The PPARα Agonist Fenofibrate Reduces the Levels of Proinflammatory Cytokines in a Maternal Immune Activation Model of Schizophrenia

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Article

The PPARα Agonist Fenofibrate Reduces the Levels of Proinflammatory Cytokines in a Maternal Immune Activation Model of Schizophrenia

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Abstract: Maternal infections during pregnancy may increase the risk of psychiatric disorders in offspring. We recently demonstrated that activation of peroxisome proliferator-activate receptor-α (PPARα), with the clinically available agonist fenofibrate, attenuates the neurodevelopmental disturbances induced by maternal immune activation (MIA) in rat offspring. We hypothesized that fenofibrate might reduce MIA-induced cytokine imbalance using a MIA model based on the viral mimetic polyriboinosinic-polyribocytidilic acid [poly (I:C)]. By using the Bio-Plex Multiplex-Immunoassay-System, we measured cytokine/chemokine levels in maternal serum and in the fetal brain of rats treated with fenofibrate, at 6 and 24 hours after poly (I:C). We found that MIA induced time-dependent changes in the levels of several cytokines/chemokines/colony-stimulating factors (CSFs). Specifically, the maternal serum of the poly (I:C)/CTRL group showed increased levels of (*i*) proinflammatory chemokine macrophage inflammatory protein 1-alpha (MIP-1α), (*ii*) tumor necrosis factor-alpha (TNF-α), the monocyte chemoattractant protein-1 (MCP-1), the macrophage (M-CSF) and granulocyte-macrophage colony-stimulating factor (GM-CSF). Conversely, in the fetal brain of the poly (I:C)/CTRL group, interleukin 12p70 and MIP-1α levels were lower than in veh/CTRL group. Notably, MIP-1α, TNF-α, GRO/KC, GM-CSF, and M-CSF levels were lower in the poly (I:C)/FEN than in poly (I:C)/CTRL rats, indicating the protective role of the PPARα agonist. PPARα might represent a therapeutic target to attenuate the consequences of MIA.

Keywords: MIA; cytokines; chemokines; colony-stimulating factors; Poly (I:C); neurodevelopmental disorders

1. Introduction

Maternal infections during pregnancy have been consistently related to neurodevelopmental disorders in offspring [1–3]. Currently, the pandemic impact of SARS-CoV-2 on pregnant women raises serious concerns. Among others, maternal immune activation (MIA) might represent a pathway by which infection with SARS-CoV-2 during gestation could influence the developing brain [4,5]. Several studies in animal models have concurred to unveil the relationship between MIA and altered fetal neurodevelopment, which might lead to long-term anatomical and behavioral impairments correlated with inflammation [6–8]. Thus, preclinical and clinical studies showed that MIA triggers a cytokine imbalance that increases the risk of neurodevelopmental disorders in the offspring [9–12]. Both rodent and non-human primate models took advantage of the polyriboinosinic-polyribocytidylic acid [poly (I:C)], a double-stranded synthetic RNA that elicits an innate immune response by simulating a viral infection. Offspring whose mothers were exposed to poly (I:C) during gestation showed schizophrenia- and autism-like traits [13–15].

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In our previous studies, we reported behavioral impairments correlated with an altered mesolimbic dopamine transmission both in the first and second generation [16,17]. We showed how inflammation caused by MIA alters the endocannabinoid signaling, which negatively influences the dopamine system, eventually leading to schizophrenia-like phenotype in adulthood [18]. In addition, we demonstrated that activation of peroxisome proliferator-activated receptor-α (PPARα) with the clinically available agonist fenofibrate attenuates the neurodevelopmental disturbances induced by MIA in rat offspring [19]. PPARα are nuclear receptor transcription factors abundantly expressed in the central nervous system, where they fine-tune several functions, such as the mesolimbic neuronal activity and other neuronal pathways involved in affective behavior [20–22]. Importantly, PPARα modulate genes involved in the anti-inflammatory response [23–25]. PPARα-mediated effects involve the nuclear factor kappa-light-chain-enhancer of activated B cells (NFkB) that leads to the inhibition of pro-inflammatory cytokines such as interleukins 1 and 6 (IL-1, IL-6) and tumor necrosis factor-α (TNF-α) [26]. Poly (I:C) acts on the toll-like-receptor 3 (TLR-3) pathway that activates the NFκB through the mitogen-activated protein kinases (MAPK) cascade [27], leading to the synthesis of several proinflammatory cytokines, such as IL-1, IL-6, and TNF-α [14,28]. Most studies reporting the effects of poly (I:C) on cytokine levels in maternal serum, placenta, or fetal brain are performed in mice, reporting conflicting results [29]. Additionally, early studies investigated the levels of proinflammatory cytokines such as IL-1, IL-6, and TNF-α [30–32]. Nevertheless, only recently, several cytokines and chemokines have been analyzed in maternal serum and in fetal brains [33]. However, to our knowledge, only two studies have examined the complete profile of cytokine, chemokine and colony-stimulating factors (CSFs) in mice [34] and Long Evans rats [35].

In a rat model of MIA, this study aimed i) to investigate the full cytokines/chemokines/CSFs profile in the maternal serum and in the fetal brain; ii) to test the hypothesis that a treatment with the PPARα agonist fenofibrate, administered during pregnancy, might reduce MIA-induced cytokine imbalance.

2. Results

Figure 1 shows the experimental protocol, illustrating the MIA model, control (CTRL) or fenofibrate treatment (FEN) during gestation and tissue collection at 6 h and 24 h after poly (I:C) or vehicle (Veh) injection.

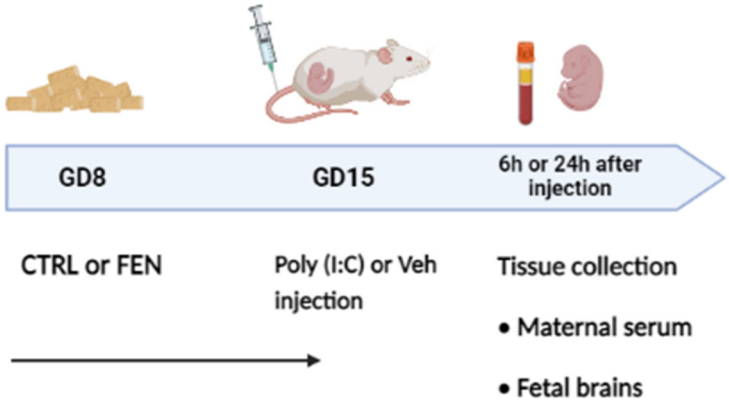

Figure 1. Representation of the experimental protocol. MIA model consisted of a single i.v. injection of poly (I:C) (4 mg/kg) or veh (sterile pyrogen-free saline) at GD15. CTRL or FEN (0.2% w/w) treatment from GD8 to the day of sacrifice at 6 h or 24 h after injection, when maternal serum and fetal brains were collected. Abbreviations: Veh = vehicle; CTRL = Control treatment; FEN = Fenofibrate treatment.

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2.1. Effect of fenofibrate treatment on MIA-induced inflammation in the maternal serum.

To examine whether FEN might rescue the inflammation induced by MIA, we analyzed cytokines, chemokines and CSFs levels in the maternal serum in both CTRL and FEN, 6 h and 24 h after poly (I:C) or veh injection.

Data showed that MIA-induced time-dependent changes in the levels of several maternal serum cytokines, chemokines and CSFs and FEN reverts some of these MIA-induced effects (Table 1).

Table 1. Cytokines, chemokines and colony-stimulating factors concentration in maternal serum. Data are mean ± SEM calculated from one experiment performed in duplicate (n = 4-5 rats per group); values are expressed as pg/ml concentrations relative to standards containing known amounts of target proteins.

Maternal serum								
	6h				24h			
	Veh/CTRL	Poly (I:C)/CTRL	Veh/FEN	Poly (I:C)/FEN	Veh/CTRL	Poly (I:C)/CTRL	Veh/FEN	Poly (I:C)/FEN
Pro- and anti-inflammatory cytokines (pg/ml)								
IL-1α	662.24 ± 29.47	524.72 ± 26.67	681.11 ± 50.14	682.17 ± 36.09	612.07 ± 44.55	786.08 ± 78.05	738.77 ± 86.65	656.81 ± 110.32
IL-1β	501.88 ± 15.28	455.14 ± 17.02	517.27 ± 39.75	516.42 ± 18.69	565.36 ± 63.49	567.05 ± 25.97	492.58 ± 103.44	554.47 ± 149.85
IL-2	1840.91 ± 32.00	1527.18 ± 122.26	2038.19 ± 173.13	1920.49 ± 114.35	6192.58 ± 620.30	7341.73 ± 722.72	7286.16 ± 997.67	5589.96 ± 1633.56
IL-4	475.85 ± 6.54	408.88 ± 21.12	499.25 ± 29.94	466.20 ± 18.30	632.96 ± 41.16	721.22 ± 65.58	731.52 ± 69.54	628.41 ± 100.95
IL-5	935.28 ± 18.51	860.29 ± 27.09	920.12 ± 38.51	858.80 ± 74.34	1271.06 ± 205.70	1509.94 ± 51.04	1443.17 ± 158.23	1389.97 ± 168.35
IL-6	765.96 ± 23.11	632.06 ± 56.62	814.45 ± 65.95	752.29 ± 52.90	2834.06 ± 208.82	3001.23 ± 413.25	2714.45 ± 428.90	2336.17 ± 672.06
IL-7	525.88 ± 16.43	470.59 ± 17.84	560.82 ± 41.89	541.17 ± 25.06	437.70 ± 42.29	566.66 ± 46.48	605.24 ± 57.26	503.07 ± 71.30
IL-10	484.67 ± 8.89	450.01 ± 20.36	518.43 ± 32.88	453.37 ± 56.79	1348.06 ± 20.62	1648.34 ± 101.43	1666.52 ± 134.54	1351.51 ± 192.95
IL-12(p70)	976.67 ± 50.03	810.81 ± 60.05	1065.58 ± 110.32	976.07 ± 77.41	1401.48 ± 208.90	1832.74 ± 134.95	1755.47 ± 240.67	1490.25 ± 269.49
IL-13	379.71 ± 13.15	301.49 ± 41.40	460.44 ± 60.05	371.99 ± 16.06	1349.80 ± 135.77	1578.60 ± 281.17	1388.52 ± 264.77	1257.93 ± 385.77
IL-17	51.58 ± 1.38	41.03 ± 3.84	48.47 ± 4.46	55.76 ± 1.70	168.95 ± 8.57	197.35 ± 17.90	218.52 ± 58.18	142.81 ± 36.68
IL-18	920.51 ± 82.08	706.49 ± 71.94	946.52 ± 38.48	1362.49 ± 187.03	1181.27 ± 145.11	1372.71 ± 69.08	1393.43 ± 141.21	1305.68 ± 82.86
IFN-γ	1092.78 ± 33.09	957.27 ± 64.16	1116.63 ± 72.31	1058.23 ± 65.99	3008.57 ± 177.01	3337.28 ± 489.82	3040.29 ± 449.30	2704.75 ± 675.73
TNF-α	1030.27 ± 71.67	922.24 ± 80.67	1170.75 ± 130.18	1171.63 ± 113.66	1323.42 ± 84.86	2118.60 ± 176.83	1492.64 ± 119.96	1177.55 ± 73.37
Chemokines (pg/ml)								
MCP-1	1654.36 ± 89.29	2138.68 ± 141.86	1522.78 ± 79.01	2370.46 ± 231.48	2194.05 ± 167.39	2730.48 ± 109.78	2351.81 ± 105.54	2586.85 ± 132.18
MIP-1α	51.35 ± 3.08	100.56 ± 14.46	42.74 ± 2.39	77.54 ± 7.43	80.34 ± 5.63	135.97 ± 5.71	99.65 ± 7.38	105.61 ± 6.67
MIP-3α	79.41 ± 4.78	100.67 ± 12.95	81.50 ± 2.95	86.18 ± 6.52	131.21 ± 11.65	175.71 ± 19.03	151.07 ± 21.32	133.88 ± 27.46
RANTES	1005.50 ± 111.25	1092.03 ± 104.73	840.24 ± 35.32	983.14 ± 81.93	879.75 ± 33.86	1014.96 ± 91.77	960.62 ± 98.43	1256.16 ± 132.65
GRO/KC	362.93 ± 8.67	585.51 ± 87.62	275.14 ± 16.59	346.89 ± 4.19	376.60 ± 32.96	482.91 ± 36.21	372.71 ± 10.99	391.68 ± 21.53
Colony-stimulating factors (pg/ml)								
GM-CSF	181.78 ± 5.42	154.16 ± 6.12	186.28 ± 14.61	185.15 ± 7.45	411.81 ± 41.50	498.83 ± 34.23	518.25 ± 43.38	297.26 ± 42.17
G-CSF	20.45 ± 2.59	18.19 ± 2.00	22.91 ± 2.25	22.93 ± 2.31	88.81 ± 7.56	114.46 ± 8.48	118.91 ± 9.78	101.02 ± 12.75
M-CSF	80.53 ± 10.94	70.96 ± 6.71	95.66 ± 8.27	89.86 ± 8.42	132.88 ± 11.41	194.21 ± 25.00	148.51 ± 8.94	105.55 ± 18.45
VEGF	311.88 ± 38.74	N.D.	427.72 ± 33.34	316.20 ± 66.24	2577.29 ± 275.38	2995.33 ± 220.37	3025.33 ± 241.53	2063.98 ± 695.48

ND: not detectable, cytokines, chemokines or colony-stimulating factors concentrations under the limit of detection. **Veh**: vehicle; **CTRL**: control treatment; **FEN**: Fenofibrate treatment.

2.1.1. Chemokines

As shown in figure 2A, at 6 h two way ANOVA showed a significant main effect of MIA for the concentration of the chemokine MIP-1α [$F_{(1,16)}$=25.27, p=0.0001] revealing a significantly higher concentration in poly (I:C)/CTRL than in veh/CTRL ($p<0.01$) and in poly (I:C)/ FEN than in veh/FEN ($p<0.05$).

At 24 h, two-way ANOVA revealed a MIA × treatment interaction [$F_{(1,12)}$=15.11, p=0.0022], and a significant main effect of MIA [$F_{(1,12)}$=23.23, p=0.0004] but not of treatment. Tukey's *post hoc* test revealed significant differences in the levels of MIP-1α between poly (I:C) and veh administration in the CTRL group ($p<0.001$), and poly (I:C)/CTRL and veh/FEN group ($p<0.01$). Remarkably, the levels of this proinflammatory chemokine are reduced in the poly (I:C)/FEN group as compared with the poly (I:C)/CTRL group ($p<0.05$).

Furthermore, at 6 h two-way ANOVA for GRO/KC showed a significant main effect of MIA [$F_{(1,15)}$=9.51, p=0.0076] and of treatment [$F_{(1,15)}$=11.70, p=0.0038] but not MIA × treatment interaction (Figure 2B). Specifically, GRO/KC displayed significantly higher concentrations in poly (I:C)/CTRL

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than in the veh/CTRL ($p<0.01$) and poly (I:C)/FEN ($p<0.01$). No differences among groups were detected at 24 h.

Finally, two way ANOVA for the concentration of the chemokine MCP-1 (Figure 2C) at 6 h and 24 h revealed a main effect of MIA [$F_{(1,16)}=20.18$, $p=0.0004$ and $F_{(1,12)}=8.67$, $p=0.0123$, respectively]. At 6 h, MCP-1 levels were higher in the poly (I:C)/FEN than in the veh/FEN group ($p<0.01$), while at 24 h levels were higher in the poly (I:C)/CTRL than in the veh/CTRL group ($p< 0.05$).

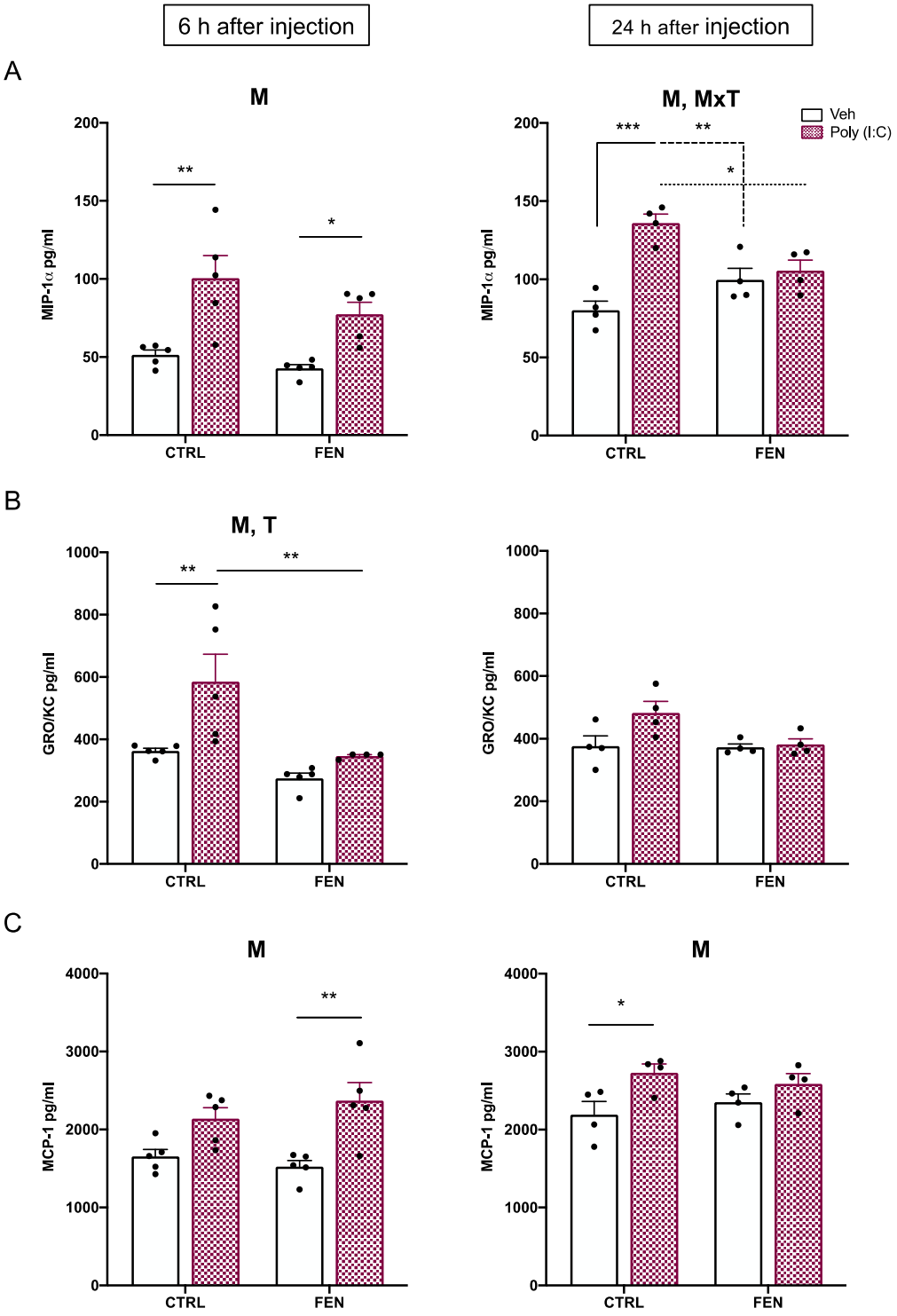

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Figure 2. Effect of fenofibrate treatment and MIA on the cytokines and chemokines expression in the maternal serum. Levels of cytokines and chemokines were evaluated by Bio-Plex ProTM Rat Cytokine Group I Panel 23-Plex (see materials and methods section) 6 h (left) and 24 h (right) after poly (I:C) injection. Data, expressed as pg/ml concentrations relative to standards containing known amounts of target proteins, are mean ± SEM for each cytokine and chemokine. *p<0.05; **p<0.01; ***p<0.001 Veh/CTRL: n = 4-5; Poly (I:C)/CTRL: n = 4-5; Veh/FEN: n = 4-5; Poly (I:C)/FEN: n = 4-5. (Two-way ANOVA; Tukey's *post hoc* test for M x T; Bonferroni's *post hoc* test for M and T). M, significant effect of MIA; T, significant effect of the treatment; M x T, significant interaction of MxT.

2.1.2. Cytokines

Among the analyzed proinflammatory cytokines, the levels of TNF-α, IL-1α, IL-17, and IL-18 were altered (Figure 3). Specifically, at 6 h after poly (I:C) injection, the two-way ANOVA revealed a MIA × treatment interaction for IL-17 [$F_{(1,15)}$=8.91, p=0.0093] and IL-18 [$F_{(1,16)}$=8.20, p=0.0112] and a significant main effect of treatment for IL-18 [$F_{(1,16)}$=9.62, p=0.0069]. Tukey's *post hoc* test revealed a significant increase of both IL-17 (p<0.05) and IL-18 (p<0.01) levels in the maternal serum of the poly (I:C)/FEN as compared to the poly (I:C)/CTRL group (Figure 3A,B).

A significant main effect of treatment was also observed for IL-1α [$F_{(1,16)}$=5.76, p=0.0289] with poly (I:C)/FEN showing higher levels than poly (I:C)/CTRL group (p<0.05) (Figure 3C).

At 24 h after the injection, no main effect of treatment, MIA nor their interaction, was observed for IL-17, IL-18 and IL-1α. The two-way ANOVA showed a MIA × treatment interaction for TNF-α [$F_{(1,12)}$=11.83, p=0.0049]. Notably, the levels of TNF-α were reduced in the poly (I:C)/FEN as compared to the poly (I:C)/CTRL group (p<0.05), indicating the protective effect of fenofibrate treatment (Figure 3D).

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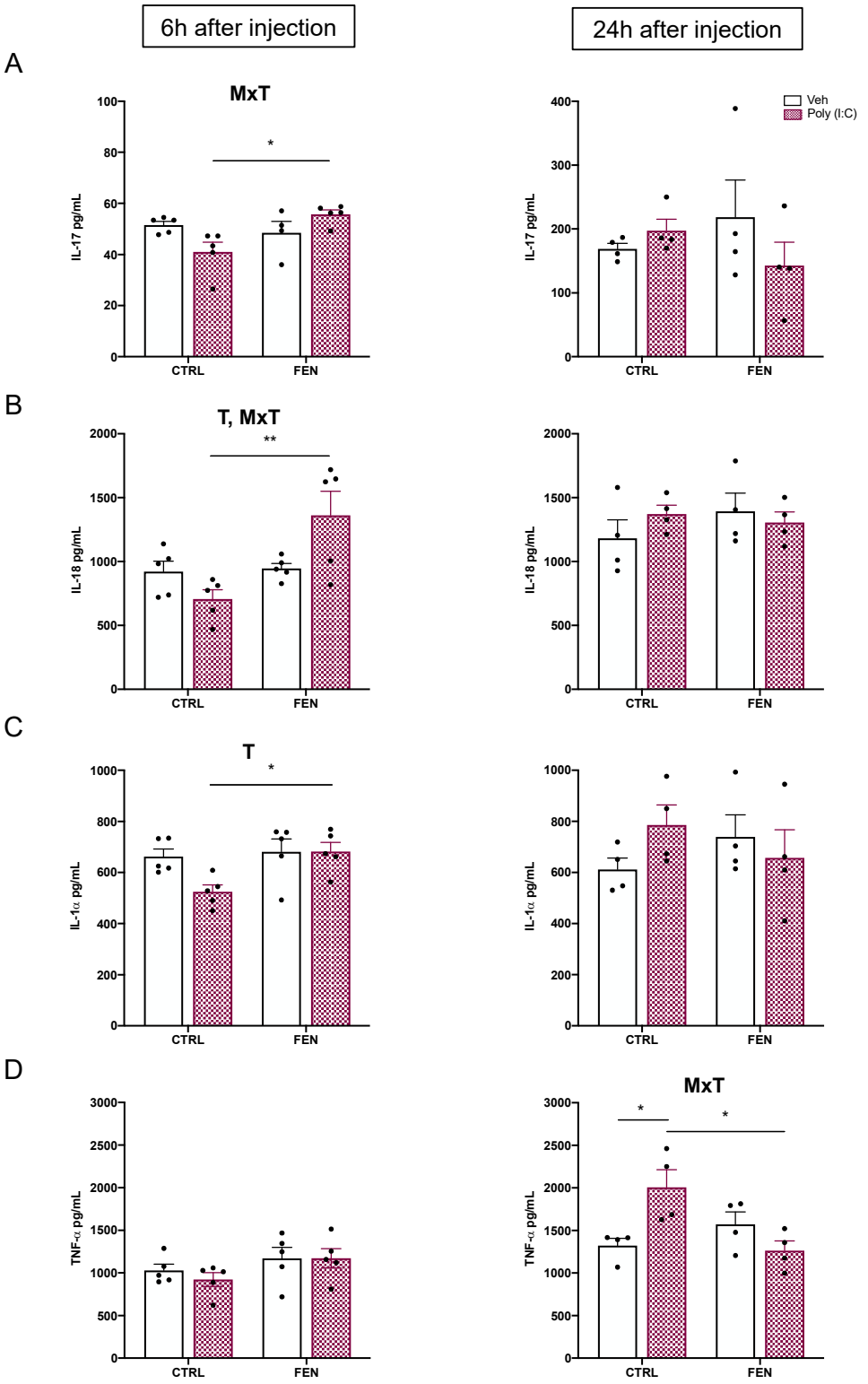

Figure 3. Effect of fenofibrate treatment and MIA on cytokines expression in the maternal serum. Levels of cytokines were evaluated by Bio-Plex ProTM Rat Cytokine Group I Panel 23-Plex (see materials and methods section) 6 h (left) and 24 h (right) after poly (I:C) injection. Data, expressed as pg/ml concentrations relative to standards containing known amounts of target proteins, are mean ± SEM for each cytokine. *p<0.05; **p<0.01; Veh/CTRL: n = 4-5; Poly (I:C)/CTRL: n = 4-5; Veh/FEN: n = 4-5; Poly (I:C)/FEN: n = 4-5. (Two-way ANOVA; Tukey's *post hoc* test for MxT; Bonferroni's *post hoc* test for T). M, significant effect of MIA; T, significant effect of the treatment; M x T, significant interaction of MxT.

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2.1.3. Colony-stimulating factors

As shown in Figure 4, no difference in the CSFs levels was observed among groups at 6 h after poly (I:C) injection, while at 24 h two-way ANOVA displayed a significant MIA x treatment interaction for GM-CSF [$F_{(1,12)}$=14.48, p=0.0025], G-CSF [$F_{(1,12)}$=4.89, p=0.0471], and M-CSF [$F_{(1,12)}$=9.25, p=0.0102]. The Tukey's *post hoc* revealed a decrease of GM-CSF ($p<0.05$) (Figure 4A) and M-CSF ($p<0.05$) (Figure 4C) in the poly (I:C)/FEN as compared to the poly (I:C)/CTRL group. Moreover, the levels of GM-CSF were reduced ($p< 0.05$) in the poly (I:C)/FEN as compared to the veh/FEN group (Figure 4A).

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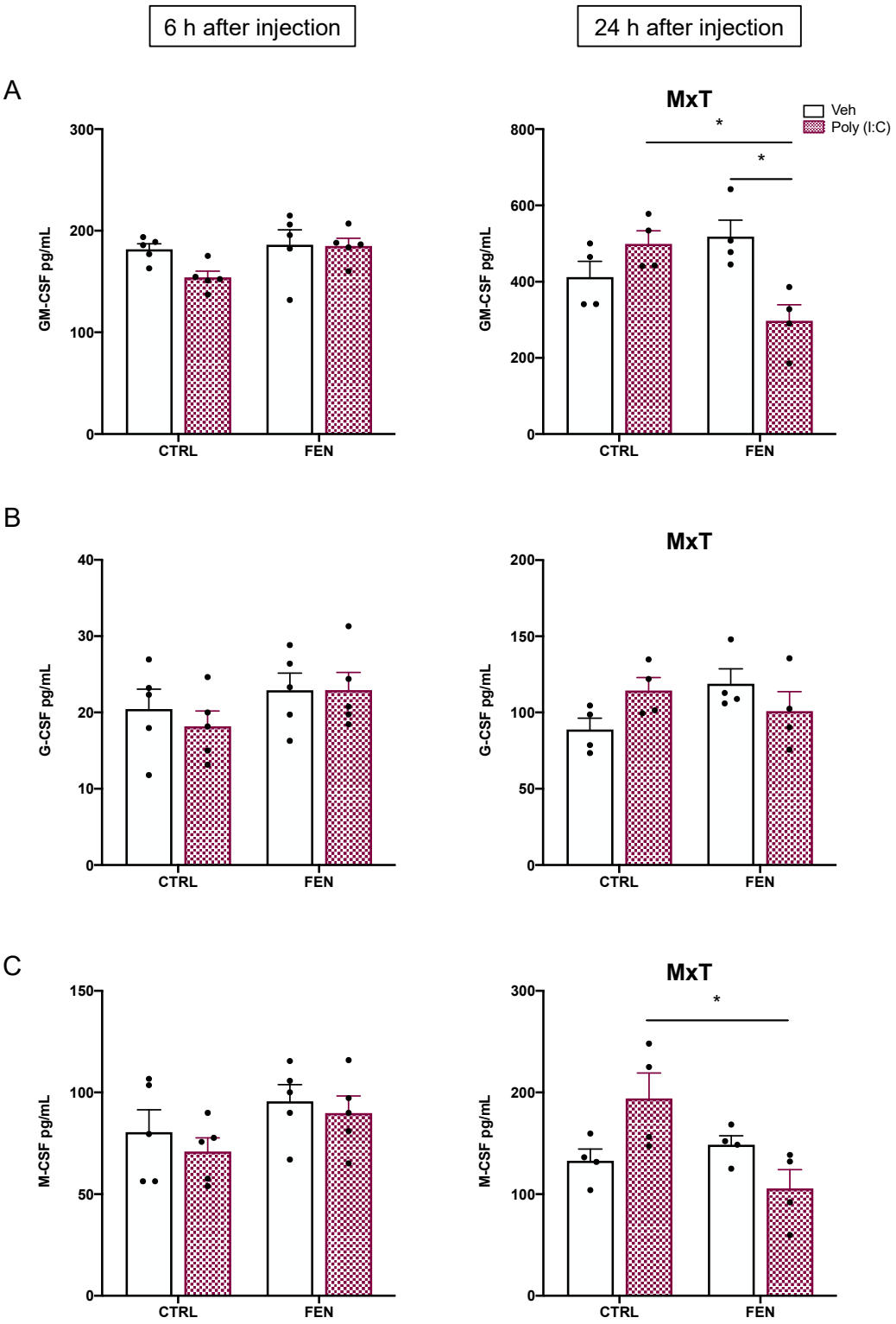

Figure 4. Effect of fenofibrate treatment and MIA on the colony-stimulating factors expression in the maternal serum. Levels of colony-stimulating factors were evaluated by Bio-Plex ProTM Rat Cytokine Group I Panel 23-Plex (see materials and methods section) 6 h (left) and 24 h (right) after poly (I:C) injection. Data, expressed as pg/ml concentrations relative to standards containing known amounts of target proteins, are mean ± SEM for each colony stimulating factor. *p<0.05 Veh/CTRL: n = 4-5; Poly (I:C)/CTRL: n = 4-5; Veh/FEN: n = 4-5; Poly (I:C)/FEN: n = 4-5. (Two-way ANOVA; Tukey's *post hoc*

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test for MxT). M, significant effect of MIA; T, significant effect of the treatment; M x T, significant interaction of MxT.

2.2. Effect of fenofibrate treatment on MIA-induced neuroinflammation in the fetal brain.

We analyzed the levels of cytokines, chemokines, and CSFs in the fetal brain from dams injected with veh or poly (I:C) at GD15 and sacrificed 6 h or 24 h later. We also examined whether the FEN treatment might rescue the MIA induced neuroinflammation.

As shown in table 2, at 6 h, all the cytokines, chemokines and CSFs, except IL-6 and VEGF, were found in controls, revealing their physiological presence in the developing brain without an immune response. All cytokines showed levels of expression ranging from 40 to 337 pg/ml, except IL-17, IL-7, and IL-10 whose concentrations were below 35 pg/ml. Chemokines and CSFs were also present in the control brain homogenates at concentrations ranging from 1.34 to 108 pg/ml.

Table 2. Cytokines, chemokines and colony-stimulating factors concentration in prenatal brain homogenates. Data, are mean ± SEM calculated from one experiment performed in duplicate (n = 4-5 rats per group); values are expressed as pg/ml concentrations relative to standards containing known amounts of target proteins.

Fetal Rat Brain								
	6h				24h			
	Veh/CTRL	Poly (I:C)/CTRL	Veh/FEN	Poly (I:C)/FEN	Veh/CTRL	Poly (I:C)/CTRL	Veh/FEN	Poly (I:C)/FEN
Pro- and anti-inflammatory cytokines (pg/ml)								
IL-1α	41.30 ± 3.14	25.90 ± 5.68	36.03 ± 3.70	36.39 ± 2.12	19.31 ± 1.62	18.59 ± 2.01	17.15 ± 1.88	20.44 ± 0.55
IL-1β	336.96 ± 17.50	394.68 ± 32.53	358.98 ± 5.68	391.34 ± 17.16	15.52 ± 1.66	14.34 ± 2.23	13.41 ± 1.68	16.65 ± 0.66
IL-2	N.D.	N.D.	N.D.	N.D.	111.36 ± 14.89	128.57 ± 12.82	96.73 ± 10.11	110.90 ± 11.76
IL-4	43.44 ± 8.02	23.71 ± 5.08	35.25 ± 3.22	31.74 ± 3.54	26.12 ± 1.44	24.79 ± 1.73	24.04 ± 2.96	27.67 ± 0.68
IL-5	96.61 ± 6.34	68.17 ± 11.60	86.03 ± 7.05	84.66 ± 4.53	92.74 ± 3.60	92.41 ± 6.98	86.89 ± 6.37	100.31 ± 2.89
IL-6	N.D.	N.D.	N.D.	N.D.	94.16 ± 5.90	94.13 ± 6.34	85.26 ± 7.08	96.07 ± 5.14
IL-7	32.92 ± 1.75	28.59 ± 2.64	31.81 ± 3.06	31.22 ± 2.15	13.85 ± 1.07	13.09 ± 0.79	12.34 ± 0.29	15.37 ± 3.00
IL-10	35.80 ± 2.52	27.24 ± 5.12	31.89 ± 3.90	33.05 ± 2.08	46.80 ± 3.48	46.60 ± 1.97	40.95 ± 3.71	40.53 ± 3.42
IL-12(p70)	88.75 ± 1.48	72.52 ± 3.23	80.14 ± 2.52	77.89 ± 1.79	61.56 ± 4.42	58.66 ± 6.08	54.09 ± 4.70	65.77 ± 1.46
IL-13	52.99 ± 7.42	43.72 ± 5.43	49.48 ± 6.19	47.16 ± 4.60	74.76 ± 5.73	70.78 ± 7.62	64.83 ± 7.43	71.22 ± 8.47
IL-17	7.65 ± 1.51	5.18 ± 1.23	6.79 ± 1.12	6.33 ± 0.99	9.20 ± 0.42	9.49 ± 0.49	8.84 ± 1.06	9.98 ± 0.87
IL-18	47.39 ± 1.19	43.46 ± 1.51	43.31 ± 3.04	42.84 ± 1.94	39.80 ± 2.85	40.96 ± 1.81	36.32 ± 1.10	39.48 ± 1.37
IFN-γ	67.40 ± 5.71	45.41 ± 6.40	58.43 ± 5.72	56.22 ± 3.47	97.21 ± 7.62	94.29 ± 11.07	84.72 ± 9.47	93.66 ± 11.47
TNF-α	124.51 ± 12.75	91.67 ± 20.91	117.90 ± 16.99	119.63 ± 9.07	126.87 ± 15.96	104.16 ± 6.24	128.10 ± 8.47	113.67 ± 9.54
Chemokines (pg/ml)								
MCP-1	108.29 ± 3.76	107.50 ± 2.12	111.03 ± 5.10	103.89 ± 4.51	99.49 ± 7.50	85.44 ± 3.72	92.67 ± 1.63	81.04 ± 2.97
MIP-1α	15.19 ± 1.10	18.41 ± 2.46	16.67 ± 0.87	16.75 ± 0.80	12.22 ± 0.29	10.80 ± 0.42	13.02 ± 0.25	10.64 ± 0.25
MIP-3α	10.28 ± 1.28	6.88 ± 1.42	9.47 ± 1.02	9.64 ± 0.59	8.62 ± 0.50	8.16 ± 0.33	9.49 ± 0.10	9.70 ± 0.97
RANTES	7.08 ± 0.37	5.88 ± 0.77	6.06 ± 0.40	5.89 ± 0.33	8.61 ± 0.51	8.10 ± 0.63	7.81 ± 0.46	8.29 ± 0.57
GRO/KC	14.76 ± 0.93	14.51 ± 0.80	14.68 ± 0.33	16.05 ± 1.40	9.70 ± 0.67	8.71 ± 0.46	8.50 ± 0.60	9.79 ± 0.46
Colony-stimulating factors (pg/ml)								
GM-CSF	16.24 ± 1.61	13.11 ± 1.60	15.38 ± 1.76	15.60 ± 1.19	22.27 ± 1.52	21.09 ± 1.04	21.33 ± 1.33	20.92 ± 1.02
G-CSF	1.34 ± 0.15	1.00 ± 0.15	1.15 ± 0.17	1.16 ± 0.08	8.65 ± 0.27	8.46 ± 0.46	8.70 ± 0.19	8.23 ± 0.26
M-CSF	7.25 ± 0.55	4.86 ± 0.74	6.15 ± 0.59	6.13 ± 0.43	7.12 ± 0.54	6.81 ± 0.63	6.17 ± 0.59	7.10 ± 0.38
VEGF	N.D.	N.D.	N.D.	N.D.	93.46 ± 6.68	85.60 ± 7.25	84.48 ± 7.13	96.96 ± 1.95

ND: not detectable, cytokines, chemokines or colony-stimulating factors concentrations under the limit of detection. **Veh**: vehicle; **CTRL**: control treatment; **FEN**: Fenofibrate treatment.

2.2.1. Cytokines

At 6 h, two-way ANOVA for the cytokine IL-12p70 revealed a MIA x treatment interaction [$F_{(1,13)}$=9.16, p=0.0097], and a significant main effect of MIA [$F_{(1,13)}$=16.00, p=0.0015]. Tukey's *post hoc* displayed a significant decrease of IL-12p70 fetal brain levels in the poly (I:C)/CTRL as compared to the veh/CTRL (p<0.01) and poly (I:C)/FEN as compared to the veh/CTRL group (p<0.05) (Figure 5A). Moreover, two-way ANOVA revealed a significant main effect of MIA for IL-4 [$F_{(1,13)}$=5.08, p=0.0421] and IFN-γ [$F_{(1,13)}$=5.28, p=0.0387] with fetus brain from poly (I:C)/CTRL dam showing lower levels than veh/CTRL dam (p<0.05) (Figure 5B,C). A significant main effect of MIA was also observed for

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IL-1β [$F_{(1,13)}$=4.90, p=0.0453] showing an upward trend of its value in fetus brain from poly (I:C)/CTRL and poly (I:C)/FEN dam (Figure 5D).

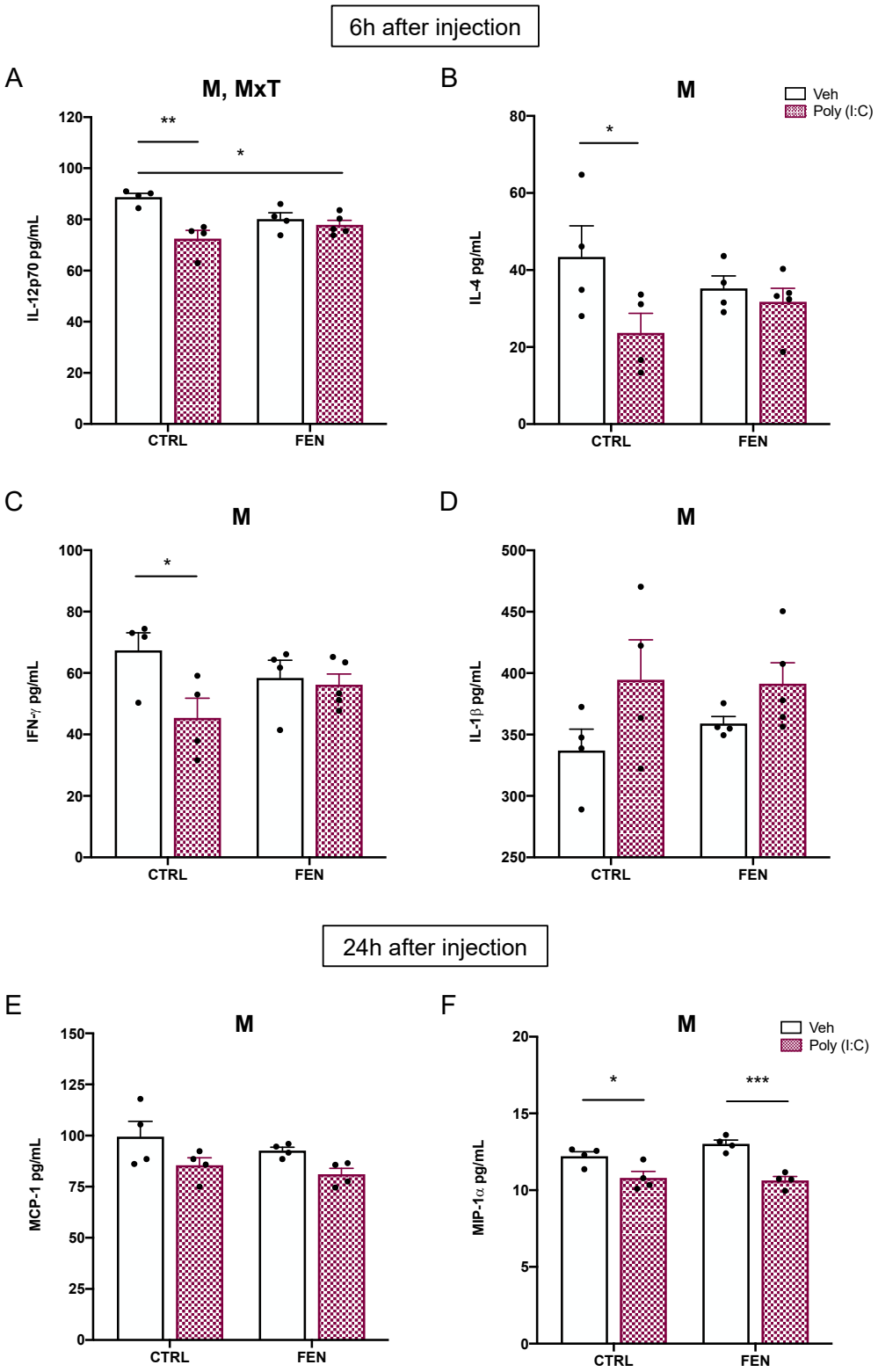

Figure 5. Effect of fenofibrate treatment and MIA on the cytokines and chemokines expression in the prenatal brain homogenates. Levels of cytokines and chemokines were evaluated by Bio-Plex ProTM Rat Cytokine Group I Panel 23-Plex (see materials and methods section) 6 h (top) and 24 h (bottom) after poly (I:C) injection. Data, expressed as pg/ml concentrations relative to standards containing known amounts of target proteins, are mean ± SEM for each cytokine and chemokine. *p<0.05; **p<0.01; Veh/CTRL: n = 4; Poly (I:C)/CTRL: n = 4; Veh/FEN: n = 4; Poly (I:C)/FEN: n = 4-5. (Two-way

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ANOVA; Tukey's *post hoc* test for M x T; Bonferroni's *post hoc* test for M) M, significant effect of MIA; T, significant effect of the treatment; M x T, significant interaction of MxT.

2.2.2. Chemokines and colony-stimulating factors

At 24 h after poly (I:C) injection two-way ANOVA showed a main effect of MIA for MCP-1 [$F_{(1,12)}$=8.08, p=0.0148] (Figure 5E) and MIP-1α [$F_{(1,12)}$=36.71, p<0.0001], showing for the latter a decrease in the poly (I:C)/CTRL and poly (I:C)/FEN group as compared to veh/CTRL (p<0.05) and veh/FEN group (p<0.001), respectively (Figure 5F).

No differences among groups were detected for CSFs both at 6 and 24 h after poly (I:C) injection.

3. Discussion

The main findings of this study are: i) MIA alters cytokines, chemokines and CSFs levels in the maternal serum and fetal brain and ii) MIA-induced effects are partially reverted by fenofibrate.

Several studies have used MIA models to induce neurodevelopmental disorders in the offspring, but only a few reported a full immune response profile to poly (I:C) injection (see [29] for a review). Here, using the multiplex assay that maximizes the simultaneous detection of cytokines, chemokines and CSFs in a single sample, we identified and monitored several molecules both in the maternal serum and fetal brain, thus providing a complete maternal and fetal immune profile.

In agreement with other groups [30,34,35], our results show that MIA produces a time-dependent increase of some cytokines in maternal serum. Specifically, the levels of chemokine GRO/KC, MCP-1, and MIP-1α showed rapid increase at 6 h, while TNF-α and CSFs levels were significantly upregulated at 24 h, suggesting that their induction required a longer time to develop and are more persistent.

Chemokines are a family of small cytokines, initially studied because of their ability to manage the migration of leukocytes in the respective anatomical locations during inflammatory and homeostatic processes (see [36,37] for a review). Besides their contribution in inflammation, chemokines also play a role in brain development [34,38] and in neurodevelopment disorders, such as autism and schizophrenia [39–42]. Our findings are in line with those obtained by Arrode-Brusés (2012) [34] in mice administered with poly (I:C) at GD16, which reported increased levels of the chemokines GRO/KC, MCP-1, and MIP-1α, and of CSFs in maternal serum at 6 or 24 h.

Additionally, in agreement with our findings, several studies described increased levels of : i) TNF-α in maternal plasma/serum after poly (I:C) or LPS challenge both in mice and rats [31,32,43]; ii) CXCL1 (GRO/KC), but not IL-6, with a 3 h delay after subcutaneous injection of poly (I:C) (1.0 mg/kg) into the tail [44].

Contrary to our results, other groups showed increased levels of IL-1β, IL-2, IL-5, IL-6, IL-10 and IL-12 in maternal plasma at 3-6 h or 24 h after poly (I:C) administration in pregnant rodents [30,33,34,43,45,46]. However, no change or even a decrease of cytokines/chemokine has been described in maternal serum after poly (I:C) injection during pregnancy [29].

These discrepancies observed among studies could depend on the wide range of protocols that vary in the type, mode of delivery, timing, and dose of the immunogen used [47]. Several studies demonstrated that the serum cytokine responses in pregnant mice after systemic administration of poly (I:C) can be either increased (IL-6, IL-12p40) [34] or unaltered (i.e.: IL-6, IL-10, IL-17A) [48].

Considering its critical role as a molecular mediator of MIA, we expected the rise of IL-6 in the maternal serum after poly (I:C) injection. Murray et al. (2019) [49] demonstrated in rats that poly (I:C) induced a variable increase of IL-6 in the plasma at 3 h post-injection, and this response is strain-dependent. Indeed, high levels of IL-6 and fewer "non-responders" were consistently observed in Wistar rats, compared to Sprague Dawley or Lister Hooded rats. Thus, other rat strain and/or sample collection timing than ours could be optimal to study IL-6.

Lipopolysaccharide (LPS) and poly (I:C) maternal administration may increase, reduce or have no effect on fetal brain and placental cytokines, chemokines and CSFs levels [31,32,50–52]. Notably, the reduction or elevation of fetal brain cytokines might originate maternally or be attributable to endogenous production.

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Out of the 14 pro- and anti-inflammatory cytokines examined in this study, only IL-12p70, IL-4, and IFN-γ were down-regulated in the fetal brain 6 h after poly (I:C), while among the chemokines analyzed, only MIP1-α and MCP-1 were decreased in the fetal brain of poly (I:C)/CTRL and poly (I:C)/FEN rats. Accordingly, maternal exposure to LPS increased placental and amniotic fluid TNF-α, but decreased it in fetal brain, indicating that maternal infection might directly impact cytokine levels in the developing fetal brain [32]. It could be argued that the observed downregulation of chemokines MIP1-α and MCP-1, together with the downregulation of the cytokine IL-12p70, IL-4, IFN-γ or TNF-α (all pro-inflammatory), may act as a compensatory mechanism occurring in the brain in response to maternal inflammation.

FEN is a clinically PPARα agonist, approved by both Food and Drug Administration (FDA) and European Medicines Agency (EMA) to treat patients with hypertriglyceridemia, primary hypercholesterolemia, or mixed dyslipidemia [53,54]. Additionally, several studies have revealed that FEN exhibits a robust anti-inflammatory effect on diseases of various systems, including the nervous system [55,56]. It exerts an anti-inflammatory effect through direct or indirect mechanisms, either secondary to its lipid-lowering effect (i.e., reduction of inflammation caused by hyperlipidemia in the circulatory system) or by activating PPARα and inhibiting downstream inflammatory signaling pathways. Specifically, FEN suppresses different inflammatory circuits, including NF-κB [57], mitogen-activated protein kinase (AMPK) [58], toll-like receptor 4 (TLR4) [59], or IL-1 or IL-6 [60]. Recently, our group demonstrated that prenatal FEN administration attenuated behavioral impairments and dopaminergic dysfunction in MIA offspring [19].

Our present findings showed that FEN treatment prevented the increase of MIA-induced cytokine TNF-α and chemokines MIP-1α and GRO/KC. Besides the neuroinflammatory processes, the above-mentioned chemokines are involved in cerebral ischemia/reperfusion injury [61,62], and in ASD [63], in which they have been associated with behavioral deficits [39,64–66].

Furthermore, MIA-induced altered CSF-s serum levels returned to basal values in the FEN group. Importantly, besides their role in the control, proliferation, and differentiation of hemopoietic cell population, evidence suggests that CSFs (GM-CSF, G-CSF and M-CSF) are also involved in inflammatory processes, including those taking place in the CNS [67–69].

By contrast, unexpectedly, we observed that FEN induced a significant increase of IL-17 and IL-18 in poly (I:C) treated dam compared to poly (I:C)/CTRL. Of note, although FEN displays anti-inflammatory effects in many diseases, it has also been reported to exert no effects [70,71] or even exacerbate the inflammatory response in an experimental mouse model of acute colitis [72] and in spontaneously hypertensive rats expressing the human C-reactive protein transgene [73]. In humans, FEN does not suppress the clinical or inflammatory response to low-dose endotoxin when administered at an effective dosage for heart disease or lipid disorders [71], suggesting that its systemic properties are limited.

Lastly, our results indicate that FEN does not reverse the observed decrease of proinflammatory cytokine and chemokine induced by MIA in the fetal brain.

To our knowledge, this is the first investigation of the effect of FEN treatment on cytokines, chemokines, and CSFs levels in the maternal serum and the fetal brain after MIA induced by poly (I:C) or LPS.

A limitation of this study could be the lack of information about the effects of FEN on cytokines, chemokines, and CSFs levels in the placenta during different time-windows during gestation (e.g., GD9 vs GD15). Further studies are needed to elucidate the cytokines transmission at the interface between the placenta and fetus. In conclusion, our findings suggest that PPARα might represent an attractive therapeutic target to attenuate the consequences of MIA.

4. Materials and Methods

4.1. Animals.

All animal care and experimental procedures were carried out in accordance with European Council directives (63/2010) and in compliance with the animal policies approved by the Italian

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Ministry of Health and the Ethics Committee for Animal Experiments (CESA, University of Cagliari). We made all efforts to minimise pain and suffering, and to reduce the number of animals used. Adult female Sprague-Dawley (SD) rats (250-300 g) (Envigo, Italy) were housed at temperature of 22 ± 2 °C and 60% humidity, under a 12 h light/dark cycle (light on at 7:00 a.m.) with food and water available *ad libitum*.

4.2. Drugs and Treatments.

Rats were mated at the age of 3 months. The first day after the copulation was defined as Gestational Day 0 (GD 0). Pregnant dams were randomly assigned to two experimental groups: the first group received a treatment consisting in a diet enriched with the PPARα agonist fenofibrate (0.2% w/w) ad libitum from GD 8 to the day of the sacrifice, while the second group received a control diet for the whole pregnancy.

Poly (I:C) was purchased from InvivoGen and dissolved in endotoxin-free saline solution. Pregnant dams on GD 15 were randomly assigned to receive either a single injection of poly (I:C) (4.0 mg/kg, i.v) or an equivalent volume of endotoxin-free saline solution (veh) in the lateral vein of the tail (for more details see SF).

4.3. Maternal blood and fetal brain collection.

6 or 24 h after poly (I:C) or saline injection, rats were deeply anesthetized with isoflurane (5%) (Merial, Toulouse, France) and sacrificed by decapitation. Maternal trunk blood was collected into 5 ml tube and allowed to clot at room temperature for 45 min. Fetuses were surgically delivered and killed by decapitation, and heads were placed in ice-cold PBS for immediate harvesting of the brains. Using a stereomicroscope, the brain was collected and immediately frozen in liquid nitrogen and stored at -80°C. An average of 15 pups per dam was obtained and a pool of 4 brain fetuses was used for cytokine/chemokine/CSFs measurements.

4.4. Blood and brain tissue processing.

Maternal blood was centrifuged at 1,000*g* for 15 min at 4°C. The supernatant was collected and recentrifuged at 10,000*g* for 10 min at 4°C and extracted serum was stored at -80°C for later cytokines/chemokines/CSFs measurements.

Tissue derived from a pool of 4 brain fetuses was processed using Bio-Plex Cell Lysis Kit (Bio-Rad Laboratories, Inc., USA), according to the manufacturer's instructions. Specifically, brain frozen tissues were disrupted in Bioplex cell lysis buffer (BioRad, Hercules, CA, USA) containing factors 1 and 2 (protease and phosphatase inhibitors, respectively; BioRad) and the protease inhibitor phenyl-methylsulfonyl fluoride (500 mM; Sigma–Aldrich). Samples were homogenized on ice with a potter and homogenate frozen for at least 30 min at -80 °C. After thawing and sonication for 15 sec, samples were centrifuged at 4°C and 4,500*g* (Thermo Scientific Micro 17R Refrigerated Centrifuge) for 8-10 min. The supernatant was collected, and the protein content of each sample was determined using the BioRad Protein Assay (BioRad), with bovine serum albumin as a standard, according to the manufacturer's protocol. The final supernatant was stored at -80°C until the day of the experiment and then used for cytokines/chemokines/CSFs measurements.

4.5. Cytokines, chemikines and CSFs measurements.

As previously described [18,74] sera and brain tissue homogenates were assayed for cytokines, chemokines and CSFs using a Luminex xMAP-based multiplex bead-based immunoassay, the Bio-Plex ProTM Rat Cytokine Group I Panel 23-Plex (Bio-Rad Laboratories, Inc., USA), which detects cytokines: [Interleukins (IL)-1α, IL-1β, IL-2, IL-4, IL-5, IL- 6, IL-7, IL-10, IL-12(p70), IL-13, IL-17A, IL-18, interferon (IFN)γ, tumor necrosis factor (TNF)α; chemokines: monocyte chemotactic protein-1 (MCP-1), macrophage inflammatory protein (MIP)1α, MIP-3α, regulated on activation normal T cells expressed and secreted (RANTES), keratinocyte derived chemokine (GRO/KC or CXCL1), and

growth factors: granulocytes macrophage colony- stimulating factor (GM)-CSF, granulocyte (G)-CSF, macrophage (M)-CSF and vascular endothelial growth factor (VEGF)].

Assays were performed in 96-well plates; briefly, each assay plate layout consisted of eight lyophilized cytokines standards in duplicate, two blank wells and up to 19 brain fetus homogenate samples run at 300 µg proteins/well and 20 maternal serum samples (diluted 1:4 prior to assay). Each tissue and serum sample were run in duplicate and samples from veh/CTRL-, poly (I:C)/CTRL-, veh/FEN- and poly (I:C)/FEN-treated animals were analyzed in the same plate. All the wash steps were performed on a Bio-Plex Pro wash station at room temperature. A Bio-Plex MagPix Multiplex System by Luminex was used to read the plate and data were analyzed using BioPlex manager 4.1 software with 5-parametric logistic regression (5PL) curve fitting to determine the standard curve (pg/ml) from 8 standards in duplicate and extrapolate the sample concentrations from the standard curve. Only standards and samples with coefficients of variance under 5% were included in the analysis, and when readings fell under the detection limit (below the background value) they were excluded from analyses.

Supplementary Materials: The following supporting information can be downloaded at: Preprints.org, SF: Maternal Immune Activation Model Reporting Guidelines Checklist.

Author Contributions: Conceptualization, M.S., R.M., M.P and M.P.C; methodology, R.M., M.S., V.O. and V.S.; validation, M.S. and R.M.; formal analysis, M.S, R.M and M.P.C.; investigation, M.S., R.M, V.O. and V.S.; writing-original draft preparation, M.S. and R.M.; writing – review and editing, M.P., M.P.C. and C.S.; supervision, M.P. and M.P.C. All authors have read and agreed to the published version of the manuscript.

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Informed Consent Statement: Not applicable.

Data Availability Statement: Data is contained within the article and supplementary material.

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Conflicts of Interest: The authors declare no conflict of interest.

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