Lipopolysaccharide preconditioning induces protection against lipopolysaccharide-induced neurotoxicity in organotypic midbrain slice culture

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Abstract: Objective To identify the protective effect of lipopolysaccharide (LPS) preconditioning against LPS-induced inflammatory damage in dopaminergic neurons of midbrain slice culture and the possible mechanisms. **Methods** After cultured *in vitro* for 14 d, the rat organotypic midbrain slices were pretreated with different concentrations (0, 1, 3, 6 or 10 ng/mL) of LPS for 24 h followed by treatment with 100 ng/mL LPS for 72 h. The whole slice viability was determined by measurement of the activity of lactic acid dehydrogenase (LDH). Tyrosine hydroxylase-immunoreactive (TH-IR) neurons and CD11b/c equivalent-immunoreactive (OX-42-IR) microglia in the slices were observed by immunohistochemical method, and tumor necrosis factor- α (TNF- α) levels in the culture media were detected by enzymelinked immunosorbent assays (ELISA). **Results** In the slices treated with 100 ng/mL LPS for 72 h, the number of TH-IR neurons reduced from 191 ± 12 in the control slices to 46 ± 4 , and the LDH activity elevated obviously ($P < 0.01$), along with remarkably increased number of OX-42-IR cells and production of TNF- α ($P < 0.01$). Preconditioning with 3 or 6 ng/mL LPS attenuated neuron loss (the number of TH-IR neurons increased to 126 ± 12 and 180 ± 13 , respectively) and markedly reduced LDH levels ($P < 0.05$), accompanied by significant decreases of OX-42-IR microglia activation and TNF- α production ($P < 0.05$). **Conclusion** Low-dose LPS preconditioning could protect dopaminergic neurons against inflammatory damage in rat midbrain slice culture, and inhibition of microglial activation and reduction of the proinflammatory factor TNF- α production may contribute to this protective effect. Further understanding the underlying mechanism of LPS preconditioning may open a new window for treatment of Parkinson's disease.

Keywords: lipopolysaccharide; preconditioning; neuroprotection; organotypic midbrain slice culture; dopaminergic neurons; inflammation; microglia; tumor necrosis factor- α

1 Introduction

Parkinson's disease (PD) is a neurodegenerative disorder characterized by progressive loss of dopaminergic neurons in the substantia nigra pars compacta (SNc) and consequent dopaminergic denervation of the striatum^[1]. Post-mortem analysis has revealed the consistent presence of inflammatory response in the brain of PD patients^[2,3], as well as increased expression of inflammatory cytokines at the site of neurodegeneration^[4,5]. All these data suggest that inflam-

matory events could be substantially involved in the pathogenesis of PD.

Microglial cells, the resident macrophages of the brain, play a role of immunosurveillance and react promptly to various insults in the central nervous system (CNS). Microglial activation is the major defense of the brain against immune challenges, but it results in the release of various proinflammatory and cytotoxic factors, including cytokines such as tumor necrosis factor- α (TNF- α) and interleukin-1 β (IL-1 β), nitric oxide (NO), and reactive oxygen intermediates^[3,6], which may contribute to neurodegeneration. However, whether microglial activation has beneficial or detrimental effects on neurons remains controversial^[7,8].

Lipopolysaccharide (LPS), a component of Gram-negative bacterial cell wall, as a powerful proinflammatory compound, induces microglial activation and causes

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neurodegeneration, thereby becomes a useful tool to study the possible link between neuroinflammation and neurodegeneration^[9]. Moreover, LPS-induced neurodegeneration appears to be relatively specific for dopaminergic neurons according to both *in vivo* and *in vitro* studies^[10-12], which may provide a useful model for studying the pathogenesis of PD. Although LPS plays major roles in inflammation, it also has the ability to induce desensitization to its own effects. A low-dose LPS exposure can induce a declined inflammatory response to the second high dose LPS challenge. This phenomenon of LPS tolerance was already well described by both *in vivo* and *in vitro* experiments^[13-16] more than a decade ago. Furthermore, studies have also revealed that pretreatment with LPS could induce cross-tolerance, wherein the protection against neuronal damage in ischemia or spinal cord injury is mediated through diminished activation of the cellular inflammatory response^[17-19]. From this point of view, we hypothesized that pretreatment with LPS was able to reduce inflammation-induced dopaminergic cell death.

To test our hypothesis, organotypic slice cultures were prepared from the midbrain (a brain region most affected in PD) to explore the neuroprotective effects of LPS preconditioning on dopaminergic neurons against LPS-induced neurodegeneration.

Organotypic slice culture system has been shown to maintain the local biochemical, physiological and structural integrity of the CNS regions^[20,21]. The method is introduced as an “intermediate” model between cell lines and *in vivo* models, and it allows for direct access and manipulation in the relevant brain tissues similar to that *in vivo* condition. It provides a potentially useful new method for the study of neurodegenerative diseases.

2 Materials and methods

2.1 Organotypic slice culture preparation Organotypic slice cultures from the midbrain were prepared following the interface method as Stoppini *et al.* described^[22] with slight modifications. Midbrain slices were prepared from the brains of 1-3 d old Sprague–Dawley rat pups under sterile conditions. Rats were anesthetized by hypothermia, and brains were quickly removed after decapitation. Coronal slices (350 μ m thick) of the mesencephalon were cut on a McIlwain tissue chopper (McIlwain, USA) and chosen under a dissection

microscope, then transferred onto humidified membranes of 0.4- μ m-pore-size cell culture plate inserts (Millicell-CM, Millipore, Billerica, USA). Five slices were put in one insert and placed in a six-well plate containing 0.75-mL of culture medium. Culture medium consisted of 50% Eagles minimal essential medium (MEM) (Gibco, Invitrogen Corporation, USA), 25% Hanks' Balanced Salt Solution (HBSS) (Sigma, USA), 25% heat inactivated horse serum (HyClone Laboratories, USA), 2 mmol/L glutamine, 20 mmol/L HEPES, 6.5 mg/mL glucose, 100 U/mL penicillin, and 100 μ g/mL streptomycin. Slices were incubated in a humidified atmosphere (5% CO₂) at 36 °C and maintained for 2 weeks before treatment. Media were refreshed the next day after preparation and subsequently refreshed every 2 d. The animal experiments were performed in accordance with the guidelines for care and use of laboratory animals of the Committee of Capital Medical University of China.

2.2 LPS treatment LPS (*E.coli* 0128:B12, L2887, Sigma, St. Louis, USA) was used for both low dose pretreatment and high dose treatment. The brain slice cultures were pretreated with low dose LPS for 24 h, and then subjected to high dose LPS for 72 h.

The choice of LPS concentration was based on our preliminary study with various doses of LPS, which showed that LPS at the concentration of 10 ng/mL did not induce neuronal damage in midbrain slice cultures, whereas at the concentration of 100 ng/mL led to both robust dopaminergic cell death and whole tissue injury (data not shown). Therefore, The concentrations of 1, 3, 6, and 10 ng/mL were used for LPS preconditioning treatment before the 100 ng/mL LPS exposure to investigate the effects of LPS preconditioning on LPS-induced neurons death.

Vehicle referred to pretreatment with culture medium before exposed to 100 ng/mL LPS. The slices with no treatment were used as control.

2.3 Lactate dehydrogenase (LDH) release assay Cytotoxic damage of the whole slice culture was quantified by measurement of the LDH released into culture medium. Cultured slices were pretreated with several concentrations of LPS for 24 h followed by treatment with 100 ng/mL LPS. Culture media were collected after treatment with 100 ng/mL LPS for 72 h, and the amount of LDH was determined by using a Cytotoxicity Detection LDH kit (Nanjin JianCheng Inc., China) according to the manufacturer's instructions. This assay sys-

tem is based on the fact that the level of LDH, which is released into the culture medium by cells, is consistent with the degree of cell death in the culture. It was used as a reliable parameter to reflect the cytotoxic damage of whole mid-brain tissues^[23].

2.4 Immunohistochemistry After pretreatment with several concentrations of LPS for 24 h, cultured slices were treated with 100 ng/mL LPS for 72 h, and then the slices were fixed and immunostained as free-floating tissue sections. Briefly, 4% formaldehyde-fixed cultures were treated with 10% hydrogen peroxide (10 min) followed by sequential incubation with blocking solution (30 min), primary antibody [monoclonal anti-tyrosine hydroxylase (TH, 1:200) antibody or anti-CD11b/c equivalent (OX-42, 1:100, both from Chemicon Inc., Temecula, CA, USA) antibody to microglial cells] (overnight, 4 °C), secondary biotinylated antibody, and streptavidin-biotin-peroxidase complex (Zymed laboratories, CA, USA). Color was developed with 3,3'-diaminobenzidine (DAB, Sigma, USA). The images were taken with an Olympus Provis AX70 microscope (Olympus, Tokyo, Japan) operated with the AnalySis (Olympus Soft Imaging) software.

All TH-immunoreactive (TH-IR) neurons in an individual slices was counted from at least 6 slices, and the results were expressed as mean $\pm$ SEM; the number of OX-42-IR cells were

counted from 6 nonoverlapping fields (400 $\times$) and described as mean $\pm$ SEM of cell counts.

2.5 TNF- α quantification The cultures were pretreated with different concentrations of LPS (vehicle, 1, 3, 6 or 10 ng/mL) for 24 h, and then subjected to 100 ng/mL LPS for 72 h. Culture medium was collected before treatment with 100 ng/mL LPS or at 24 h, 48 h, and 72 h after the treatment. The concentration of TNF- α was measured by using commercially available ELISA kits (R&D Systems, USA) according to the manufacturer's protocol.

2.6 Statistical analysis All data are expressed as mean $\pm$ SEM. Comparisons between groups were carried out using the analysis of variance (ANOVA), coupled with the L-S-D *post hoc* test, where appropriate. $P < 0.05$ was considered statistically significant.

3 Results

3.1 Neuroprotective effects of LPS preconditioning in mid-brain slice cultures After pretreatment with LPS (vehicle, 1, 3, 6 or 10 ng/mL) for 24 h, slices were treated with 100 ng/mL LPS for 72 h, and then levels of LDH released into the culture media and the number of dopaminergic neurons were examined.

Figure 1 shows that in the slices pretreated with vehicle

Fig. 1 Protective effects of LPS-pretreatment against LPS-induced neurotoxicity in midbrain slice cultures. Cultured slices were pretreated with different concentrations of LPS for 24 h followed by treatment with 100 ng/mL LPS for 72 h. Culture media were collected and the degree of cell death was assessed by LDH activity assay. Experiments were conducted in triplicate, and the results are shown as mean $\pm$ SEM (U/L) of two independent experiments. Control: without both pretreatment and 100 ng/mL LPS treatment; vehicle: pretreatment with culture medium followed by treatment with 100 ng/mL LPS; PC: preconditioning. # $P < 0.01$ vs control, * $P < 0.05$ vs vehicle.

Fig. 2 Protective effects of LPS-pretreatment on LPS-induced degeneration of dopaminergic neurons in midbrain slice cultures. Cultures were pretreated with various concentrations of LPS for 24 h followed by treatment with 100 ng/mL LPS. After 72 h, cultures were immunocytochemically stained with anti-TH antibody. Treatment with 100 ng/mL LPS for 72 h led to remarkable loss of tyrosine hydroxylase immunoreactive (TH-IR) neurons in vehicle-treated slices, and the remaining TH-IR neurons exhibited shortened and damaged neurites and shrunk cell bodies. LPS pretreatment blocked the subsequent high dose LPS-induced morphological changes and loss of dopaminergic neurons. (a) Images of TH-IR neurons in slice cultures under different treatments. A: control, without both pretreatment and 100 ng/mL LPS treatment; B: vehicle, pretreatment with culture medium followed by 100 ng/mL LPS; C: pretreatment with 1 ng/mL LPS followed by 100 ng/mL LPS; D: pretreatment with 3 ng/mL LPS followed by 100 ng/mL LPS; E: pretreatment with 6 ng/mL LPS followed by 100 ng/mL LPS; F: pretreatment with 10 ng/mL LPS followed by 100 ng/mL LPS. Scale bar, 200 μ m. (b) Number of dopaminergic neurons in different groups. Results are expressed as mean $\pm$ SEM of TH-IR cells per slice obtained from at least six slices. PC: preconditioning. # P < 0.01 vs control, * P < 0.05 vs vehicle.

Fig. 3 Inhibitory effects of LPS-pretreatment on LPS-induced activation of microglia in midbrain slice cultures. Cultures were pretreated with various concentrations of LPS for 24 h followed by treatment with 100 ng/mL LPS for 72 h. Slices were fixed and immunocytochemical stained for OX-42. In controlled slices, microglial cells were stained rarely and weakly. Treatment with 100 ng/mL LPS for 72 h led to an intensive microglial activation in vehicle-treated slices. LPS-pretreatment could attenuate the activation of microglia. (a) Images of the microglia immunostained with OX-42 antibody under different conditions. A: control, without both pretreatment and 100 ng/mL LPS treatment; B: vehicle, pretreatment with culture medium followed by 100 ng/mL LPS; C: pretreatment with 1 ng/mL LPS followed by 100 ng/mL LPS; D: pretreatment with 3 ng/mL LPS followed by 100 ng/mL LPS; E: pretreatment with 6 ng/mL LPS followed by 100 ng/mL LPS; F: pretreatment with 10 ng/mL LPS followed by 100 ng/mL LPS. Scale bar, 100 μ m. (b) Number of OX-42-IR cell bodies in midbrain slice cultures in different groups. Data are expressed as mean $\pm$ SEM of cell counts obtained from six nonoverlapping 400 $\times$ fields. PC: preconditioning. # P < 0.01 vs control, * P < 0.05 vs vehicle.

followed by treatment with 100 ng/mL LPS, the LDH levels were much higher than that in the control ($P < 0.01$). The augment of LDH release induced by 100 ng/mL LPS was markedly reduced by pretreating the slices with 3 or 6 ng/mL LPS ($P < 0.05$). However, 10 ng/mL LPS pretreatment failed to decrease the LDH release.

Immunocytochemical analysis revealed that application of LPS (100 ng/mL) for 72 h induced a remarkable decrease in the number of visible dopaminergic neurons in the slices without LPS preconditioning (vehicle-treated slices) compared with the control ($P < 0.01$, Fig. 2b). The number of neurons per slice decreased from 191 ± 12 (mean $\pm$ SEM, $n = 6$) in the control cultures to 46 ± 4 in the vehicle-treated cultures. Moreover, the surviving neurons exhibited shortened and damaged neurites and shrunk cell bodies, in contrary to the healthy TH-IR neurons with extensive neurites in the control slice cultures (Fig. 2a). Pretreatment with 3 and 6 ng/mL LPS significantly prevented LPS-induced dopaminergic cell death compared with that in vehicle-treated slices ($P < 0.05$, Fig. 2b): the number of dopaminergic neurons per slice increased to 126 ± 12 (3 ng/mL LPS) and 180 ± 13 (6 ng/mL LPS), respectively. Besides, the remaining dopaminergic neurons exhibited relatively healthy and extended neurites (Fig. 2a). In contrast, 10

ng/mL LPS pretreatment did not show protective effect. After 100 ng/mL LPS treatment for 72 h, more extensive loss of dopaminergic neurons and rare emergence of TH-IR neurons could be seen in the slices pretreated with 10 ng/mL LPS (Fig. 1).

3.2 Attenuation of microglial activation in LPS-pretreated midbrain slice cultures Since microglial activation is observed during the pathogenesis of PD and has been thought to play an important role in the progression of neurodegeneration, the effects of LPS preconditioning on LPS-induced microglial activation were examined in the midbrain slice cultures. Slices were pretreated with different concentrations of LPS (vehicle, 1, 3, 6 or 10 ng/mL) for 24 h followed by application of 100 ng/mL LPS. After 72 h, microglia was detected by immunohistochemistry with OX-42 antibody, and both the morphology and number of OX-42-IR cells were assessed. In the control slices, few immunopositive cells were recognized; whereas in the vehicle-pretreated slices, 100 ng/mL LPS treatment for 72 h caused a large increase of OX-42-IR cells ($P < 0.01$, Fig. 3b) along with pronounced morphological transformation of microglia into its active form (Fig. 3a), which was characterized by amoeboid and round cell bodies with increased OX-42 immunoreactivity. Pretreatment with LPS significantly attenuated 100 ng/mL LPS-induced micro-

Fig. 4 Effects of LPS-pretreatment on TNF- α release before and after 100 ng/mL LPS treatment in midbrain slice cultures. Cultured slices were pretreated with several concentrations of LPS (1, 3, 6, and 10 ng/mL) for 24 h followed by treatment with 100 ng/mL LPS; culture media were collected at the indicated time (before 100 ng/mL LPS treatment and at 24 h, 48 h, 72 h after 100 ng/mL LPS treatment). TNF- α was assayed with commercial ELISA kits. Results are shown as mean $\pm$ SEM (pg/mL) of triplicate experiments. Control: without both pretreatment and 100 ng/mL LPS; vehicle: pretreatment with culture medium followed by treatment with 100 ng/mL LPS; PC: preconditioning. * $P < 0.01$ vs control, * $P < 0.05$ vs vehicle.

glial activation in all preconditioned groups as compared with vehicle-treated group ($P < 0.05$, Fig. 3b).

3.3 Alteration of TNF- α production in LPS-pretreated midbrain slice cultures Among cytokines, TNF- α is the most interested candidate in the process of LPS preconditioning. To assess the involvement of TNF- α in LPS preconditioning, we examined the level of TNF- α in the culture medium at various time points. TNF- α levels increased markedly right after 24-h pretreatment with 3, 6 or 10 ng/mL LPS compared with the vehicle group ($P < 0.05$, Fig. 4). TNF- α level was also tested at 24 h, 48 h, and 72 h after treatment with 100 ng/mL LPS. We observed that the amount of TNF- α induced by 100 ng/mL LPS reached the maximal level at 24 h post-stimulation, and constantly declined during the next 48 h. At all the three time points, vehicle-treated slices showed obviously increased TNF- α levels compared with the control ($P < 0.01$), and LPS pretreatment significantly reduced TNF- α production at the concentrations of 3 and 6 ng/mL ($P < 0.05$), but no further reduction was detected at the concentration of 10 ng/mL (Fig. 4).

4 Discussion

4.1 Organotypic brain slice culture system for PD study Studying mechanisms of neuroinflammation involved in PD and developing rational strategies to prevent neurodegeneration are highly dependent on accurate and reliable *in vivo* and *in vitro* models. In this study, we chose organotypic brain slice culture as the model. The organotypic brain slice culture is characterized by preservation of different neural cell types and complex three-dimensional organization of nervous tissues, thus can be served as a useful experimental model system for understanding the mechanisms involved in inflammation-mediated neurodegeneration. Using tissues from midbrain—the most affected region in PD, we could prepare and maintain a large number of cultures under *in vitro* condition, allowing changes to be easily monitored by analysis of culture media or cultured tissues. Previous studies using toxic compounds, such as 6-hydroxydopamine (6-OHDA) and rotenone, have successfully established *in vitro* models of PD in slice culture system^[24,25]. Here, we recreated the pattern of neuronal loss seen in other models of PD by using LPS, which could also induce decrease of TH-IR neurons in midbrain slice cultures. Therefore, LPS-treated midbrain slice cultures should be considered as

an excellent model for *in vitro* investigation of the process involved in inflammatory-mediated dopaminergic neurodegeneration. More importantly, this model system would be valuable in determining the effects of some treating methods such as LPS preconditioning, which might be effective to protect against neurodegeneration.

4.2 Effects of LPS preconditioning on microglial activation It is no doubt that microglia is involved in the process of LPS preconditioning. However, the exact role of microglia (harmful or beneficial) is highly debated. The contradiction mainly results from lack of adequate experimental model. Most information about microglial activation is derived from the *in vitro* studies that use monolayer dissociated cell cultures or drawn from the *in vivo* studies without differentiation between resident microglial cells and peripheral macrophage/monocyte cells entering the brain. For example, in researches about the effect of LPS preconditioning on ischemic brain injury, one study observed that LPS-pretreatment provided neuroprotective effect along with the attenuation of microglial activation^[17], whereas another indicated that LPS-pretreatment reduced ischemic injury but increased the number of inflammatory cells in the brain^[26]. This paradox may be due to the difficulty to distinguish between microglia and other hematogenous macrophages/monocytes from periphery. Taking advantage of organotypic slice culture, we could address the role of CNS-resident microglia in inflammation progress excluding the interference from peripheral monocytes/macrophages. The results of our study showed that there seemed a negative correlation between microglial activation and neuronal damage.

LPS-induced dopaminergic neurotoxicity was associated with the activation of microglia, and the neuroprotective effect of LPS preconditioning was dependent on the degree of suppression of microglial response. Pretreatment with LPS at concentration of 1 ng/mL could not attenuate the LPS-induced neuron loss, which may due to insufficient suppression of microglial activation. Although higher concentration (10 ng/mL LPS) pretreatment greatly decreased the number of microglia, it provided no protective effect. The reason may be that at this concentration, LPS damaged not only neurons but also microglial cells, which was consistent with the features of dying microglia (Fig. 3a). These results further indicated that inhibition of microglial activation might provide protection against inflammation-mediated dopaminergic

neurodegeneration, since microglia was essential for LPS-induced dopaminergic cell death.

4.3 Effect of LPS preconditioning on TNF- α production Beyond microglia, cytokines involved in the neuroprotective effect of LPS preconditioning remain elusive. Among all these cytokines, TNF- α is the most interesting. Since TNF- α is known for its destructive effects in a variety of neurological diseases^[30,31], suppression of TNF- α production, which has been characterized as a common feature of LPS tolerance for a long time^[27-29], should be responsible for the neuroprotection elicited by LPS preconditioning.

As expected, we observed that preconditioning with LPS at the concentration of 3 ng/mL or 6 ng/mL elicited significant neuroprotective effect on midbrain slice cultures ($P < 0.05$), and remarkably decreased TNF- α release ($P < 0.05$). The results suggest that LPS preconditioning may partly mediate its protective effects through inhibition of TNF- α production.

However, several other studies presented a conflicting view that TNF- α was required for LPS-preconditioning induced protection, which was supported by the evidences that neutralization of TNF- α in the systemic circulation could abolish the protective effect of LPS preconditioning against subsequent cerebral ischemic damage^[32], and TNF- α knockout mice could not be protected against heart infarction by ischemia preconditioning^[33].

Here, by measuring the amount of TNF- α released into culture media after 24 h LPS pretreatment, we proved that the increase of TNF- α production induced by LPS preconditioning was associated with the decrease of TNF- α production in response to lethal LPS challenge. Vehicle-treated cultures did not induce TNF- α release, and had no inhibitory effect on TNF- α production after 100 ng/mL LPS challenge. In contrast, preconditioning with 3 or 6 ng/mL LPS triggered detectable increase ($P < 0.05$) of TNF- α , and dramatically inhibited TNF- α production following 100 ng/mL LPS treatment ($P < 0.05$); whereas preconditioning with 1 ng/mL LPS did not induce notable increase of TNF- α level, and failed to inhibit the subsequent release of TNF- α (Fig. 4). This potential neuroprotective role of TNF- α in acquisition of tolerance has also been reported by another study, in which pretreatment of mice with TNF- α induced their tolerance to a subsequent ischemic brain damage^[34]. However, the further increase of TNF- α induced by 10 ng/mL LPS pretreatment showed no inhibitory effect on subsequent TNF- α production. These

results implied that the neuroprotective effect of LPS pretreatment depended not only on previous production of TNF- α , but also on the degree of TNF- α increase, and overproduction of TNF- α was harmful rather than beneficial to the host.

Taken together, all these data highlighted a dual role of TNF- α in LPS preconditioning process. Although excessive production of TNF- α played a deleterious role in the CNS inflammation, low concentration of this cytokine might have the capacity to inhibit the production of TNF- α in inflammatory response, which may contributed to the neuroprotective effects of LPS preconditioning. The phenomena observed in our midbrain slice cultures was consistent with data from another study^[35] that investigated LPS-induced ischemic tolerance in a mice model of stroke. It showed that LPS preconditioning increased circulating levels of TNF- α before middle cerebral artery occlusion, while significantly reduced TNF- α release after the stroke.

4.4 Other mechanisms under LPS preconditioning Our study demonstrated that preconditioning with low dose LPS provided neuroprotection on neurons in midbrain slice culture, which may be due to that the preconditioning inhibit LPS-induced microglia activation and TNF- α over production. However, other mechanisms may underlie this neuroprotective processes. It has been manifested that in macrophages, LPS tolerance is usually accompanied by suppression of many proinflammatory cytokines (e.g., TNF- α and IL-1 β), while anti-inflammatory mediators (e.g., IL-10, TGF- β , and IL-1R antagonist) are not inhibited^[36,37]. Similar to LPS-tolerant macrophages, pretreatment with low dose of LPS may shift the balance between pro- and anti-inflammatory cytokine productions by activated microglia. In addition, superoxide dismutase (SOD), which is postulated to play a beneficial role in LPS-induced brain ischemic tolerance^[38], may also contribute to the mechanism of neuroprotection in our midbrain slice cultures.

4.5 The significance of the study Since there is no optimal concentration for LPS preconditioning been reported by previous studies, several concentrations (1, 3, 6 or 10 ng/mL) were used to investigate the effect of LPS preconditioning. Interestingly, we observed that low concentrations (3 and 6 ng/mL) of LPS preconditioning significantly attenuated LPS-induced neurodegeneration in cultured slices, whereas vehicle or high concentration of LPS (10 ng/mL) provided no

protection. It appears that the neuroprotective effect of preconditioning depends on the dose of LPS: only appropriate dose has the power to reverse the deleterious inflammatory processes, while preconditioning under the low-threshold or over the high-threshold cannot obtain the neuroprotective effect. It is noteworthy to investigate the potential mechanisms involved in this phenomenon, because further to determine the thresholds of beneficial stimulation, better understanding of the cellular and molecular cross-talks linking neurons to glial cells, may allow modulation of the inflammatory responses in dopaminergic neurodegeneration, thus prompt the development of effective methods to prevent PD.

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脂多糖预处理改善脂多糖诱导的中脑脑片神经元损伤

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摘要: 目的 探讨脂多糖(lipopolysaccharide, LPS)预处理对脂多糖所致中脑脑片多巴胺能神经元炎症损伤的影响及其可能的机制。方法 建立大鼠中脑脑片体外培养体系, 于体外培养14 d后以不同剂量脂多糖(0、1、3、6及10 ng/mL)预处理24 h, 然后用大剂量脂多糖(100 ng/mL)作用72 h, 观察脂多糖预处理对脑片乳酸脱氢酶(lactic acid dehydrogenase, LDH)活性的影响。通过免疫组化检测酪氨酸羟化酶(Tyrosine hydroxylase, TH)和OX-42的阳性细胞数变化, 应用酶联免疫吸附法测定培养液上清肿瘤坏死因子- α (tumor necrosis factor- α , TNF- α)水平。结果 100 ng/mL脂多糖作用72 h后引起脑片TH阳性细胞数从对照组的 191 ± 12 减少到 46 ± 4 , LDH活性明显升高($P < 0.01$), 小胶质细胞大量激活, TNF- α 水平显著增高($P < 0.01$)。脂多糖预处理能减少神经细胞的丢失(3 ng/mL和6 ng/mL的LPS处理后TH阳性细胞数分别为 126 ± 12 和 180 ± 13), 降低脑片LDH活性($P < 0.05$), 并有效地抑制小胶质细胞的激活, 明显减少TNF- α 的生成($P < 0.05$)。结论 小剂量脂多糖预处理可改善脂多糖对大鼠中脑脑片多巴胺能神经元的损伤, 其作用机制可能是通过抑制小胶质细胞的激活, 减少TNF- α 的释放, 减轻炎症反应对神经元的损伤。这种保护作用可为帕金森氏病的治疗提供新思路。

关键词: 脂多糖; 预处理; 神经保护; 中脑器官型脑片培养; 多巴胺能神经元; 炎症反应; 小胶质细胞; 肿瘤坏死因子- α