Poly(I:C) increases the expression of mPGES-1 and COX-2 in rat primary microglia

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Abstract

Background: Microglia recognize pathogen-associated molecular patterns such as double-stranded RNA (dsRNA) present in some viruses. Polyinosinic-polycytidylic acid [poly(I:C)] is a synthetic analog of dsRNA that activates different molecules, such as retinoic acid-inducible gene I, melanoma differentiation-associated gene 5, and toll-like receptor-3 (TLR3). Poly(I:C) increases the expression of different cytokines in various cell types. However, its role in the regulation of the production of inflammatory mediators of the arachidonic acid pathway by microglia is poorly understood.

Methods: In the present study, we evaluated the effect of poly(I:C) on the production of prostaglandin E2 (PGE2) and the inducible enzymes cyclooxygenase-2 (COX-2) and microsomal prostaglandin E synthase-1 (mPGES-1) in primary rat microglia. Microglia were stimulated with different concentrations of poly(I:C) (0.1–10 μg/ml), and the protein levels of COX-2 and mPGES-1, as well as the release of PGE2, were determined by western blot and enzyme immunoassay (EIA), respectively. Values were compared using one-way ANOVA with post hoc Student-Newman-Keuls test.

Results: Poly(I:C) increased the production of PGE2, as well as mPGES-1 and COX-2 synthesis. To investigate the mechanisms involved in poly(I:C)-induced COX-2 and mPGES-1, we studied the effects of various signal transduction pathway inhibitors. Protein levels of COX-2 and mPGES-1 were reduced by SB203580, SP600125, and SC514 (p38 mitogen-activated protein kinase (MAPK), c-Jun N-terminal kinase (JNK), and IκB kinase (IKK) inhibitors, respectively), as well as by PD98059 and PD0325901 (mitogen-activated protein kinase kinase (MEK) inhibitors). Rapamycin, a mammalian target of rapamycin (mTOR) inhibitor, enhanced the synthesis of COX-2. Inhibition of phosphatidylinositol 3-kinase (PI3K) by LY294002 or dual inhibition of PI3K/mTOR (with NVP-BEZ235) enhanced COX-2 and reduced mPGES-1 immunoreactivity. To confirm the data obtained with the inhibitors, we studied the phosphorylation of the blocked kinases by western blot. Poly(I/C) increased the phosphorylation of p38 MAPK, extracellular signal-regulated kinase (ERK), JNK, protein kinase B (Akt), and IκB.

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Taken together, our data demonstrate that poly(I:C) increases the synthesis of enzymes involved in PGE₂ synthesis via activation of different signaling pathways in microglia. Importantly, poly(I:C) activates similar pathways also involved in TLR4 signaling that are important for COX-2 and mPGES-1 synthesis. Thus, these two enzymes and their products might contribute to the neuropathological effects induced in response to dsRNA, whereby the engagement of TLR3 might be involved.

**Keywords:** Polyninosinic-polycytidylic acid, TLR3, COX-2, mPGES-1, Microglia

**Background**

Microglia express receptors that recognize pathogen-associated molecular patterns leading to various immunological responses. For instance, the toll-like receptor-3 (TLR3) is an intracellular receptor that recognizes double-stranded RNA produced by some viruses [1, 2]. TLR3 might also be activated by endogenous messenger RNA (mRNA) released from apoptotic cells [3], and activation of this receptor has been shown to increase the expression of different cytokines in various cell types, including microglia [4, 5].

Polyinosinic-polycytidylic acid [poly(I:C)] is a ligand of TLR3 and is able to activate different immune cells in a toll/interleukin (IL)-1 receptor domain-containing adaptor inducing IFN-β (TRIF)-dependent manner [6]. Activation of this receptor is important to protect and fight against infections, since its activation induces the production of type I interferon and other cytokines involved in antiviral responses [7]. Furthermore, poly(I:C) increases the phagocytosis and the intracellular killing of *Escherichia coli* by primary microglia [8].

Besides its role in infections, TLR3 activation might be involved in neurodegeneration, psychiatric disorders, and pain [2, 9–12]. Considering that RNA released from necrotic cells could activate TLR3 [3], it may be assumed that the binding of endogenous nucleic acid released from dying neurons could activate TLR3 in other cell types, such as microglia, and promote an inflammatory process in the brain. Systemic administration of poly(I:C) increases apoptosis and exacerbates an existing chronic neurodegenerative process in a ME7 model of prion disease [9]. Furthermore, injection of poly(I:C) enhances neuronal loss in the substantia nigra pars compacta and striatum induced by 6-hydroxydopamine and paraquat [13, 14]. Additionally, challenge of mice aged 5 to 7 days with poly(I:C) induces schizophrenia-like signs, as well as a progressive microglia activation [15]. Indeed, prenatal injection of poly(I:C) in rodents is used as a neurodevelopmental model of schizophrenia [2, 16].

Although different studies have demonstrated that the effects of poly(I:C) might be dependent on TLR3, it is currently known that this compound acts via other targets. To date, it has been shown that poly(I:C) activates retinoic acid-inducible gene 1 (RIG-I) and melanoma differentiation-associated gene 5 (MDA5), which are also pattern recognition receptors (PRRs) that recognize pathogen-specific molecular patterns [17, 18]. Interestingly, the involvement of these PRRs in neurodegeneration has also been suggested [19, 20].

Although the pathological conditions induced by poly(I:C) might be a consequence of an inflammatory process that leads to neurodevelopmental abnormalities, neurodegenerative processes, or pain, the underlying mechanisms are still unknown. These effects might be associated with microglia activation [21], which results in the release of neurotoxic molecules such as the lipid inflammatory mediators from the arachidonic acid cascade. Since cyclooxygenase-2 (COX-2), microsomal prostaglandin E synthase-1 (mPGES-1), and prostaglandin E₂ (PGE₂) are involved in neurodegeneration, psychiatric disorders, and pain [22–26], these molecules may mediate the pathological effects induced by dsRNA.

Thus, it is necessary to unveil molecular mechanisms induced by a viral mimetic in isolated brain microglial cells, since these cells are the main source of various inflammatory mediators. Different studies use lipopolysaccharide (LPS) as a gold standard to activate microglia, but the main receptor of this substance is the TLR4. However, although it has been shown that TLR3 ligands increase the production of cytokines in microglia [27, 28], the role of this receptor in the production of inflammatory lipid mediators in microglia is poorly understood. In the present study, we evaluated the effect of poly(I:C) in the synthesis of molecules involved in the arachidonic acid cascade (i.e., COX-2, mPGES-1, and PGE₂), as well as the intracellular mechanisms involved in these responses in rat primary microglia.

**Methods**

The following inhibitors were purchased from Calbiochem (Bad Soden, Germany): PD 98059 (2′-amino-3′-methoxyflavone), an inhibitor of mitogen-activated protein kinase kinase (MEK); SB 203580 [4-(4-fluorophenyl)-2-(4-methylsulfinylphenyl)-5-(4-pyridyl)1H-imidazole], an inhibitor of p38 mitogen-activated protein kinase (MAPK); SP600125 [anthra(1,9-cd)pyrazol-6(2H)-one 1,9-pyrazoloanthrone], an inhibitor of c-Jun N-terminal kinase (JNK); rapamycin [(3S,6R,7E,9R,10R,12R,14S,15E,17E,19E,21S,23S,26R,27R,29S)-3β,6β,21α,23β,26α,27α,29α-7,14,21,23,26,27,29-septahydroxy-8,9,12,14,17,21,23,26,27,29-decahydro-1H-28-anthracen-1,4-diene-23-carboxylic acid], an inhibitor of 3′-5′ cyclic nucleotide phosphodiesterase (PDE); and Z-VAD-FMK [(3α,5α)-1H-benzimidazole-2-carboxylic acid, (5α)-1,2,3,4-tetrahydrocarbazole-3-carboxylic acid, (4S)-4-[(2S)-2-methyl-2-[[(2S)-1-methyl-2,3-dihydrobenzofuran-3-yl]methyl]amino]-3-butenoic acid], an inhibitor of caspase-3, caspase-8, and caspase-9.
Primary microglia cultures
Primary microglia cultures were prepared from cerebral cortices of 1-day neonatal Wistar rats [29, 30]. In brief, forebrains were minced and gently dissociated by repeated pipetting in Hank’s balanced salt solution (PAA Laboratories GmbH, Cölbe, Germany). Dissociated cells were then passed through nylon cell strainer (BD biosciences, Heidelberg, Germany). Cells were collected by centrifugation, re-suspended in Dulbecco’s modified Eagle’s medium (DMEM) containing 10 % fetal calf serum (FCS) and antibiotics and cultured on 10-cm cell culture dishes (Falcon, 5 \times 10^5 cells/plate) in 5 % CO2 at 37 °C. Floating microglia were harvested from 12 to 14-day-old mixed (astrocyte-microglia) primary cultures and re-seeded into cell culture plates at the density of 2 \times 10^5 cells/ml to give pure microglial cultures. On the next day, cells were washed to remove non-adherent cells, and fresh medium was added. After 1 h, cells were used for the different experiments.

Western blot analysis
Thirty minutes, 24 or 48 h after stimulation with poly(I:C), microglial cells were washed with phosphate-buffered saline (PBS) and lysed in 1.3 \times SDS (sodium dodecyl sulfate)-containing sample buffer without dithiothreitol (DTT) or bromophenol blue containing 100 μM orthovanadate [31]. Cell lysates were homogenized by repeated passage through a 26-gauge needle. Protein contents were measured using the bicinchoninic acid (BCA) method (Thermo Fischer Scientific, Waltham, MA, USA). Bovine serum albumin (BSA) was used as a protein standard at concentrations ranging from 0.2 to 4 μg/μl, and the optical density was read at 570 nm using a microplate reader. Immediately before electrophoresis, bromophenol blue and DTT (final concentration, 10 mM) were added to the samples. For mPGES-1, COX-2, p-p38 MAPK, p-JNK, p-ERK, p-IκB-α, p-Akt, and α-tubulin immunoblotting, 30 to 50 μg of protein from each sample was subjected to SDS-PAGE (polyacrylamide gel electrophoresis) on a 10 % (for p-p38 MAPK, p-JNK, p-ERK, p-IκB-α, p-Akt) or 12 % (for COX-2 and mPGES-1) gel under reducing conditions. Proteins were then transferred onto a polyvinylidene fluoride (PVDF) membrane (Millipore, Bedford, MA, USA) by semi-dry blotting. The membrane was blocked for 1 or 2 h at room temperature using Rotiblock (Roth, Karlsruhe, Germany) for COX-2 or 5 % blocking milk (BioRad, München, Germany) for the other proteins, before the overnight incubation at 4°C with the primary antibody. Primary antibodies were goat anti-COX-2 (M-19, Santa Cruz, Heidelberg, Germany); rabbit anti-mPGES-1 (Cayman Chemical, USA; 1:500); rabbit anti-actin and anti-p-ERK, anti-p-JNK, anti-p-IκB-α, anti-p-Akt, and anti-α-tubulin (all from Cell Signaling Technology). After extensive washing (three times for 15 min each in TBS containing 0.1 % Tween 20), proteins were detected with horseradish peroxidase (HRP)-coupled rabbit anti-goat IgG (Santa Cruz, 1:100,000) by semi-dry blotting. The membrane was then dried before digitizing using a microplate reader.

Enzyme immunoassay (EIA)
Twenty-four hours after stimulation, supernatants were harvested, centrifuged at 10,000×g for 10 min, and levels of PGES in the media were measured by enzyme immunoassay (EIA) (Biotrend, Köln, Germany) according to the manufacturer’s instructions. Standards from 39 to 2500 pg/ml were used; sensitivity of the assay was 36.2 pg/ml.
Statistical analysis
At least three independent experiments were used for data analysis. Original data were converted into % values of LPS or poly(I:C) controls, and mean ± S.E.M. were calculated. Values were compared using t test (two groups) or one-way ANOVA with post hoc Student-Newman-Keuls test (multiple comparisons). For each experiment, P value < 0.05 was considered statistically significant. Significant effects are indicated by asterisks (*P < 0.05, **P < 0.01, ***P < 0.001).

Results
Poly(I:C) increases the expression of mPGES-1 and COX-2 and PGE2 production
We first evaluated the effect of poly(I:C) on the production of PGE2, a prostanoid involved in neuroinflammatory conditions. Poly(I:C) increased the production of PGE2 at concentrations of 5 and 10 μg/ml in rat primary microglia (Fig. 1a; P < 0.05 and 0.001, respectively). In order to investigate the mechanism by which poly(I:C) increases PGE2, we investigated the synthesis of mPGES-1 and COX-2 after treatment with poly(I:C). 10 μg/ml of poly(I:C) increased the synthesis of mPGES-1 and COX-2 proteins at 24 (Fig. 1b, c; P < 0.05)- and 48-h (Fig. 1d, e; P < 0.05) post-stimulation. Interestingly, poly(I:C) increased mPGES-1 synthesis even at lower concentrations (1–10 μg/ml, Fig. 1d, e, P < 0.05) at 48 h. In the same conditions, LPS (10 ng/ml), used as a positive control, also increased the synthesis of mPGES-1 and COX-2 (Fig. 1b–e).

Inhibition of IKK-2, MEK, JNK, and p38 MAPK reduces the expression of mPGES-1 and COX-2 induced by poly(I:C)
As described in our previous studies, mPGES-1 and COX-2 are regulated in microglia by various signal transduction pathways [29, 33] such as protein kinase C, NF-κB, MEK, JNK, and p38 MAPK. We therefore evaluated whether the increase in COX-2 and mPGES-1 induced by poly(I:C) could be altered by the inhibition of different kinases. As shown in Fig. 2a, b, inhibition of IKK-2, JNK, MEK, and p38 MAPK with SC514, SP600125, PD98059, and SB203580, respectively, reduced the levels of mPGES-1 and COX-2 proteins 24 h after stimulation with poly(I:C) (P < 0.05). A similar pattern of inhibition of these kinases was also observed 48 h after stimulation (Fig. 2c, d; P < 0.05). Considering that PD98059 might have direct inhibitory effects on COX-2 [34], we evaluated the effect of other MEK inhibitor on the expression of this enzyme, as well as on mPGES-1. We confirmed that PD0325901, a selective MEK1/2 inhibitor, reduced COX-2 and mPGES-1 protein levels induced by poly(I:C) (P < 0.05; Fig. 2e, f).

Inhibition of PI3K and mTOR differently regulates the expression of mPGES-1 and COX-2 induced by poly(I:C)
We have previously demonstrated that inhibition of PI3K and mTOR differently regulates the expression of mPGES-1 and COX-2 in LPS-stimulated rat primary microglia [29, 33]. Thus, we decided to investigate the effect of PI3K and mTOR inhibition on microglia stimulated with a TLR3 agonist. As observed in Fig. 3a–c, LY294002, a PI3K inhibitor, increased the immunoreactivity of COX-2 at 24 and 48 h after stimulation (P < 0.001 and P < 0.05, respectively), although mPGES-1 was reduced only at 48 h (P < 0.05). A similar pattern of the modulation of production of COX-2 and mPGES-1 was also obtained with NVP-BEZ235, a PI3K dual inhibitor (Fig. 3a–c, P < 0.05). On the other hand, inhibition of mTOR with rapamycin enhanced the immunoreactivity of COX-2 at 24 and 48 h, although a strong tendency towards induction of mPGES-1 was observed at 48 h after stimulation with poly(I:C) (P < 0.05; Fig. 3b, c).

Poly(I:C) increases the phosphorylation of ERK, JNK, p38 MAPK, Akt, and IκB-α
We next studied whether poly(I:C) directly activated the intracellular signaling molecules described above. Thirty minutes after stimulation, poly(I:C) increased the phosphorylation of JNK and p38 MAPK in all concentrations used (Fig. 4a, b, P < 0.001). Phosphorylation of IκB-α were also obtained with the same concentrations (0.1–10 μg/ml; Fig. 4e, f, P < 0.001). ERK (Fig. 4c, d, P < 0.05) and protein kinase B (Akt) (Fig. 4e, f, P < 0.001) were activated by poly(I:C) only at a higher concentration (10 μg/ml). As a control for the experiments, we demonstrated that LPS (10 ng/ml) increased the phosphorylation of IκB-α and all kinases studied (Fig. 4a, c, e) at the same time point.

Discussion
In the present study, we evaluated the effects of the TLR3 ligand poly(I:C) on the synthesis of enzymes of the arachidonic acid cascade, as well as the intracellular signaling pathways involved in their expressions in rat primary microglia. We demonstrate here that poly(I:C) increases the synthesis of COX-2 and mPGES-1 in rat primary microglia and that these effects are dependent on the activation of MAPKs, PI3K, mTOR, and NF-κB.

Various studies have been performed to elucidate the roles of both mPGES-1 and COX-2, since these enzymes are induced during inflammatory processes. The increased production of PGE2 by microglia during neuroinflammatory processes might be important for the development of psychiatric and neurodegenerative processes [22, 35, 36]. Thus, we decided to investigate the effect of a TLR3 agonist on the production of these molecules of the arachidonic acid
cascade in microglia. Importantly, poly(I:C) might be considered as an alternative stimulus to LPS to activate microglial cells.

We first demonstrated that poly(I:C) increased the immunoreactivity of both COX-2 and mPGES-1, as well as the production of PGE$_2$. In contrast to our results,

**Fig. 1** Effect of poly(I:C) on the production of PGE$_2$, and mPGES-1 and COX-2 protein levels in primary microglia. 

*a* Effect of poly(I:C) (0.1–10 μg/ml) on PGE$_2$ production after 24 h of stimulation in rat primary microglia. 

*b, d* Quantitative densitometric analysis of COX-2 and mPGES-1 protein levels normalized to actin loading control at 24 (b) and 48 h (d). 

*c, e* Immunoblot analysis of protein levels of COX-2, mPGES-1, and actin in poly(I:C)-activated microglia at 24 (c) and 48 h (e). *P < 0.05 and ***P < 0.001 with respect to negative control.
Gutierrez-Venegas and Rodriguez-Perez [37] demonstrated that poly(I:C) reduced the expression of COX-2 and the production of PGE\(_2\) induced by histamine in human gingival fibroblasts. These differences observed in the two studies might be due to the different cell type and stimulation agents used. However, another study has shown that, in RAW264.7 cells, poly(I:C) increased the synthesis of COX-2 and PGE\(_2\) production via TLR3 [38].
Besides, in microglia, poly(I:C) increased the production of TNF-α, IL-6, and IFN-β in a TLR3-dependent manner [28]. In accordance with our data, it has been shown that poly(I:C) increases the expression of mPGES-1 and COX-2 in mouse glial cells [39–41]. However, in these studies, the authors did not investigate whether the effects of poly(I:C) in microglia were mediated by TLR3 and did not elucidate the signaling cascades involved in their syntheses. The increased expression of inflammatory mediators, such as cytokines and prostanoids, induced by dsRNA stimulation, could contribute to the development of the neuropathological processes promoted by the activation of some cytosolic PRRs.

In order to investigate the signaling pathways involved in the synthesis of mPGES-1 and COX-2, we evaluated the effect of poly(I:C) in the phosphorylation of kinases and IκB-α. We demonstrated here that poly(I:C) increased the activation of all MAPKs. These results are in accordance with the study of Steer et al. [42], which demonstrated that poly(I:C) induces MAPK activation in macrophages. Jing et al. [43] showed that activation of TLR3 recruits the complex formed by TRAF6 (TNF receptor-associated factor 6)-TAK1 (TGF-β-activated kinase 1)-TAB2 (TAK1-binding protein 2), which thereafter translocates to the cytosol and interacts with dsRNA-dependent protein kinase (PKR), inducing TAK1 activation. This activation leads to further activation of MAPK and NF-κB. This work was corroborated by another study, which showed that poly(I:C) induced the phosphorylation of MKK3/6 and p38 MAPK in human natural killer cells [44], MKK4/7, and JNK in fibroblast-like synoviocytes [45] and ERK in bone marrow-derived macrophages [46]. However, in human dendritic cells, poly(I:C) failed to induce phosphorylation of JNK, ERK, and p38 MAPK, albeit this effect was observed in rheumatoid arthritis synovial fibroblasts [4]. In accordance with our data, activation of these kinases has also been demonstrated in mouse microglia although the concentrations of poly(I:C) necessary for these effects were higher [28].

MAPKs are important regulators of the expressions of inflammatory mediators. Activation of ERK by poly(I:C) controls the production of nitric oxide and IL-1-β in macrophages [47, 48]. Inhibitors of JNK and p38 MAPK reduced the production of CXCL10 in a human bronchial epithelial cell line and human natural killer cells.
stimulated with poly(I:C), respectively [44, 49]. JNK and p38 MAPK induce the transcription of genes via activation of different downstream molecules [50, 51]. Moreover, different studies have demonstrated that JNK and p38 MAPK stabilize mRNA, leading to an enhanced transduction. In monocyte-derived dendritic cells, poly(I:C) induced stabilization of IFN-β mRNA, and this effect is mediated by TRIF, MK2, and p38 MAPK [52]. Considering that JNK and p38 MAPK might be involved in the stabilization of COX-2 mRNA [53–55], it is possible that, in our conditions, the reduction in COX-2 observed with the incubation of the cells with JNK and p38 MAPK inhibitors might be due to a reduced stability of the COX-2 mRNA, although a direct effect on gene transcriptions cannot be discarded.

The NF-κB is involved in the expression of different inflammatory mediators. We and others have previously demonstrated that NF-κB regulates the expression of COX-2 and mPGES-1 induced by LPS [33, 56]. However, it has been demonstrated that poly(I:C) and LPS induce distinct NF-κB signaling [57]. Thus, we aimed to investigate whether poly(I:C) induces NF-κB activation in microglia. We have demonstrated that poly(I:C) increased IκB phosphorylation and that SC514, a IKK-2
inhibitor, reduced the expression of both COX-2 and mPGES-1. Thus, we were able to attest that NF-κB is involved in the expression of COX-2 and mPGES-1 induced by poly(I:C). In contrast to our previous results, SC-514 abolished the expression of COX-2 induced by poly(I:C), albeit higher concentrations of SC-514 only partially reduced the expression of this enzyme induced by LPS in microglia [33]. Thus, it might be possible that the mechanism or the kinetics of activation of NF-κB induced by LPS and poly(I:C) differ.

The PI3K/Akt/mTOR pathway controls a wide range of physiological and pathological events. These enzymes are involved in the production of inflammatory mediators. Here, we showed that inhibition of mTOR with rapamycin enhanced the synthesis of COX-2. On the other hand, inhibition of PI3K or a dual inhibition of PI3K and mTOR increased the expression of COX-2, albeit the expression of mPGES-1 was partially reduced only at 48 h. We have previously demonstrated that inhibition of PI3K or dual inhibition of PI3K/mTOR increased the expression of COX-2 and reduced the expression of mPGES-1 in LPS-activated microglia [29]. Importantly, Akt is downstream of TRIF and TANK-binding kinase 1 and regulates the expression of IFN-β mRNA in RAW264.7 cells induced by poly(I:C) through interferon regulatory factor 3 (IRF3) activation [58]. Interestingly, Tarassishin and colleagues [59] have recently shown that IRF3 also activates Akt, and inhibition of PI3K with LY294002 reduced the expression of the immunoregulatory cytokines IL-10, IL-1ra, and IFN-β, suggesting an anti-inflammatory role of this kinase. Figure 5 summarizes all the intracellular molecules investigated in this study that contribute to the regulation of COX-2 and mPGES-1 protein levels induced by poly(I:C) in rat primary microglia.

We have previously demonstrated that LPS, a TLR4 ligand, increased the expression of COX-2 and mPGES-1 in primary microglia, and different kinases were involved in the regulation of these two enzymes [29, 33]. Whereas activation of TLR4 utilizes both MyD88 and TRIF, TLR3 signals only via TRIF [7]. Although TLR3 does not stimulate signals via the MyD88 adapter molecule, the signals promoted from TLR3 and TLR4 converge to TRIF, which could lead to further activation of different downstream molecules necessary for COX-2 and mPGES-1 expression.

In the present study, we were not able to prove that the effects shown by poly(I:C) are mediated by the TLR3 in our rat microglia model. It might also be possible that the effects of poly(I:C) are mediated via a TLR3-independent pathway. For example, it has been demonstrated that the effects of poly(I:C) on the expression of COX-2 were not dependent on TLR3 [42]. Furthermore, deficiency of TLR3 also did not avoid the CD8 T cell expansion induced by poly(I:C) [60]. In this same study, it was demonstrated that injection of poly(I:C) increased
the levels of serum IL-6 in WT and TLR3−/− mice, albeit the levels of TNF-α were reduced, demonstrating a differential regulation of poly(I:C) on both cytokines [60]. Poly(I:C) might have other targets, such as the melanoma differentiation-associated gene 5-deficient mice (MDA5), which is a cytosolic PRR that recognizes viral RNA. It has been shown that in MDA5−/− mice, poly(I:C) administration did not increase the sera levels of IFN-γ as compared with those in WT mice. The production of IL-6 and IL-12p40 was also impaired in MDA5−/− mice [61]. Moreover, poly(I:C) may also activate the NLRP3 inflammasome in TLR3- and MDA5-independent pathways [62].

Conclusions

In conclusion, we provide evidences that the signaling cascades involved in the expression of COX-2 and mPGES-1 induced by poly(I:C) are similar with the pathways induced by LPS in microglia. Finally, considering that activation of PRRs, such as TLR3, might be associated with pain and psychiatric and neurodegenerative diseases [2, 9–11], it could be speculated that COX-2, mPGES-1, and PGE2 induced by this stimulus could partially contribute to the development of neuropathological conditions.

Abbreviations

IKK: IκB kinase; Akt: protein kinase B; COX: cyclooxygenase; ERK: extracellular signal-regulated kinase; GSK-3: glycogen synthase kinase 3; IL: interleukin; JNK: c-Jun N-terminal kinase; LPS: lipopolysaccharide; mPGES: microsomal prostaglandin E synthase; MAPK: mitogen-activated protein kinase; MEK: mitogen-activated protein kinase kinase; mTOR: mammalian target of rapamycin; poly(I:C): polyinosinic-polycytidylic acid; Pgc: prostaglandin; PPAR: peroxisome proliferator-activated receptor; PGE2: prostaglandin E2; TNF: tumor necrosis factor.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

ACPD0, NMY, and BLF participated in the research design. The experiments were performed by ACPD0, NMY, and JH. The data were analyzed by ACPD0, NMY, HSB, and JH. ACPD0, NMY, HSB, and BLF wrote or contributed to the writing of the manuscript. In addition, ACPD0, NMY, HSB, MH, and BLF reviewed the data and discussed the manuscript. All authors have read and approved the final version of the manuscript.

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