Adiponectin attenuates splenectomy-induced cognitive deficits by alleviating neuroinflammation and oxidative stress via the TLR4/MyD88/NF-κb signaling pathway in aged rats

Zhijing Zhang
Dongguan People's Hospital
Lideng Guo
Guangdong Medical University
Fei Yang
Southern Medical University
Shanpan Peng
Dongguan People's Hospital
Di Wang
Guangdong Medical University
Xiawei Lai
Southern Medical University
Baiqin Su
Dongguan People's Hospital
Haihui Xie (xhh900@163.com)
Dongguan People's Hospital

Research Article

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Abstract

Background

Perioperative neurocognitive disorder (PND) is a common adverse event after surgical trauma in elderly patients. The pathogenesis of PND is still unclear. Adiponectin (APN) is a plasma protein secreted by adipose tissue. We have reported that decreased APN expression is associated with PND patients. APN may be a promising therapeutic agent for PND. However, the neuroprotective mechanism of APN in PND is still unclear.

Methods

Eighteen month-old male Sprague Dawley rats were assigned to six groups: the sham, sham + APN (intragastric (i.g.) administration of 10 μg/kg/day for 20 days before splenectomy), PND (splenectomy), PND + APN, PND + TAK-242 (intraperitoneal (i.p.) administration of 3 mg/kg TAK-242) and PND + APN + LPS (i.p. administration of 2 mg/kg LPS). The cognitive function of the rats was assessed with the Morris water maze (MWM) test. Immunohistochemistry/ immunofluorescence, western blotting and ELISA were used to evaluate the activation of the TLR4/NF-κb axis, oxidative stress-mediated apoptosis, microglial activation and proinflammatory cytokine expression in the hippocampus.

Results

We first found that APN treatment significantly improved learning and cognitive function in the MWM test after surgical trauma. Further experiments showed that APN could inhibit the TLR4/MyD88/NF-κb p65 pathway to decrease the degree of oxidative damage (MDA, SOD and caspase 3) and microglia-mediated neuroinflammation (IBA1, TNF-α, IL-1β and IL-6). The TLR4 antagonist TAK-242 had a similar effect as APN, while the TLR4 agonist LPS abolished the beneficial effect of APN.

Conclusions

APN exerts a neuroprotective effect against cognitive deficits induced by peripheral trauma, and the possible mechanisms include inhibition of oxidative stress and neuroinflammation, which is mediated by suppression of the TLR4/MyD88/NF-κb signaling pathway. We propose that APN is a promising candidate for PND treatment.

Introduction

There are 313 million major surgical procedures performed worldwide every year[1]. Perioperative neurocognitive disorder (PND) is a severe complication that can affect attention, memory, orientation, executive function, and language fluency[2]. PND may emerge several days to several months after

surgery, and some patients, especially elderly patients, may even develop permanent cognitive impairment[3]. Notably, PND occurs in 52% of senior patients. PND is associated with decreased quality of life, increased mortality and the need for social financial assistance[4–6]. However, the mechanisms and pathogenesis of PND in elderly individuals remain largely unknown.

A large number of studies have demonstrated that the Toll-like receptor 4 (TLR4) and nuclear factor-kappa B (NF-κb) signaling pathways play important roles in oxidative stress and microglia-mediated neuroinflammation during the progression of PND[7–9]. The TLR4/NF-κb signaling pathway can induce the transcription activation of various inflammatory factors, ultimately leading to apoptosis of nerve cells[7]. TAK-242 inhibits LPS-mediated activation of TLR4/NF-κb pathway, which is related to protection against neuroinflammation and transient cognitive deficits in aged animals[8, 10]. With the progression of time following splenectomy, the activation of the TLR4 pathway and increases in the levels of inflammatory factors as well as cognitive decline are gradually reversed[11]. Early management of neuroinflammation and oxidative stress may prevent the occurrence of some neurological disorders[12–15]. However, Terrando found that the TLR4 pathway is seemingly not necessary for the occurrence of surgical trauma-induced cognitive deficits[16]. Considering these controversial results, the role of the TLR4 pathway in PND and its potential as a therapeutic target need to be further investigated.

Adiponectin (APN), a plasma protein that belongs to the complement 1q family, is secreted by adipose tissue. Previous studies have indicated that APN protects against diabetes-related cognitive dysfunction[17, 18]. Moreover, APN can attenuate amyloid-β (Aβ) neurotoxicity by promoting adenosine monophosphate-activated protein kinase (AMPK) expression and suppressing NF-κb expression[19, 20]. In the clinic, a decreased APN level is a risk factor for cognitive dysfunction in patients with spinal cord injury and Alzheimer's disease[21, 22]. However, in nonobese individuals, APN expression appears to be weakly associated with cognitive impairment[23]. Although the incidence of peripheral trauma-mediated cognitive impairment is increasing, little is known about the role of APN in this condition. Our previous studies found that the level of APN is decreased in elderly patients after peripheral operation[24, 25]. Pretreatment of APN may alleviate cognitive deficits. However, the detailed functions and mechanisms of APN in PND have not yet been elucidated. In this study, the effects of APN treatment on memory, inflammation, and oxidative stress and the underlying mechanism were investigated. Our results indicate that APN may be a promising therapeutic agent for cognitive decline.

Methods And Materials

Animals and experimental design

All animal experiments were approved by the Southern Medical University Institutional Animal Care and Use Committee (approval number: AF-97-04). Male Sprague Dawley rats (18 months old, specific pathogen-free) were used for this study. The animals were kept in stainless steel cages and housed in an animal facility at a temperature of 24 ± 2°C and relative humidity of 55 ± 5% on a 12 h light/dark cycle. The animals were provided food and water ad libitum and allowed to adjust to the laboratory conditions

one week before the commencement of the experiment. All experimental procedures were performed in accordance with the Institutional Health Guide for Care and Use of Laboratory Animals.

The animals were randomly allotted to six groups. APN (ApexBio, A1009) was dissolved in 1% DMSO in sterile saline and administered via intragastric (i.g.) gavage at a dosage of 10 μg/kg/d for 20 consecutive days before splenectomy. The TLR4 agonist LPS (Millipore, Sigma, L2630) was dissolved in sterile saline and given by a single intraperitoneal (i.p.) injection at a dose of 2 mg/kg before splenectomy. The TLR4 antagonist TAK-242 (ApexBio, A3850) was dissolved in 1% DMSO in sterile saline and given via a single i.p. injection at a dose of 3 mg/kg[26] before splenectomy. The study design is shown in Fig. 1 and Table 1.

TABLE 1 | Grouping of experimental animals.

Group	Splenectomy	APN 10ug/kg.d, i.g. 20 days before surgery	TAK-242 3mg/kg, i.p. before surgery	LPS 2mg/kg, i.p. before surgery
Sham	-	-	-	-
Sham+APN	-	+	-	-
PND	+	-	-	-
PND+APN	+	+	-	-
PND+TAK-242	+	-	+	-
PND+APN+LPS	+	+	-	+

Surgical Procedure

Rats in groups 3–6 received isoflurane anesthesia (induction with 3.0% isoflurane followed by maintenance with 1.5% isoflurane carried by 100% oxygen[27]) during splenectomy. The body temperature of the animals was maintained at 37°C by a warming blanket during surgery. During splenectomy, a small incision was made in the abdomen, and the spleen was mobilized, isolated and removed. After surgery, the skin was sutured, and the animals were placed in a warmed cage[8]. Cefoperazone (5%) was administered by i.p. injection at a dose of 50 mg/kg after surgery.

Morris Water Maze (Mwm) Test

Seven days after splenectomy, all rats underwent Morris water maze (MWM) training and testing to assess spatial memory and learning[28]. The apparatus consisted of a circular pool (120 cm diameter and 50 cm deep) filled with water and divided into 4 equal quadrants (I–IV). A platform was submerged 1 cm below the water surface in the center of quadrant II. The temperature was kept at 23 ± 1°C. The rats

were allowed to swim freely for 60 s and guided to the platform to ensure that they were aware of its existence. Training trials were performed for 5 days. The escape latency of each rat (the time required to find the platform) was recorded every day. If a rat failed to locate the platform within 60 s, it was placed on it for 10 s, and the escape latency was recorded as 60 s. In the probe trial, each rat was placed in quadrant IV, i.e., the quadrant opposite the target quadrant. The time spent in the target quadrant and the time spent in quadrant II were recorded. After each training trial, the animals were dried with a towel and returned to their home cages.

Histopathological Examination

Seven days postsurgery, the rats were sacrificed by i.p. injection of an overdose of ketamine (120 mg/kg) and xylazine (10 mg/kg) and then transcardially perfused with precooled saline followed by 4% paraformaldehyde (PFA) for fixation. Brain tissue sections were heated at 60°C for 30 min, deparaffinized in xylene (5 min × 3 times) and dehydrated with 100%, 95% and 70% ethanol 3 times. Hydrogen peroxide (3%) in methanol was used to inhibit endogenous peroxidase activity. The tissues were blocked in 5% BSA at room temperature for 1 hour. Each block was cut (20 μm) and frozen at 4°C before staining. Images were taken with an Olympus BX53 upright microscope. The sections were analyzed manually by two researchers blinded to treatments at 400× magnification.

Immunofluorescence Analysis

Immunofluorescence was performed as described previously[27]. The slides were incubated in 5% BSA for 1.5 h at room temperature and then with anti-ionized calcium binding adapter molecule-1 (Iba1) antibody (1:250, Proteintech, 10904-01-AP) in 0.5% BSA overnight at 4°C. Next, the slides were rinsed in 0.1 mol/L PBS three times, incubated with secondary antibody (1:200, Proteintech, SA00013-2) at 37°C for 1 h, and mounted with glass coverslips. The average number of stained cells in three visual fields of each section was determined, and the average number of stained cells over three sections was taken as the final value for each animal. A researcher blinded to the treatments conducted the data analysis.

Immunohistochemical Analysis

Nine sections per group (three sections each from three rats per group) were selected. The sections were incubated with primary antibodies against p-p63 (1:100, Santa Cruz, sc-166748) and caspase3 (1:100, Proteintech, 19677-1-AP) diluted in 5% BSA overnight at 4°C. Next, the slides were rinsed in PBS (5 min × 3 times), incubated in horseradish peroxidase-conjugated secondary antibody at 37°C for 30 min, and developed with 0.025% DAB.

ELISA

Seven days postsurgery, the hippocampus was removed, frozen rapidly on powdered dry ice and stored at -80°C until processing. Malondialdehyde (MDA), superoxide dismutase (SOD), IL-1β, IL-6, and TNF-α levels in the brain homogenates were determined. Analysis was carried out with an ELISA kit (Cusabio Technology, CSB-E04640r) following the manufacturer's instructions.

Western Blot Analysis

Western blotting was performed as described previously[27]. The blots were incubated overnight at 4°C with anti-TLR4 (1:500, Proteintech, 19811-1-AP), anti-MyD88 (1:1000, Proteintech, 23230-1-AP), anti-p-p65 (1:100, Santa Cruz, sc-166748), anti-p65 (1:1000, Santa Cruz, 10745-1-AP), anti-Cleaved-caspase3 (1:500, Proteintech, 19677-1-AP), anti-pro-caspase3 (1:500, Proteintech, 19677-1-AP), and anti-β-actin (1:5000, Proteintech, 66009-1-Ig) primary antibodies and then with HRP–conjugated secondary antibodies (Proteintech, SA00001-1, SA00001-2). A Tanon 5200 imaging system was used for visualization of the protein bands. Densitometric analysis was performed using Quantity One Software.

Statistical analysis

For all statistical analyses, Prism 5.0 software (GraphPad, La Jolla, CA, USA) was used. Escape latency data from the place navigation phase of the MWM test were analyzed using repeated-measures two-way ANOVA. The other data were analyzed by one-way ANOVA followed by Bonferroni tests for multiple comparisons. Significance levels were set at $P < 0.05$.

Results

APN treatment ameliorates learning and memory impairment in aged rats

The MWM test is one of the most commonly used tools in the field of behavioral neuroscience for assessing hippocampus-dependent spatial learning and memory in rodents[29, 30]. From the first day to the fifth day of the place navigation phase, splenectomy markedly impaired spatial learning and reference memory, as indicated by an increase in the escape latency ($^{***}p < 0.001$ vs. the sham group; Fig. 2A). APN treatment and TAK-242 treatment significantly decreased the escape latency of PND model rats ($^{\#\#\#}p < 0.001$ vs. the PND group; Fig. 2A). However, LPS reversed the effect of APN in PND model rats ($^{\&\&\&}p < 0.001$ vs. the PND + APN group; Fig. 2A).

In the spatial probe trial, the number of platform crossings and the time spent in the target quadrant were markedly reduced in the PND group compared to the sham group ($^{*}p < 0.05$, $^{**}p < 0.01$ vs. the sham group; Fig. 2B-C). Meanwhile, the PND rats treated with APN and TAK-242 show significantly increased number of target crossings and the time spent in the target quadrant compared to that of the PND group ($^{\#}p <$

0.05, $^{\#\#}p < 0.01$ vs. the PND group; Fig. 2B-C). LPS reversed the beneficial effect of APN on cognitive function in PND model rats ($^{\&}p < 0.05$, $^{\&\&}p < 0.01$ vs. the PND + APN group; Fig. 2B-C).

Apn Treatment Downregulates The Tlr4/myd88/nf-κb Signaling Pathway In Hippocampus

The TLR4/MyD88/NF-κb pathway is traditionally considered the primary target of LPS and plays a pivotal role in peripheral trauma-meditated cognitive deficits[11, 31]. Phosphorylation of NF-κb results in its activation and translocation into the nucleus, where it mediates a series of downstream reactions. Phosphorylation of p65 is a marker of NF-κb activation[32]. As shown in Fig. 3, the expression levels of TLR4, MyD88 and NF-κb p65 were increased in the PND group ($^{**}p < 0.01$, $^{***}p < 0.001$ vs. the sham group; Fig. 3A-F), while the activation of the TLR4/MyD88/NF-κb pathway was reduced to varying degrees in the PND + APN and PND + TAK-242 groups ($^{\#}p < 0.05$, $^{\#\#}p < 0.01$ vs. the PND group; Fig. 3A-F). LPS could activate the TLR4/MyD88/NF-κb pathway to reverse the effect of APN ($^{\&}p < 0.05$ vs. the PND + APN group; Fig. 3A-C).

Apn Treatment Inhibits Oxidative Stress And Apoptosis In Hippocampus

Oxidative stress can lead to mitochondrial dysfunction and eventually activate the caspase-mediated apoptotic cascade in neurons[33]. Data related to oxidative stress-induced production of MDA, SOD and the level of the apoptosis marker caspase 3 are shown in Fig. 4. Splenectomy promoted the transformation of pro-caspase 3 to cleaved-caspase 3 ($^{***}p < 0.001$ vs. the sham group; Fig. 4A-D), increased the production of MDA and decreased the activity of SOD ($^{*}p < 0.05$, $^{**}p < 0.01$ vs. the sham group; Fig. 4E-F) in aged rats. APN treatment ($^{\#}p < 0.05$ vs. the PND group; Fig. 4A-C) and TAK-242 treatment ($^{\#\#}p < 0.01$ vs. the PND group; Fig. 4A-C) led to significant inhibition of caspase 3 activation. Moreover, the MDA concentration was markedly reduced($^{\#}p < 0.05$ vs. the PND group; Fig. 3E), and SOD activity was elevated by 20.3% and 22.98% in the PND + APN and PND + TAK-242 groups ($^{\#}p < 0.05$ vs. the PND group; Fig. 4F) respectively. In rats treated with APN, these inhibitory effects were markedly reversed after pretreatment with LPS ($^{\&}p < 0.05$, $^{\&\&}p < 0.01$, $^{\&\&\&}p < 0.001$ vs. the PND + APN group; Fig. 4A-F).

Apn Treatment Relieves Microglia-mediated Neuroinflammation In The Hippocampus

Activation of the TLR4/MyD88/NF-κb signaling pathway enhances microglia-mediated neuroinflammation, which aggravates nerve damage[11]. We therefore investigated the impact of APN on microglial activation and the expression of proinflammatory factors in the hippocampus. The data showed that neuroinflammation was aggravated in the PND group ($^{*}p < 0.05$ vs. the sham group; Fig. 5A-

C). Furthermore, APN or TAK-242 treatment inhibited this change in microglia-mediated neuroinflammation ($^{\#}p < 0.05$, $^{\#\#}p < 0.01$ vs. the PND group; Fig. 5A-C), while LPS reduced this improvement in the levels of these inflammatory factors ($^{\&\&}p < 0.01$ vs. the PND + APN group; Fig. 5B).

Microglia are usually activated early in response to a variety of pathological stimuli. The degree of microglial activation can reflect the severity of trauma[34]. To determine whether APN exerts a protective effect against microglial overactivation in aged rats, immunostaining of hippocampal tissue with an antibody against the microglial marker IBA1 was performed. We found that the level of microglial activation in the PND group was increased ($^{*}p < 0.05$ vs. the sham group; Fig. 4D-E). Additionally, APN or TAK-242 treatment markedly decreased the number of microglia in the hippocampus ($^{\#}p < 0.05$ vs. the PND group; Fig. 5D-E). However, the PND + APN + LPS group showed higher levels of microglial activation than the PND + APN group ($^{\&\&}p < 0.01$ vs. the PND + APN group; Fig. 5D-E).

Discussion

To the best of our knowledge, this is the first study to investigate the overall mechanisms underlying the effect of APN on splenectomy-induced cognitive deficits, TLR4 signaling pathway activation, oxidative stress, microgliosis and neuroinflammation. The role of APN, a plasma protein secreted by adipose tissue, in diabetes and Alzheimer's disease (AD) has been studied extensively [18, 19, 22]. Moreover, we have reported that decreased APN expression is associated with peripheral trauma-induced PND[24, 25], it suggests that pretreatment of APN may alleviate cognitive deficits. Furthermore, in the current study, the results demonstrated that APN exerts neuroprotective effects against cognitive deficits induced by peripheral trauma and revealed the possible mechanisms, including down-regulation of oxidative stress and neuroinflammation mediated by TLR4/MyD88/NF-κb signaling pathway inhibition.

Hippocampus-related cognitive processes includes exploratory behavior, global cognition and working memory[35]. Surgery may induce deficits in learning and working memory, which can be observed in the MWM test. In this research, we confirmed that splenectomy leads to cognitive deficits in aged rats. Similar results were reported by Lu et al.[8]. Furthermore, it has been reported that APN deficiency can exacerbate hippocampus-related cognitive dysfunction, neuroinflammation, microglial activation, and neuronal loss in a model of vascular dementia[36]. Regarding surgical trauma-induced cognitive decline in aged rats, we found that pretreatment with APN could significantly decrease the escape latency from the first to fifth days of the place navigation phase and increase the number of platform crossings and time spent in the target quadrant in the spatial probe trial. No relevant research has been reported to date. This result strongly suggests that APN may be a promising candidate for the treatment of PND after peripheral trauma.

TLR4 agonists can activate MyD88-dependent intracellular signaling pathways, leading to the translocation of NF-κb. Phosphorylation of NF-κb results in its activation and translocation into the nucleus, where it mediates a series of downstream reactions, especially oxidative stress and neuroinflammation[37–39]. Phosphorylation of p65 is a marker of NF-κb activation. Inhibition of the

TLR4/MyD88/NF-κb signaling pathway contributes to alleviating cognitive dysfunction by reducing oxidative stress, apoptosis[7, 38], and neuroinflammation[8]. The TLR4/NF-κb pathway is a critical targeting for preventing and treating PND. Furthermore, we found that APN-induced TLR4/NF-κb signaling inhibition in the hippocampus is accompanied by suppression of neuroinflammation and oxidative stress and amelioration of cognitive dysfunction. The TLR4 antagonist TAK-242 had similar effects as APN, while the TLR4 agonist LPS inhibited the beneficial effects of APN. However, whether other TLRs are involved in APN-induced improvement of cognitive function needs to be further researched.

MDA and SOD are the products of oxidative stress, while caspase-3 is the ultimate executor of apoptosis[7]. Analysis of MDA, SOD and caspase-3 levels in this study revealed that oxidative stress and apoptosis were induced. Our previous results confirmed that the level of oxidative stress increases with aging[27]. Aging amplifies oxidative stress and apoptosis induced by peripheral trauma. Here, we found that splenectomy aggravated oxidative stress and apoptosis in aged rats, whereas APN treatment reversed these alterations in the brain. The marked reduction in the activity of MDA and caspase-3 suggests that APN exerted antioxidant and antiapoptotic effects. Although there was no statistic difference in SOD activity between PND group and PND + APN group, we considered that it may be due to the optimal dose selected by APN. SOD activity showed an upward tendency in PND + APN group. APN may mitigate the activation of NF-κb and consequently blunted subsequent oxidative stress-related neuronal apoptosis.

Activation of the TLR4/MyD88/NF-κb pathway can trigger the expression of inflammatory factors, which is related to the degree of cognitive deficits[8, 27, 40]. The induction of microglia-mediated neuroinflammation accompanied by oxidative stress can perpetuate a vicious cycle that ultimately causes behavioral dysfunction[41, 42]. Several studies have shown that proinflammatory factor synthesis inhibitors can ameliorate hippocampus-dependent cognitive decline induced by neuroinflammation[35, 43]. In our study, we confirmed that APN treatment could attenuate the expression of proinflammatory factors downstream of the TLR4/NF-κb signaling pathway.

Excess inflammatory factor production in the aged brain following peripheral trauma is dependent on the activation of microglia[44–46]. Microglia, which are important immune cells in the CNS, are regarded as tissue macrophages in the brain[47]. Our previous research found that microglia in the aged brain remain in a preactivated state and that peripheral surgery further enhances hippocampal microglial activation[27]. Microgliosis, defined as an increase in the number of microglia, is an important neuroinflammation-related response. Inhibiting the TLR4/MyD88/NF-κb pathway can attenuate microgliosis[48, 49]. IBA1 is a protein expressed on the surface of microglia/macrophages. We found that the number of IBA1-positive cells in the hippocampal region was increased in aged rats after splenectomy, consistent with the excessive secretion of proinflammatory factors in the hippocampus and the sharp decline in cognitive ability. Moreover, APN treatment reversed the overactivation of microglia induced by splenectomy. These data are consistent with previous studies showing that APN deficiency aggravates microglial activation and neuroinflammation in AD mice[20]. APN deficiency enhances the

responsiveness of microglia to proinflammatory stimuli, resulting in higher sensitivity to neuroinflammation [50]. APN treatment significantly inhibited microglia-mediated neuroinflammation downstream of the NF-κb pathway in aged rats after peripheral trauma.

It has been confirmed that a single i.p. injection of 3 mg/kg TAK-242 can inhibit TLR4 activation to attenuate cognitive deficits[26]. Similar results were obtained in the current study. However, the i.g. route is more in line with the needs of clinical patients, and there have been no relevant studies on the optimal dose of APN. Here, we confirmed that i.g. administration of APN at a dosage of 10 μg/kg/d for 20 days before peripheral trauma had a similar neuroprotective effect as TAK-242. These results strongly suggest that oral administration of APN may alleviate cognitive impairment in clinical patients at high risk for PND. Moreover, APN may be found to have even better efficacy as the effects of other doses are evaluated.

This study also has some limitations that have to be acknowledged. First, Lu et al found that splenectomy can increase the mRNA expression of both TLR4 and TLR2 in the hippocampus[8]. This suggests that there may be other potential pathways through which APN improves cognition. Further experiments will be performed to explore the interaction between APN and TLR2 signaling activation. Second, the origin of microgliosis during neuroinflammation is the subject of controversy. The contributions of CNS-resident microglial proliferation and bone marrow-derived progenitors to splenectomy-induced reactive microgliosis[16, 51] are still unclear. Third, we only demonstrated the treatment effect of APN on cognitive impairment less than 7 days postsurgery. The long-term effect and optimal dose of APN in improving cognitive function need to be further confirmed.

Abbreviations

PND
perioperative neurocognitive disorder
APN
adiponectin
MWM
Morris water maze
TLR4
Toll-like receptor 4
MyD88
myeloid differentiation factor 88
NF-κb
nuclear factor kappa B
TNF-α
tumor necrosis factor IL-1β:interleukin-1β
IL-6
interleukin-6

LPS
lipopolysaccharide
IBA1
ionized calcium binding adapter molecule 1
Aβ
amyloid-β
AMPK
adenosine monophosphate-activated protein kinase
PFA
paraformaldehyde
MDA
malondialdehyde
SOD
superoxide dismutase.

Declarations

Acknowledgments

Not applicable.

Author contributions

HHX conceived and designed the experiments. ZJZ performed ELISA and Western blotting. LDG performed the immunofluorescence and immunohistochemical assays. FY performed all injections and behavioral testing. ZJZ, LDG and FY performed the surgery. SPP, DW, XWL and BQS analyzed the data. ZJZ, LDG and FY wrote the paper. All authors read and approved the final manuscript.

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Data availability

All data used in this study are available upon reasonable request.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Author details

[1] Department of Anesthesiology, Affiliated Dongguan Hospital, Southern Medical University, No. 3 South Wandao Road, Wanjiang District, Dongguan, China

[2] Guangdong Medical University, No. 2 East Wenming Road, Xiashan District, Zhanjiang, China

[3] Southern Medical University, No. 1023, South Sha Tai Road, Jingxi Street, Baiyun District Guangzhou, China

Conclusions

Our observations strongly indicated that APN could attenuate cognitive deficits induced by splenectomy in aged rats. The underlying mechanism may be alleviation of neuroinflammation and oxidative stress resulting from inhibition of the TLR4/MyD88/NF-κb signaling pathway.

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Figures

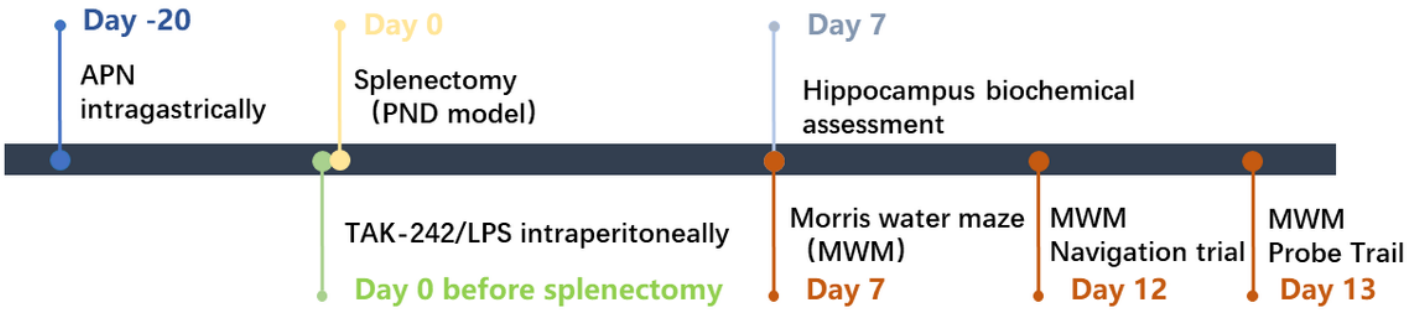

Figure 1

Experimental schedule.

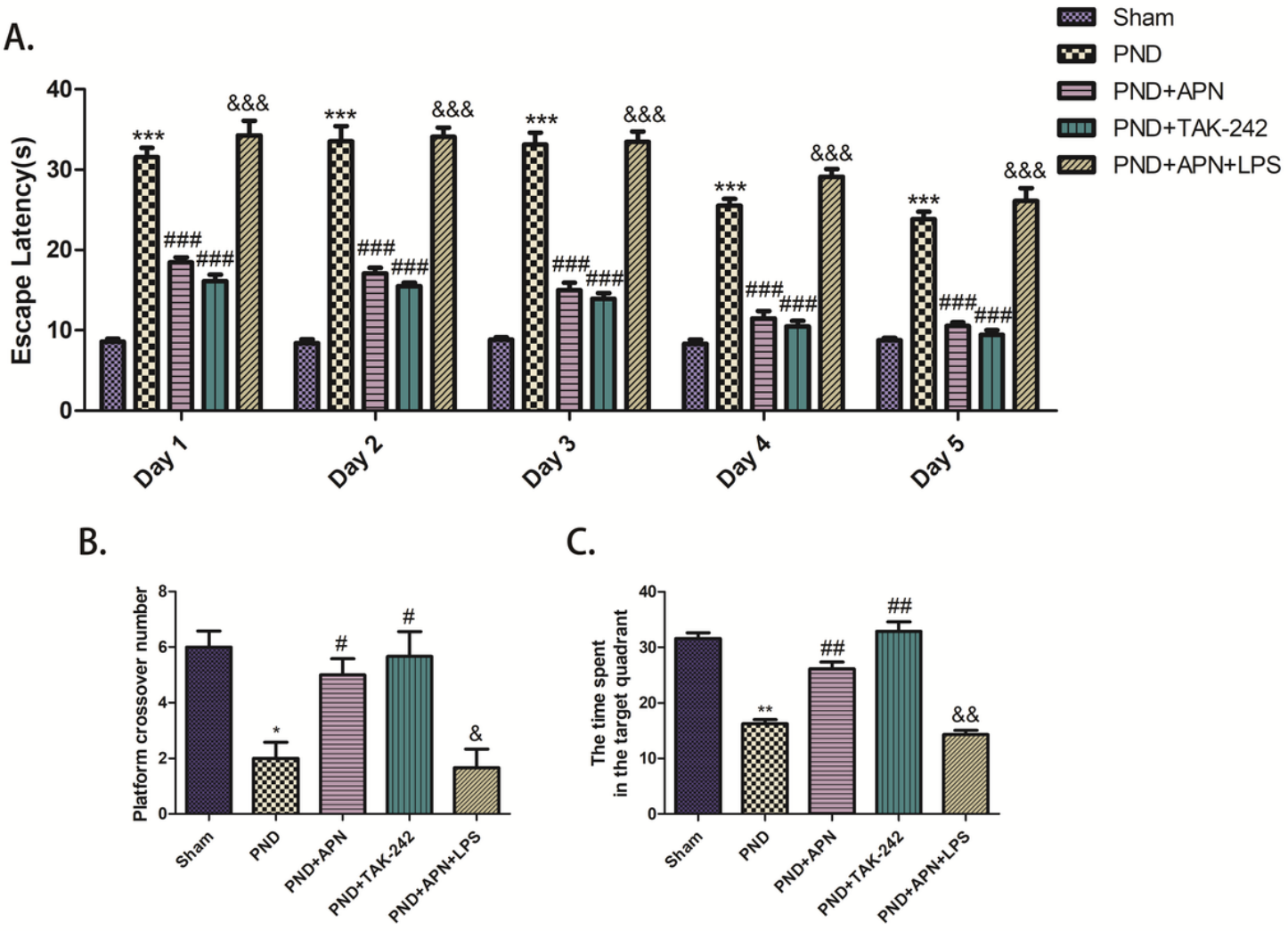

Figure 2

Effects of APN on learning and memory impairment in aged rats. Escape latency (A), number of platform crossings (B), and time spent in the target quadrant (C) on the fifth day of the MWM test. The data are shown as the mean ± SD (n = 3); $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ vs. the sham group; $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$ vs. the PND group; $^{\&}p< 0.05$, $^{\&\&}p < 0.01$, $^{\&\&\&}p < 0.001$ vs. the PND+APN group.

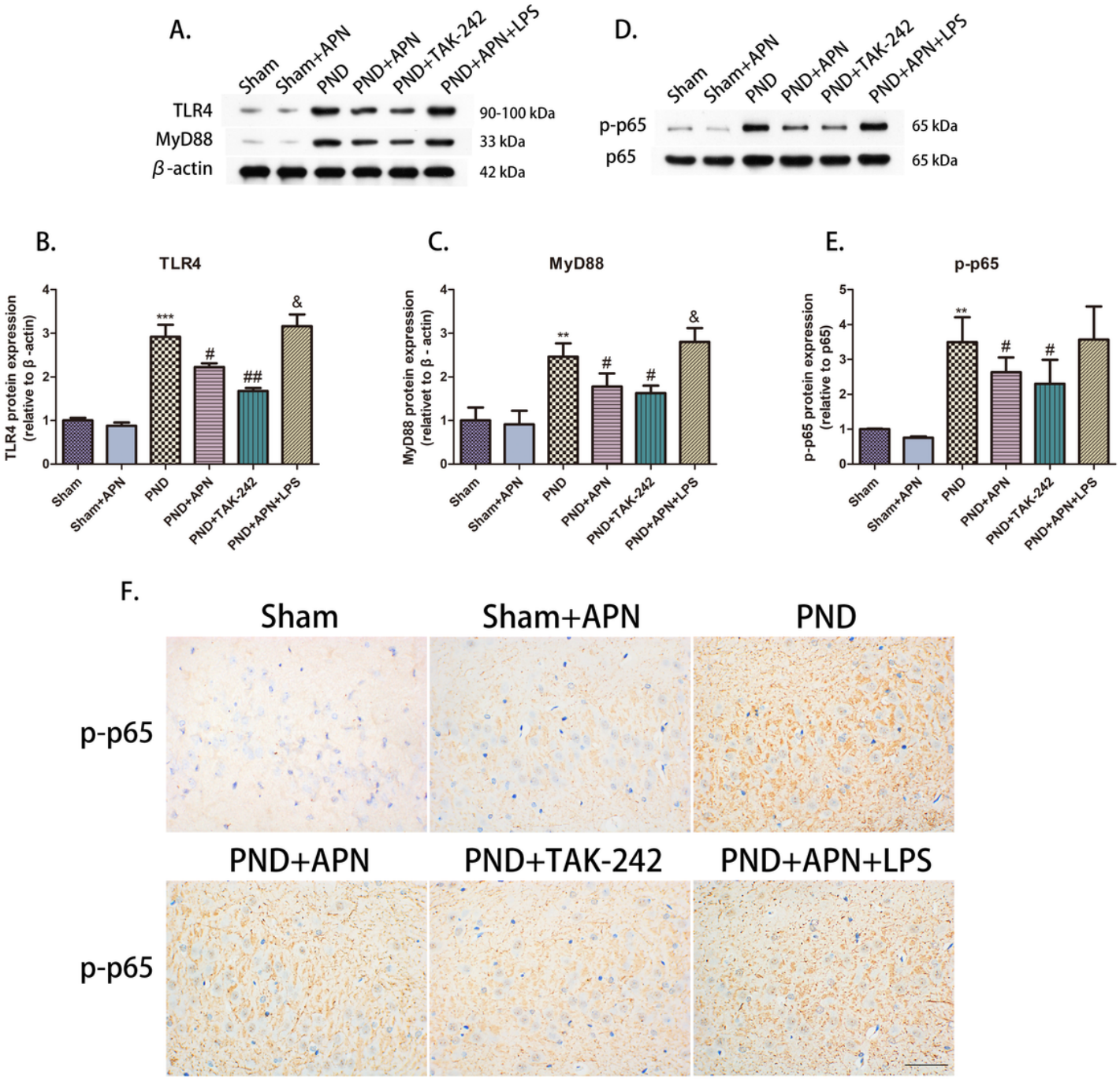

Figure 3

Effects of APN on the TLR4/MyD88/NF-κb p65 signaling pathway. (A) Representative western blot images of hippocampal TLR4 and MyD88. (B-C) Protein expression of TLR4 and MyD88. (D) Representative western blot images of hippocampal p-p65. (E) Protein expression of p-p65. (F) Representative immunohistochemical staining showing p-p65 localization in the hippocampus after splenectomy (scale bar, 100 μm). The data are shown as the means ± SEMs (n = 4). $^{**}p < 0.01$, $^{***}p < 0.001$ vs. the sham group; $^{\#}p < 0.05$, $^{\#\#}p < 0.01$ vs. the PND group; $^{\&}p< 0.05$ vs. the PND+APN group.

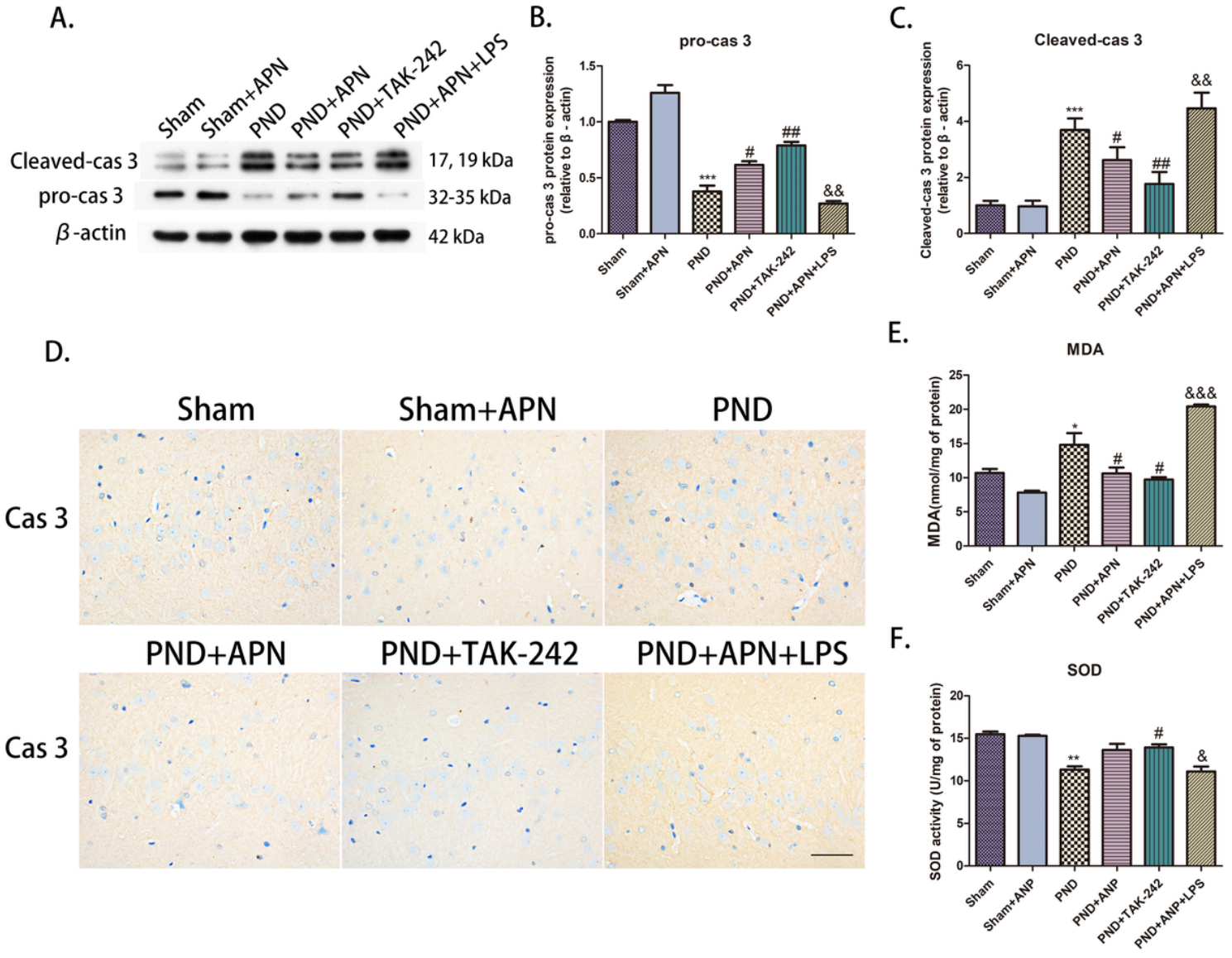

Figure 4

Effects of APN on oxidative stress and apoptosis downstream of the NF-κb signaling pathway. (A) Representative western blot images of hippocampal pro-caspase 3 and cleaved-caspase 3. (B-C) Protein expression of pro-caspase 3 and cleaved-caspase 3. (D) Representative immunohistochemical staining showing caspase 3 localization in the hippocampus after splenectomy (scale bar, 100 μm). (E) MDA levels in the hippocampus. (F) SOD activity in the hippocampus. The data are shown as the means ± SEMs (n = 4). $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ vs. the sham group; $^{\#}p < 0.05$, $^{\#\#}p < 0.01$ vs. the PND group; $^{\&}p< 0.05$, $^{\&\&}p< 0.01$, $^{\&\&\&}p < 0.001$ vs. the PND+APN group.

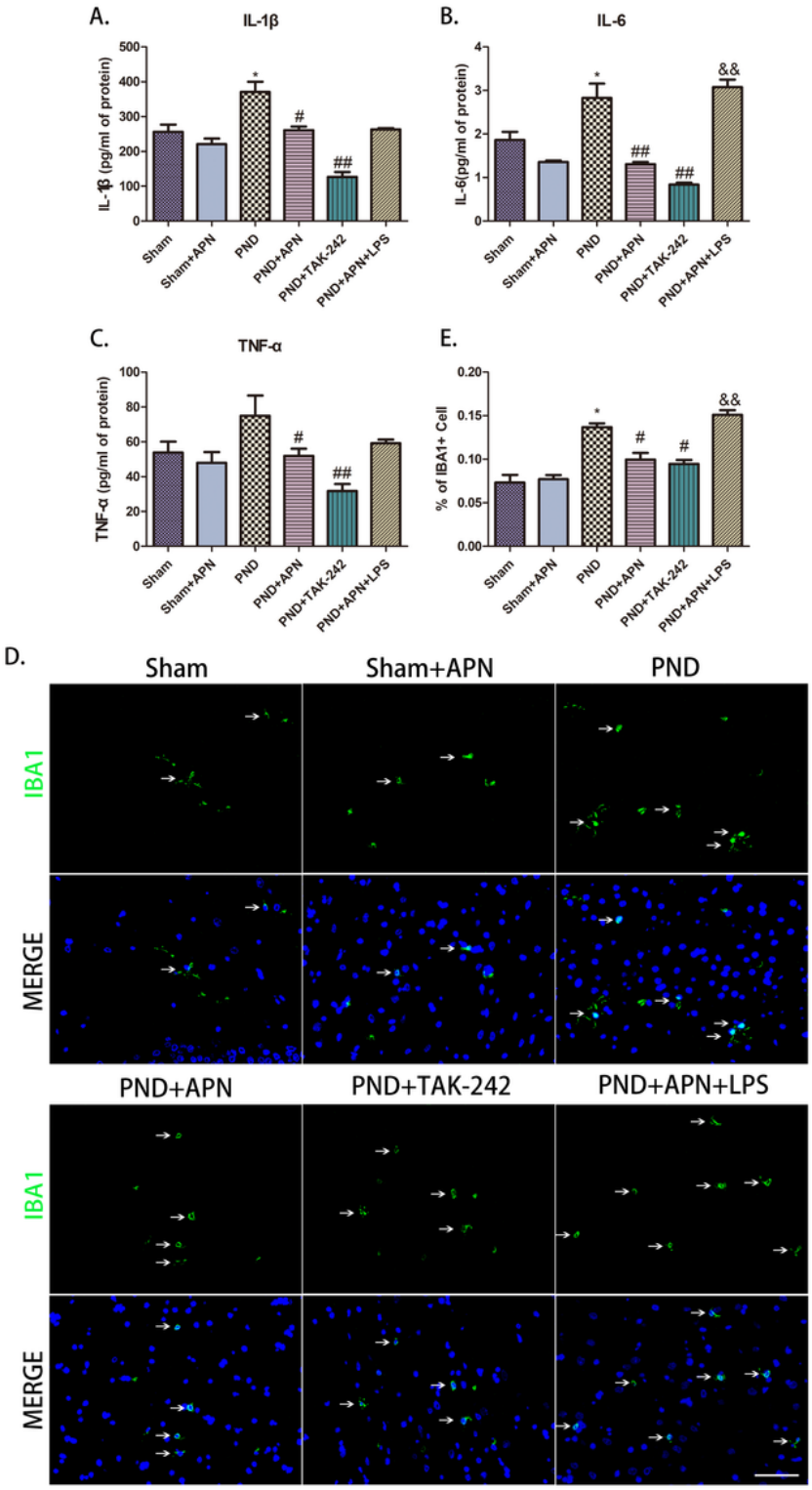

Figure 5

Effects of APN on microglia-mediated neuroinflammation downstream of the NF-κb signaling pathway. Expression levels of IL-1β (A), IL-6 (B), and TNF-α (C) in the hippocampus. (D) Representative immunofluorescence staining images of the hippocampus (scale bar, 100 μm). Brain sections were stained for IBA1. (E) Analysis of the percentage of IBA1-positive cells in the hippocampus. The data

shown are the means ± SEMs (n = 3-4). *p < 0.05 vs. the sham group; #p < 0.05, ##p < 0.01 vs. the PND group; &&p< 0.01 vs. the PND+APN group.

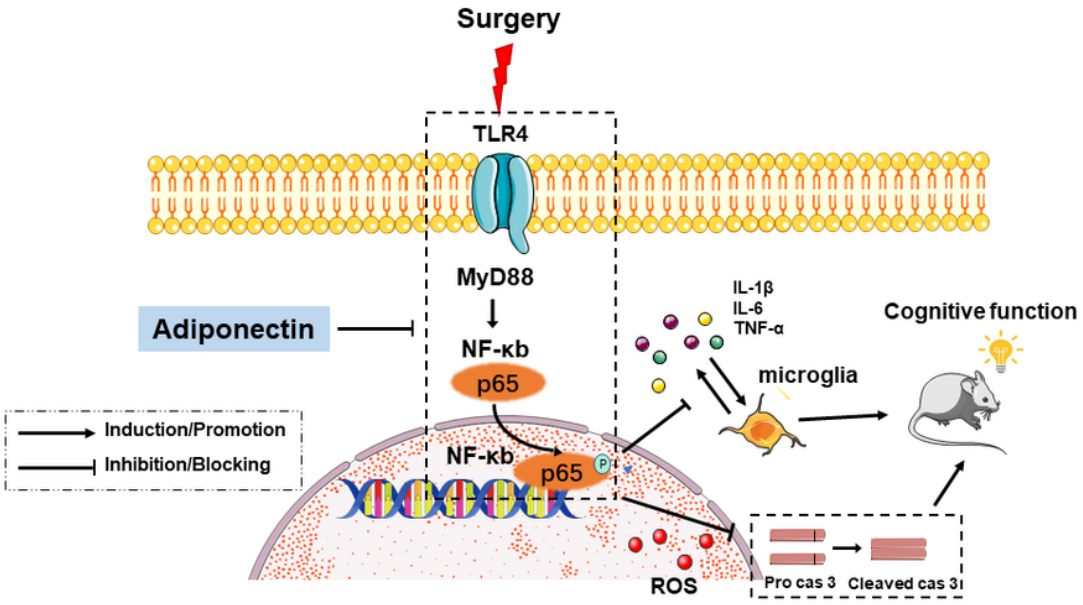

Figure 6

Schematic of the potential mechanisms underlying the neuroprotective effect of APN. APN attenuates cognitive deficits induced by splenectomy in aged rats. The mechanism may involve inhibition of the TLR4/MyD88/NF-κb signaling pathway, which alleviates neuroinflammation and oxidative stress-mediated apoptosis activation.

Supplementary Files

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