厚生労働科学研究費補助金

障害者対策総合研究事業(神経・筋疾患分野)

筋萎縮性側索硬化症患者由来疾患モデル細胞を用いた病態解明と治療法開発

平成 22 年度　総括・分担研究報告書

主任研究者　高橋　良輔

分担研究者　井上　治久

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厚生労働科学研究費補助金（障害者対策総合研究事業）
（総括）研究報告書

筋萎縮性側索硬化症由来疾患モデル細胞を用いた病態解明と治療法開発に関する研究

研究代表者　高橋　良輔　京都大学大学院医学研究科臨床神経学　教授

研究要旨：変異 SOD1 を有する遺伝性筋萎縮性側策硬化症（amyotrophic lateral sclerosis: ALS）においては、変異 SOD1 を有するアストロサイトによる運動ニューロン変性加速（非自律性神経変性）が生じる。これまでの研究から、アストロサイトの有する SOD1 転写抑制が治療効果を有する可能性がある。本研究では、ヒトアストロサイト細胞株を用いて SOD1 転写活性を抑制する低分子化合物・既存薬を同定した。同定した低分子化合物の1つは、SOD1 の転写因子 Nrf2 のリン酸化を抑制し、SOD1 の転写抑制効果を示した。また、本年度 ALS モデルマウスを用いたパイロット実験により、同定した既存薬は ALS モデルマウス脊髄で SOD1 の転写を抑制した。

研究分担者：井上治久
京都大学 iPS 細胞研究所　准教授

A. 研究目的

神経変性疾患では、特定の神経系が脆弱性を有し、選択的神経変性を生じる。最近の研究において、グリア細胞がその神経変性に寄与していることが明らかになりつつある。変異SOD1を有する遺伝性筋萎縮性側策硬化症（amyotrophic lateral sclerosis: ALS）においては、変異SOD1を有するアストロサイトが運動ニューロン変性を加速すると考えられている。また、アストロサイトのSOD1タンパク質量を減少させることが治療効果を有する可能性がある。本研究では、アストロサイトのSOD1タンパク質量を減少させる可能性のある低分子化合物・既存薬候補を同定し、最終的に、ALS治療薬を発見することを目的としている。

B. 研究方法

ヒトSOD1のゲノムを用いて、SOD1のpromoter制御下に分泌型ルシフエラーゼを発現するベクターを構築する。これまでの研究で、ヒトアストロサイト細胞株にそのベクターを導入し、恒常的に分泌型ルシフエラーゼを発現するクローンを樹立する。上清のルシフエラーゼを測定し、SOD1転写活性を抑える低分子化合物および既存薬を同定している。

同定した低分子化合物については、SOD1の転写因子の1つであるNrf2との関連について解析した。

さらに、同定した既存薬については、変異SOD1マウスに4週間経口投与した後、脊髄におけるSOD1mRNA量を定量化した。

（倫理面への配慮）

本研究は京都大学医学部倫理委員会により、課題名『ヒト疾患特異的 iPS 細胞の作製とそれを用いた疾患解析に関する研究』（承認番号第 824 番）および『ヒト疾患特異的 iPS 細胞を用いた遺伝子解析研究』（承認番号第 G259）として承認されている。ヒトゲノム・遺伝子解析研究に関する倫理指針（平成 13 年 3 月 29 日文部科学省・厚生労働省・経済産業省告示第 1 号）を尊守するものである。

C. 研究結果

約 10,000 種類の低分子化合物のうち、濃度依存性に SOD1 の発現量を減少させる 177 種類の化合物といくつかの既存薬を一次スクリーニングで同定した（Z'-factor は 0.5~1.0）。WST-1 アッセイでその効果が細胞毒性によるものであることを除外し、ELISA で SOD1 タンパク質量の実際の発現低下を 177 のヒットの内 10 化合物で確認した。

その化合物の中で、化合物 052C9 が SOD1 転写を制御する機序として、SOD1 の転写因子の一つ Nrf2 のリン酸化を 052C9 が抑制することを見いだした。

さらに、最も効果の高い 2 つの化合物に構造式類似の既存薬を変異 SOD1 マウスに 4 週間経口投与したところ、脊髄において SOd1 の mRNA 量が約 70%に低下（$p<0.05$）することを見いだした。

D. 考察

変異 SOD1 による遺伝性 ALS モデルマウスの研究から、変異 SOD1 を有するアストロサイトが毒性を有し、運動ニューロン変性を加速させると考えられ、治療薬スクリーニングの標的細胞となりうると考えられている。本研究により、遺伝性 ALS 標的細胞アストロサイトの有する標的分子 SOD1 の発現モニタリングシステムとそれを用いたアッセイ系を確立した。今後、同定した既存薬が SOD1 転写を抑制するメカニズムを検討すると同時に、ALS モデルマウスの生存期間等を含む治療効果について解析を行う。
する可能性がある。

E. 結論

ALS モデルマウスにおいて、SOD1 転写活性を低下させる既存薬を同定した。

F. 健康危険情報

なし

G. 研究発表

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H. 知的財産権の出願・登録状況（予定を含む。）

1. 特許取得
　　なし。
2. 実用新案登録
　　なし。
3. その他
　　なし。

別紙3

厚生労働科学研究費補助金（障害者対策総合研究事業）
（分担）研究報告書

筋萎縮性側索硬化症由来疾患モデル細胞のアストロサイトへの分化誘導とその治療的応用

研究分担者 井上 治久 京都大学 iPS 細胞研究所 臨床応用研究部門 准教授

研究要旨：変異 SOD1 による遺伝性筋萎縮性側索硬化症（amyotrophic lateral sclerosis: ALS）モデルマウスを用いた研究から、変異 SOD1 を有するアストロサイトの SOD1 タンパク質量を低下させることが治療効果を有することが考えられる。本研究では、ALS モデルマウスアストロサイトとヒト iPS 細胞由来運動ニューロンとの共培養で運動ニューロン数減少を認めた。新たな ALS モデル開発につながる可能性がある。

A. 研究目的

本研究は筋萎縮性側索硬化症（amyotrophic lateral sclerosis: ALS）患者由来 iPS 細胞を用いることにより、これまで入手困難であった患者由来の神経系細胞を作製し、それらの細胞を用いて、創薬スクリーニングを行い、治療薬候補を同定することを目標としている。最終的には、ALS の克服を目的としている。

B. 研究方法

本申請研究では、患者さんへの適切な説明及びそれに基づく同意取得の下、皮膚線維芽細胞を培養し、レトロウィルスを用いてiPS細胞を作製した。さらにアストロサイトへ分化誘導しSOD1変異を有するALS患者由来iPS細胞を分化誘導し、アストロサイトを入手した。また、変異SOD1トランスジェニックマウス（ALSモデルマウス）由来プライマリーアストロサイトを入手した。それぞれを用いて、ヒトiPS細胞由来運動ニューロンとの共培養を用いてALS細胞モデル系の再現を試みた。

（倫理面への配慮）

本研究は京都大学医学部倫理委員会により、課題名『ヒト疾患特異的 iPS 細胞の作製とそれを用いた疾患解析に関する研究』（承認番号第 824 番）および『ヒト疾患特異的 iPS 細胞を用いた遺伝子解析研究』（承認番号第 G259）として承認されている。ヒトゲノム・遺伝子解析研究に関する倫理指針（平成 13 年 3 月 29 日文部科学省・厚生労働省・経済産業省告示第 1 号）を尊守するものである。

C. 研究結果

変異 SOD1 トランスジェニックマウス（ALS モデルマウス）由来プライマリーアストロサイトとコントロールヒト iPS 細胞由来運動ニューロンの共培養において、運動ニューロン数の減少を共培養開始 1 ヶ月後に認めた。SOD1 変異を有する ALS 患者由来アストロサイトとヒト iPS 細胞由来運動ニューロンの共培養は現在解析中である。

D. 考察

今後、変異 SOD1 を有するヒトアストロサイトとヒト iPS 細胞由来運動ニューロンの共培養における運動ニューロン変性を解析する。

変異 SOD1 による遺伝性 ALS モデルマウスの研究で有効性が確認された治療薬のうち、ヒト ALS において有効であった治療薬はこれまでに同定されていない。モデルマウスがヒトの細胞と異なることが原因の 1 つと考えられ、本研究で使用したヒト iPS 細胞由来の細胞がこれまでの治療薬スクリーニング方法を補完する可能性がある。

E. 結論

新たな ALS モデル開発の可能性を示した。

G. 研究発表

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H. 知的財産権の出願・登録状況(予定を含む。)
1. 特許取得
なし。
2. 実用新案登録
なし。
3.その他
なし。

研究成果の刊行に関する一覧表

別紙４

研究成果の刊行に関する一覧表

書籍

著者氏名	論文タイトル名	書籍全体の編集者名	書　籍　名	出版社名	出版地	出版年	ページ

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発表者氏名	論文タイトル名	発表誌名	巻号	ページ	出版年
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Egawa N, Yamamoto K, Inoue H, Hikawa R, Nishi K, Mori K, Takahashi R.	The endoplasmic reticulum stress sensor, ATF6{alpha}, protects against neurotoxin-induced dopaminergic neuronal death.	J Biol Chem	286	7947-57	2011
Murakami G, Inoue H, Tsukita K, Asai Y, Amagai Y, Aiba K, Shimogawa H, Uesugi M, Nakatsuji N, Takahashi R	Chemical library screeningidentifies a small molecule that downregulates SOD1 transcription for drugs to treat ALS, J Biomol Screen	J Biomol Screen	in press		

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雑誌

発表者氏名	論文タイトル名	発表誌名	巻号	ページ	出版年
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Inoue H, Yamanaka S.	The Use of Induced Pluripotent Stem Cells in Drug Development.	Clinical Pharmacology & Therapeutic	in press		2011
Inoue H.	Neurodegenerative disease-specific induced pluripotent stem cell research.	Journal of Pharmacological Sciences	115	Supplement 1	2011
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Nonhypotensive Dose of Telmisartan Attenuates Cognitive Impairment Partially Due to Peroxisome Proliferator-Activated Receptor-{gamma} Activation in Mice With Chronic Cerebral Hypoperfusion * Supplemental Methods

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Nonhypotensive Dose of Telmisartan Attenuates Cognitive Impairment Partially Due to Peroxisome Proliferator-Activated Receptor-γ Activation in Mice With Chronic Cerebral Hypoperfusion

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Background and Purpose—The effect of telmisartan, an angiotensin II Type 1 receptor blocker with peroxisome proliferator-activated receptor-γ-modulating activity, was investigated against spatial working memory disturbances in mice subjected to chronic cerebral hypoperfusion.

Methods—Adult C57BL/6J male mice were subjected to bilateral common carotid artery stenosis using external microcoils. Mice received a daily oral administration of low-dose telmisartan (1 mg/kg per day), high-dose telmisartan (10 mg/kg per day), or vehicle with or without peroxisome proliferator-activated receptor-γ antagonist GW9662 (1 mg/kg per day) for all treatments for 30 days after bilateral common carotid artery stenosis. Cerebral mRNA expression of monocyte chemoattractant protein-1 and tumor necrosis factor-α was measured 30 days after bilateral common carotid artery stenosis, and postmortem brains were analyzed for demyelinating change with Klüver-Barrera staining and immunostained for glial, oxidative stress, and vascular endothelial cell markers. Spatial working memory was assessed by the Y-maze test.

Results—Mean systolic blood pressure and cerebral blood flow did not decrease with low-dose telmisartan but significantly decreased with high-dose telmisartan. Low-dose telmisartan significantly attenuated, but high-dose telmisartan provoked, spatial working memory impairment with glial activation, oligodendrocyte loss, and demyelinating change in the white matter. Such positive effects of low-dose telmisartan were partially offset by cotreatment with GW9662. Consistent with this, low-dose telmisartan reduced the degree of oxidative stress of vascular endothelial cells and the mRNA levels of monocyte chemoattractant protein-1 and tumor necrosis factor-α compared with vehicle.

Conclusions—Anti-inflammatory and antioxidative effects of telmisartan that were exerted in part by peroxisome proliferator-activated receptor-γ activation, but not its blood pressure-lowering effect, have protective roles against cognitive impairment and white matter damage after chronic cerebral hypoperfusion. **(*Stroke*. 2010;41:1798-1806.)**

Key Words: chronic cerebral hypoperfusion ■ oligovascular niche ■ oxidative stress ■ PPAR-γ ■ telmisartan

Drugs that target the renin–angiotensin system seem to have particular potential for prevention of dementias, including Alzheimer disease and vascular dementia. The Perindopril Protection Against Recurrent Stroke Study (PROGRESS) has suggested a protective effect of angiotensin-converting enzyme inhibitors on cognitive function in patients with stroke.[1] Moreover, the Study on Cognition and Prognosis in the Elderly (SCOPE) trial demonstrated a positive effect of the angiotensin II Type 1 receptor blocker (ARB), candesartan, in a subgroup of elderly hypertensive patients with mild cognitive impairment.[2] Notably, a prospective cohort analysis of 819 491 participants suggested that ARBs are associated with a significant reduction in the incidence and progression of dementia, even compared with angiotensin-converting enzyme inhibitors.[3]

No benefit was found in cognitive performance after administration of the ARB, telmisartan, at the subacute stage (within 15 days) after stroke in the Prevention Regimen for Effectively Avoiding Second Strokes (PRoFESS) study.[4] However, in vitro studies have suggested that telmisartan, the strongest peroxisome proliferator-activated receptor-γ (PPAR-γ) activator among ARBs,[5] may protect oligodendrocytes and neurons through a reduction of brain inflammation through PPAR-γ activation and AT_1 receptor blockade.

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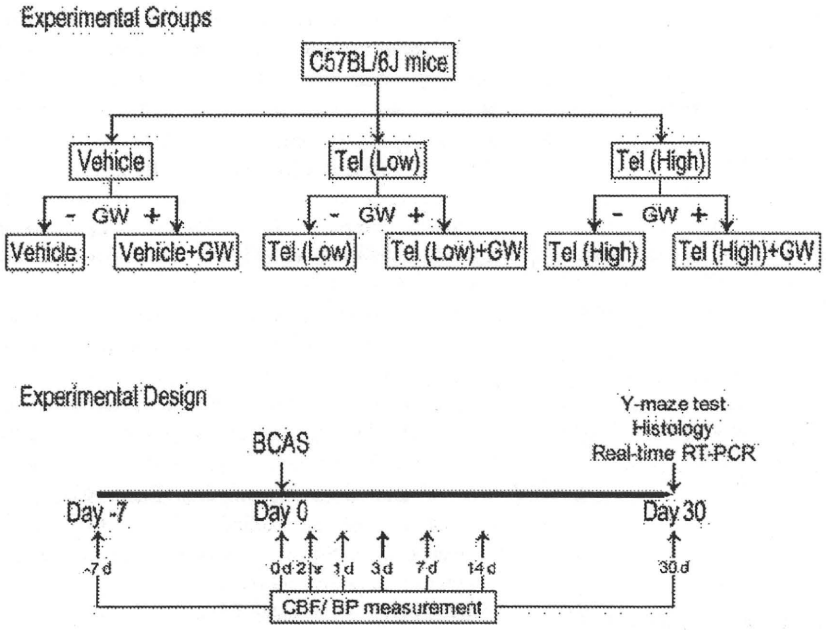

Figure 1. Experimental protocol. Tel (Low) indicates low-dose telmisartan (1 mg/kg per day); Tel (High), high-dose telmisartan (10 mg/kg per day); GW, GW9662 (1 mg/kg per day); RT-PCR, reverse transcriptase–polymerase chain reaction.

Telmisartan has structural similarities to the PPAR-γ ligand pioglitazone and thus could act as a partial agonist of PPAR-γ.[5] PPAR-γ, one of the nuclear receptors, plays a critical role in a variety of biological processes, including angiogenesis, inflammation, oxidative stress, glucose metabolism, and adipogenesis.[5] Moreover, PPAR-γ activation in the brain has been suggested as a protective effect against Alzheimer disease through its multifaceted effects, including anti-inflammation and amyloid-β clearance.[6] Therefore, in the PRoFESS study, excessive lowering of blood pressure (BP) in the period with cerebrovascular autoregulatory dysfunction may have affected the cerebral circulation and neuronal function, although other factors could also be involved.

The present study is therefore designed to explore the multifaceted effects of telmisartan on cognitive disturbances in a mouse model of vascular dementia by administering a hypotensive or a nonhypotensive dose of telmisartan. This model of chronic cerebral hypoperfusion, which is produced by placing microcoils bilaterally on the common carotid arteries, invariably exhibits glial activation, oxidative stress, inflammation, demyelinating change, and axonal loss in the white matter with resultant spatial working memory deficits.[7,8] This in vivo system will help determine whether telmisartan affects vascular autoregulatory function and whether and how telmisartan exerts its protective effect against cognitive impairment related to white matter damage.

Materials and Methods

Experimental Protocol

The experimental protocol is shown in Figure 1. Nine-week-old male C57BL/6J mice (weighing 24 to 29 g; CLEA, Tokyo, Japan) were fed with the pelleted chow (MF) containing low-dose telmisartan (1 mg/kg per day), high-dose telmisartan (10 mg/kg per day), or vehicle with or without PPAR-γ antagonist GW9662 (1 mg/kg per day; Sigma-Aldrich) for all treatments, beginning from 7 days before the bilateral common carotid artery stenosis (BCAS) surgery until 30 days post-BCAS. Immediately after spatial working memory was assessed by the Y-maze test, mice were euthanized for histological and real-time reverse transcriptase–polymerase chain reaction examination 30 days post-BCAS.

Surgical Procedure of BCAS

Under anesthesia with halothane (2%), both common carotid arteries were exposed through a midline cervical incision, and a microcoil with an inner diameter of 0.18 mm was applied to the bilateral common carotid arteries. See Supplemental Method I for details (available at http://stroke.ahajournals.org).[7,8]

Systolic BP and Cerebral Blood Flow Measurements

Various doses of telmisartan (0 to 100 mg/kg per day) were administered and the BP measured for determining nonhypotensive and hypotensive doses of telmisartan. Then, in mice receiving the predetermined nonhypotensive or hypotensive dose of telmisartan or vehicle, systolic BP and cerebral blood flow (CBF) were monitored at 7 days before BCAS (before starting telmisartan treatment), immediately before BCAS, and 2 hours, 1 day, 3 days, 7 days, 14 days, and 30 days after BCAS. See Supplemental Method II for details.

Histochemical Evaluation of White Matter Lesions, Glial Activation, and Oxidative Stress

The mouse brains were analyzed for demyelinating change with Klüver-Barrera staining and immunostained for glial fibrillary acidic protein (a marker of astrocyte), ionized calcium binding adaptor molecule-1 (Iba-1; microglia), glutathione S-transferase-π (GST-π; oligodendrocyte), 8-hydroxy-deoxyguanosine (8-OHdG; oxidative stress), and CD31 (vascular endothelial cell). See Supplemental Method III for details.[7]

Quantitative Real-Time Reverse Transcriptase–Polymerase Chain Reaction

Cerebral mRNA levels of monocyte chemoattractant protein-1 (MCP-1) and tumor necrosis factor-α (TNF-α) were assessed by quantitative real-time reverse transcriptase–polymerase chain reaction pre-BCAS and 30 days post-BCAS. Detailed procedures are described in Supplemental Method IV.

Y-Maze Test for Spatial Working Memory Assessment

Spatial working memory was assessed by the Y-maze test. The detail of the Y-maze test protocol is described in Supplemental Method V.

Blood Concentration of Telmisartan

See Supplemental Method VI for details.

Statistical Analysis

All values are expressed as means±SEM in the text and figures. One-way analysis of variance was used to evaluate significant differences among groups except when otherwise stated. When a statistically significant effect was found, a post hoc Tukey test or Tukey-Kramer test was performed to detect the difference between the groups. Temporal profiles of systolic BP and CBF were analyzed by 2-way repeated-measures analysis of variance followed by a post hoc Tukey test. Differences with $P<0.05$ were considered statistically significant in all statistical analyses used.

Results

Systolic BP and CBF After Telmisartan Administration

Treatment with telmisartan, ≤1 mg/kg per day, did not result in a significant reduction in BP, whereas treatment with telmisartan ≥3 mg/kg per day resulted in a significant reduction in BP (Figure 2A). CBF was not significantly reduced ≤1 mg/kg per day but began to decrease at 3 mg/kg per day (Figure 2B). A nonhypotensive dose of 1 mg/kg per day or a hypotensive dose of 10 mg/kg per day was subsequently administered. Temporal profiles of systolic BP and CBF were not affected by administration of a nonhypotensive dose of telmisartan or addition of GW9662 (Figure 2C–E). CBF gradually recovered after BCAS in mice with vehicle or a nonhypotensive dose of telmisartan but not in those with a hypotensive dose of telmisartan (Figure 2E).

The mortality rates were 10% at ≤1 mg/kg per day in the telmisartan-treated group after BCAS surgery. The mortality rate increased to 30% at 3 mg/kg per day in the telmisartan-treated group and 50% at 10 mg/kg per day in the telmisartan-treated group. Eighty percent at 50 or 100 mg/kg per day in the telmisartan-treated group died within 3 days post-BCAS.

Effects of Telmisartan on Glial Activation, Oligodendrocyte Restoration, and White Matter Lesion in Mouse Brain With Chronic Cerebral Hypoperfusion

Immunohistochemical analysis showed that in response to ischemic insults, resting astrocytes and microglia appeared to enter a reactive state due to apparent morphological changes characterized by thick dendritic formation. Such morphological changes, however, were attenuated by a nonhypotensive dose of telmisartan (Figure 3; compare 3A and 3C and compare 3I and 3K). Both the number of glial fibrillary acidic protein-positive astrocytes and Iba-1-positive microglia were significantly reduced in both the corpus callosum and anterior commissure from a nonhypotensive dose of telmisartan-treated BCAS mice compared with the vehicle-treated BCAS mice (Figure 3G–H, O–P). Such effects of low-dose telmisartan were partially offset by cotreatment with GW9662 (Figure 3; compare 3C and 3D and compare 3K and 3L).

Next, a hypotensive dose of telmisartan was examined to assess whether it ameliorated glial activation in BCAS-treated mice. In contrast to a nonhypotensive dose, a hypotensive dose of telmisartan caused substantial glial activation in the white matter (Figure 3; compare 3C and 3E and compare 3K and 3M). Cotreatment with GW9662 did not lead to additional glial changes in the white matter of mice with vehicle (Figure 3; compare 3A and 3B and compare 3I and 3J) or high-dose telmisartan (Figure 3; compare 3E and 3F and compare 3M and 3N).

Klüver-Barrera staining showed that white matter lesions were significantly attenuated in the nonhypotensive group compared with the vehicle group (Figure 4; compare 4A and 4C). Although patterns in oligodendrocytes arrangement could not be seen in the vehicle-treated mice (Figure 4A), alignment in a row formation could be seen in the group given a nonhypotensive dose of telmisartan (Figure 4C). Such effects of low-dose telmisartan were also partially offset by GW9662 (Figure 4; compare 4C and 4D) with significant differences (Figure 4G–H). In contrast, high-dose telmisartan did not attenuate white matter lesions (Figure 4; compare 4A and 4E). There were no significant histological differences between vehicle and high-dose telmisartan-treated mice with or without GW9662. In addition, administration of GW9662 had no effects on morphology of the white matter in sham-operated mice (data not shown), vehicle-treated, BCAS-operated mice (Figure 4; compare 4A and 4B), and high-dose telmisartan-treated, BCAS-operated mice (Figure 4; compare 4E and 4F). White matter lesion Grade 3 (disappearance of myelinated fibers) was only partially (approximately 10% of the white matter) observed in mice with vehicle or high-dose telmisartan.

In addition, the number of GST-π-positive oligodendrocytes of the vehicle-treated mice were significantly decreased in the white matter compared with that of mice treated with a nonhypotensive dose of telmisartan (Figure 4I–K).

Telmisartan Attenuates mRNA Expression of Inflammatory Cytokines in Mouse Brain With Chronic Cerebral Hypoperfusion

Cerebral mRNA expression of inflammatory cytokines such as MCP-1 and TNF-α was significantly increased after the BCAS but significantly attenuated by a nonhypotensive dose of telmisartan 30 days post-BCAS (Figure 4L–M).

Vascular Endothelial Oxidative Stress in Mouse Brain With Chronic Cerebral Hypoperfusion Was Ameliorated by Telmisartan

To further explore the antioxidative effect of telmisartan, 8-OHdG-positive vascular endothelial cells of the brain were assessed. The number of CD31-positive vascular endothelial cells positive for 8-OHdG was markedly reduced by a nonhypotensive dose of telmisartan (Figure 5; compare 5A and 5C). The difference was statistically significant as assessed by 8-OHdG/CD31-positive area (%; the percentage of 8-OHdG-positive area to CD31-positive area; Figure 5J). Such antioxidative effects of low-dose telmisartan were partially offset by cotreatment with GW9662 (Figure 5; compare 5C and 5D). By contrast, high-dose telmisartan showed an attenuated antioxidative effect in comparison to low-dose telmisartan (Figure 5;

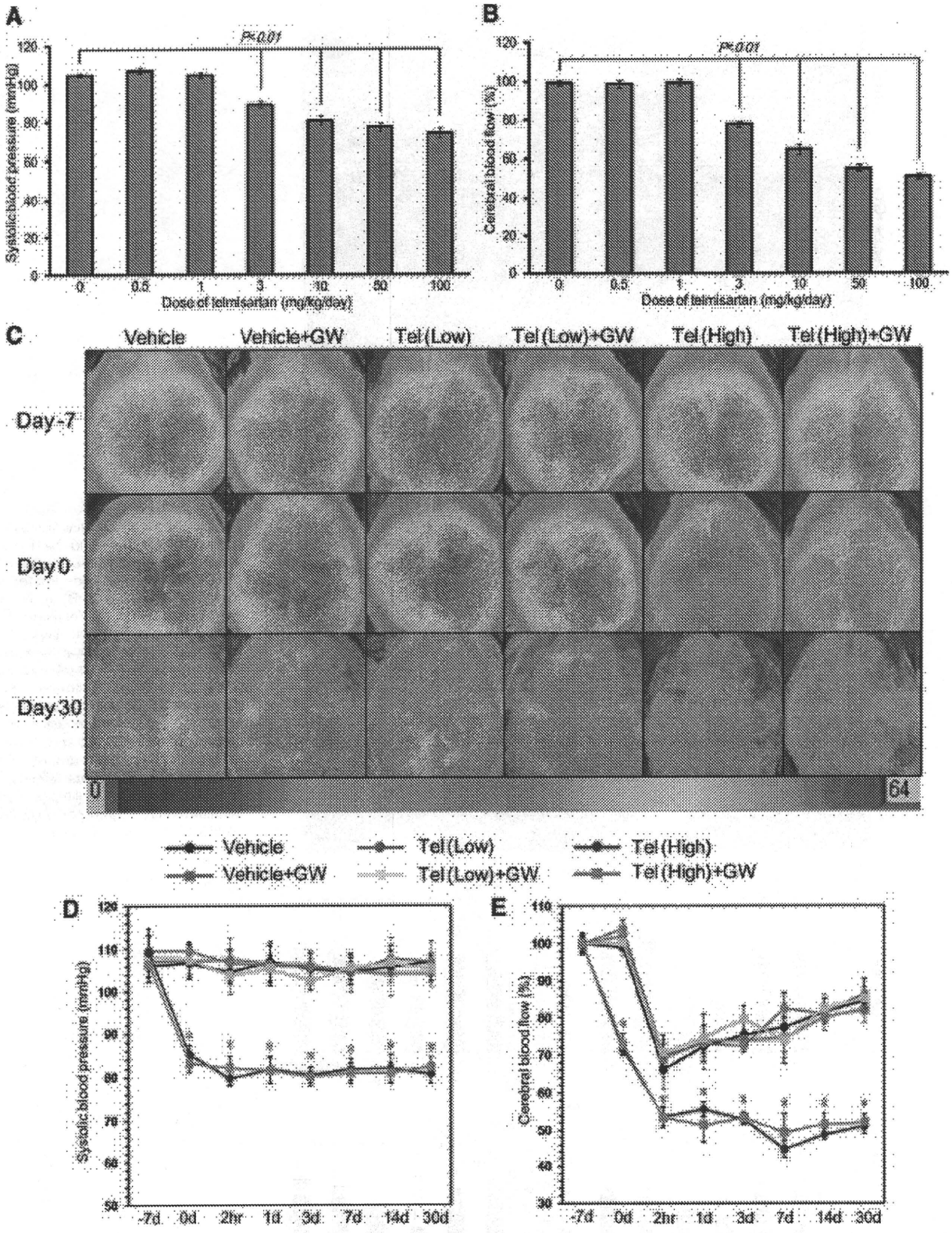

Figure 2. Systolic BP (A) and CBF (B) in mice treated with various doses of telmisartan (n=10 each). Representative CBF images of the 6 groups of mice as assessed by laser speckle flowmetry 7 days before (Day −7) and immediately before (Day 0) BCAS and 30 days post-BCAS (Day 30; C). Temporal profiles of systolic BP (D) and CBF (E) of the 6 groups of mice (n=5 each). CBF was expressed as a percentage of baseline flow. $F_{5,24}=84.100$ (D), $F_{5,24}=37.441$ (E), *$P<0.01$ versus vehicle.

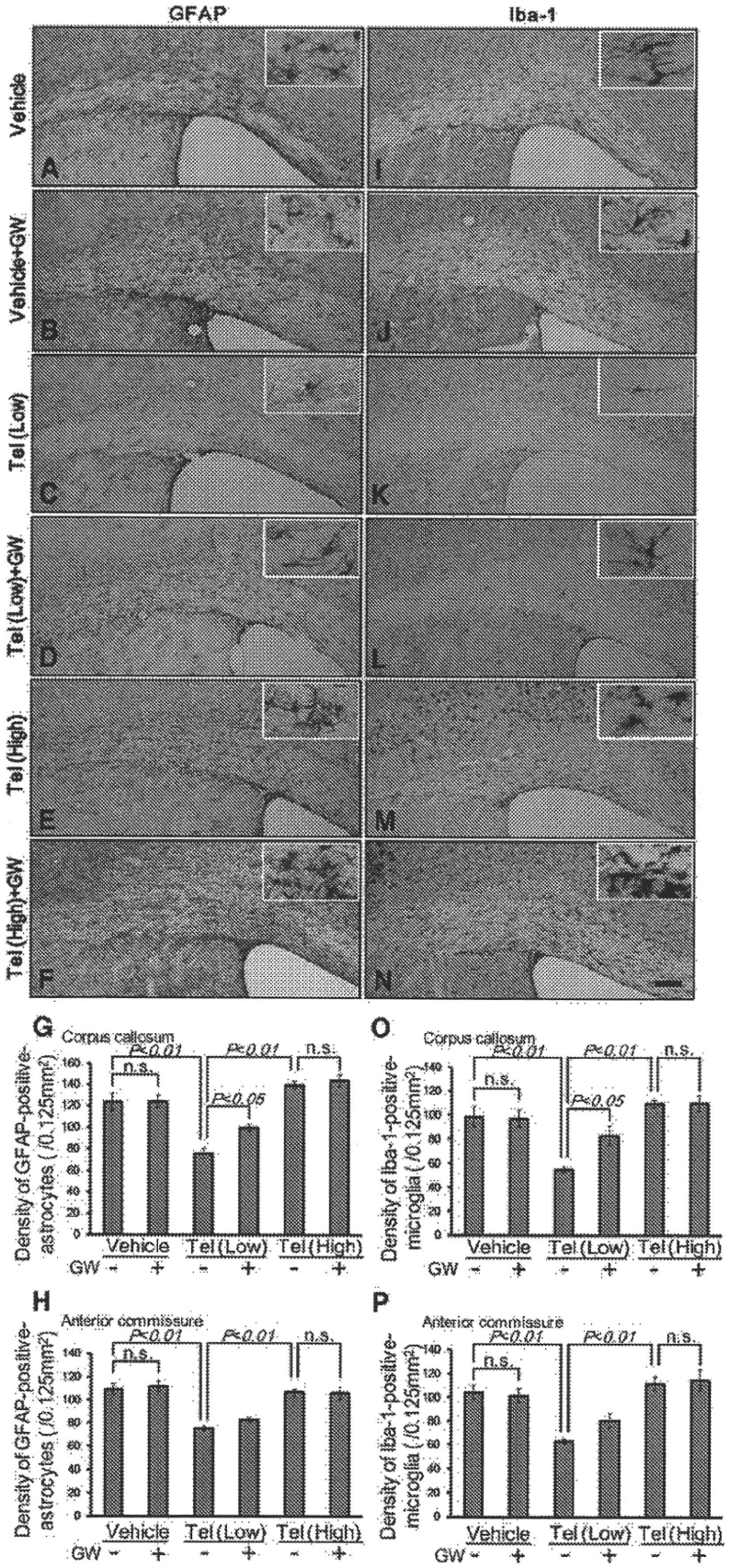

Figure 3. Representative images of immunohistochemistry for glial fibrillary acidic protein (GFAP; A–F) and Iba-1 (I–N) in the paramedian parts of the corpus callosum of the BCAS-operated mice treated with vehicle (A, I), vehicle+GW (B, J), Tel (Low; C, K), Tel (Low)+GW (D, L), Tel (High; E, M), and Tel (High)+GW (F, N) 30 days post-BCAS (n=7 each). Insets indicate enlarged images of astrocytes (A–F) and microglia (I–N). Scale bar, 100 μm. Histogram showing the density of GFAP-positive astrocytes (G–H) and Iba-1-positive microglia (O–P) in the corpus callosum (G, O) and anterior commissure (H, P) of the 6 groups of mice. Tel (Low) indicates low-dose telmisartan (1 mg/kg per day); Tel (High), high-dose telmisartan (10 mg/kg per day); GW, GW9662 (1 mg/kg per day).

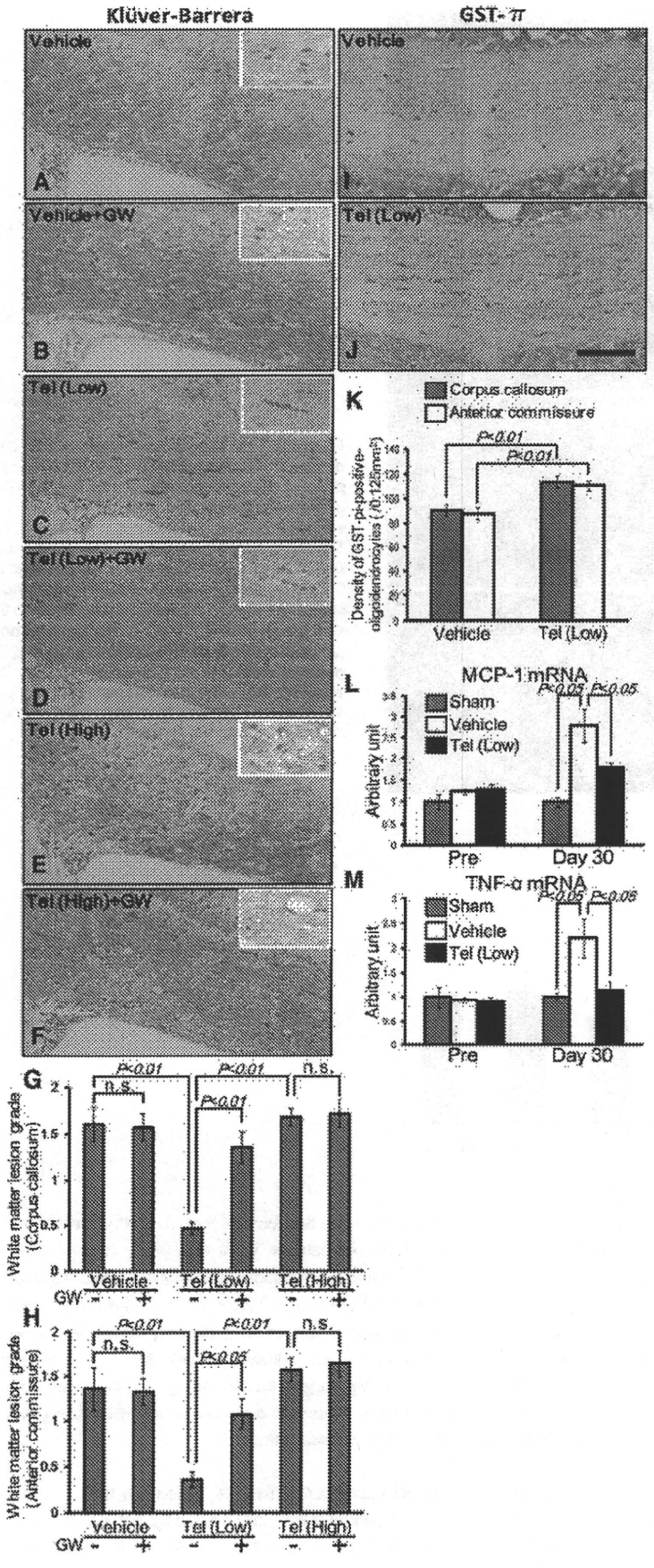

Figure 4. Representative images of the Klüver-Barrera staining in the paramedian parts of the corpus callosum of the BCAS-operated mice treated with vehicle (A), vehicle+GW (B), Tel (Low; C), Tel (Low)+GW (D), Tel (High; E), and Tel (High)+GW (F) 30 days post-BCAS (n=7 each). Insets indicate enlarged images of oligodendrocytes. Histogram showing the grading of the white matter lesions of the 6 groups of mice (G–H; see Supplemental Method III for details). Representative images of immunohistochemistry for GST-π-positive oligodendrocytes in the medial parts of the corpus callosum of the BCAS-operated mice treated with vehicle (I) and Tel (Low; J) 30 days post-BCAS (n=7 each). Scale bar, 100 μm. Histogram showing the density of GST-π-positive oligodendrocytes (K) of the 2 groups of mice. Cerebral mRNA expressions of MCP-1 (L) and TNF-α (M) pre-BCAS and 30 days post-BCAS in the sham-operated or BCAS-operated mice treated with vehicle or Tel (Low; n=5 each). Tel (Low) indicates low-dose telmisartan (1 mg/kg per day); Tel (High), high-dose telmisartan (10 mg/kg per day); GW, GW9662 (1 mg/kg per day).

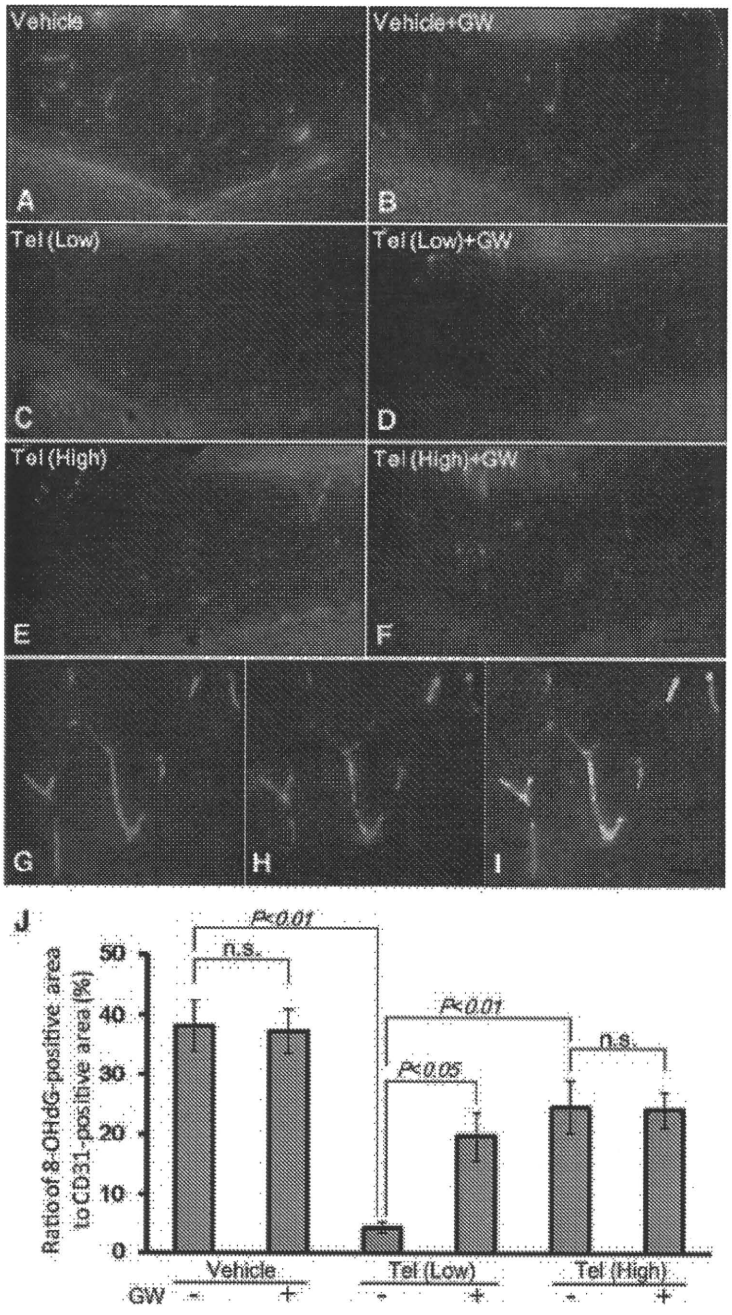

Figure 5. Representative images of the immunofluorescent staining for 8-OHdG (red) in the medial parts of the corpus callosum of the BCAS-operated mice treated with vehicle (A), vehicle+GW (B), Tel (Low; C), Tel (Low)+GW (D), Tel (High; E), and Tel (High)+GW (F) 30 days post-BCAS (n=7 each). Capillaries double-positive for CD31 (G, green) and 8-OHdG (H, red) and merged image (I) in vehicle-treated mice. Scale bars, 100 μm (A–F), 50 μm (G–I). Histogram showing the percentage of 8-OHdG-positive area to CD31-positive area of the 6 groups of mice (J). Tel (Low) indicates low-dose telmisartan (1 mg/kg per day); Tel (High), high-dose telmisartan (10 mg/kg per day); GW, GW9662 (1 mg/kg per day).

compare 5A, 5C, and 5E). Cotreatment with GW9662 did not lead to additional histological changes in mice with vehicle (Figure 5; compare 5A and 5B) or high-dose telmisartan (Figure 5; compare 5E and 5F).

Spatial Working Memory in Mice With Chronic Cerebral Hypoperfusion Was Restored by a Nonhypotensive Dose of Telmisartan

Finally, we analyzed spatial working memory of BCAS mice by the Y-maze test as the final functional output. The percentage of alternation behaviors significantly decreased in vehicle-treated BCAS mice compared with the sham-operated mice but significantly increased in a nonhypotensive dose of telmisartan-treated mice. Such effects of low-dose telmisartan were partially offset by cotreatment with GW9662. Hypotensive doses of telmisartan-treated mice manifested in further impaired working memory (Figure 6A). There were no significant differences in the number of entries to each arm, which was considered to reflect locomotor activity, among the 5 groups (Figure 6B). These results suggest that a nonhypotensive dose of telmisartan, but not a hypotensive dose, improved spatial working memory of BCAS-operated mice.

Blood Concentration of Telmisartan

Plasma concentration after 1 mg/kg per day administration of telmisartan for 7 days was 142.86±14.85 ng/mL (n=4, values