# Mdivi-1 improves postoperative neurocognitive disorders in aged rats undergoing splenectomy by inhibiting dynamin-related protein-1

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**Background:** The regulatory role of mitochondria in the inflammatory response of the nervous system postoperatively remains unclear. This study explored the relationship between mitochondria and postoperative neurocognitive dysfunction (PNCD) by regulating mitochondrial function in aged rats undergoing splenectomy.

**Methods:** A total of 120 aged rats were randomly divided into five groups (n=24) as follows: Control group (not subjected to any form of treatment), Sham group (subjected only to sham-splenectomized operation after anesthesia), Splenectomy group (only underwent splenectomy after anesthesia), Synonyms Mitochondrial division inhibitor 1 (Mdivi-1) group [treated with Mdivi-1, a dynamin-relatedprotein 1 (Drp1) inhibitor], and Dimethyl Sulfoxide (DMSO) group (treated with DMSO, a solvent). Inflammatory markers, namely interleukin-1β (IL-1β) and tumor necrosis factor α (TNF-α), were measured in the plasma and brains of the rats. Cognitive function was assessed using the Morris water maze test.

**Results:** During the perioperative period, the physiological parameters did not differ among the five groups (P>0.05). The results of the Morris water maze experiments showed that the memory of the rats was significantly impaired after splenectomy, which was alleviated by Mdivi-1 administration (P=0.04). Postoperatively, the proinflammatory cytokine levels in the serum and hippocampus tissue were upregulated, while Mdivi-1 administration reduced this increase. The electron microscopy and hematoxylin-eosin (HE) staining results indicated that the structure of neurons and mitochondria was minimally impaired in the Mdivi-1 group.

**Conclusions:** Aged rats that underwent splenectomy exhibited significant postoperative cognitive impairments. The selective inhibitor of Drp1, Mdivi-1, exerted protective effects against PNCD by ameliorating mitochondrial dysfunction and reducing the inflammatory response.

**Keywords:** Postoperative neurocognitive dysfunction (PNCD); aged rats; splenectomy; mitochondria; Mdivi-1

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## Introduction

Postoperative neurocognitive dysfunction (PNCD) is a frequent postoperative complication in elderly patients and can lead to prolonged hospitalization and additional postoperative complications, and imposes a serious medical and economic burden on both families and society. Despite the continuous improvement of surgical and anesthesia techniques, the incidence of PNCD remains high, especially among elderly patients, and its pathogenesis is not yet fully understood (1-4). Several pathogenic mechanisms have been proposed for PNCD, including oxidative and nitrosative stress, protein misfolding and aggregation, apoptosis, and glutamatergic excitotoxicity (5,6).

Previous studies demonstrated that acute peripheral inflammation was caused by anesthesia or surgery, which can lead to hippocampus-dependent cognitive damages (7-9). The pro-inflammatory cytokines can destroy the blood–brain barrier by entering the central nervous system and induce the generation of free radicals, which result in oxidative stress and damage neurocognitive function (10,11). Recent studies have reported that mitochondrial dysfunction followed by inflammation in the central nervous system plays a key role in the pathogenesis of PNCD (5,12,13).

Mitochondrial dynamics are necessary for maintaining the physiological function of cells (14). Mitochondrial dysfunction can lead to insufficient cellular energy metabolism, the destruction of cell membrane integrity, and the release of numerous inflammatory factors, thereby resulting in excessive local inflammation and promoting necrosis or apoptosis. The mitochondrial dynamin-related protein-1 (Drp1), as a cytosolic dynamin-like guanosine triphosphate lipase (GTPase), is crucial to mitochondrial dynamics and acts as a key regulator of mitochondrial injury.

Mdivi-1 is a cell permeable quinazolinone originally described by Cassidy-Stone *et al.* as an inhibitor of Drp1 function in 2008 (15). And mdivi-1 likely impairs Drp1 function by acting allosterically and preventing Drp1 oligomerization which is in turn required for GTPase activity (15). Excessive Drp1 upregulation or its abnormal distribution eventually results in mitochondrial dysfunction. However, blocking Drp1 phosphorylation through pharmacological inhibition can relieve mitochondrial injury, restore mitochondrial function, and improve cellular function (16,17). Chronic central nervous system inflammation can induce neuronal necrosis or increased apoptosis and even lead to cognitive dysfunction, whereas inhibiting Drp1 phosphorylation can delay the occurrence and development of chronic neurodegenerative diseases (18).

The present study explored the correlation between Drp1 and PNCD via the inflammatory response by utilizing the Drp1 blocker, Mdivi-1, in elderly post-splenectomy rats and evaluating the mitochondrial injury, inflammatory responses in the hippocampal CA1, and postoperative neurocognitive function. We aimed to clarify the regulatory mechanisms involved in mitochondrial function to improve postoperative neurocognitive function. We present the following article in accordance with the ARRIVE reporting checklist (available at https://atm.amegroups.com/article/view/10.21037/atm-22-5496/rc).

### Highlight box

**Key findings**

- Mdivi-1 ameliorate mitochondrial dysfunction and reducing the inflammatory response.

**What is known and what is new?**

- Aged rats that underwent splenectomy exhibited significant postoperative cognitive impairments.
- The selective inhibitor of Drp1, Mdivi-1, exerted protective effects against PNCD.

**What is the implication, and what should change now?**

- Mdivi-1 against PNCD by ameliorate mitochondrial dysfunction and reducing the inflammatory response.

## Methods

### *Ethics statement*

All experimental procedures were approved by the Institutional Animal Care and Use Committee of Fujian Medical University (ethics review approval No. FJMU-IACUC-2019-0124; animal laboratory facility permit No. SYXK2020-0004) and carried out in accordance with the relevant laws and regulations in China. All of the procedures were conducted according to the Chinese Guidelines for the Care and Use of Laboratory Animals.

### *Experimental protocol*

Aged male Sprague Dawley rats (22–24 months, 450–600 g) were individually housed in standard cages at 22±2 ℃ and a 12-hour light/dark cycle and had access

to water and food *ad libitum*. Rats purchased from Experimental Animal Centre of Fujian Medical University. First, the Morris water maze test was conducted on 15 rats for six consecutive days, which were then used for various tests as the baseline. Next, 120 rats were randomly divided into five groups (n=24/group) as follows: Control group (not subjected to any form of treatment), Sham group (subjected only to sham-splenectomized operation after anesthesia), Splenectomy group (only underwent splenectomy after anesthesia), Mdivi-1 group (treated with Mdivi-1, Drp1 inhibitor); and Dimethyl Sulfoxide (DMSO) group (treated with DMSO, solvent). Mdivi-1 (20 mg/kg; Sigma-Aldrich St. Louis, MO, USA) was intraperitoneally (i.p.) injected into the rats after splenectomy every 12 hours for 3 days. The dose of Mdivi-1 was basing on the previous study (19). After surgery, all of the rats were housed separately for the remainder of the study. The Morris water maze tests were performed during the 3$^{rd}$ or 7$^{th}$ day after splenectomy, after which the rats were sacrificed under deep anesthesia (n =12/time point in each group). Finally, the rats' brain tissue and blood were extracted and stored in liquid nitrogen. A protocol was prepared before the study without registration.

### *Anesthesia and procedures for splenectomy*

The rats were anesthetized using 2% sodium pentobarbital (50 mg/kg, i.p.), after which they respired spontaneously in a nose mask delivering 100% oxygen. We monitored mean arterial pressure, heart rate, and blood oxygen via the rat tail artery while maintaining the temperature at 38.0±0.5 ℃ through fluorescent lamp irradiation. Once the surgical levels of anesthesia were attained (assessed by the absence of hind leg withdrawal when pinched), surgery was performed as previously described by Wan *et al.* (20).

Briefly, the left subcostal margin of the rats was chosen as the site of the surgical incision (2 cm) and 0.25% bupivacaine was used as the local infiltration anesthesia before skin resection. After opening the abdominal cavity, the spleen was located and dissociated from the pedicle. Next, the splenic arteries and veins were ligated and the spleen was completely removed to stop the bleeding. Finally, 10,000 U of gentamicin (Sichuan Xinghua Pharmaceutical Co., Ltd, Sichuan, China) was given i.p. and the abdomen was closed.

### *Morris water maze test*

The spatial learning and memory ability of the rats were evaluated using the Morris water maze test (Smart Junior Software, Panlab, Spain) by a researcher who was blinded to the treatments, as previously described (21). On day 7, the rats underwent general anesthesia and/or surgery. Five rats randomly selected from each group were subjected to a reversal test on the 3$^{rd}$ and 7$^{th}$ days postoperatively, in which the platform was moved to the opposite quadrant of the pool (northwest). The time spent to reach the platform (latency) and the distance were recorded by a video track system (Logitech, Suzhou, China).

### *Hematoxylin-eosin (HE) staining and immunostaining*

HE staining was performed in the previous studies (22,23). Brains that were previously fixed in 4% formaldehyde were embedded in OCT, and 20-μm sections were cut using a cryostat. Another four sections of the hippocampus were randomly selected from four sets of serial sections at 3.6 to 4.16 mm anteroposterior to the Bregma from each rat for Ionized Calcium Binding Adaptor Molecule 1 (IBA1) immunostaining. After three washes, the sections were blocked with a 5% bovine serum albumin (BSA), 0.1% Triton X-100 in 0.01 M Phosphate Buffer Saline (PBS) solution for 1 hour at room temperature and then incubated with the primary antibodies at 4 ℃ overnight (rabbit polyclonal antibody Iba1: 1:1,000, Wako, Japan, #019-19,741; rabbit polyclonal antibody AMPK: 1:200, Proteintech, USA, #10,929-2-AP; mouse monoclonal antibody Neun: 1:1,000, Millipore, USA, #MAB377). After three additional washes, the sections were incubated with secondary antibodies (goat anti-rabbit antibody: 1:500, Jackson; goat anti-mouse antibody: 1:500, Jackson) for 1 hour at room temperature and then washed three times. Finally, the sections were covered using a mounting medium with 4,6-diamidino-2-phenyiindole 2 hci (DAPI).

Photographs of the CA1 and dentate genus (DG) regions in all sections were taken with a microscope (DS-Ri1) under the same magnification (40× objective lens) and the same light intensity by a researcher who was blinded to the treatments. Based on the Iba1 staining results, the percentage of activated microglia in CA1 and DG were quantified by a researcher who was blinded to the treatments, as per the method reported by Cerbai *et al.* (24). Briefly, the microglia were determined to be resting when the cell bodies were small and round and the branches were thin, highly ramified, and equally distributed around them. In contrast, activated microglia were defined by bigger, pleomorphic bi- or tripolar, or spindle/rod-shaped cell

 

bodies and by branches that were shortened, twisted, or without ramification (24).

### *Transmission electron microscopy (TEM)*

After completing the Morris water maze test on the 3rd or 7th day postoperatively, five animals from each group were sacrificed under deep anesthesia using 20 g/L pentobarbital sodium. Their hippocampi were quickly dissected, fixed in 2.5% glutaraldehyde in 0.1 mol/L cacodylic acid buffer (pH 7.3) overnight, and postfixed in 2% Osmic Acid for 2 h. The fixed samples were dehydrated using an ethanol series soaked in the mixed liquor of epoxy resin and acetone for 24 h, 37 ℃, after which they were embedded in Epon 812, dodecenyl succinic anhydride (DDSA), and 2,4,6-Tris (dimethylaminomethyl) phenol (DMP-30) for 24 h, cut into semi-thin sections, and stained with toluidine blue. Tissue slices were then cut into ultra-thin sections using an ultramicrotome (LKB-III, Sweden), mounted on copper grids, and stained with uranyl acetate and plumbi nitras. The specimens were then observed under a transmission electron microscope (Hitachi H-7500; Hitachi, Tokyo, Japan).

### *Western blot*

Western blotting was used to assess the expressions of Drp1, p-Drp1, and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) in the hippocampus. Briefly, the frozen hippocampi were homogenized in a lysis buffer containing protease inhibitor cocktails (Roche, Germany, #P8340) and phenyl methanesul fonyl fluoride (PMSF, Sigma, USA, #p7626). The total protein in the samples was determined using a BCA protein assay kit (Wellbio, China) according to the manufacturer's instructions. Equal amounts of protein samples were applied to each lane, separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), and transferred to polyvinylidene fluoride membranes. After washing, the membranes were blocked with 10% skim milk in Tris Buffered Saline with Tween-20 (TBST) buffer for 1 h and then incubated with the primary antibodies (rabbit polyclonal antibody Drp1: 1:500, Cell Signaling Technology, USA, #2532; rabbit monoclonal antibody p-Drp1: 1:500, Cell Signaling Technology, USA, #2535; rabbit polyclonal antibody to GAPDH: 1:2,000, Proteintech, China, #10494-1-AP) overnight at 4 ℃. After three washes, the membranes were incubated with the secondary antibody (Goat Anti-Rabbit immunoglobulin G (IgG), Horseradish Peroxidase (HRP) Conjugated, 1:2,000, CWBIO, China, #CW0103, Goat Anti-Mouse IgG, HRP Conjugated,1:2,000, CWBIO, China, #CW0102) at room temperature for 2 h. Finally, the proteins were visualized using an enhanced chemiluminescence detection kit (Pierce; Thermo Scientific, Shanghai, China) and the intensity of each band was quantified by densitometry. The relative protein expression levels of each targeted protein (Drp1, p-Drp1) were normalized to GAPDH. The activation level of Drp1 was measured by the (p-Drp1/GAPDH)/(Drp1/GAPDH) ratio.

### *Plasma cytokine measurement by enzyme linked immunosorbent assay (ELISA)*

The levels of inflammatory cytokines, IL-1β and tumor necrosis factor-α (TNF-α), were measured in blood extracts using specific ELISA kits [IL-1β (ab235346) and TNF-α (ab144243); Abcam, USA] according to the manufacturer's instructions. The optical density was determined using a microplate reader at 450 nm (Olympus, Hamburg, Germany).

### *Statistical analysis*

Data were presented as mean ± standard deviation (mean ± SD). The training trail performance in the water maze was analyzed using one-way repeated measures analysis of variance (ANOVA), with treatment as the between-subjects factor and measured time as the with-in subject's factor, followed by Tukey honesty significant difference (HSD) comparisons. The probe trail performance in the water maze and the biochemical data among groups were analyzed using one-way ANOVA followed by the Bonferroni test. Data were analyzed using SPSS 18.0 for Windows (IBM Corp, USA). A two-tailed P value <0.05 was considered statistically significant.

## Results

### *Physiological variables*

The physiological parameters are summarized in *Table 1*. Among the five groups, there was no significant difference in rectal temperature and respiratory frequency before, during, and after the operation (P>0.05). Hypotension (mean arterial blood pressure <70% baseline), bradycardia (heart rate <70% baseline), or hypoxemia (partial pressure of

 

**Table 1** Physiological variables before and after splenectomy (n=12, mean ± SD)

| Group | Control | Sham | Splenectomy | Mdivi-1 | DMSO |
|---|---|---|---|---|---|
| Before operation | | | | | |
| RT (℃) | 38.08±0.51 | 37.78±0.81 | 38.03±0.53 | 37.93±0.72 | 30.08±0.63 |
| HR (BPM) | 561.33±113.45 | 524±105.16 | 563.5±101.52 | 589±95.15 | 593.42±90.07 |
| RF (BPM) | 106.58±33.88 | 113.92±28.24 | 114.42±33.15 | 136±27 | 128±36.78 |
| MABP (mmHg) | 103.83±13.45 | 99.08±12.16 | 105.75±13.57 | 101.33±12 | 94.5±13.12 |
| During operation | | | | | |
| RT (℃) | 37.90±0.56 | 38.14±0.72 | 38.03±0.61 | 38.23±0.63 | 38.03±0.58 |
| HR (BPM) | 601.92±74.84 | 562±107.83 | 603.25±118.81 | 603.08±98.46 | 583.08±102.61 |
| RF (BPM) | 128.75±40.25 | 128.92±42.84 | 110.33±33.33 | 126.67±32.61 | 124.17±38.43 |
| MABP (mmHg) | 96.25±14.3 | 97.33±14.12 | 95.5±12.36 | 96.17±13.62 | 92.58±13.42 |
| After operation | | | | | |
| RT (℃) | 37.97±0.66 | 38.08±0.71 | 38.11±0.55 | 37.73±0.53 | 37.78±0.56 |
| HR (BPM) | 547±71.47 | 558±108.52 | 588.25±118.73 | 617±97.15 | 583.08±83.77 |
| RF (BPM) | 117±30.74 | 105.75±26.65 | 132.75±39.71 | 131.83±33.46 | 118.08±31.07 |
| MABP (mmHg) | 102.42±12.15 | 93.25±11.25 | 95.08±11.98 | 96.33±15.05 | 89.92±11 |

RT, rectal temperature; HR, heart rate; RF, respiratory frequency; MABP, mean arterial blood pressure; BPM, beat per minute; DMSO, dimethyl sulfoxide.

oxygen, $PaO_2$ <60 mmHg) was not registered in any of the groups regardless of the operation (*Table 1*).

### *Morris water maze memory test*

As shown in *Figure 1* and *Table 2*, the results of the water maze experiments showed that the memory of rats in the Splenectomy and Mdivi-1 groups was significantly impaired after splenectomy. However, the memory of rats in these two groups could be restored to a certain degree with the extension of postoperative time. Compared with the Control and Sham groups, the swimming distance of rats in Splenectomy and Mdivi-1 groups was significantly longer. Mdivi-1 administration could alleviate the memory impairment after splenectomy (P=0.04; Splenectomy *vs.* Mdivi-1 group). Also, the time to locate the submerged platform after splenectomy was significantly longer in both Splenectomy and Mdivi-1 groups, but not in Control or Sham groups. Compared with the Mdivi-1 group, the time needed to locate the submerged platform was markedly longer than that in the Splenectomy group (P=0.047; Splenectomy *vs.* Mdivi-1 group). The swimming speed among the four groups showed no statistical differences before or after anesthesia/splenectomy.

### *HE staining and immunostaining*

HE staining and immunostaining showed that the degree of neuron injury in the Splenectomy group was significantly increased compared with that in the Sham and Control groups. The degree of neuron injury in the Mdivi-1 group was lower than that in the Splenectomy and Sham groups (*Figure 2*). We found evidence of inflammatory cell infiltration in the Splenectomy group.

To further characterize these inflammatory cell infiltrations, we employed immunostaining for Iba1 in the brain tissue, as shown in *Figure 3*. The results demonstrated that the inflammatory cell infiltration in the Splenectomy group was markedly increased compared with that in the Sham and Control groups. In addition, the inflammatory cell infiltration in the Mdivi-1 group was lower than that in the Splenectomy and Sham groups.

### *Analysis of TEM*

The mitochondrial damage observed in the Splenectomy

 

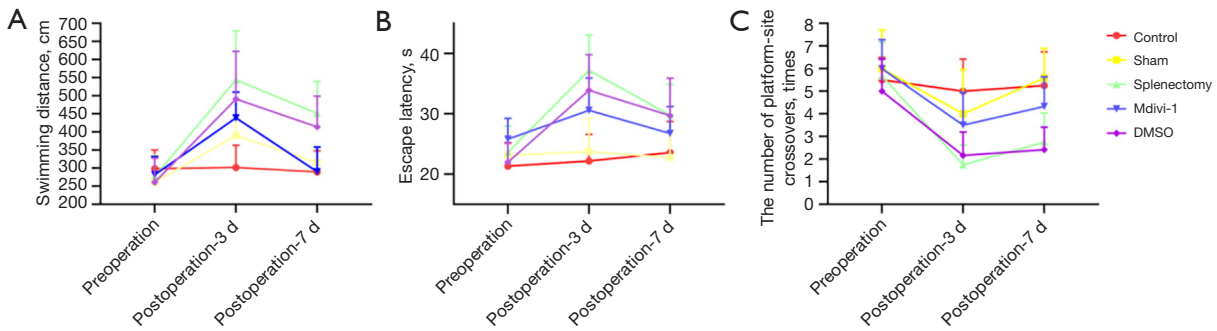


**Figure 1** Morris water maze memory test. (A) Swimming distance of the five groups at different time points (preoperatively, 3 days postoperatively, and 7 days postoperatively). (B) Escape latency of the five groups at different time points (preoperatively, 3 days postoperatively, and 7 days postoperatively). (C) The number of platform-site crossovers of the five groups at different time points (preoperatively, 3 days postoperatively, and 7 days postoperatively). DMSO, dimethyl sulfoxide.

**Table 2** Morris water maze memory test before and after splenectomy (n=12, mean ± SD)

| Group | Control | Sham | Splenectomy | Mdivi-1 | DMSO |
|---|---|---|---|---|---|
| Preoperatively | | | | | |
| Distance (cm) | 298.58±51.78 | 261.21±74.39 | 277.77±56.02 | 281.76±50.29 | 261.19±66.68 |
| Escape latency (s) | 21.33±3.80 | 23.13±3.75 | 23.67±4.31 | 25.82±3.44 | 21.99±3.26 |
| Number of platform-site crossovers (times) | 5.50±1.00 | 6.00±1.71 | 5.67±1.50 | 6.00±1.28 | 5.00±1.41 |
| 3 days postoperatively | | | | | |
| Distance (cm) | 301.67±61.31 | 390.21±56.77 | 543.85±135.19 | 439.05±71.01 | 491.12±131.50 |
| Escape latency (s) | 22.18±4.41 | 23.73±5.56 | 37.20±5.89 | 30.58±5.37 | 33.91±5.90 |
| Number of platform-site crossovers (times) | 5.00±1.41 | 4.00±1.95 | 1.75±0.87 | 3.50±1.45 | 2.17±1.03 |
| 7 days postoperatively | | | | | |
| Distance (cm) | 290.00±57.90 | 316.23±40.36 | 451.67±87.78 | 290.02±68.18 | 413.90±84.86 |
| Escape latency (s) | 23.59±5.16 | 22.77±3.96 | 29.80±5.08 | 26.76±4.45 | 29.70±6.22 |
| Number of platform-site crossovers (times) | 5.25±1.48 | 5.58±1.31 | 2.75±1.29 | 4.33±1.3 | 2.42±1 |

DMSO, dimethyl sulfoxide.

group was notably higher than that in the Control group (*Figure 4*). As evaluated by TEM, mitochondrial morphology in the Control and Sham groups showed slight mitochondrial swelling burst, unclear structures, mitochondrial cristae disappearing, as well as the presence of small air bubbles with round, oval, or irregular shapes (*Figures 4A,4B*). Additionally, the mitochondrial morphology in the Splenectomy group displayed mitochondrial swelling burst, mitochondrial cristae shorter fracture less side move, inner and outer membranes, outer membrane damage, mitochondria vacuoles, matrix dissolution, and decreased electron density (*Figure 4C*). Moreover, the Mdivi-1 group showed mitochondrial swelling burst, unclear structures, mitochondrial cristae disappearing, as well as the presence of small air bubbles with round, oval, or irregular shapes (*Figure 4D*). In the DMSO group, the degree of mitochondrial swell was comparable to that in the Splenectomy group (*Figure 4E*).

 

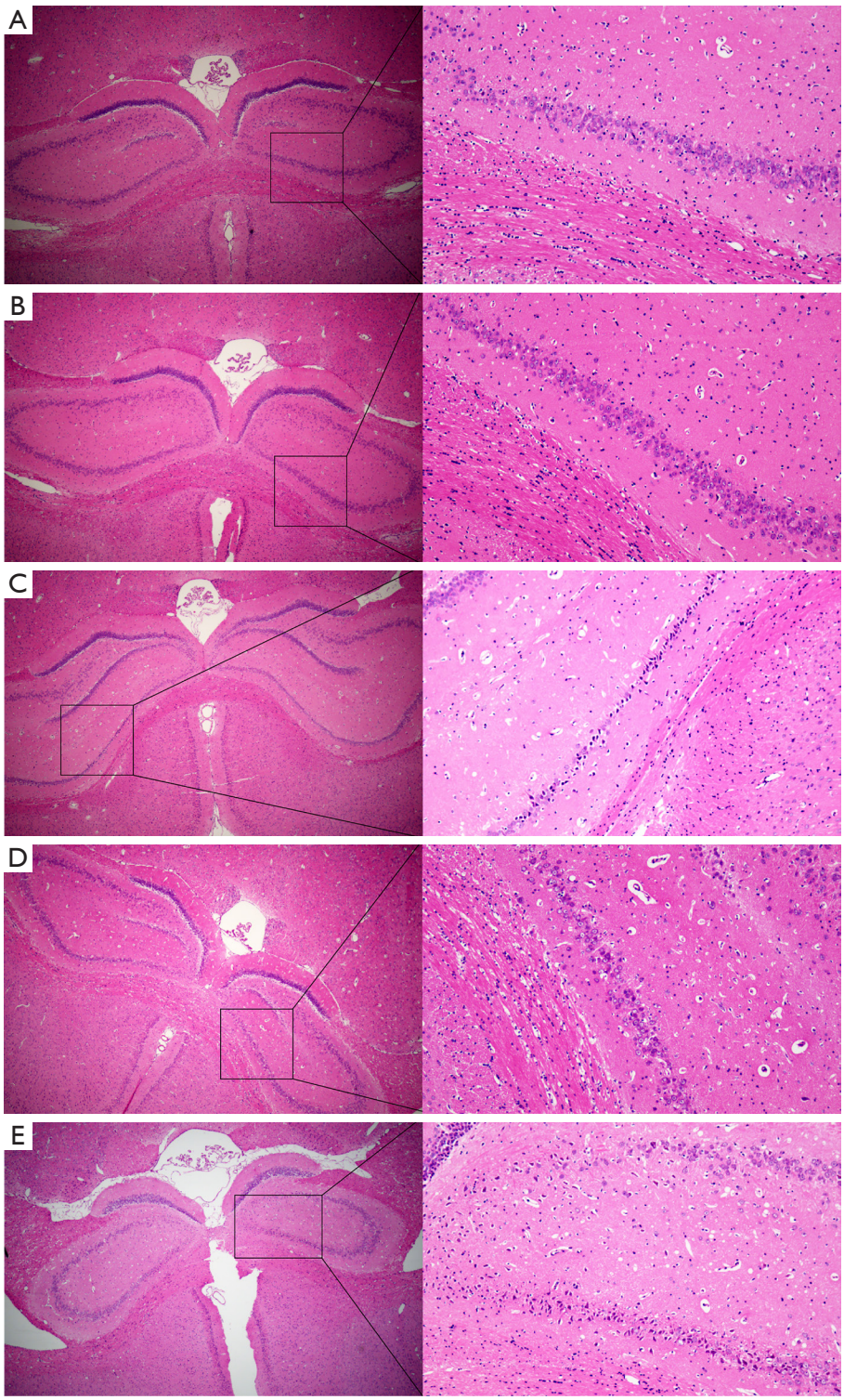


**Figure 2** HE Staining. (A) Control group; (B) Sham group; (C) Splenectomy group; (D) Mdivi-1 group; (E) DMSO group. The HE staining showed neuronal injury in the brain tissues. In the Splenectomy and DMSO groups, we observed more inflammatory cell infiltration compared with that in the Sham and Control groups. Moreover, this parameter was lower in the Mdivi-1 group than in the Splenectomy and Sham groups. The magnification, left: 10×10; right: 40×10. HE, hematoxylin-eosin; DMSO, dimethyl sulfoxide.

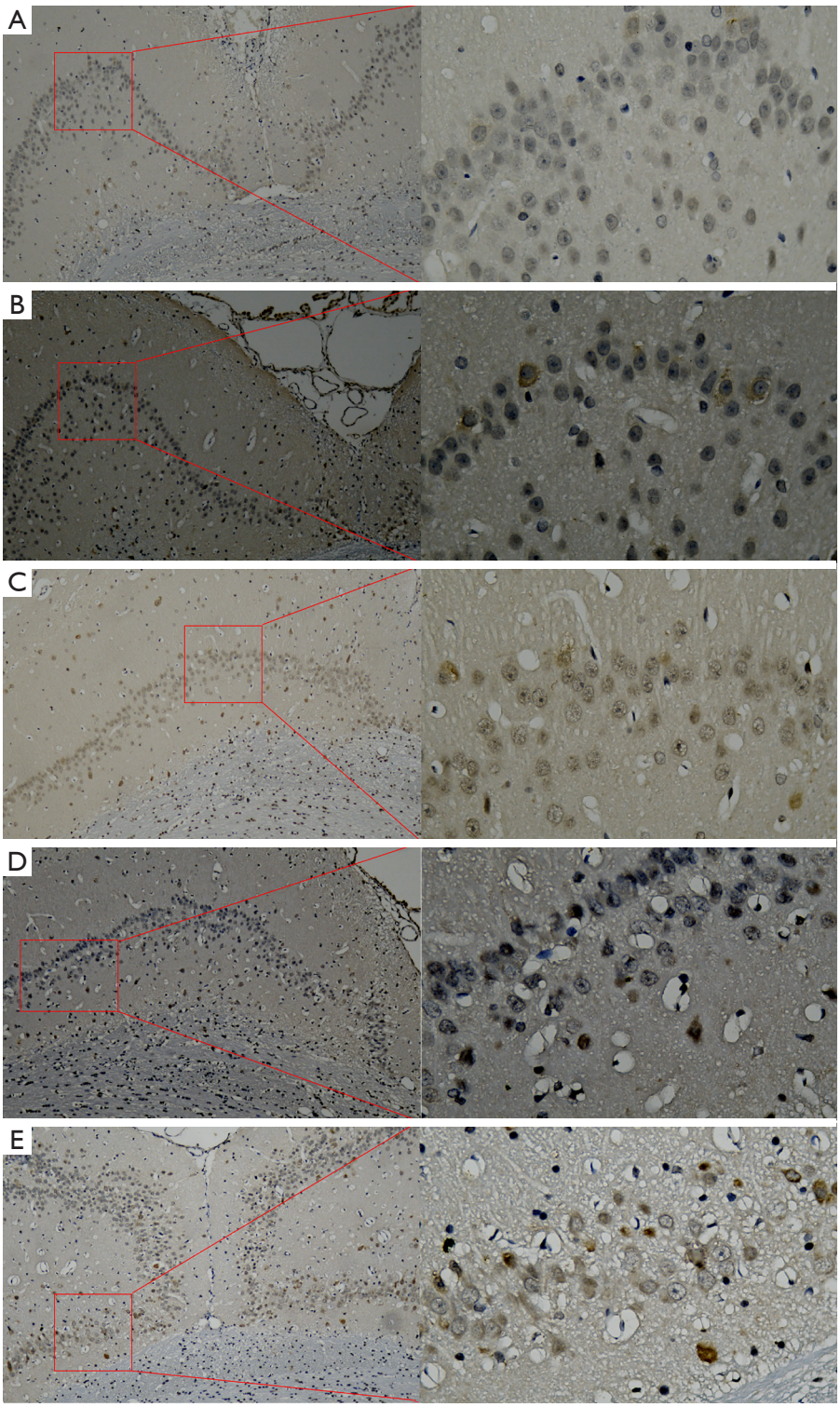


**Figure 3** Representative immunostaining images. (A) Control group; (B) Sham group; (C) Splenectomy group; (D) Mdivi-1 group; (E) DMSO group. Immunostaining against Iba1 revealed that the infiltration of inflammatory cells in the Splenectomy group was significantly increased compared with that in the Sham and Control groups. Additionally, inflammatory cell infiltration in the Mdivi-1 group was lower than that in the Splenectomy and Sham groups. The magnification, left: 10×10; right: 40×10. DMSO, dimethyl sulfoxide.

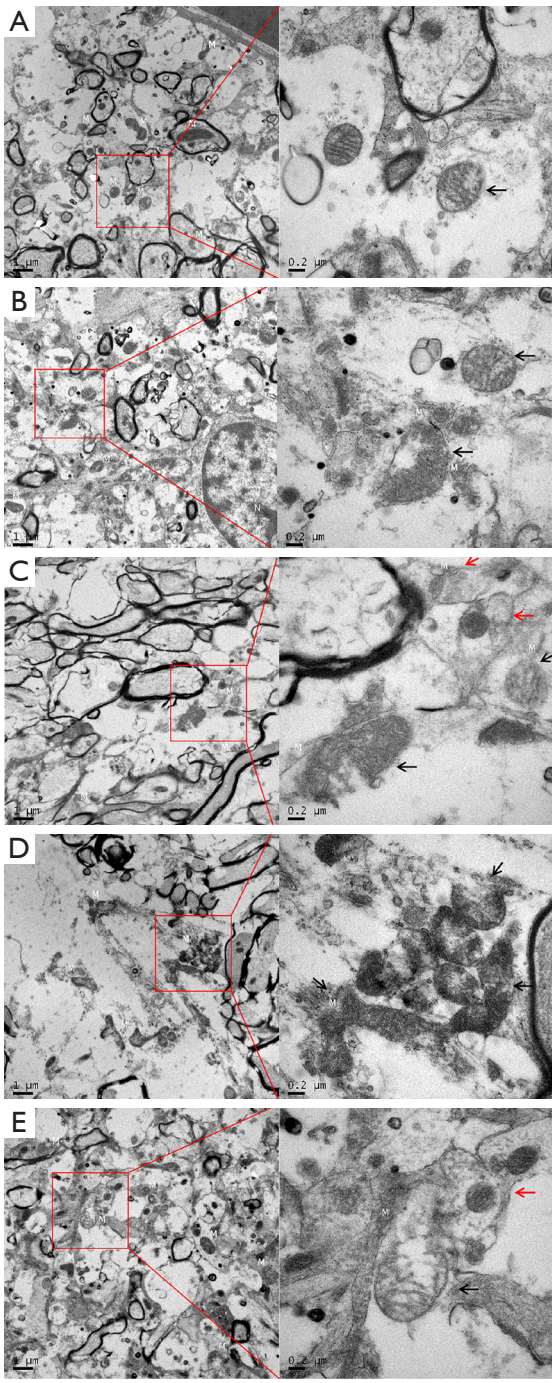


**Figure 4** Representative electron microscopy images. (A) Control group; (B) Sham group; (C) Splenectomy group; (D) Mdivi-1 group; (E) DMSO group. The black arrows represent the slight mitochondrial swelling. The red arrows represent the mitochondria swelling burst, mitochondrial cristae shorter fracture less side move, inner and outer membranes, outer membrane damage, mitochondria vacuoles, matrix dissolution, and decreased electron density. M, mitochondria; N, nuclear; DMSO, dimethyl sulfoxide.

### *Western blot analysis*

To further explore the mechanisms of Mdivi-1 and how they affect neurocognitive disorders, we analyzed several protein levels, as shown in *Figure 5A*,*5B*. Our results demonstrated that Drp1 expression was similar in the five groups. In addition, the Splenectomy group showed higher levels of p-DPR1 compared with the Control and Sham groups. Furthermore, p-Drp1 expression in the Mdivi-1 group was decreased compared with the Splenectomy group. The above-mentioned results indicated that Mdivi-1 administration influenced p-Drp1 expression, thereby contributing to the decrease in neurocognitive disorders.

### *Serum levels of TNF-α and IL-1β*

No significant differences between the levels of TNF-α were observed among the groups preoperatively. However, when analyzing samples taken on the 3$^{rd}$ and 7$^{th}$ day after surgery, we found that the TNF-α content in the Sham, Splenectomy, and Mdivi-1 groups increased markedly compared with that in the Control group. In addition, the increase in the Sham and Splenectomy groups was statistically significant compared with that in the Mdivi-1 group. Also, the changes in IL-1 were similar to those observed for TNF-α (*Table 3*, *Figure 6*).

## Discussion

Our experimental results demonstrated that aged rats that underwent splenectomy exhibited significant cognitive impairments on the 3$^{rd}$ day postoperatively, which was alleviated on the 7$^{th}$ day. The observed damage to the hippocampal $CA_1$ neurons, mitochondria, and the central inflammatory response were consistent with the changes in neurocognitive function. Also, the selective inhibitor of Drp1, Mdivi-1, exerted protective effects against PNCD by ameliorating mitochondrial dysfunction and inflammation.

PNCD is currently a poorly understood disorder with an undefined etiology and no available treatments. In addition, anesthesia has been historically associated with cognitive decline and neurotoxicity (25). Isoflurane, a commonly used inhalation anesthetic agent, has been shown to cause cognitive dysfunction in adult rats (26). Nevertheless, isoflurane or sevoflurane preconditioning induces neuroprotection in rats exposed to cerebral ischemia-reperfusion (27,28). Inhalation anesthetics have been described as a “double-edged sword”, as they play a

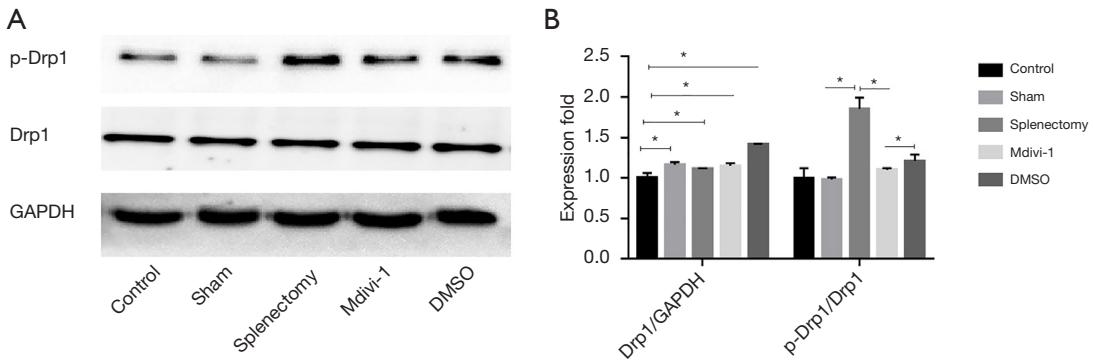


**Figure 5** Western-blot. Western blot analysis of the Drp1 and p-Drp1 expressions in the five groups. (A) GAPDH was used as the protein load control, and the relative expression levels of the Drp1 were normalized to GAPDH. Drp1 was used as the protein load control, and the relative expression levels of the p-Drp1 in each group were normalized to Drp1. (B) The statistics figure of the western blot. *, P<0.05. DMSO, dimethyl sulfoxide.

**Table 3** Serum levels of TNF-α and IL-1β before and after splenectomy (n=12; mean ± SD)

| Group | Control | Sham | Splenectomy | Mdivi-1 | DMSO |
|---|---|---|---|---|---|
| Preoperatively | | | | | |
| TNF-α (ng/L) | 10.38±3.2 | 11.3±3.05 | 9.8±2.47 | 9.02±2.88 | 11.03±4.38 |
| IL-1β (ng/L) | 7.36±2.69 | 7.89±2.82 | 6.73±1.75 | 7.57±2.69 | 6.98±2.06 |
| 3 days postoperatively | | | | | |
| TNF-α (ng/L) | 9.18±2.8 | 10.42±1.81 | 15.35±4.56 | 12.76±3.44 | 14.66±1.73 |
| IL-1β (ng/L) | 7.87±2.01 | 8.37±3.32 | 11.77±4.14 | 9.27±1.61 | 10.67±1.73 |
| 7 days postoperatively | | | | | |
| TNF-α (ng/L) | 10.15±2.6 | 10.63±3.11 | 14.04±3.33 | 11.04±1.68 | 13.53±1.97 |
| IL-1β (ng/L) | 7.22±2.67 | 7.53±1.78 | 10.14±2.22 | 8.1±2.04 | 10.21±2.35 |

TNF-α, tumor necrosis factor α; IL-1β, interleukin-1β; DMSO, dimethyl sulfoxide.

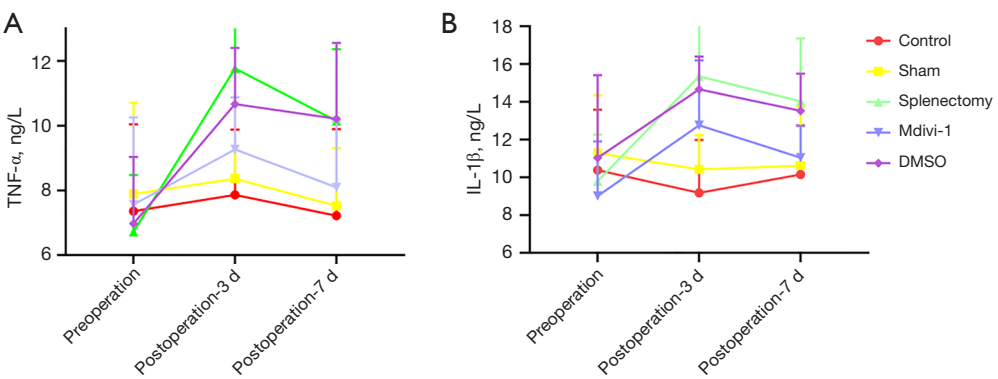


**Figure 6** Serum levels of TNF-α and IL-1β. (A) The serum level of TNF-α in the five groups at different time points (preoperatively, 3 days postoperatively, and 7 days postoperatively). (B) The serum level of IL-1β in the five groups at different time points (preoperatively, 3 days postoperatively, and 7 days postoperatively). DMSO, dimethyl sulfoxide.

pivotal role in protecting against harmful organisms but also lead to potentially lethal effects when uncontrolled and dysregulated. In our previous study of 213 elderly patients subjected to total knee replacement, patients who were given peripheral nerve block anesthesia showed faster postoperative recovery, including neurocognitive function, than those who were given general anesthesia (29). Therefore, it can be inferred that PNCD affects postoperative surgical patients irrespective of the anesthetic technique used (30).

Surgical trauma could be another factor influencing postoperative cognitive decline. PNCD is particularly prevalent in patients after cardiac or major orthopedic surgery (31,32). Besides anesthesia and surgery, other factors have also been associated with sickness behavior and cognitive disturbances, such as perioperative hypotension, hypothermia, and hypoxemia (33,34). In the present study, we monitored a few physiological parameters of the animals, including the mean arterial pressure, heart rate, rectal temperature, and blood oxygen, during the perioperative period. However, we did not observe significant hypotension, bradycardia, or hypoxemia in the rats before, during, or after surgery.

A study has found that inflammatory responses in the central nervous system play an important role in PNCD (35). Surgical stimulation can lead to immune system activation, produce excessive inflammatory factors, and cause tissue damage (36). Simultaneously, trauma and stress can temporarily open the blood-brain barrier, allowing peripheral inflammatory mediators to migrate to the central nervous system, activate microglial cells, and induce the secretion of various inflammatory mediators, thus leading to neuron injury and cognitive dysfunction. The present study indicated that the serum inflammatory factors of rats in the Splenectomy and Mdivi-1 groups were significantly increased on the 3$^{rd}$ day postoperatively and then gradually decreased. This is consistent with the results of the Morris water mazes experiments and certain a previous study (37). However, the inflammatory factors in the hippocampus in the Splenectomy group were still relatively high on the 7$^{th}$ day postoperatively. In our previous study on rats undergoing focal cerebral ischemia, we reported that the peripheral inflammatory response gradually returned to the normal baseline value 7 days after the operation, although the inflammatory response in the central nervous system remained for more than 2 weeks (38).

Further studies have reported that mitochondria are critical to the inflammatory response (14,39). Under normal conditions, mitochondria produced by neurons are transported in both directions along the microtubules of nerve fibers and distributed to the synaptic sites, where they play a role in substance metabolism, productivity, and calcium ion buffering. Increased local TNF-α caused by surgical trauma can activate PGAM5, which then triggers the phosphorylation of its downstream mitochondrial Drp1 protein, resulting in the imbalance of mitochondrial division and fusion, increased mitochondrial permeability, and the cascade release of inflammatory factors, eventually leading to mitochondrial division and necrosis. A *in vitro* study has shown that the Drp1 blocker Mdivi-1 could reduce the damage to mitochondrial function and the inflammatory response (40). In animal models of ischemia-reperfusion injury and neuropathic pain, researchers also found that the application of a Drp1 blocker or Drp1 gene knockout could reduce mitochondrial injury to the kidney, heart, and brain (16,41,42). In animal models of Parkinson's and Alzheimer's diseases, intraperitoneal administration of a Drp1 blocker or Drp1 gene knockout can also reduce mitochondrial function damage and improve the pathological status (43). Hence, the regulation of mitochondrial Drp1 has become a new target for the treatment of neurodegenerative diseases (18,44). Our study further corroborated that the selective inhibitor of Drp1, Mdivi-1, exerts protective effects against postoperative neurocognitive decline by ameliorating mitochondrial dysfunction and inflammation in aged rats after splenectomy.

Based on these results, we speculated that PNCD may have a common pathogenesis with cognitive dysfunction caused by ischemic brain injury and chronic inflammatory diseases of the central nervous system. The inflammatory response caused by various factors may lead to mitochondrial damage and increased neuron necrosis or apoptosis, generating cognitive dysfunction. In this study, we observed changes in neuronal morphology as well as neuron numbers in the hippocampal $CA_1$ area, accompanied by damage to the neuronal mitochondria, a central inflammatory response, and impaired cognitive function of aged rats that underwent splenectomy. In addition, Mdivi-1 administration inhibited Drp1 phosphorylation, reduced neuronal and mitochondrial injury, and significantly decreased the expression level of inflammatory factors, such as TNF-α and IL-1β, thereby significantly improving the postoperative neurocognitive function.

## Conclusions

Our results confirmed a correlation between the occurrence

 

of PNCD and mitochondrial functional injury. Hence, inhibiting the phosphorylation of neuronal mitochondrial Drp1 and reducing mitochondrial injury and the central nervous system inflammatory response, could considerably improve neurocognitive function postoperatively.

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## Footnote

*Reporting Checklist:* The authors have completed the ARRIVE reporting checklist. Available at https://atm.amegroups.com/article/view/10.21037/atm-22-5496/rc

*Data Sharing Statement:* Available at https://atm.amegroups.com/article/view/10.21037/atm-22-5496/dss

*Conflicts of Interest:* All authors have completed the ICMJE uniform disclosure form (available at https://atm.amegroups.com/article/view/10.21037/atm-22-5496/coif). The authors have no conflicts of interest to declare.

*Ethical Statement:* The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. All experimental procedures were approved by the Institutional Animal Care and Use Committee of Fujian Medical University (ethics review approval No. FJMU-IACUC-2019-0124) and carried out in accordance with the relevant laws and regulations in China. All of the procedures were conducted according to the Chinese Guidelines for the Care and Use of Laboratory Animals.



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