Current Research in Pharmacology and Drug Discovery 8 (2025) 100221

Contents lists available at ScienceDirect

Current Research in Pharmacology and Drug Discovery

journal homepage: www.journals.elsevier.com/current-research-in-pharmacology-and-drug-discovery

Carnosine modulates Aβ-induced transcriptional aberrations in murine microglial cells

Veronica Rivi [a,b,1], Giuseppe Carota [c,1], Fabio Tascedda [b,d,e], Johanna M.C. Blom [a,b], Filippo Caraci [f,g], Cristina Benatti [a,b,2], Giuseppe Caruso [h,i,*,2]

[a] Department of Biomedical, Metabolic and Neural Sciences, University of Modena and Reggio Emilia, Modena, Italy
[b] Centre of Neuroscience and Neurotechnology, University of Modena and Reggio Emilia, Modena, Italy
[c] Department of Biomedical and Biotechnological Sciences, University of Catania, Italy
[d] Department of Life Sciences, University of Modena and Reggio Emilia, Modena, Italy
[e] CIB, Consorzio Interuniversitario Biotecnologie, Trieste, Italy
[f] Department of Drug and Health Sciences, University of Catania, Catania, Italy
[g] Unit of Neuropharmacology and Translational Neurosciences, Oasi Research Institute-IRCCS, Troina, Italy
[h] Departmental Faculty of Medicine, UniCamillus—Saint Camillus International University of Health and Medical Sciences, Rome, Italy
[i] IRCCS San Camillo Hospital, Venice, Italy

ARTICLE INFO

Keywords:
Carnosine
Microglia
Neurodegeneration
Neuroinflammation
Alzheimer's disease

ABSTRACT

Carnosine (β-alanyl-L-histidine) is an endogenous dipeptide known for its anti-inflammatory and antioxidant effects, making it a promising agent for neurodegenerative diseases like Alzheimer's disease (AD). Carnosine has shown protective effects against amyloid beta (Aβ)-induced oxidative stress and inflammation in murine microglial cells, yet its full immunomodulatory impact on these cells, particularly in terms of transcriptional regulation and cytokine interplay, remains underexplored. This study examined carnosine's effects on immune response markers in BV-2 cells exposed to Aβ oligomers. Specifically, gene expression changes in anti-inflammatory mediators (CXCL2 and IL-10) and phagocytic markers (CD11b, CD68, TNFα, IL-1β) were assessed. Notably, carnosine increased CXCL2 and IL-10 expression, promoting an anti-inflammatory response and enhancing microglial phagocytosis. Additionally, carnosine restored CX3CR1 expression, a receptor implicated in Aβ- effects in murine macrophages, and upregulated TGF-β1 and its receptor, supporting its neuroprotective role. These results underscore carnosine's potential to modulate immune responses, enhance microglial activity, and provide neuroprotection in Aβ-induced conditions. The findings highlight carnosine's therapeutic promise for AD treatment, offering a pathway for future research on its use in neurodegenerative disease interventions.

1. Introduction

Carnosine, a naturally occurring dipeptide synthesized by carnosine synthase 1 from β-alanine and L-histidine (Boldyrev et al., 2013; Quinn et al., 1992), is prevalent in mammalian tissues, especially in the brain, muscle, and heart (Hipkiss et al., 2002). Given its antioxidant, anti-inflammatory, and anti-aggregation properties (Rivi et al., 2024; Aloisi et al., 2013; Caruso et al., 2019a), carnosine can exert neuroprotective effects through a multimodal mechanism of action (Rajanikant et al., 2007; Kulebyakin et al., 2012). These include prevention of neuroinflammation, modulation of microglia functions and polarization, and reduction of intraneuronal amyloid-β (Aβ) aggregation and accumulation (Caruso et al., 2019b, 2021), all of which are pathological hallmarks of Alzheimer's disease (AD) (Guo et al., 2020). Besides inducing direct toxic effects on neuronal and synaptic functions, Aβ peptides (especially 1–42) (Bondareff et al., 1989; Cheng et al., 2020), serve as inflammatory stimuli for aberrant microglia activation and promotion of chronic inflammation (Affram et al., 2017; Hambardzumyan et al., 2016; Ding et al., 2021). In particular, microglia can adopt either an M1 pro-inflammatory phenotype, characterized by

* Corresponding author. Departmental Faculty of Medicine, UniCamillus—Saint Camillus International University of Health and Medical Sciences, Rome, Italy.
E-mail address: giuseppe.caruso@unicamillus.org (G. Caruso).
[1] These authors contributed equally to this work and shared the first authorship.
[2] These authors contributed equally to this work and shared senior authorship.

https://doi.org/10.1016/j.crphar.2025.100221
Received 1 February 2025; Received in revised form 1 April 2025; Accepted 30 April 2025
Available online 6 May 2025

the release of cytokines such as IL-1β, IL-6, and TNF-α as well as reactive oxygen and nitrogen species, or an M2 anti-inflammatory phenotype, which produces protective and trophic factors such as IL-4, IL-10, and TGF-β1 (Caruso et al., 2019b; Affram et al., 2017; Ding et al., 2021; Guo et al., 2022; Bolós et al., 2017; Hanslik and Ulland, 2020; Garrison et al., 2018; Ciani et al., 2024). Thus, immunomodulators, which modulate microglial activation without suppressing its activity, represent potential therapeutics for AD. To the best of our knowledge, this is the first study to comprehensively investigate the transcriptional and immunomodulatory effects of carnosine in BV-2 microglial cells exposed to Aβ1-42 oligomers. Specifically, we focused on the expression levels of pro- and anti-inflammatory cytokines and microglial markers related to phagocytosis. Additionally, we evaluated the ability of carnosine to modulate the microglial Nod-like receptor protein 3 (NLRP3) inflammasome complex, which is an emerging key contributor to neuroinflammation during neurodegeneration (Hanslik and Ulland, 2020), as well as the fractalkine (CX3CL1)/CX3CR1 signaling (Bolós et al., 2017), which has proven to act as a regulator of microglia activation in response to inflammation (Merino et al., 2016).

2. Materials and methods

2.1. Materials and reagents

All the materials were purchased from Sigma-Aldrich Corporate (St. Louis, MO, USA) (e.g., carnosine, Cat# C9625-5G) or Thermo Fisher Scientific Inc. (Pittsburgh, PA, USA) unless specified otherwise.

2.2. Preparation of Aβ1-42 oligomers

HFIP-treated Aβ1-42 monomers were dissolved in dimethyl sulfoxide, diluted to a final concentration of 100 μM in DMEM/F12 (1:1) medium, and left to incubate for 72 h at 4 °C. At the end of this incubation step, the obtained oligomers (oAβ1-42) were immediately used or aliquoted and stored at −20 °C until use (Caruso et al., 2019b).

2.3. Cell culture and treatment

BV-2 cells (ICLC ATL03001, Interlab Cell Line Collection, Genova, Italy) were cultured as previously described (Caruso et al., 2019a, 2021). The day before treatment, cells were harvested, counted, and seeded in 6-well plates at a density of 3.5×10^5 cells/well. The next day, BV-2 cells were treated with oAβ1-42 for 6 or 24 h, in the absence or the presence of carnosine (Car) at the final concentration of 20 mM, a concentration already employed in previous studies with murine immune cells (Caruso et al., 2019a, 2021).

2.4. Total RNA extraction, reverse transcription, qRT-PCR, and statistical analysis

RNA extraction and DNase treatment were performed using the GenElute™ Mammalian Total RNA Miniprep Kit and the DNase70-On-Column DNase I Digestion Set (Merck KGaA). Two micrograms of total RNA were reverse transcribed with the High-Capacity cDNA Reverse Transcription Kit as previously described (Rivi et al., 2025). Real-time PCR was conducted on a Bio-Rad CFX Connect thermocycler using Bio-Rad SsoAdvanced Universal SYBR Mix, with specific forward and reverse primers at a final concentration of 300 nM. The selected target genes were: IL-1β (Fw: TGAAAGCTCTCCACCTCAATG; Rv:CCAAGGCCACAGGTATTTTG); IL-6 (Fw: CTTCACAAGTCGGAGGCTTA; Rv: CAAGTGCATCATCGTTGTTC); TNFα (Fw:GGCCTCCCTCTCATCAGTTC; Rv: CACTTGGTGGTTTGCTACGA); IL-10 (Fw:GAAGCATGGCCCAGAAATCAAG; Rv: AAATCACTCTTCACCTGCTCCAC); IL-4 (Fw: CCAAGGTGCTTCGCATATTT; Rv:ATCGAAAAGCCCGAAAGAGT); TGFβR (Fw:CAAGGGCTACCATGCCAACTT; Rv: GTTGTGTTGGTTGTAGAGGGC); CXCL1 (Fw: TGGCTGGGATTCACCTCAAG; Rv: CAAGCCTCGCGACCATTCTT); CXCL2 (Fw: TCAATGCCTGAAGACCCTGC; Rv:TTTGACCGCCCTTGAGAGTG); CXCR2 (Fw:AAGCCTTGAGTCACAGAGAGTTG; Rv: TTATCCACCTTGAATTCTCCCA); CX3CR1 (Fw: CGTGAGACTGGGTGAGTGACT; Rv: TCAGCAGAATCGTCATACTCAAA); IL-18 (Fw: TGAAGAAAATGGAGACCTGGA; Rv: CAGTCCTCTTACTTCACTGTC); IL-18bp (Fw:TGCCACTGAATGGAACTCTG; Rv: CTGGGAGGTGCTCAATGAAG); NLRP3 (Fw: AGAAGAGTGGATGGGTTTGCT; Rv: GCGTTCCTGTCCTTGATAGAG) and its downstream caspase CASP-1 (Fw: CCGTGGAGAGAAACAAGGAG; Rv: AATGAAAAGTGAGCCCCTGA); ASC (Fw:CAAACGACTAAAGAAGAGTCTG; Rv:AGAGCTTCCTCATCTTGTCT); CD11b (Fw:ACGCCATCTACATGATTGTCAC; Rv: AAGACTACACTGACAGGGAGGC); CD14 (Fw: AGATGTGGAATTGTACGGCG; Rv:CGTAAGCCGCTTTAAGGACA); CD68 (Fw:CTTATGGACAGCTTACCTTT; Rv: AGCTCTCGAAGAGATGAAT); CD86 (Fw: GTAGACGTGTTCCAGAACTTA; Rv:TGTTTTGAGCCTTTGTAAAT). Cycle threshold (Cq) values were determined using CFX Maestro software. The reference genes selected were GAPDH (Fw: CAAGGTCATCCATGACAACTTTG; Rv: GGGCCATCCACAGTCTTCTG), ribosomal protein L27 (RPL27) (Fw: AAGCCGTCATCGTGAAGAACA; Rv: CTTGATCTTGGATCGCTTGGC), and cyclophilin A (CypA) (Fw: AGCATACAGGTCCTGGCATC; Rv: TTCACCTTCCCAAAGACCAC), which were identified as the most stable combination by NormFinder (Wang et al., 2012). Their geometric mean Cq values served as the calibrator. Gene expression changes were quantitatively evaluated using the comparative $2^{-\Delta\Delta Cq}$ method, with the average expression levels of resting cells at 6 and 24 h serving as the calibrator.

2.5. Statistical analysis

Relative gene expression data were analyzed separately for each exposure time (6 and 24 h) using One-way ANOVA, followed by Tukey's *post hoc* test. Extreme outliers were excluded before statistical analysis using the boxplot tool in SPSS (defined as values more than 3 times the interquartile range outside the end of the interquartile box). A type I error rate of 0.05 was used for all analyses. Data were presented as mean ± standard error (SEM). All statistical analyses were performed using SPSS software ver. 29.0 (IBM Corp., Armonk, NY, USA), and graphs were created using GraphPad Prism v. 10.00 for Windows® (GraphPad Software, Inc., La Jolla, CA, USA).

3. Results

3.1. Effects of a 6- and 24-h exposure to oAβ1-42, oAβ1-42 + Car, or Car alone on expression levels of immune-related targets in BV-2 cells

We assessed the expression of immune-related targets (IL-1β, IL-6, TNFα, IL-10, IL-4, and CXCL2) in BV-2 microglial cells exposed to oAβ1-42 with or without Car for 6 or 24 h (Fig. 1). One-way ANOVA revealed significant treatment effects for IL-1β [$F(3,15) = 15.129$; $p < 0.0001$ for 6 h; $F(3,15) = 7.155$; $p = 0.006$ for 24 h; Fig. 1A], IL-6 [$F(3,15) = 33.205$; $p < 0.0001$ for 6 h; $F(3,15) = 5.615$; $p = 0.014$ for 24 h; Fig. 1B], and TNFα [$F(3,15) = 55.746$; $p < 0.0001$ for 6 h; $F(3,15) = 11.013$; $p = 0.001$ for 24 h; Fig. 1C]. After 6 h, oAβ1-42 treatment, alone or in the presence of Car, significantly increased IL-1β, while a decrease of IL-6 and TNFα levels compared to resting, untreated cells or those treated with Car alone was observed. After 24 h, co-treatment with oAβ1-42 and Car significantly boosted IL-1β and TNFα levels compared to oAβ1-42 alone, while IL-6 expression significantly decreased after Car exposure, regardless of oAβ1-42 treatment (Fig. 1A–C). At 6 h, there were no significant changes in IL-10 levels among the different experimental conditions [$F(3,15) = 1.552$; $p = 0.252$; Fig. 1D]. However, after 24 h, co-treatment significantly increased IL-10 levels compared to oAβ1-42 alone or untreated cells [$F(3,15) = 8.979$; $p = 0.002$; Fig. 1D]. A significant effect for IL-4 was noted at both time points [$F(3,15) = 5.399$; $p = 0.014$ for 6 h; $F(3,15) = 24.286$; $p < 0.0001$ for 24 h; Fig. 1E]. After 6 h, IL-4 expression was lower in co-treated cells compared to

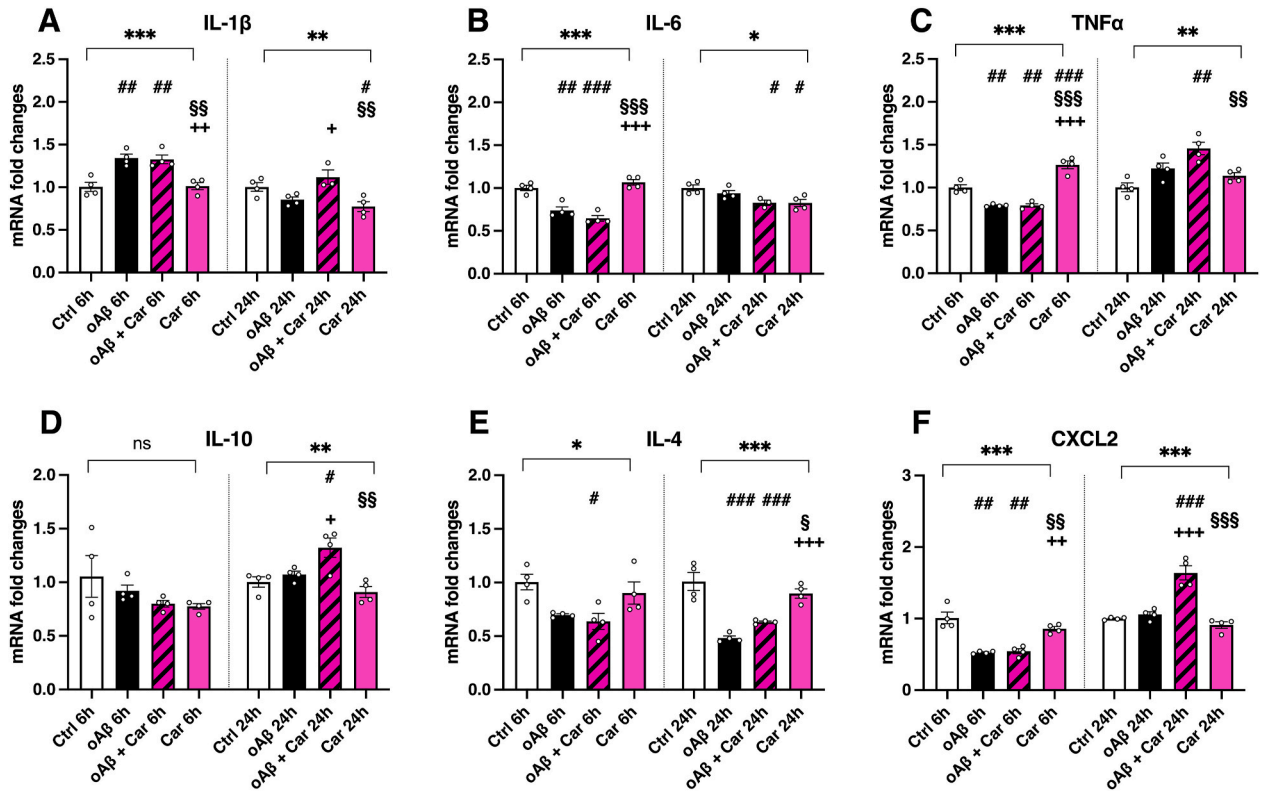

Fig. 1. Effect of a 6- and 24-h exposure to oAβ1-42, oAβ1-42 + Car, or Car alone on the expression of immune-related targets in BV-2. Cells were treated with oAβ1-42 (1 μM), in the absence or presence of Car (20 mM), or Car alone, after which mRNA expression of (A) IL-1β, (B) IL-6, (C) TNFα, (D) IL-10, (E) IL-4, and (F) CXCL2, with GAPDH/CypA/RPL27 as endogenous control, were measured by qRT-PCR. Data are represented as means ± S.E.M. and were analyzed for each time with one-way ANOVA [* = $p < 0.05$, ** = $p < 0.01$; *** = $p < 0.0001$] followed by Tukey's *post hoc* test: # = $p < 0.05$, ## = $p < 0.01$; ### = $p < 0.001$ vs resting cells at matching time; + = $p < 0.05$, ++ = $p < 0.01$, +++ = $p < 0.0001$ vs oAβ1-42 at matching time; § = $p < 0.05$; §§ = $p < 0.01$; §§§ = $p < 0.0001$ vs oAβ1-42 + Car at matching time (n = 4 per group).

resting cells, a trend that persisted at 24 h, with oAβ1-42 significantly decreasing IL-4 levels regardless of Car presence. Resting BV-2 cells showed low CXCL1 (KC) levels but abundant CXCL2 mRNA. CXCL2 levels significantly decreased after 6 h of oAβ1-42 exposure, either alone or with Car [$F(3,15) = 25.825$; $p < 0.0001$; Fig. 1F]. After 24 h, CXCL2 mRNA levels in cells co-treated with Car and oAβ1-42 were significantly higher compared to all other experimental conditions [$F(3,15) = 32.658$; $p < 0.0001$; Fig. 1F]. When assessing TGFβR, CXCR2, and CX3CR1 expression (Fig. 2), Car significantly increased TGFβR mRNA levels compared to all groups [$F(3,15) = 45.171$; $p < 0.0001$; Fig. 2A] due to 6 h treatment, while no significant effects were observed at 24 h [$F(3,15) = 2.044$; $p = 0.161$; Fig. 2A]. CXCR2 expression significantly decreased after a 6-h exposure to oAβ1-42, regardless of Car treatment [$F(3,15) = 27.830$; $p < 0.0001$; Fig. 2B]. After 24 h, CXCR2 levels significantly increased in the presence of oAβ1-42, but decreased with Car treatment compared to resting cells [$F(3,15) = 26.473$; $p < 0.0001$;

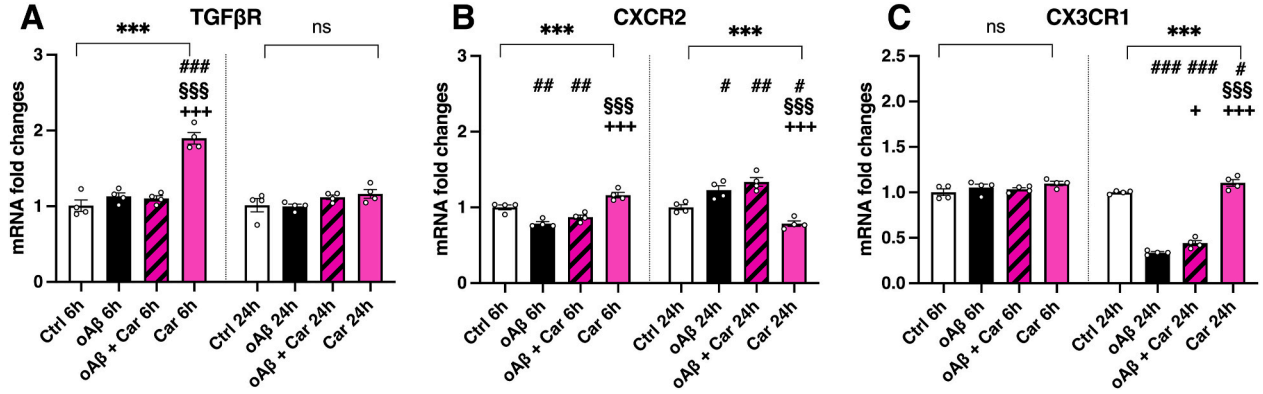

Fig. 2. Effect of a 6- and 24-h exposure to oAβ1-42, oAβ1-42 + Car, or Car alone on the expression of immune-related targets in BV-2. Cells were treated with oAβ1-42 (1 μM), in the absence or presence of Car (20 mM), or Car alone, after which mRNA expression of (A) TGFβR, (B) CXCR2, and (C) CX3CR1 with GAPDH/CypA/RPL27 as endogenous control, was measured by qRT-PCR. Data are represented as means ± S.E.M. and were analyzed for each time with one-way ANOVA [* = $p < 0.05$, ** = $p < 0.01$; *** = $p < 0.0001$] followed by Tukey's *post hoc* test: # = $p < 0.05$, ## = $p < 0.01$; ### = $p < 0.001$ vs resting cells at matching time; + = $p < 0.05$, ++ = $p < 0.01$, +++ = $p < 0.0001$ vs oAβ1-42 at matching time; § = $p < 0.05$; §§ = $p < 0.01$; §§§ = $p < 0.0001$ vs oAβ1-42 + Car at matching time (n = 4 per group).

Fig. 2B]. CX3CR1 expression showed no significant changes after 6 h [F (3,15) = 1.849; p = 0.192; Fig. 2C]. However, after 24 h, oAβ1-42 significantly decreased CX3CR1 levels compared to untreated or Car-only groups, including those co-treated with Car [F(3,15) = 259.159; p < 0.0001; Fig. 2C]. Finally, we examined IL-18 and NLRP3 inflammasome mRNA levels (Fig. 3). IL-18 levels remained stable across all the conditions, with no significant treatment effects at either time point [F (3,15) = 0.838; p = 0.499 for 6 h; F(3,15) = 2.968; p = 0.075 for 24 h; Fig. 3A]. IL-18bp expression significantly decreased after 6 h of oAβ1-42 exposure compared to untreated or Car-treated cells [F(3,15) = 6.518; p = 0.007; Fig. 3B]. After 24 h, IL-18bp levels increased in the presence of oAβ1-42 compared to other groups [F(3,15) = 6.938; p = 0.006; Fig. 3B]. NLRP3 mRNA levels were unaffected by any treatment at either time point [F(3,15) = 1.299; p = 0.320 for 6 h; F(3,15) = 1.976; p = 0.172 for 24 h; Fig. 3C]. CASP-1 expression significantly increased after a 6-h exposure to Car compared to resting cells [F(3,15) = 5.010; p = 0.018; Fig. 3D], even though the same effect was not seen at 24 h. Despite that, after 24 h co-treatment (oAβ1-42 + Car) led to significantly increased CASP-1 levels compared to untreated BV-2 cells [F(3,15) = 7.739; p = 0.004; Fig. 3D]. Car treatment for 6 h also significantly raised ASC mRNA levels compared to all other treatments [F(3,15) = 24.180; p < 0.0001; Fig. 3E], but no significant effects were observed at the 24-h time point [F(3,15) = 2.161; p = 0.146; Fig. 3E].

3.2. Effects of a 6- and 24-h exposure to oAβ1-42, oAβ1-42 + Car, or Car alone on expression levels of microglia markers in BV-2 cells

Incubation with Car for 6 h significantly increased CD11b expression levels compared to cells exposed to oAβ1-42 or untreated controls [F (3,15) = 8.081; p = 0.003; Fig. 4A]. This enhancement persisted at 24 h, where CD11b expression remained significantly higher in Car-treated cells compared to those exposed to oAβ1-42, which exhibited reduced CD11b levels relative to resting cells [F(3,15) = 9.186; p = 0.002; Fig. 4A]. A similar trend was observed for CD14; in fact, after 6 h CD14 mRNA levels were significantly upregulated in Car-treated cells compared to those exposed to oAβ1-42 [F(3,15) = 5.414; p = 0.014; Fig. 4B]. However, after 24 h, both oAβ1-42 treatment and co-treatment with Car resulted in a significant decrease in CD14 expression compared to untreated cells and microglia treated with Car alone [F(3,15) = 34.582; p < 0.0001; Fig. 4B]. Statistical analysis with one-way ANOVA indicated significant effects for CD68 at 24 h and for CD86 at 6 h. In particular for CD68, significant differences were observed at 24 h [F (3,15) = 13.605; p < 0.0001; Fig. 4C], while CD86 expression showed significant effects at 6 h [F(3,15) = 20.578; p < 0.0001; Fig. 4D]. At the 6-h time point, oAβ1-42 treatment, regardless of Car presence, significantly decreased CD86 expression compared to resting cells. In contrast, after 24 h of exposure to oAβ1-42, CD68 mRNA levels were significantly reduced compared to both resting cells and those co-treated with Car.

4. Discussion

In this pivotal study, we investigated the neuroprotective effects of Car in BV-2 microglial cells exposed to oAβ and found that carnosine significantly increased IL-1β mRNA levels while not affecting pro-inflammatory cytokines IL-6 and TNFα. Furthermore, carnosine significantly increased the expression levels of anti-inflammatory mediators such as IL-10 and CXCL2 compared to oAβ alone, with a trend toward increased IL-4 levels (Fig. 1). This is consistent with previous studies on carnosine's role in enhancing anti-inflammatory responses in immune cells (Rivi et al., 2024; Caruso et al., 2019b, 2021). Increased CXCL2

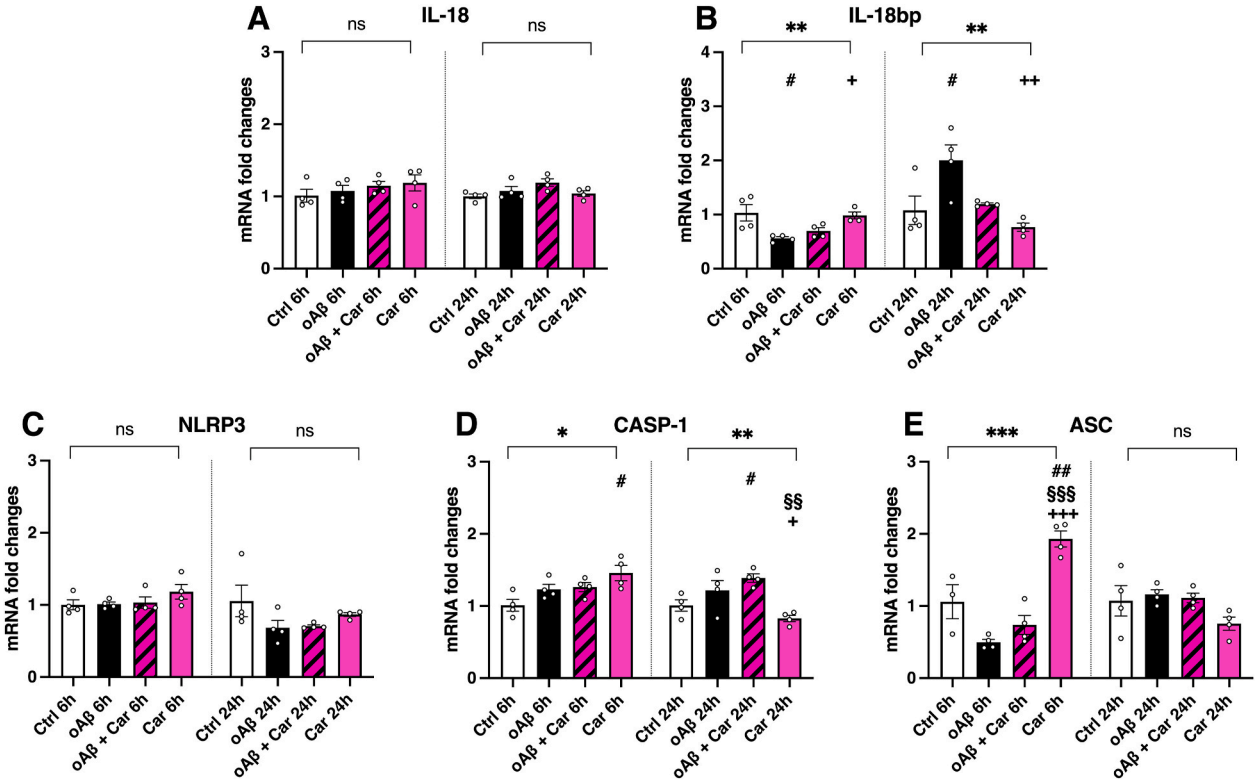

Fig. 3. Effect of a 6- and 24-h exposure to oAβ1-42, oAβ1-42 + Car, or Car alone on the expression of immune-related targets in BV-2. Cells were treated with oAβ1-42 (1 μM), in the absence or presence of Car (20 mM), or Car alone, after which mRNA expression of (A) IL-18, (B) IL-18bp, (C) NLRP3, (D) CASP-1, and (E) ASC with GAPDH/CypA/RPL27 as endogenous control, were measured by qRT-PCR. Data are represented as means ± S.E.M. and were analyzed for each time with one-way ANOVA [* = p < 0.05, ** = p < 0.01; *** = p < 0.0001] followed by Tukey's *post hoc* test: # = p < 0.05, ## = p < 0.01; ### = p < 0.001 vs resting cells at matching time; + = p < 0.05, ++ = p < 0.01, +++ = p < 0.0001 vs oAβ1-42 at matching time; § = p < 0.05; §§ = p < 0.01; §§§ = p < 0.0001 vs oAβ1-42 + Car at matching time (n = 4 per group).

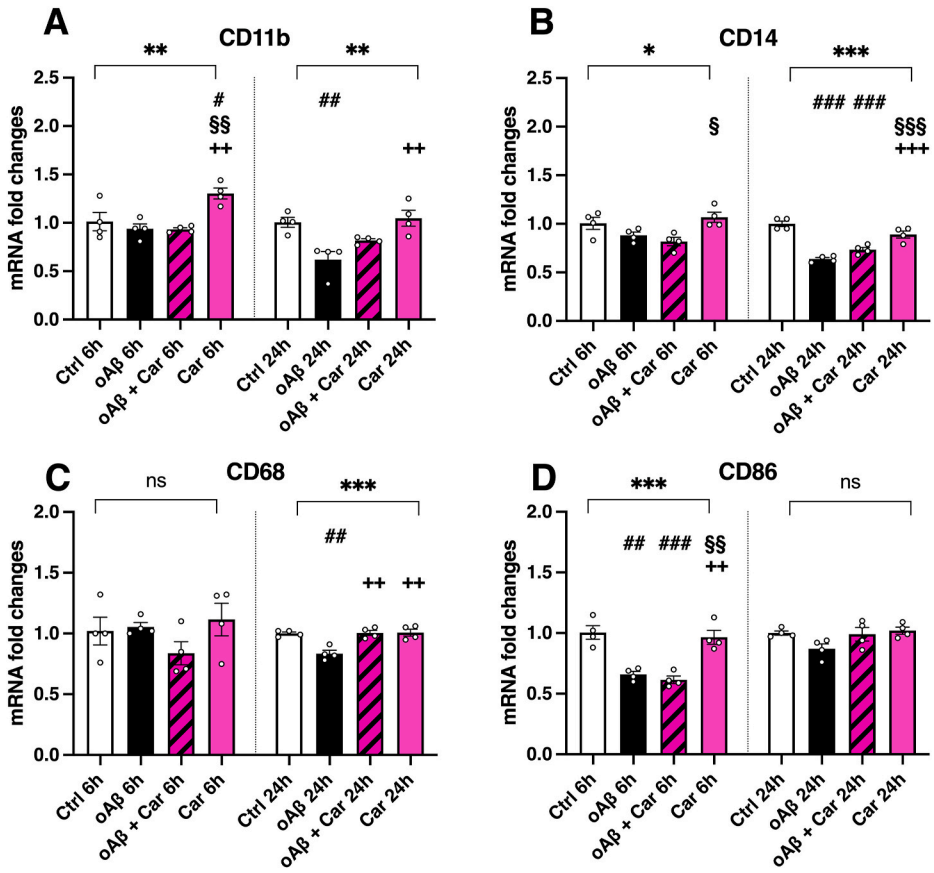

Fig. 4. Effect of a 6- and 24-h exposure to oAβ1-42, oAβ1-42 + Car, or Car alone on the expression of immune-related targets in BV-2. Cells were treated with oAβ1-42 (1 μM), in the absence or presence of Car (20 mM), or Car alone, after which mRNA expression of (A) CD11b, (B) CD14, (C) CD68, and (D) CD86 with GAPDH/CypA/RPL27 as endogenous control, were measured by qRT-PCR. Data are represented as means ± S.E.M. and were analyzed for each time with one-way ANOVA [* = $p < 0.05$, ** = $p < 0.01$; *** = $p < 0.0001$] followed by Tukey's *post hoc* test: # = $p < 0.05$, ## = $p < 0.01$; ### = $p < 0.001$ vs resting cells at matching time; + = $p < 0.05$, ++ = $p < 0.01$, +++ = $p < 0.0001$ vs oAβ1-42 at matching time; § = $p < 0.05$; §§ = $p < 0.01$; §§§ = $p < 0.0001$ vs oAβ1-42 + Car at matching time (n = 4 per group).

expression is particularly notable, as it acts as a potent neutrophil chemoattractant induced by pro-inflammatory stimuli like LPS or Aβ (Filippo et al., 2013). Additionally, carnosine's modulation of inflammatory mediators may relate to its capacity to enhance phagocytosis, as evidenced by elevated levels of phagocytic markers CD11b and CD68 (Bergman et al., 2002; Koenigsknecht-Talboo and Landreth, 2005) (Fig. 4). Carnosine treatment significantly increased CD11b levels at 6 h, surpassing all other experimental conditions. Our study also demonstrated that carnosine increased the expression of TGF-β1 and its type I receptor (TGFβR), promoting TGF-β signaling (Duan and Derynck, 2019) (Fig. 2), which contributes to neuroprotection against Aβ oligomers (Caruso et al., 2021; Cheng et al., 2020; Koenigsknecht-Talboo and Landreth, 2005). Moreover, carnosine counteracted the downregulation of CX3CR1, the receptor for fractalkine, induced by oAβ, which is vital for neuron-microglia interactions in AD (Bolós et al., 2017; Merino et al., 2016) (Fig. 2). This suggests that carnosine may play a role in regulating the CX3CL1/CX3CR1 axis. The depletion of CX3CR1 is linked to abnormal microglial proliferation around amyloid plaques (Lee et al., 2014). Thus, carnosine's ability to rescue CX3CR1 expression may have implications for drug discovery in AD. Growing evidence is emerging on the involvement of the NLRP3 inflammasome's components, CASP-1 and ASC, in neurodegenerative diseases, particularly in AD progression (Fang et al., 2014; Fang et al., 2014; Guo et al., 2023). Interestingly, NLRP3-independent activation of CASP-1 and ASC has been linked to improved immune responses (Fang et al., 2014). Carnosine's potential to enhance basal CASP-1 and ASC mRNA expression merits further exploration. Among inflammasome-dependent molecules, IL-18 is pivotal in regulating inflammatory and metabolic responses, with its activity modulated by IL-18 binding protein (IL-18bp) to maintain free IL-18 levels (Sutinen et al., 2012). Elevated IL-18 levels have been reported in the brains of AD patients and transgenic models. Conversely, higher IL-18bp levels correlate negatively with cognitive impairment in AD patients (Sutinen et al., 2012). Notably, carnosine counteracted the increase in IL-18bp expression in BV-2 cells treated with oAβ (Fig. 3), underscoring its modulatory effect on the IL-18 system. Overall, our pilot study highlights carnosine's neuroprotective effects on microglial function in the context of Aβ exposure, positioning it as a potential therapeutic agent in AD. Our comprehensive analysis establishes a solid foundation for future research into the mechanisms underlying carnosine's effects. However, limitations exist, as *in vitro* models may not fully replicate microglial behavior in vivo. Further studies are necessary to assess carnosine's effects in animal models of AD and investigate the long-term impacts of carnosine treatment on microglial function and its interactions with other neuroinflammatory pathways.

In conclusion, our study presents novel insights into carnosine's neuroprotective effects on microglial function in the context of Aβ-induced oxidative stress and inflammation, revealing its potential to modulate critical inflammatory pathways and enhance phagocytosis. The findings underscore carnosine's therapeutic potential in AD, offering a promising direction for mitigating neuroinflammation and promoting neuroprotection.

CRediT authorship contribution statement

Veronica Rivi: Methodology, Software, Validation, Formal analysis, Investigation, Data curation, Writing – original draft, Writing – review & editing, Visualization. **Giuseppe Carota:** Methodology, Validation, Investigation, Writing – original draft, Writing – review & editing, Visualization. **Fabio Tascedda:** Validation, Writing – review & editing, Project administration, Funding acquisition. **Johanna M.C. Blom:** Validation, Writing – review & editing, Project administration. **Filippo Caraci:** Validation, Resources, Writing – review & editing, Project administration, Funding acquisition. **Cristina Benatti:** Conceptualization, Software, Formal analysis, Investigation, Resources, Data curation, Writing – original draft, Writing – review & editing, Visualization, Supervision, Project administration. **Giuseppe Caruso:** Conceptualization, Validation, Investigation, Resources, Writing – original draft, Writing – review & editing, Visualization, Supervision, Project administration, Funding acquisition.

Fundings

This research was funded by the Italian Ministry of Health Research Program, grant number RC2022-2024 and by FONDO DI ATENEO PER LA RICERCA ANNO 2020, Department of Life Sciences, UNIMORE.

Declaration of competing interest

The author is an Editorial Board Member/Editor-in-Chief/Associate Editor/Guest Editor for this journal and was not involved in the editorial review or the decision to publish this article.

Acknowledgments

The authors would like to thank the BRIT laboratory at the University of Catania (Italy) for the valuable technical assistance and use of their laboratories.

Data availability

Data will be made available on request.

References

Affram, K.O., Mitchell, K., Symes, A.J., 2017. Microglial activation results in inhibition of TGF-β-regulated gene expression. J. Mol. Neurosci. 63, 308–319. https://doi.org/10.1007/s12031-017-0980-3.

Aloisi, A., Barca, A., Romano, A., Guerrieri, S., Storelli, C., Rinaldi, R., Verri, T., 2013. Anti-aggregating effect of the naturally occurring dipeptide carnosine on aβ1-42 fibril formation. PLoS One 8, 68159. https://doi.org/10.1371/journal.pone.0068159.

Bergman, M., Salman, H., Bessler, H., Omanski, M., Punsky, I., Djaldetti, M., 2002. Interaction between phagocytosis and IL-1beta production by rat peritoneal macrophages. Biomed. Pharmacother. 56, 159–162. https://doi.org/10.1016/s0753-3322(02)00166-x.

Boldyrev, A.A., Aldini, G., Derave, W., 2013. Physiology and pathophysiology of carnosine. Physiol. Rev. 93, 1803–1845. https://doi.org/10.1152/physrev.00039.2012.

Bolós, M., Llorens-Martín, M., Perea, J.R., Jurado-Arjona, J., Rábano, A., Hernández, F., Avila, J., 2017. Absence of CX3CR1 impairs the internalization of Tau by microglia. Mol. Neurodegener. 12, 59. https://doi.org/10.1186/s13024-017-0200-1.

Bondareff, W., Mountjoy, C.Q., Roth, M., Hauser, D.L., 1989. Neurofibrillary degeneration and neuronal loss in Alzheimer's disease. Neurobiol. Aging 10, 709–715. https://doi.org/10.1016/0197-4580(89)90007-9.

Caruso, G., Fresta, C.G., Fidilio, A., O'Donnell, F., Musso, N., Lazzarino, G., Grasso, M., Amorini, A.M., Tascedda, F., Bucolo, C., Drago, F., Tavazzi, B., Lazzarino, G., Lunte, S.M., Caraci, F., 2019a. Carnosine decreases PMA-induced oxidative stress and inflammation in murine macrophages. Antioxidants (Basel 8, 281. https://doi.org/10.3390/antiox8080281.

Caruso, G., Fresta, C.G., Musso, N., Giambirtone, M., Grasso, M., Spampinato, S.F., Merlo, S., Drago, F., Lazzarino, G., Sortino, M.A., Lunte, S.M., Caraci, F., 2019b. Carnosine prevents aβ-induced oxidative stress and inflammation in microglial cells: a key role of TGF-β1. Cells 8, 64. https://doi.org/10.3390/cells8010064.

Caruso, G., Benatti, C., Musso, N., Fresta, C.G., Fidilio, A., Spampinato, G., Brunello, N., Bucolo, C., Drago, F., Lunte, S.M., Peterson, B.R., Tascedda, F., Caraci, F., 2021. Carnosine protects macrophages against the toxicity of aβ1-42 oligomers by decreasing oxidative stress. Biomedicines 9, 477. https://doi.org/10.3390/biomedicines9050477.

Cheng, Y., Tian, D.-Y., Wang, Y.-J., 2020. Peripheral clearance of brain-derived Aβ in Alzheimer's disease: pathophysiology and therapeutic perspectives. Transl. Neurodegener. 9, 16. https://doi.org/10.1186/s40035-020-00195-1.

Ciani, M., Rigillo, G., Benatti, C., Pani, L., Blom, J.M.C., Brunello, N., Tascedda, F., Alboni, S., 2024. Time- and region-specific effect of vortioxetine on central LPS-induced transcriptional regulation of NLRP3 inflammasome. Curr. Neuropharmacol. https://doi.org/10.2174/1570159X22666240705143649.

Ding, Z., Guo, S., Luo, L., Zheng, Y., Gan, S., Kang, X., Wu, X., Zhu, S., 2021. Emerging roles of microglia in neuro-vascular unit: implications of microglia-neurons interactions. Front. Cell. Neurosci. 15, 706025. https://doi.org/10.3389/fncel.2021.706025.

Duan, D., Derynck, R., 2019. Transforming growth factor-β (TGF-β)-induced up-regulation of TGF-β receptors at the cell surface amplifies the TGF-β response. J. Biol. Chem. 294, 8490–8504. https://doi.org/10.1074/jbc.RA118.005763.

Fang, R., Zhao, X., Smalley, C., Saito, T., Brouwer, D., Ismail, N., Walker, D., 2014. ASC inflammasome mediates host protection against rickettsiae via a NLRP3-independent signaling pathway (INC7P.403. J. Immunol. 192, 186. https://doi.org/10.4049/jimmunol.192.Supp.186.4, 4.

Filippo, K., Dudeck, A., Hasenberg, M., Nye, E., Rooijen, N., Hartmann, K., Gunzer, M., Roers, A., Hogg, N., 2013. Mast cell and macrophage chemokines CXCL1/CXCL2 control the early stage of neutrophil recruitment during tissue inflammation. Blood 121, 4930–4937. https://doi.org/10.1182/blood-2013-02-486217.

Garrison, A.M., Parrott, J.M., Tuñon, A., Delgado, J., Redus, L., O'Connor, J.C., 2018. Kynurenine pathway metabolic balance influences microglia activity: targeting kynurenine monooxygenase to dampen neuroinflammation. Psychoneuroendocrinology 94, 1–10. https://doi.org/10.1016/j.psyneuen.2018.04.019.

Guo, T., Zhang, D., Zeng, Y., Huang, T.Y., Xu, H., Zhao, Y., 2020. Molecular and cellular mechanisms underlying the pathogenesis of Alzheimer's disease. Mol. Neurodegener. 15, 40. https://doi.org/10.1186/s13024-020-00391-7.

Guo, S., Wang, H., Yin, Y., 2022. Microglia polarization from M1 to M2 in neurodegenerative diseases. Front. Aging Neurosci. 14, 815347. https://doi.org/10.3389/fnagi.2022.815347.

Guo, M.-L., Roodsari, S.K., Cheng, Y., Dempsey, R.E., Hu, W., 2023. Microglia NLRP3 inflammasome and neuroimmune signaling in substance use disorders. Biomolecules 13, 922. https://doi.org/10.3390/biom13060922.

Hambardzumyan, D., Gutmann, D.H., Kettenmann, H., 2016. The role of microglia and macrophages in glioma maintenance and progression. Nat. Neurosci. 19, 20–27. https://doi.org/10.1038/nn.4185.

Hanslik, K.L., Ulland, T.K., 2020. The role of microglia and the Nlrp3 inflammasome in Alzheimer's disease. Front. Neurol. 11, 570711. https://doi.org/10.3389/fneur.2020.570711.

Hipkiss, A.R., Brownson, C., Bertani, M.F., Ruiz, E., Ferro, A., 2002. Reaction of carnosine with aged proteins: another protective process? Ann. N. Y. Acad. Sci. 959, 285–294. https://doi.org/10.1111/j.1749-6632.2002.tb02100.x.

Koenigsknecht-Talboo, J., Landreth, G.E., 2005. Microglial phagocytosis induced by fibrillar β-amyloid and IgGs are differentially regulated by proinflammatory cytokines. J. Neurosci. 25, 8240–8249. https://doi.org/10.1523/JNEUROSCI.1808-05.2005.

Kulebyakin, K., Karpova, L., Lakonsteva, E., Krasavin, M., Boldyrev, A., 2012. Carnosine protects neurons against oxidative stress and modulates the time profile of MAPK cascade signaling. Amino Acids 43, 91–96. https://doi.org/10.1007/s00726-011-1135-4.

Lee, S., Xu, G., Jay, T.R., Bhatta, S., Kim, K.-W., Jung, S., Landreth, G.E., Ransohoff, R.M., Lamb, B.T., 2014. Opposing effects of membrane-anchored CX3CL1 on amyloid and tau pathologies via the p38 MAPK pathway. J. Neurosci. 34, 12538–12546. https://doi.org/10.1523/JNEUROSCI.0853-14.2014.

Merino, J.J., Muñetón-Gómez, V., Alvárez, M.-I., Toledano-Díaz, A., 2016. Effects of CX3CR1 and fractalkine chemokines in amyloid beta clearance and p-tau accumulation in Alzheimer's disease (AD) rodent models: is fractalkine a systemic biomarker for AD? Curr. Alzheimer Res. 13, 403–412. https://doi.org/10.2174/1567205013666151116125714.

Quinn, P.J., Boldyrev, A.A., Formazuyk, V.E., 1992. Carnosine: its properties, functions and potential therapeutic applications. Mol. Aspect. Med. 13, 379–444. https://doi.org/10.1016/0098-2997(92)90006-l.

Rajanikant, G.K., Zemke, D., Senut, M.-C., Frenkel, M.B., Chen, A.F., Gupta, R., Majid, A., 2007. Carnosine is neuroprotective against permanent focal cerebral ischemia in mice. Stroke 38, 3023–3031. https://doi.org/10.1161/STROKEAHA.107.488502.

Rivi, V., Caruso, G., Caraci, F., Alboni, S., Pani, L., Tascedda, F., Lukowiak, K., Blom, J.M.C., Benatti, C., 2024. Behavioral and transcriptional effects of carnosine in the central ring ganglia of the pond snail Lymnaea stagnalis. J. Neurosci. Res. 102, e25371. https://doi.org/10.1002/jnr.25371.

Rivi, V., Batabyal, A., Benatti, C., Tascedda, F., Blom, J.M.C., Lukowiak, K., 2025. Quercetin, the new stress buster: investigating the transcriptional and behavioral effects of this flavonoid on multiple stressors using *Lymnaea stagnalis*. Comp. Biochem. Physiol. C Toxicol. Pharmacol. 287, 110053. https://doi.org/10.1016/j.cbpc.2024.110053.

Sutinen, E.M., Pirttilä, T., Anderson, G., Salminen, A., Ojala, J.O., 2012. Pro-inflammatory interleukin-18 increases Alzheimer's disease-associated amyloid-β production in human neuron-like cells. J. Neuroinflammation 9, 199. https://doi.org/10.1186/1742-2094-9-199.

Wang, Q., Ishikawa, T., Michiue, T., Zhu, B.-L., Guan, D.-W., Maeda, H., 2012. Stability of endogenous reference genes in postmortem human brains for normalization of

quantitative real-time PCR data: comprehensive evaluation using geNorm, NormFinder, and BestKeeper. Int. J. Leg. Med. 126, 943–952. https://doi.org/10.1007/s00414-012-0774-7.