiple comparison test (Tukey–Kramer Multiple Comparison Test). Statistical significance was considered at p < 0.05.

**RESULTS**

**Captopril Treatment Does Not Show Any Cytotoxic Effect in BV2 Microglial Cells**

We first investigated the possible cytotoxic effect of a 24 h captopril treatment on the BV2 microglial cell line using the XTT assay (Figure 1: F(5,18) = 123, p < 0.0001). The effect of captopril was compared to that of the already known cytotoxic drug actinomycin D. As indicated in Figure 1, while 0.25 µg/ml actinomycin D reduced cell viability by 95%, as compared to non-treated cells (control), captopril (0.1–3 mM) did not show any cytotoxic effect.

**Captopril Dually Regulates NO Production by LPS-Treated BV2 Microglia**

We investigated the effect of captopril on NO production levels by BV2 cells treated with two different LPS doses (7 and 100 ng/ml). As shown in Figure 2A, a significant increase in NO production was observed following 7 ng/ml LPS treatment.
Captopril Dually Regulates TNF-α (Pro-inflammatory) and IL-10 (Anti-inflammatory) Production From LPS-Treated BV2 Microglial Cells

The effect of captopril on the secretion of pro-inflammatory TNF-α, and anti-inflammatory IL-10 from BV2 microglial cells was assessed (Figure 3). TNF-α levels were significantly increased by more than 97% in 7 ng/ml LPS-treated BV2 microglial cells, as compared to controls (Figure 3A: $F_{(5,283)} = 202.4, p < 0.0001$). Incubation with low doses of captopril (0.1 and 0.3 mM) did not affect LPS-induced TNF-α production levels. When administered at a 1 mM concentration, captopril elicited a 32% increase over that observed following treatment with LPS alone (Figure 3A).

By contrast, captopril provided at 3 mM abrogated the effect of LPS on TNF-α production by about 50% (Figure 3A). Basal TNF-α levels were significantly reduced upon captopril treatment (Figure 3B: $F_{(4,132)} = 4.488, p < 0.0001$).

A robust increase in IL-10 production was observed following treatment with higher doses of captopril (1 and 3 mM). Captopril alone significantly affected IL-10 production levels in non-stimulated BV2 cells (Figure 3D: $F_{(4,57)} = 35.9$, $p < 0.0001$).
Captopril Decreases NO and TNF-α Production Levels by LPS-Treated Neonatal Mixed Glial Cells

The effect of captopril on NO production levels by primary mixed glial cells was investigated in 100 ng/ml and 0.5 µg/ml LPS-treated cells. Treatment with 100 ng/ml (Figure 4A) and 0.5 µg/ml (Figure 4B) LPS resulted in robust induction of NO production levels. 3 mM captopril treatment significantly reduced the NO production levels by approximately 50% compared to 100 ng/ml LPS-treated cells (Figure 4A: $F(5,51) = 14.55, p < 0.0001$). While lower captopril doses (0.1 and 0.3 mM) reduced the LPS (0.5 µg/ml)-induced NO production by 11% and 24%, respectively, higher doses of the inhibitor (1 mM and 3 mM) resulted in 40% and 50% reduction of NO production by LPS-treated primary mixed glial cells, respectively (Figure 4B: $F(5,265) = 293.7, p < 0.0001$). Moreover, 30% reduction in TNF-α production levels were observed following 3 mM captopril treatment of 0.5 µg/ml LPS-treated primary mixed glial cells (Figure 4C: $F(5,92) = 101.6, p < 0.0001$).

Dual Effect of Captopril on LPS-Induced iNOS Protein Expression Levels in BV2 Microglial Cells

We previously showed a 50% reduction in iNOS expression upon treatment with higher concentrations of captopril in LPS-treated BV2 cells, as compared to cells treated with LPS (7 ng/ml) alone for 24 h (Torika et al., 2016). In contrast, iNOS expression was amplified 2-fold by adding 1 mM captopril over the level of enzyme expression in cells treated solely with LPS (Figure 5: $F(3,15) = 96.22, p < 0.0001$).
FIGURE 6 | Intranasal administration of captopril reduces amyloid burden and CD11b expression in the cortex of 5XFAD mice. Eight-week-old wild type (WT) or 5XFAD mice were treated intranasally with either saline or captopril (cap; 5 mg/kg/day) for 2 months. At the end of treatment, the mice were anesthetized and cardiac perfusion with cold PBS was performed. Brains were fixed in 4% paraformaldehyde (PFA) and 30% sucrose solutions. Then, 40 µm-thick brain sagittal sections were stained for Aβ (red) and CD11b (green) proteins using target-specific antibodies and counterstained with mounting solution containing DAPI (blue). Representative cortical layers from the three mice groups are presented. The experiment included 11 mice per group (n = 33). The calculated average sums of Aβ- (A,B) and CD11b-stained (C,D) cortical areas are represented as mean percentage ± SEM of the stained area in the saline-treated group in five repeats. Merged images of anti-Aβ and anti-CD11b staining are presented (E). One-way ANOVA and a Tukey–Kramer multiple comparison test were used to determine statistical significance. The scale bar is 200 µm. ***p < 0.001 vs. WT+cap; *p < 0.05 vs. 5XFAD+saline; ##p < 0.01 vs. 5XFAD+saline.

A 2-Month Intranasal Captopril Treatment Ameliorates Gliosis and Aβ-Pathology in Cortical Layers of 5XFAD Mice

The effects of captopril, given at a clinically relevant dose via intranasal administration procedure, on CD11b expression and amyloid burden in 5XFAD mice cortex were studied, as was brain immunohistochemistry (Figure 6). Cortical sections of 4 month-old WT mice showed low CD11b expression (Figures 6C,D), with no Aβ formation (Figures 6A,B). By contrast, cortical section of age-matched 5XFAD mice exhibited increased levels of Aβ plaques (Figures 6A,B) and the CD11b marker (Figures 6C,D), when compared to WT-treated mice. Intranasal administration of 5 mg/kg/day captopril significantly reduced the areas stained for CD11b (Figures 6C,D: F(2,43) = 515.8, p < 0.0001) and Aβ (Figures 6A,B: F(2,43) = 272.3, p < 0.0001) proteins in the cortex of 4 month-old 5XFAD mice, when compared to saline-treated 5XFAD mice brain sections.

Different Time-Dependent Effects of Intranasally Administered Captopril on Gliosis and Aβ Pathology in the Cortical Layers of 5XFAD Mice

We compared the effects of intranasal exposure of mice to captopril (5 mg/kg/day) for different periods of time on gliosis and amyloid burden expression in the cortical areas of 5XFAD mice (Table 1). As indicated in Table 1, the decrease of Aβ burden in captopril-treated mice over time (125.5%–75.1%) was paralleled by increased microglial CD11b expression (67.8%–101%).

DISCUSSION

Although microglia comprise only 10% of the CNS cell population, much of the innate immune response in the CNS is mediated by these cells (Disabato et al., 2016). The microglial inflammatory response can be mimicked by the use of LPS endotoxin, which triggers microglia to secrete a wide variety of inflammatory cytokines (Pardon, 2015).

In this study, a robust inflammatory response by BV2 microglial cells was observed following LPS treatment, and resulted in the enhanced release of TNF-α, and NO, as well as elevated levels of iNOS expression (Figures 2–5). The present study also provides evidence for the first time that ACE inhibition by captopril serves a dual role in microglia-mediated neuroinflammation.

Dual regulation of neuroinflammation was also observed by us with kinins. Stimulation of the bradykinin 2 receptor (BK2R) enhanced glial inflammation in a manner that was blocked by BK2R antagonist. By contrast, a BK 1 receptor
TABLE 1 | Overview of the time-dependent effects of intranasal captopril on glial and Aβ expression in 5XFAD mouse cortical areas.

| Captopril dosage | Treatment period | Amyloid burden stained area (% of Saline-treated 5XFAD) | CD11b stained area (% of Saline-treated 5XFAD) |
|------------------|------------------|--------------------------------------------------------|---------------------------------------------|
| 5 mg/kg/day       | 2 months         | 15.8 ± 3.15                                            | 3.61 ± 1.06                                 |
| 5 mg/kg/day       | 3.5 weeks        | 12.4 ± 3.15                                            | 3.61 ± 1.06                                 |
| 5 mg/kg/day       | 7 months         | 14.66 ± 7.34                                           | 67.82 ± 3.61                                |

Asraf et al. Captopril Modulates Microglial Activation

Effects of perindopril (1 nM–1 mM), a centrally active ACEI, in the brain. In contrast to the proposal that captopril acts as an anti-inflammatory drug, captopril has been shown to enhance therapeutic delivery to the CNS by promoting the intranasal delivery of AD-related proteins. In this study, we demonstrate that captopril promotes intranasal delivery of proteins by promoting the intranasal delivery of proteins by promoting the intranasal delivery of proteins. In addition, captopril was shown to enhance the expression of Aβ in a dose-dependent manner.

In conclusion, captopril potentiates the delivery of therapeutic proteins to the CNS and may be a beneficial treatment for AD.
clearance of Aβ via phagocytic microglial cells (Doens and Fernández, 2014), variations in Aβ-degrading enzyme expression (Nalivaeva et al., 2012) or changes in the generation of Aβ peptides followed by lowered brain inflammation (Griffin, 2006).

Table 1 summarizes what we have observed (Torika et al., 2016) with respect to the effects of intranasal captopril treatment on cortical Aβ and CD11b expression in the brain of 5XFAD mice over three different time periods. Interestingly, our findings show decreased burden in captopril-treated mice over time which was paralleled by increased microgial activation. In AD patient brain, the amyloid burden is accompanied by a clustering of activated microgria around the amyloid plaques. Reduced Aβ depositions, alongside microglial activation and enhanced phagocytic ability by angiotensin-related drugs, was shown to potentially improve cognitive performance in AD mice (Tsukuda et al., 2009; Shindo et al., 2012; Torika et al., 2017). It is worth noting that reduced amyloid burden can also involve other mechanisms which are not necessarily related to changes in microgial activation. Further studies are required to conclude whether intranasally administered captopril alters Aβ-degrading enzyme expression or influences other mechanisms involved in Aβ production.

AUTHOR CONTRIBUTIONS

KA, NT and SF-B designed the experiments. KA, RNA and NT performed cell culture experiments. KA performed in vivo experiments and analyzed the data. SF-B secured funds for this work. NT and SF-B wrote the manuscript. All authors read the manuscript and approved its final content.

FUNDING

This work was supported by the Israel Science Foundation (grant no. 101/11-16).

ACKNOWLEDGMENTS

We wish to thank Prof. Abraham Danon for careful reading of the manuscript and useful advice.

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**Conflict of Interest Statement:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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