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Short communication

Time-course of striatal Toll-like receptor expression in neurotoxic, environmental and inflammatory rat models of Parkinson's disease

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ABSTRACT

Because Toll-like receptors (TLRs) are emerging as potential targets for anti-inflammatory intervention in neurodegenerative diseases, the aim of this study was to characterise the time-course of TLR expression in neurotoxic, environmental and inflammatory Parkinson's disease models. Male Sprague Dawley rats were given intra-striatal injections of 6-hydroxydopamine (10 μg), rotenone (1.25 μg), LPS (10 μg) or Poly I:C (20 μg) and were sacrificed on Days 1, 4, 14 and 28 post surgery. Changes in the expression of several inflammatory markers, including TLR3, TLR4 and selected cytokines, were examined using qRT-PCR. We found pronounced changes in the bacterial responsive TLR4 and the viral responsive TLR3 receptors in the inflamed striatum in all models, regardless of whether the challenge was neurotoxic, environmental or inflammatory in nature. However, the magnitude and time-course of changes in expression was different between the different models. This study highlights the pattern of changes in TLR expression in models of Parkinson's disease, and further strengthens the rationale for targeting TLRs for anti-inflammatory intervention in this neurodegenerative disease.

1. Introduction

Toll-like receptors (TLRs) are pattern recognition receptors which recognise moieties associated with pathogens (pathogen-associated molecular patterns (PAMPs)) or cellular damage/death (damage-associated molecular patterns (DAMPS)) leading to induction of an innate immune response. Because of their pivotal role in the induction of innate immunity, TLRs are emerging as potential targets for anti-inflammatory intervention in neurodegenerative diseases, such as Parkinson's disease, in which neuroinflammation plays a key pathogenic role. Indeed, there is now growing evidence supporting the role of TLRs in the pathogenesis of several neurodegenerative diseases including Parkinson's disease with studies reporting upregulation of TLR2 and TLR4 in the human Parkinson's disease brain *post mortem* (Drouin-Ouellet et al., 2014; Dzamko et al., 2016).

To facilitate research into the role of TLRs in the pathogenesis of Parkinson's disease, as well as investigations into the potential of targeting TLRs for anti-inflammatory/neuroprotective intervention in Parkinson's disease, the expression of TLRs in animal models of the condition needs to be explored. Therefore, the aim of this study was determine the time-course of changes in striatal expression of TLR4 and TLR3 in a several animal models of Parkinson's disease induced by neurotoxic (6-hydroxydopamine (6-OHDA)), environmental (rotenone), bacterial (lipopolysaccharide (LPS)) or viral (Poly I:C) mechanisms.

2. Methods

Adult male Sprague Dawley rats (Charles Rivers, UK) were used in this experiment. All procedures were completed by licensed individuals in accordance with European Union Directive 2010/63/EU and S.I. No. 543 of 2012 and with institutional ethical approval. Animals were group housed, four per cage on a 12:12 light:dark cycle, and water and food was provided *ad libitum* throughout the course of the study. Animals were randomly assigned to receive unilateral intra-striatal infusions of 6-OHDA (10 μg), rotenone (1.25 μg), LPS (10 μg) or Poly I:C (20 μg), with corresponding vehicle on the other side. Animals were sacrificed on Days 1, 4, 14 and 28 (n = 6–7 per group per time point) and their brains were processed for *post mortem* assessment of expression of TLR4, TLR3, TNFα, IL-6 and IL-10 using qRT-PCR. The mRNA used in this study was extracted from the brains of rats used in Concannon et al. (2015, 2016), and therefore all methods were completed as described therein. All data were analysed using 2-way ANOVA followed by a *post-hoc* Bonferroni test. Results were deemed statistically significant if $P < 0.05$. Data was expressed as mean ± S.E.M.

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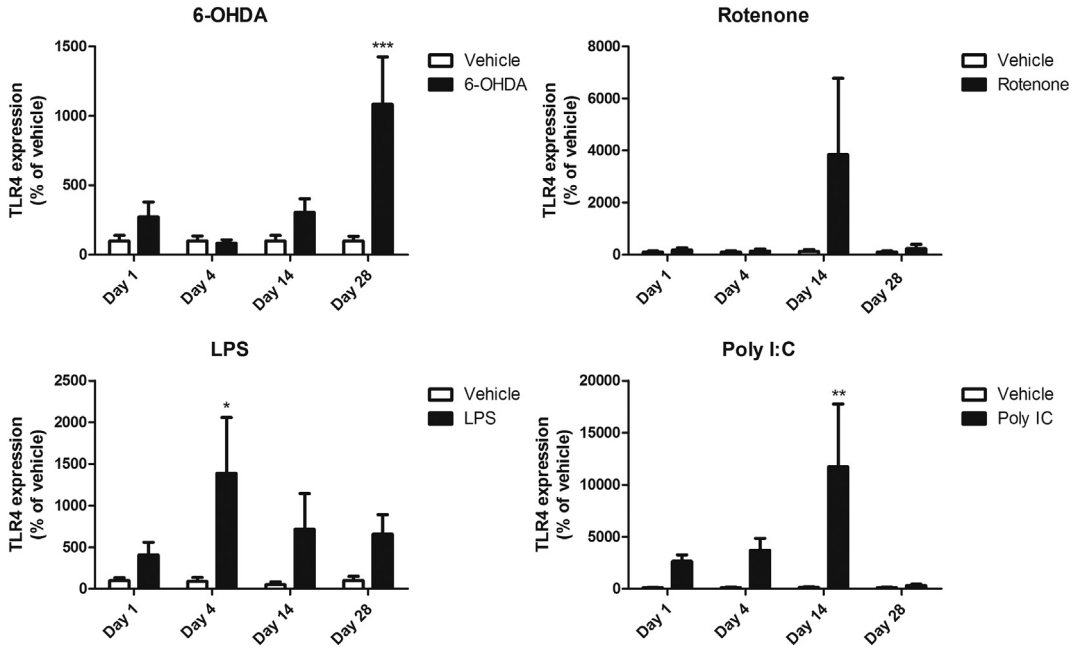

Fig. 1. TLR4 expression.
Striatal TLR4 was significantly increased at Day 28 after 6-OHDA, Day 4 after LPS, and Day 14 after Poly I:C. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ *vs.* Vehicle.

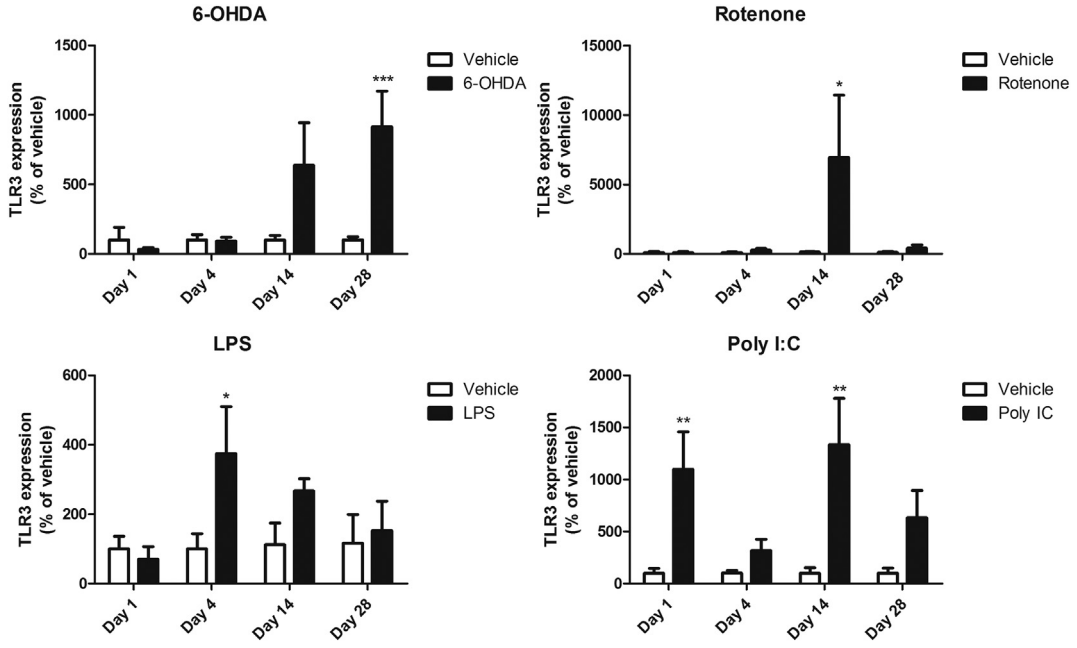

Fig. 2. TLR3 expression.
Striatal TLR3 was significantly increased at Day 28 after 6-OHDA, Day 14 after rotenone, Day 4 after LPS and Days 4 & 14 after Poly I:C. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ *vs.* Vehicle.

3. Results

3.1. TLR4 and TLR3 expression

We first sought to determine the time-course of changes in striatal expression of the bacterial responsive TLR4 after intra-striatal infusion of the different neurotoxins/inflammagens (Fig. 1). We found that 6-OHDA (Group × Time, $F_{(3,45)} = 11.65$, $P < 0.01$), LPS (Group × Time, $F_{(3,31)} = 9.221$, $P < 0.001$) and Poly I:C (Group × Time, $F_{(3,37)} = 9.63$, $P < 0.01$), but not rotenone, induced significant changes in expression of this bacterial-responsive pattern recognition receptor. With respect to time, *post hoc* analyses revealed significant elevation in expression at Day 28 after 6-OHDA, Day 4 after LPS and Day 14 after Poly I:C.

We then investigated changes in expression of the viral responsive TLR3 after administration of the neurotoxins/inflammagens (Fig. 2). This revealed a significant upregulation after infusion of 6-OHDA (Group × Time, $F_{(3,43)} = 10.57$, $P < 0.01$), rotenone (Group × Time, $F_{(3,40)} = 2.49$), LPS (Group × Time, $F_{(3,40)} = 5.10$, $P < 0.05$) and Poly I:C (Group × Time, $F_{(3,36)} = 21.72$, $P < 0.001$). Again *post hoc* analyses revealed the temporality of the changes with significant elevation in expression at Day 28 after 6-OHDA, Day 14 after rotenone, Day 4 after LPS and Days 4 & 14 after Poly I:C.