Carboxy-PTIO Increases the Tetrahydrobiopterin Level in Mouse Brain Microvascular Endothelial Cells

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ABSTRACT—The aim of the present study was to characterize the increase in tetrahydrobiopterin (BH4), which is a cofactor for nitric oxide synthase (NOS), by carboxy-PTIO, a scavenger of nitric oxide (NO), in vascular endothelial cells. BH4 level was determined by oxidation under acidic conditions as biopterin. Addition of lipopolysaccharide (LPS) to endothelial cells increased mRNA levels of inducible NOS (iNOS) and GTP-cyclohydrolase I (GTPCH), which is a rate-limiting enzyme for BH4 synthesis, and the biopterin level. NOS inhibitors, NO-donors and L-arginine, a substrate of NOS, did not affect the increase in the biopterin level induced by LPS, suggesting that BH4 synthesis is unlikely to be modulated by NO produced by iNOS during LPS treatment. However, carboxy-PTIO increased the biopterin level in the absence and the presence of LPS. Carboxy-PTIO did not affect the expression of GTPCH mRNA level. Moreover, 2,4-diamino-6-hydroxypyrimidine, an inhibitor of GTPCH, inhibited only about 30% of the carboxy-PTIO-induced increase in the biopterin level. Whereas, *N*-acetylserotonin, an inhibitor of sepiapterin reductase, strongly inhibited the increase in biopterin level. Carboxy-PTIO inhibited the accumulation of pterin, a decomposition product of BH4 in endothelial cells. These findings suggest that carboxy-PTIO accumulates BH4 under basal and LPS-treated conditions in vascular endothelial cells due to both inhibition of the decomposition of BH4 to pterin and activation of the salvage pathway of BH4 synthesis via sepiapterin reductase.

Keywords: Carboxy-PTIO, Lipopolysaccharide, Tetrahydrobiopterin, Nitric oxide, GTP-cyclohydrolase I

Nitric oxide (NO), a potent vasodilator, is synthesized by the conversion of L-arginine to L-citrulline by nitric oxide synthase (NOS) (1–4). There are three isoforms of NOS, i.e., two constitutive NOSs, neuronal NOS and endothelial NOS, and inducible NOS (iNOS). iNOS has been shown to be induced by lipopolysaccharide (LPS) and/or cytokines in various cell types, including smooth muscle cells, macrophages and endothelial cells (5–8), and produces large amounts of NO. The over-produced NO by iNOS within the vascular wall appears to be responsible for the pathogenesis of vascular failure, including vascular contractility and endothelial cell functions, in endotoxin-induced shock (9–11). Reactive oxygen species (ROS) are also implicated in the systemic inflammatory response induced by LPS (12, 13). NO has been shown to react with ROS, such as superoxide and H₂O₂, to produce more toxic

species such as peroxynitrite, hydroxyl radical and singlet oxygen (14–16). Reactions between ROS and NO appear to be related to the development of inflammatory diseases (17, 18).

The tetrahydrobiopterin (BH4) level is increased along with induction of iNOS by treatment with LPS and/or cytokines in several types of cells (19–21). The biosynthesis of BH4 can occur via a de novo pathway from guanosine triphosphate (GTP), in which the enzyme GTP-cyclohydrolase I (GTPCH) catalyzes the rate-limiting step, or via a salvage pathway that utilizes sepiapterin during an intermediate step (22, 23). Expression of the GTPCH mRNA is also increased by LPS and/or cytokines (19–21). iNOS is active as a polypeptide homodimer (24). Dimerization of iNOS requires the concomitant presence of heme and BH4. Gross and Levi (19) reported that BH4 synthesis is an absolute requirement for induction of NO synthesis by LPS in vascular smooth muscle cells. Nakayama et al. (21) also suggested that the intracellular BH4 content was

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closely correlated with induction of iNOS in vascular smooth muscle cells, and the intracellular availability of BH4 has been shown to limit the expression of active iNOS dimers (25, 26). Thus, BH4 has been shown to be an essential cofactor for NO synthesis. BH4 has another function as a protective agent against NO- and/or ROS-induced cell injury in various cell types, including vascular endothelial cells and HL-60 cells (27–30). The protective effect of BH4 appears to involve antioxidative and radical scavenging activity, since BH4 prevents H₂O₂-induced lipid peroxidation and scavenges superoxide measured by electron spin resonance spectroscopy (27). Moreover, we recently found that excess BH4 is present during NO production in LPS-treated rat aorta (31). Thus, BH4 is likely to function not only as a cofactor for NOS but also a protector against cytotoxicity of over-produced NO and ROS under inflammatory diseases. Therefore, it is important to determine the regulation of BH4 synthesis under inflammatory diseases. Although iNOS has been shown to be negatively regulated by NO (32), the regulation of BH4 synthesis by NO during LPS treatment has not been evaluated.

In the present study, we first examined whether NO released by iNOS during LPS treatment affects BH4 synthesis in vascular endothelial cells. In the process of the study, we found that carboxy-PTIO, a scavenger of NO, markedly increases BH4 synthesis during treatment with LPS. Carboxy-PTIO has been shown to attenuate septic shock (33–35). Therefore, to determine the effect of carboxy-PTIO on the regulation of BH4 level is important. The goal of this study was to characterize the increase in the BH4 level by carboxy-PTIO in vascular endothelial cells.

MATERIALS AND METHODS

Cell culture

Mouse brain microvascular endothelial cells (MBMECs) were purchased from Toyobo Co. (Osaka). The cells were cultured in Dulbecco's modified Eagle medium containing 10% fetal bovine serum, 100 U/ml penicillin and 100 µg/ml streptomycin. Cells were finally grown in 60- or 100-mm culture dishes to extract total mRNA, or in 6-well plates to measure BH4 content, and used for experiments when they reached confluence. The cells at 5 to 10 passages after purchase were used throughout all the experiments.

Measurement of mRNA levels of iNOS and GTPCH by reverse transcriptase (RT)-PCR

Confluent endothelial cells in 60-mm dishes were washed three times with physiological saline solution (PSS, pH 7.4) containing 118.5 mM NaCl, 4.74 mM KCl, 2.5 mM CaCl₂, 1.18 mM MgSO₄, 1.18 mM KH₂PO₄,

2.5 mM NaHCO₃, 11 mM glucose and 10 mM *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (HEPES). The washed cells were then treated with LPS at 37°C in 2 ml of PSS. Total RNA was extracted from the cells by a modified guanidinium isothiocyanate method with ISOGEN™ Reagent (Nippon Gene Co., Ltd., Tokyo). Reverse transcription and PCR amplification from 0.2 µg of total RNA were performed using *rTth* DNA polymerase (RT-PCR high Plus; Toyobo Co., Osaka). The primer pairs used for amplifications of iNOS and GTPCH were 5'-CGCTACACTTCCAACGCAAC-3' and 5'-AGGAAGTAGGTGAGGGCTTG-3' (AC#: D14051), and 5'-GGA TACCAGGAGACCATCTCA-3' and 5'-TAGCATGGTGCTAGTGACAGT-3' (AC#: M58364), respectively. The thermocycler was programmed to give an initial cycle consisting of 60°C reverse transcription for 30 min and 94°C denaturation for 2 min, followed by 28 cycles of 94°C denaturation for 1 min and 58°C annealing/extension for 1.5 min. To control the amounts of total RNA, parallel RT-PCR of glyceraldehyde-3-phosphate-dehydrogenase (GAPDH) mRNA was performed as a reference, using the primer pairs 5'-TCCACCACCCTGTTGCTGTA-3' and 5'-ACCACAGTCCATGCCATCAC-3', respectively. PCR products were electrophoresed on a 3% agarose (NuSieve 3:1 agarose; FMC Co., Rockland, ME, USA) gel containing ethidium bromide and visualized by UV-induced fluorescence.

Measurement of mRNA level of GTPCH by Northern blot hybridization

Confluent endothelial cells in 100-mm dishes were washed three times with PSS. The washed cells were then treated with LPS and/or various reagents at 37°C in 10 ml of PSS. Total RNA was extracted from the cells by a modified guanidinium isothiocyanate method with ISOGEN™ Reagent (Nippon Gene Co., Ltd., Tokyo). Twenty micrograms of total RNA from each sample was treated with the solution containing 2.2 M formaldehyde, 50% formamide, 5 mM sodium acetate, 1 mM EDTA and 20 mM 3-morpholinopropanesulfonic acid (MOPS, pH 7.0) for 15 min at 65°C, and the RNA was separated by electrophoresis through a 1% agarose gel containing 2.2 M formaldehyde in MOPS buffer containing 5 mM sodium acetate, 1 mM EDTA and 20 mM MOPS (pH 7.0). The RNA samples were transferred to Hybond-N nylon membranes (Amersham Pharmacia Biotech, Buckinghamshire, UK), and hybridized to the indicated random prime-labeled cDNA probe (Takara Shuzo Co., Ltd., Otsu). The cDNA probe of GTPCH was prepared by RT-PCR as shown in the previous section in the Methods. Hybridization reactions were carried out for 1 h at 68°C in ExpressHyb™ hybridization solution (Clontech Lab., Inc., Palo Alto, CA, USA). The membrane was washed in 20 mM Na₂HPO₄ (pH 7.2),

1 mM EDTA (pH 8.0) and 1% SDS. The washed membrane was exposed to Kodak Biomax™ film at -80°C for 24 h. The membrane was re-hybridized by GAPDH cDNA, which is a constitutive gene. The cDNA probe of GAPDH was prepared by RT-PCR as shown in the previous section in the Methods.

Measurement of biopterin and pterin

Biopterin (BH4 and more oxidized species) and pterin were measured by reversed-phase high performance liquid chromatography (HPLC) with fluorometric detection, as previously described (36, 37). Confluent endothelial cells in 6-well plates were washed three times with PSS and then treated with LPS at 37°C in 2 ml of PSS. After the treatment with LPS, the supernatant and the remaining cells were separately treated with 10% trichloroacetic acid. Each sample was oxidized with MnO_2 and then injected into the HPLC.

Materials

Lipopolysaccharide (*E. coli* type, Serotype 055:B5), N^{G} -nitro-L-arginine and N^{G} -methyl-L-arginine were obtained from Sigma Chemical Co. (St. Louis, MO, USA). Carboxy-PTIO and TMA-PTIO were obtained from Dojindo (Osaka). L-Arginine was purchased from Wako Pure Chemicals (Osaka). All other reagents were of the highest grade commercially available.

Statistical analyses

Values are presented as means $\pm$ S.E.M. of *n* observations. Differences between groups were assessed using analysis of variance followed by Bonferroni's method. Differences among means were considered significant at $P < 0.05$.

RESULTS

Induction of iNOS and GTPCH mRNAs by LPS

Changes in GTPCH and iNOS mRNA levels by the treatment with LPS were measured by RT-PCR (Fig. 1). Both expression levels of iNOS and GTPCH mRNAs were increased from 2 h after the addition of $10 \mu\text{g/ml}$ LPS. The peak of induction was observed at approximately 6 h after the addition of LPS and then decreased over time.

Changes in BH4 level by LPS

We next examined whether LPS ($10 \mu\text{g/ml}$) increased the intracellular and extracellular biopterin levels in MBMECs. As shown in Fig. 2, both intracellular and extracellular biopterin levels were increased after at least a 4-h delay. The intracellular biopterin level was increased until 12 h after the addition of LPS (Fig. 2A), whereas the extracellular biopterin level was increased dependent

Fig. 1. Induction of iNOS and GTPCH mRNAs by LPS in MBMECs. Cells were incubated with LPS ($10 \mu\text{g/ml}$) for various periods, and then total RNA was extracted from the cells. Total RNA ($0.2 \mu\text{g}$) was amplified by RT-PCR using primers for iNOS, GTPCH or GAPDH. The product sizes for iNOS, GTPCH and GAPDH are 407, 371 and 450 bp, respectively.

on the incubation period until 24 h after the addition of LPS (Fig. 2B). The total biopterin level (intracellular plus extracellular) was also increased dependently on the incubation period and saturated at 12 h after the addition of LPS (Fig. 2C). Moreover, the intracellular, extracellular and total biopterin levels by the treatment with LPS less than 100 ng/ml were increased in a concentration-dependent manner, and higher concentrations of LPS decreased the biopterin levels to the upper levels of the untreated condition (Fig. 3).

Effects of NOS inhibitors, L-arginine, NO-donors and PTIO analogues on BH4 level

To determine whether NO released by iNOS modulates BH4 synthesis, the effects of NOS inhibitors, L-arginine, NO-donors and carboxy-PTIO on the increases in the biopterin levels induced by LPS were investigated. The LPS ($10 \mu\text{g/ml}$)-induced increase in the biopterin level was not affected by the treatment with N^{G} -nitro-L-arginine (Fig. 4A) and N^{G} -methyl-L-arginine (Fig. 4B), both inhibitors of NOS, and L-arginine, a substrate of NOS (Fig. 4C). Moreover, SNAP and NOC18, both NO-donors, also did not affect the LPS-induced increase in the biopterin level. These findings suggest that NO released by iNOS is unlikely to be implicated in the modulation of BH4 synthesis during LPS treatment in MBMECs. On the other hand, the biopterin level during treatment with LPS was concentration-dependently increased by co-treatment with carboxy-PTIO (Fig. 5A). The carboxy-PTIO-induced increase in

Fig. 2. Time-course of the increase in the biopterin level by LPS in MEMECs. Cells were incubated with LPS ($10 \mu\text{g/ml}$) for various periods. At the indicated times after addition of the LPS, biopterin levels were determined. A: intracellular, B: extracellular, C: total (intracellular plus extracellular) biopterin levels. Values are the means $\pm$ S.E.M. of 4 experiments.

the biopterin level was not affected by the co-treatment with N^G -nitro-L-arginine (Fig. 5B). Moreover, carboxy-PTIO also increased the biopterin level in the absence of LPS (Fig. 5C). However, TMA-PTIO, which is an analog of carboxy-PTIO and also an NO scavenger, had no effect on the basal biopterin level (Fig. 5D). Carboxy-PTIO, but not TMA-PTIO appeared to increase the BH4 level via

Fig. 3. Concentration-dependent increase in the biopterin level by LPS in MEMECs. Cells were incubated with various concentrations of LPS for 12 h. A: intracellular, B: extracellular, C: total (intracellular plus extracellular) biopterin levels. Values are the means $\pm$ S.E.M. of 4 experiments.

a different mechanism(s) from NO scavenging.

Does carboxy-PTIO stimulate BH4 synthesis through the GTPCH pathway?

We examined whether carboxy-PTIO stimulated the expression of the GTPCH mRNA level during LPS treatment. As shown in Fig. 6, carboxy-PTIO did not affect the

Fig. 4. Effect of NO on LPS-induced increase in the bioppterin level. A: Cells were incubated with LPS (10 μ g/ml) in the presence or absence of N^G -nitro-L-arginine (10–1,000 μ M) for 12 h. B: Cells were incubated with LPS (10 μ g/ml) in the presence or absence of N^G -methyl-L-arginine (10–1,000 μ M) for 12 h. C: Cells were incubated with LPS (10 μ g/ml) in the presence or absence of L-arginine (10–1,000 μ M) for 12 h. D: Cells were incubated with LPS (10 μ g/ml) in the presence or absence of SNAP (100 and 1,000 μ M) or NOC18 (100 and 1,000 μ M) for 12 h. Values are the means $\pm$ S.E.M. of 4 experiments.

GTPCH mRNA level during the treatment with LPS. Although 2,4-diamino-6-hydroxypyrimidine (1 mM, DAHP), an inhibitor of GTPCH, strongly inhibited the LPS-induced increase in bioppterin level (Fig. 7A), the increase in the bioppterin level by carboxy-PTIO alone was attenuated only by about 30% (Fig. 7B). These findings suggest that both the induction and activation of GTPCH are not implicated in the increase in BH4 level by carboxy-PTIO. However, *N*-acetylserotonin (NAS), an inhibitor of sepiapterin reductase, strongly inhibited the increase in the bioppterin level induced by LPS alone (Fig. 7C) or carboxy-PTIO alone (Fig. 7D). Cycloheximide, an inhibitor of protein synthesis, did not affect carboxy-PTIO alone-induced bioppterin synthesis (Fig. 8).

Effect of carboxy-PTIO on BH4 and pterin levels

The degradation of BH4 would be an important factor in the level of cellular BH4. The enzyme which catalyzes BH4 to pterin was not determined, and BH4 was suggested

to be non-enzymatically degraded to pterin (38). Therefore, the effect of carboxy-PTIO on the pterin level during LPS treatment was investigated. As shown in Fig. 9, carboxy-PTIO markedly increased the bioppterin level in the presence or absence of LPS (Fig. 9A), whereas the pterin level was decreased by the treatment with carboxy-PTIO compared with control or LPS alone, respectively (Fig. 9B). Moreover, the amount of totally synthesized bioppterin (bioppterin plus pterin) was increased by the treatment with carboxy-PTIO in the presence or absence of LPS (Fig. 9C).

DISCUSSION

LPS and/or cytokines have been shown to induce the NO production and BH4 synthesis through the induction of iNOS and GTPCH mRNAs in various cells including macrophages, vascular smooth muscle cells and vascular endothelial cells (19–21). In the present study, LPS stimu-

Fig. 5. Effects of carboxy-PTIO and its analog on the BH4 level. A: Cells were incubated with 10 μ g/ml LPS in the presence or absence of carboxy-PTIO (10–300 μ M) for 12 h. B: Cells were incubated with 10 μ g/ml LPS and 100 μ M carboxy-PTIO in the presence or absence of 1 mM *N*^G-nitro-L-arginine for 12 h. C: Cells were incubated with various concentrations of carboxy-PTIO (0–300 μ M) in the absence of LPS for 12 h. D: Cells were incubated with various concentrations of TMA-PTIO (0–300 μ M) in the absence of LPS for 12 h. Values are the means $\pm$ S.E.M. of 6 experiments. [#]Significantly different from the LPS alone group ($P < 0.05$). ^{*}Significantly different from the control group ($P < 0.05$).

lated the expression of iNOS and GTPCH mRNAs and increased the biopterin level in MBMECs. These findings strongly suggested that LPS increases NO production and BH4 synthesis via the induction of iNOS and GTPCH in MBMECs, respectively.

Although iNOS has been shown to be inhibited by NO (32), it has not been determined whether BH4 synthesis is modulated by NO. In the present study, the biopterin level during LPS treatment was not affected by the treatment with NOS inhibitors. The concentrations of the NOS inhibitors used in the present study have been shown to completely inhibit iNOS activity (39). Although the availability of extracellular L-arginine can be rate-limiting to

obtain a maximal generation of NO by iNOS (40), the addition of L-arginine did not affect the biopterin level during LPS treatment. Moreover, co-addition of NO-donors also did not affect LPS-induced increase in biopterin level. These findings strongly suggest that NO has no regulatory role for BH4 synthesis during LPS treatment in MBMECs.

Despite the lack of an effect with NOS inhibitors, NO-donors and L-arginine on the biopterin level during LPS treatment, carboxy-PTIO, but not TMA-PTIO, both NO scavengers, markedly increased the biopterin level under both basal and LPS treatment conditions. Increases in the biopterin level in MBMECs by carboxy-PTIO during LPS treatment is unlikely to be dependent on its NO scavenging

Fig. 6. Effect of carboxy-PTIO on the mRNA level of GTPCH during LPS treatment. Cells were incubated with various concentrations of carboxy-PTIO (1–300 μ M) in the presence of LPS (10 μ g/ml) for 6 h before RNA extraction. After electrophoresis of 20 μ g RNA per sample and transfer to a nylon membrane, the blot was sequentially hybridized with 32 P-labeled GTPCH cDNA and GAPDH cDNA.

activity. Carboxy-PTIO produces NO_2 by the reaction with NO (41). However, NO_2 produced by the reaction with NO and carboxy-PTIO is also unlikely to be implicated in the increase in the biopterin level by carboxy-PTIO, since the carboxy-PTIO-induced increase in biopterin level was not affected by the co-treatment with NOS inhibitor and NO-donors (data not shown).

BH4 is synthesized from GTP via GTPCH, which catalyzes the first and the rate-limiting step (22, 23). Moreover, BH4 can also be produced via a salvage pathway that utilizes sepiapterin as an intermediate step (22, 23). It is well known that LPS and/or cytokines induce BH4 synthesis through the induction of GTPCH in various types of cells (19–21, 31). However, the increase in the biopterin level by carboxy-PTIO is unlikely to be mediated by the induction of GTPCH, since GTPCH mRNA level during LPS treatment was unchanged by the treatment with carboxy-PTIO and an inhibitor of GTPCH only slightly re-

Fig. 7. Effects of DAHP and NAS on the LPS- or carboxy-PTIO-induced increase in BH4 level. A and C: Cells were incubated with LPS (10 μ g/ml) in the presence or absence of DAHP (100 and 1,000 μ M) or NAS (100 and 1,000 μ M) for 12 h. B and D: Cells were incubated with carboxy-PTIO (100 μ M) in the presence or absence of DAHP (100 and 1,000 μ M) or NAS (100 and 1,000 μ M) for 12 h. Values are the means $\pm$ S.E.M. of 4 experiments. #Significantly different from the LPS-treated group ($P < 0.05$). *Significantly different from the carboxy-PTIO alone group ($P < 0.05$).

Fig. 8. Effect of cycloheximide on the carboxy-PTIO-induced increase in BH4 level. Cells were incubated with LPS (10 μg/ml) or carboxy-PTIO (100 μM) in the presence or absence of cycloheximide (1 μg/ml) for 12 h. Values are the means ± S.E.M. of 4 experiments. [#]Significantly different from the LPS alone group ($P < 0.05$).

duced the carboxy-PTIO alone-induced increase in the biopterin level. Interestingly, the inhibition of sepiapterin reductase strongly inhibited the carboxy-PTIO alone-induced increase in the biopterin level. These findings suggest that carboxy-PTIO may increase the biopterin level by stimulation of the salvage pathway of BH4 synthesis via sepiapterin reductase. Moreover, BH4 is non-enzymatically degraded to pterin (38). Carboxy-PTIO reduced the pterin level during both basal and LPS treatment conditions. The inhibition of BH4 degradation to pterin may also be involved in the increase of the biopterin level which reflects the BH4 level during LPS treatment. Thus, carboxy-PTIO appears to increase the BH4 level by stimulation of the salvage pathway of BH4 synthesis and inhibition of BH4 degradation.

BH4 has antioxidative and scavenging activity for ROS. Shen and Zhang (42) first demonstrated that BH4 has antioxidative activity by showing that BH4 inhibits dopamine autoxidation. Kojima et al. (27) found that BH4 acts directly as a scavenger of superoxide anion generated by xanthine/xanthine oxidase or by rat macrophage/phorbol myristate acetate radical-generating systems and that paraquat-induced cytotoxicity of cultured hepatocytes is suppressed by BH4. The superoxide anion-scavenging activity of BH4 appears to be stronger than that of ascorbic acid (27). Using 2',7'-dichlorofluorescein, a hydroperoxide-sensitive dye, we previously showed that BH4 reduced hydrogen peroxide-induced intracellular oxidative stress, and protected vascular endothelial cells against H₂O₂-induced cytotoxicity (29).

Fig. 9. Effect of carboxy-PTIO on the biopterin and pterin levels in basal and LPS treatment conditions. Cells were incubated with carboxy-PTIO (100 μM), LPS (10 μg/ml) or LPS (10 μg/ml) plus carboxy-PTIO (100 μM). A: BH4 level, B: pterin level, C: total (BH4 plus pterin) level. Values are the means ± S.E.M. of 4 experiments. ^{*}Significantly different from the control group ($P < 0.05$). [#]Significantly different from the LPS alone group ($P < 0.05$).

Thus, BH4 has strong antioxidative activity and protects various cells against oxidative stress. Moreover, it has been shown that BH4 reduces cytotoxicity by large amounts of NO in vascular endothelial cells and HL-60 cells (28, 31). ROS and NO are implicated in the development of inflammatory diseases (43, 44). Moreover, there are several studies showing that carboxy-PTIO prevents NO-induced cell injury via NO scavenging (45, 46). Carboxy-PTIO

also has a protective effect against NO-donors-induced endothelial cell injury (30). In the present study, it was not shown whether increased BH4 by carboxy-PTIO treatment had a protective effect against LPS-induced cytotoxicity, because MBMECs were not injured by LPS treatment. However, under inflammatory diseases, vascular endothelial cells may be exposed to large amounts of ROS and NO released from macrophages and neutrophils. Therefore, carboxy-PTIO may protect the cells under inflammatory diseases, by scavenging NO and increasing the BH4 level.

In conclusion, under stimulation with LPS in MBMECs, NO produced by iNOS is unlikely to modulate BH4 synthesis. However, carboxy-PTIO, an NO scavenger, increases the BH4 level during LPS treatment in MBMECs due to both stimulation of the salvage pathway of BH4 synthesis via sepiapterin reductase and the inhibition of the decomposition of BH4 to pterin. Carboxy-PTIO is a unique substance to treat inflammatory diseases, since it can scavenge over-produced NO and increase the BH4 level, which has a protective effect against NO and/or ROS toxicities.

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