Neuroprotective effect of Estrogen Receptor α against neuroinflammation induced by hypoxia/ischemia via SIRT1-dependent AMPK pathway

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Abstract

Background: Stroke-related damage in rats is protected against by estrogen which also has an anti-cerebral ischemia role mostly conducted via its association with estrogen receptor (ER) α .However,processes governing ER α-mediated neuroprotection are poorly comprehended. This study sought to establish whether ERα's control of neuroinflammation caused by NLRP3 inflammasome activation emanating from SIRT1-AMPK signaling allowed ERα's improvement of hypoxia/ischemic damage.

Methods: The intraperitoneal administration of estrogen was performed to ovariectomized (bilaterally) (OVA) SD rats prior to middle cerebral artery occlusion (MCAO). The strong rise in NLRP3 inflammasome activation including caspase-1, ASC, IL-1β and IL-18 occurred following OVA and were specifically decreased following estrogen treatment. Moreover, the expression of Silent Information Regulator 1 (SIRT1) and ERα were reversed. The association between ERα-led inhibition of the NLRP3 inflammasome in conditions of hydrogen peroxide (H_2O_2) and SIRT1-AMPK signaling were also examined.

Results: Findings confirmed the prevention of NLRP3 inflammasome activation instigated by H_2O_2 and the in vitro production of IL-1β, IL-18 together with the enhancement of this impact by SIRT1. Additionally, ERα's neuroprotective impact was prevented by inhibiting of AMPK. The synergistic impact of SIRT1 on ERα increased AMPK activation; however, SIRT1 knockout eliminated this.

Conclusions: The findings indicate that inhibition of the NLRP3 inflammasome by ERα results in neuroprotection against hypoxia/ischemic injury and that ERα's neuroprotection can be highly improved by the SIRT1-dependent AMPK pathway.

Background

High mortality and disability rates are associated with ischemic cerebral/reperfusion [1,2], with complex processes governing the pathological mechanisms of ischemic cerebral/reperfusion damage [3−5], including neurotoxin, neuroinflammation, oxidative stress and neuronal apoptosis. Till today the primary role of the neuroinflammatory reaction in cortical ischemic-reperfusion damage has been noted [6,7]. The CNS highly expresses NLRP3 (NOD-like receptor family, pyrin domain-containing 3); a complex of NLRP3 scaffold, caspase−1 and the ASC (PYCARD) adaptor [8,9]. Caspase−1 activation results from NLRP3 inflammasome activation leading to the production of the pro-inflammatory cytokines interleukin−1β (IL−1β) and interleukin−18 (IL−18) and initiation of prompt cell death [10]. Numerous studies linked cerebral ischemic-reperfusion development with the NLRP3 inflammasome [11,12]. Thus, possible therapeutic targets are found in the prevention of the neuronal inflammatory response.

The NAD+-dependent class III histone deacetylase SIRT1 (Silent information regulator 1) has a significant part in numerous biological functions such as oxidative stress, inflammation, metabolism and aging [13]. Recent data has indicated the inhibition of NFκ Band p53-induced neuronal apoptosis and inflammatory

responses by SIRT1 indicating SIRT1's key role in neuroprotection [14–16]. Furthermore, the prevention of NLRP3 activation and its anti-inflammatory impact is also mediated by SIRT1 [17–18]. Therefore, much notice has been given to the possibility of using the NLRP3 inflammasome-mediated by SIRT1 signaling pathway as a therapeutic target concerning cerebral ischemia.

The typical genomic reactions in target tissues born of estrogen release are mediated by two receptors of the steroid hormone receptor superfamily; namely estrogen receptor (ER) α and β [19]. Previous research indicated the need for ERβ in cortical neuronal migration during development but it was absent in decreasing ischemia-mediated injury [20–22]. Furthermore, these studies indicated ERα's facilitating of estrogen's neuroprotective impacts in the cerebral cortex in animals both old and young [23], thus indicating ERα's principal role in protecting against cerebral ischemia-reperfusion injury. Interestingly, earlier research alluded to the inhibitory effect of estrogen on the expression of the NLRP3 inflammasome gene in the cerebral cortex following cerebral ischemia [24], thereby proposing estrogen's regulation of inflammasome activation. Nevertheless, evidence is still required for estrogen-directed ERα expression suppression of the NLRP3 inflammasome via regulation of SIRT1 following cerebral ischemia.

This research aims to examine ERα processes and it's neuroprotective impact on neuroinflammation instigated by cerebral ischemia initiated by the SIRT1-dependent pathway.

Methods

Reagents

PC12 cells were obtained from the Cell bank of the Chinese Academy of sciences.

Genechem company (Shanghai, China) constructed all plasmids including wild type ERα, wild-type SIRT1 and SIRT1 siRNA. NLRP3 antibody was purchased from Adipogen (San Diego, USA). IL- 1β, IL- 18, β-actin and Caspase–1 p10 antibodies were purchased from Santa Cruz Biotechnology (Dallas, USA). Fu GENE HD, FCM Assay System Kit was obtained from Roche (Diagnostics, Indianapolis).

Animals

The Laboratory Animal Center of Hubei Province supplied the female Sprague-Dawley (SD) rats with weights ranging between 280 to 320g. Rats were kept in pathogen-free environments and separated into sham, M&OVA, MCAO and M&E$_2$ groups.10% chloral hydrate anesthesia was used prior to bilateral ovariectomization of the M&OVA group by insertion of nylon monofilament into the anterior cerebral artery. Group prior to MCAO, M&OVA groups: 7 days before OVA, the rats were administered estrogen (0.5mg/kg) intraperitoneally before MCAO. Numbers of animals utilized and animal suffering was kept to a minimum. The protocol was approved by the Institutional Animal Care and Use Committee and the Medical ethics committee of wuhan university of science and technology (No:201961).

Middle cerebral artery occlusion/reperfusion model (MCAO)

350mg/kg i.p. of 10%chloral hydrate was used to anesthetize rates by insertion of nylon monofilament (0.25−0.28mm diameter) into the anterior cerebral artery via the external carotid artery for 2h prior to recovering perfusion. Focal cerebral ischemia was induced by occlusion of the right MCAO with a nylon monofilament (diameter criteria,0.25−0.28mm) with a heparin-coated tip (Huang et al., 1994).This coated filament was introduced into the internal carotidartery through the external carotid artery, up to the origin of the anterior cerebral artery, and occluded the MCA andanterior cerebral artery for 2 h. Rats with a neurological score <3 were removed from the study. All rats were euthanized at ischemic 2h-reperfusion 24h. After the MCAO building, once rats lack the alertness of physical or mental that result from the symptoms of unconscious or bleeding, rats would be euthanasia by an overdose of pentobarbital (120 mg/kg intraperitoneal injection) to produce unconsciousness and death [25,26]. The humane method we chosen is to induce a rapid, painless, and distress-free death.

Infarct volume evaluation

Coronal sectioning was performed on brains followed by TTC (Sigma, USA) staining for quantification by incubation in 2% TTC solution at 37°C for 20min prior to paraformaldehyde (4%) fixation. Infarct volume was calculated by totaling infarct area per section and multiplying it through the distance between section as indicated by image analysis software (Version 1.61, NIH image).

Cell culture and Treatment

PC12 cells were cultured in Ham's F12K Medium supplemented with FBS (2.5%), horse serum (15%), penicillin and streptomycin (100μg/ml) and incubated in a humidified atmosphere containing 5% CO_2 at 37 °C. Wild-type ERα, SIRT1 or SIRT1 siRNA plasmids were used to transfect PC12 cells following their seeding and coating with poly-L-Lysine. Transfection was performed in Ham's F12K-Medium with DNA (0.5μg) and Fu GENE (2.5μl) prior to adding culture medium consisting of 400μM hydrogen peroxide.

HE stains

Brain pieces were fixed in Bouin's solution following washing in physiological saline. They were then dehydrated using increasing ethanol concentrations, paraffin embedded and cut to 2mm. Brain sections were hematoxylin-eosin (HE) stained and examined under the light microscope.

Flow cytometric

PC12 cells' viability was examined using flow cytometry (FCM) following PI and Annexin V-FITC staining.

Detection of LDH release

The LDH-release kit (Nanjing Jincheng Bioengineering Institute) was used to quantify LDH as per manufacturer's instructions.

Western blot analysis

A cell pellet containing 50μg cells or cerebral cortex was collected and electrophoresed in sodium dodecyl sulfate-polyacrylamide gel (15%) prior to membrane probing with antibodies (NLRP3, caspase−1 p10, IL-1β, IL- 18, ERα and SIRT1 antibody) and incubated overnight at 4°C. An ECL chemiluminescence system was used to visualize signals using a computerized image processing software (Sunnyvale, CA).

Statistical

Assays were repeated three to five times and represented as mean + standard deviation (S. D.). ANOVA (analysis of variance) was used to examine significant differences of varied groups. A P-value below 0.05 was considered statistically significant.

Results

Estrogen supplement attenuates MCAO-induced cerebral neuron injury in rats

The MCAO group attained a mean score of 3.40 ± 0.55 with mean infarct volume of 22.46 ± 1.90 mm^3 thus both scores confirming positive formation by MCAO of cerebral ischemia-reperfusion damage. The M&OVA group attained a mean score of 3.60 ± 0.55 with mean infarct volume of 25.33 ± 1.81 mm^3 with a score of 1.40 ± 0.89 for the estrogen treatment group with a mean infarct volume of 7.98 ± 0.53 mm^3 when compared to the MCAO group (Figures 1A and 1C). In support of these results, LDH production measured by the LDH assay was noted in the MCAO group ischemic hemisphere but was higher in the M&OVA group and decreased in the estrogen group (Figure 1B). Moreover, the increased number of neurons attaining a normal morphology in the estrogen treatment group coincided with greater numbers of neurons dying in the M&OVA group compared to those in the MCAO group (Figure 1D). Overall, these results indicated that the reduction in estrogen levels following ovariectomization can exacerbate cerebral ischemia-reperfusion damage and that this damage would be improved on by estrogen supplements.

Estrogen supplement inhibits NLRP3 inflammasome activation during cerebral ischemia-reperfusion injury

Western blot analysis employed for the examination of inflammation-associated cytokines expression per group after MCAO in rats to determine ERα's role in neuroinflammation. Significant reduction in ERα expression noted following MCAO as illustrated in Figure 2A and 2B. When compared against the MCAO group, the M&OVA group demonstrated highly reduced levels of ERα protein, whereas, these levels reached high levels following estrogen supplementation (Figures 2A and 2B). Moreover, MCAO and M&OVA treatment led to high increases in NLRP3 inflammasome production (including ASC, NLRP3, IL−1β and IL−18) and activation of caspase−1. This was particularly true following ovariectomy. Moreover, when estrogen supplementation was given after MCAO treatment in rats, NLRP3 inflammasome and caspase−1 p10 levels were inhibited. Simultaneously, apoptotic cell percentages were observed to be comparable to expression levels of NLRP3 (Figure 2E and 2F). These findings demonstrate the crucial part for ERα in MCAO damage-induced neuroinflammation.

ERα rescuing hydrogen peroxide-induced neuronopathies via SIRT1-dependent in vitro

PC12 cell transfection with ERα plasmid was performed to examine potential processes occurring due to ERα activation and neuron damage. Neuronopathies were initiated by hydrogen-peroxide treatment after 24hrs and ERα levels examined by Western blot analysis. This indicated efficacious elevation of ERα levels following transfection of cultured PC12 cells (Figures 3A and 3B). Moreover, additional findings of the FCM assay (Figures 3C and 3d) and the LDH assay (Figure 3E) on PC12 cells demonstrated that when compared to the hydrogen-peroxide treatment group, hydrogen peroxide-instigated cell death and LDH production were prevented by ERα overexpression. Thus, indicating ERα neuroprotective impact on neuronal damage *in vitro.*

Previous studies performed in this laboratory demonstrated significant SIRT1 level reductions following MCAO. When contrasted against MCAO, the M&OVA group showed greatly decreased SIRT1 protein expression levels which were efficaciously elevated with estrogen supplementation (Figure 3A and 3B). This demonstrated the close association between estrogen anti-neuronopathies and SIRT1. Furthermore, co-transfection of ERα with either SIRT1 or SIRT1 siRNA was performed to demonstrate the involvement of the SIRT1-dependent pathway *in vivo* in ERα's protection against neuronopathies. Figure 3 illustrates the outcome indicating that SIRT1 overexpression with ERα in a hydrogen-peroxide environment, resulted in decreases in neuron apoptosis and LDH production continuously and this was eradicated via SIRT1 siRNA and ERα co-transfection. Thus, these findings demonstrate neuronal activity regulation by ERα via the SIRT1-dependent pathway and as a response to hydrogen peroxide treatment.

SIRT1 cooperates with ERα to counteract NLRP3 inflammasome activation

ERα overexpression was achieved by transfection of cells with an ERα wildtype plasmid which was performed to examine ERα response to NLPR3-dependent neuroinflammation and caspase−1 activation. PC12 cell's expression of cleaved caspase−1 and NLRP3 inflammasome was highly decreased following ERα treatment. Next, PC12 cells SIRT1 levels were raised by transfection using wildtype SIRT1 plasmid in the presence of ERα overexpression. This was performed to establish whether maintenance of neuroinflammatory-associated gene on anti-nerve damage mediated by hydrogen peroxide requires SIRT1. From the caspase−1 activity, it was evident that downregulation of NLRP3 inflammasome expression was attained in PC12 cells compared to those transfected as described. Contrastingly, SIRT1 siRNA transfection resulted in SIRT1 knockdown in the presence of ERα overexpression, and consequently, this resulted in higher inflammasome expression. Overall, these findings indicate the crucial part for SIRT1 in ERα's impact on the anti-neuroinflammatory factor expression.

ERα-evoked anti-neuronopathies though SRIT1 is mediated by AMPK

Earlier research indicated regulation of AMPK activation by estrogen *in vivo* [40]. Compound C was employed to obstruct the AMPK pathway to allow evaluation of whether an association existed between the AMPK pathway and ERα's anti-neuro damage impact. Examining PC12 cells exposed to hydrogen-peroxide found that ERα decreased neuronal damage levels. This was further backed by elevated cell viability levels and lower apoptotic-positive cell percentages and LDH production. Thus, compound C was found to suppress ERα' neuroprotective properties (Figure 5A and 5B), thereby indicating the principal role for AMPK in neuron survival due to ERα. Earlier studies in this lab indicated the need for SIRT1 in ERα-mediated neuroprotection. Consequently, this led to the hypothesis that ERα's neuroprotection was facilitated by the SIRT1-AMPK pathway with AMPK forming a strong relationship between ERα and SIRT1. Moreover, increased AMPK activation resulted from SIRT1 overexpression and AMPK phosphorylation mediated by ERα. Conversely, neurons where SIRT1 was suppressed demonstrated reduced AMPK expression thereby demonstrating that in hydrogen peroxide-treated neurons AMPK activation was damaged in conditions of SIRT1 absence.

Discussion

This study demonstrated the effect of ERα treatment and transfection using ERα plasmid in conferring neuroprotectivity against hypoxia/ischemic damage *in vitro* and *in vivo.* Findings indicated great improvements in neurological scores and lower infarct volumes following higher ERα and SIRT1 expression levels resulting from estrogen treatment. Moreover, estrogen treatment was found to inhibit NLRP3 inflammasome activation resulting in reduced cerebral ischemic/reperfusion-instigated neuronal damage. Contrastingly, ERα and SIRT1 deficit caused by ovariectomization led to direct elevation in neurological shortfalls, lessened *in vivo* infarct volume and initiated NLRP3 pathway factors expression indicating the involvement of ERα and SIRT1 activation in post-brain damage neuroinflammation. Outcomes of this study also indicated how abolishing SIRT1 ended the impact of ERα in inflammasomes,

thus pointing to the SIRT1-dependent pathway of ERα's neuroprotective properties. Overall, this indicates that less hypoxia/ischemic damage would ensue through the action of ERαvia the SIRT1-dependent pathway and by inhibition of NLRP3 inflammasome activation.

The ERα and ERβ receptors through which estrogen manifests its actions are part of the nuclear receptor (NR) superfamily [27]. The binding of estrogen to ER led to the ER's interaction with a particular DNA response element leading to the transcription of specific genes [28]. Notwithstanding the significance of both ERα and ERβ actions, various studies have indicated contrasting outcomes in relation to their role in ischemic protection, with increasing levels of evidence pointing towards copious ERα at the RNA and protein levels [29−31] and its central status in neuronal survival. One study indicated that lower ERα, not ERβ levels, led to loss of E2 protection in MCAO models [32]. Other research has alluded to the significant role of ERα rather than ERβ in ischemic protection [33] and that certain ERα, not ERβ was also neuroprotective [34]. These studies outline the greater part ERα holds concerning cerebral ischemic/reperfusion damage compared to ERβ.

In support of past research, the findings of this study demonstrated that ERα shortage following ovariectomization led to *in vivo* decrease in infarct volume and elevated neurological insufficiency. Contrastingly, estrogen treatment resulted in enhancement of ERα leading to smaller cerebral infarct volume and better neurobehavioral results following stimulation by cerebral ischemia/reperfusion. Thus, this demonstrates the direct association between ERα activation and cerebral ischemia/reperfusion damage. Protection against hydrogen peroxide-induced nerve damage facilitated through ERα overexpression protection was examined to determine if ERα is a primary element conferring neuroprotection for neuronal survival using PC12 cells. Findings indicated efficacious enhancement of cell viability and suppressed LDH production *in vitro* by ERα, thereby demonstrating the protective impacts of ERα against hydrogen peroxide-induced neuronal injury.

Neuroinflammation is a byproduct of cerebral ischemia-reperfusion that provokes neuronal death together with elevated levels of IL−1β and IL−18 in the ischemic hemisphere [35,36]. Earlier research alluded to the progress of ischemia/reperfusion-induced brain damage because of the crucial part played by the NLRP3 inflammasome [11,12]. Ischemic stroke leads to elevation of NLRP3 inflammasome associated proteins' expression [37], whilst reducing NLRP3 inflammasome expression can decrease the likelihood of cerebral ischemia-induced neuronal injury [38]. As caspase−1 activation and IL−1β production are impacted by the NLRP3 inflammasome, these inflammasomes were assessed for partaking in cerebral ischemic/reperfusion damage and if NLRP3 inflammasome activation is inhibited by ERα. The finding of this study indicated significant elevation in NLRP3 inflammasome expression in brains at times of cerebral ischemia/reperfusion, whilst ERα shortage following ovariectomization enhances caspase−1 activation and generation. Contrastingly, significant decrease in NLRP3 inflammasome (e.g. NLRP3, caspase−1 p10, ASC *in vivo* and *invitro)* expression was brought about by transfection with ERα plasmid or estrogen treatment. Furthermore, higher levels of ERα decreased IL−18

and IL−1β levels. Overall, these findings indicated an efficient increase in neuronal survival by ERα against neuroinflammation.

The crucial role played by SIRT1 in the inflammatory response is widely acknowledged with several studies suggesting SIRT1 overexpression highly suppressed NLRP3 inflammasome activation in vascular endothelial cells [18] and dextran sodium sulfate-related rhapontin increased NLRP3 inflammasome protein expression by the SIRT1-dependent pathway [39]. Large research data also indicates a vital need for SIRT1 as a neuroprotective element combatting cerebral ischemic/reperfusion damage. This study's findings were in keeping with this demonstrating elevation of SIRT1 expression together with NLRP3 inflammasome suppression following estrogen treatment. Interestingly, the neuroprotective impact of ERα against damage can be eliminated by SIRT1 silencing. SIRT1 silencing was also implicated in annulling the lowering of NLRP3 inflammasome activation induced by ERα, thereby signifying a relationship between SIRT1 signaling and ERα protective impacts.

Neuronal cell energy levels are depleted as a result of brain ischemia and the presence of barriers to energy depletion enhances the outcome of brain damage resulting from ischemic stress [40]. AMPK functions to preserve energy and is, therefore, a master metabolic switch with one study signifying the crucial part played by the SIRT1-AMPK signaling pathway in resisting ischemic stroke [41]. Whilst a separate study demonstrated ERα's suppression of neuronal injury facilitated by ischemic stroke using the SIRT1-dependent AMPK pathway [42]. Findings of this study further strengthened this argument by demonstrating the increase of AMPK activation by ERα and the elimination of ERα's neuroprotective qualities following the action of AMPK inhibitors. Most significantly, the findings concerning PC12 cells established that AMPK and SIRT1 took part in ERα neuroprotection processes and that ERα-induced AMPK activation was reliant on SIRT1. Overall, these findings indicate the significance of the SIRT1-AMPK signaling pathway in ERα's neuroprotection.

In summary, suppression of ischemic-induced NLRP3 inflammasome expression and protection against cerebral ischemic damage both resulted from ERα activation together with SIRT1-AMPK pathway activation. These findings contribute new insights into ERα processes involved in ischemic stroke damage, thereby indicating possible therapeutic targets for stroke inhibition in postmenopausal women.

Conclusion

The findings indicate that inhibition of the NLRP3 inflammasome by ERα results in neuroprotection against hypoxia/ischemic injury and that ERα's neuroprotection can be highly improved by the SIRT1-dependent AMPK pathway.

Abbreviations

ERα:estrogen receptorα MCAO:middle cerebral artery occlusion OVA:ovariectomized bilaterally SIRT1: Silent Information Regulator 1 H_2O_2:hydrogen peroxide IL−1β:pro-inflammatory cytokines interleukin−1β

IL−18:interleukin−18 ⁣SD rats:Sprague-Dawley rats ⁣HE: hematoxylin-eosin ⁣FCM: flow cytometry ⁣ANOVA:analysis of variance⁣NR: nuclear receptor

Declarations

Ethics approval and consent to participate

The study was approved by the Medical ethics committee of Wuhan university of science and technology for scientific research projects. ⁣Audit section no.(201961)⁣

Ethical review opinions as follows: By the hospital ethics committee review, the research of experimental design and scheme fully considering the safety and fairness principle, fully embodies the experiment by replacing, reduction and optimization of three principles, rights and interests to protect animals and will ease the pain of animals to the greatest extent, pain and stress, there is no conflict of interest between research content and research results.

Consent for publication

Not applicable.

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

Ze Zhong designed the study, participated to data analysis；Wenqi He participated to animals feeding and behavior test; Xiaolu Cao took part to build animal models and protein detection; Bei Tang participated to animals feeding and morphological detection; Qianya Fan took part to cell culture and flow cytometry test; Minghui Zhao took part to cell culture and plasmid transfection experiment; Yajun Chen participated to the writing of the manuscript, data analysis and marking of the final version.

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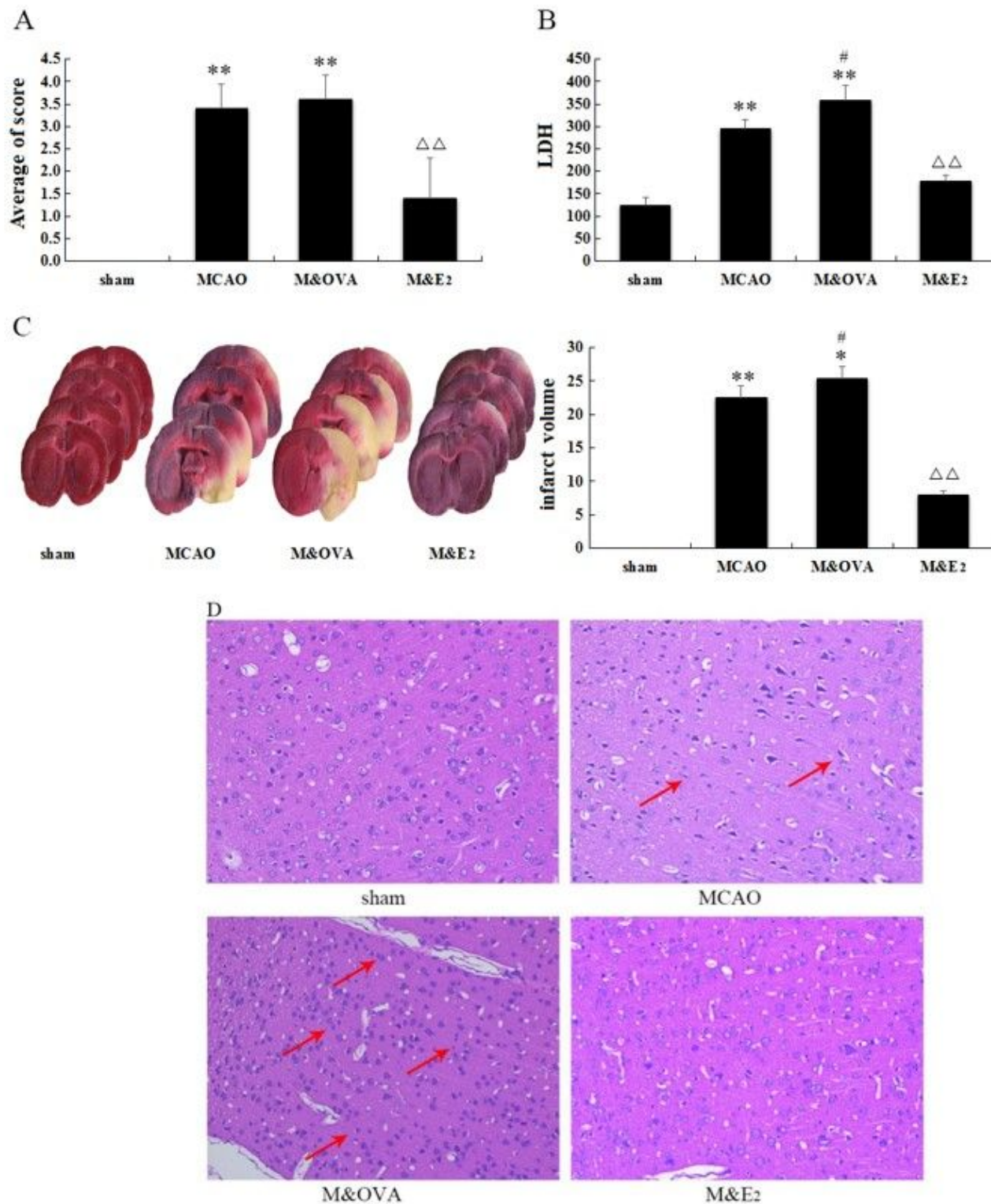
Figures

Figure 1

Illustration of Longa's behavioral analyses (A), LDH assay (B), TTC stained infarct volume assessment (C) and HE staining (D) per group. M&E2:estrogen supplement prior to MCAO, M&OVA: ovariectomized prior to MCAO. Dead neurons indicated by red arrows. Data are given as mean ± SD for triplicate assays, n=5, *P<0.05, **P<0.01 vs sham group, #P<0.05 vs MCAO group, △△P<0.01 vs M&OVA group.
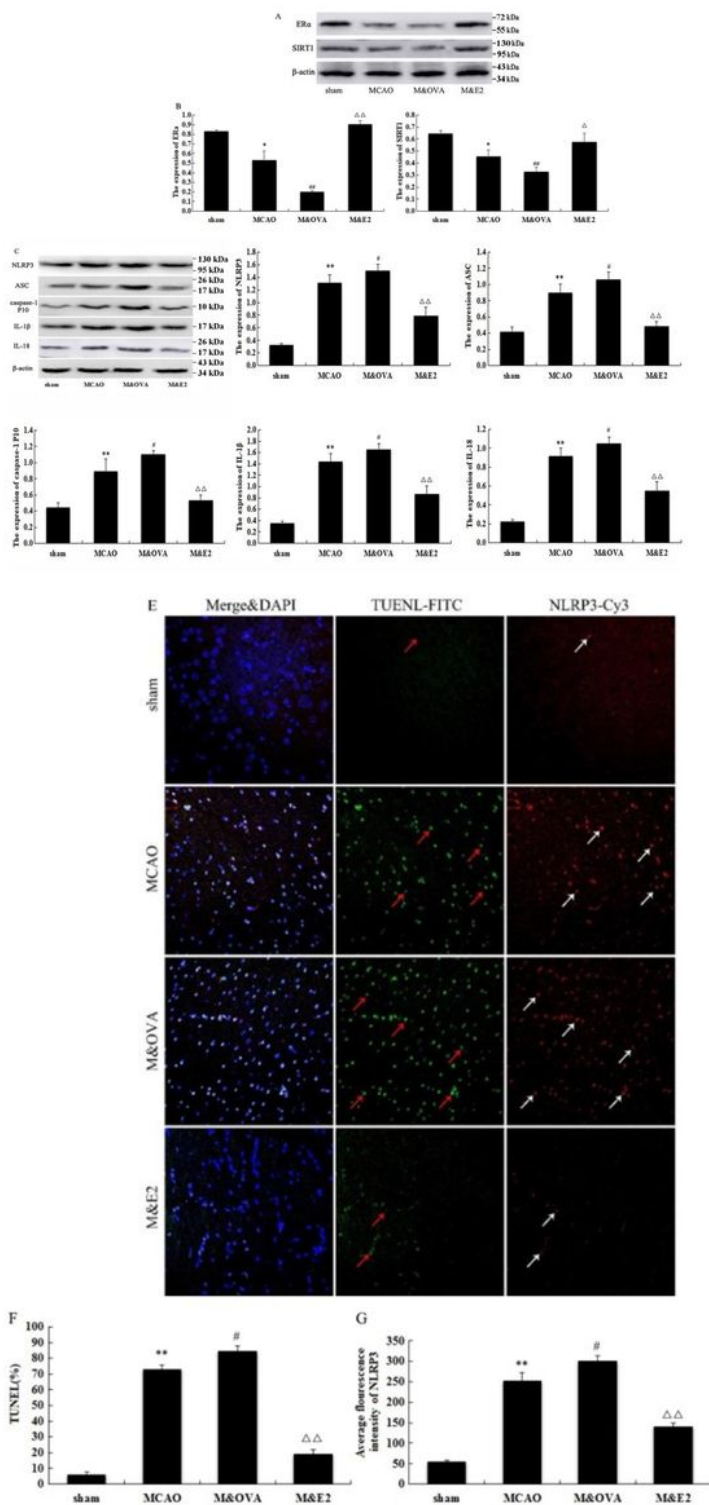
Figure 2

Western blot analysis of expression of ERα (A and B) and NLRP3, ASC, caspase-1 P10, IL-1β and IL-18 (C and D). Protein expression levels are given arbitrarily and results normalized to the related β-actin protein quantity. Immunofluorescent image of TUNEL stained apoptotic cell percentage (green) (E and F) and expression of NLRP3 (red) (E and G). Positive apoptotic cells shown by red arrows, whilst white arrows are indicative of NLRP3 protein expression as observed in the same field. M&E2:estrogen supplement

before MCAO, M&OVA: ovariectomized before MCAO. Data are given as mean ± SD for triplicate assays, n=5, *P<0.05, **P<0.01 vs sham group, ##P<0.05, #P<0.05 vs MCAO group, △△P<0.01 vs M&OVA group.
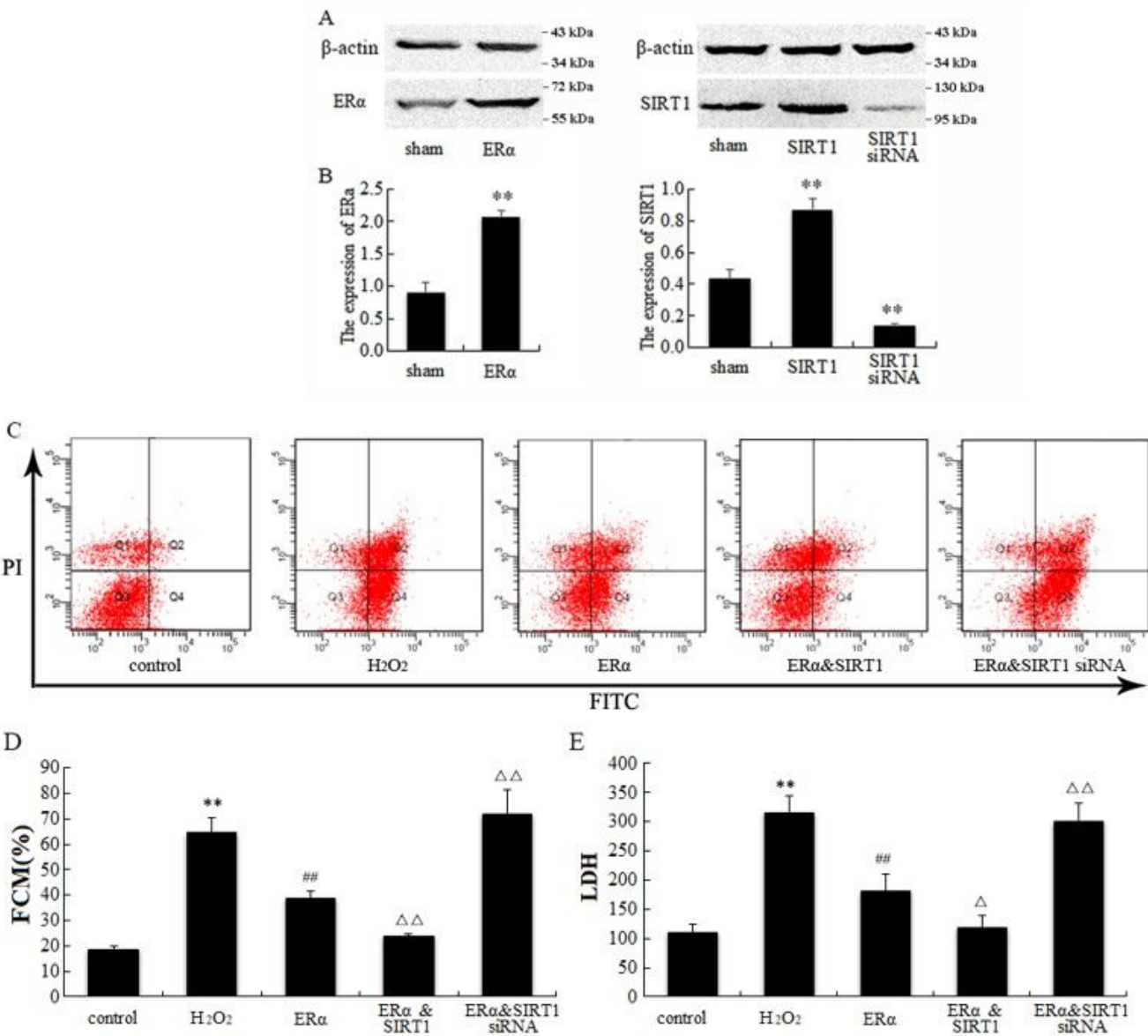

Figure 3

PC12 cells Western blot analysis of ERα and SIRT1 expression (A and B) and FCM assay examination of PC12 apoptotic cells (C and D). LDH production demonstrates LDH levels of culture media. The findings are of three individual assays and are mutually supportive. ERα: transfected with a wild-type Estrogen Receptor α (0.5μg) before H2O2 treatment (400μM for 12h), ERα&SIRT1: co-transfected a wild-type SIRT1 (0.5μg) with Estrogen Receptor α (0.5μg) before H2O2 treatment, ERα&SIRT1 siRNA: co-transfected the SIRT1 small interference RNA (0.5μg) with Estrogen Receptor α (0.5μg) before H2O2 treatment, H2O2: hydrogen peroxide. *P<0.05 vs sham group, ## P<0.01 vs H2O2 group, △P<0.05, △△P<0.01 vs ERα group.
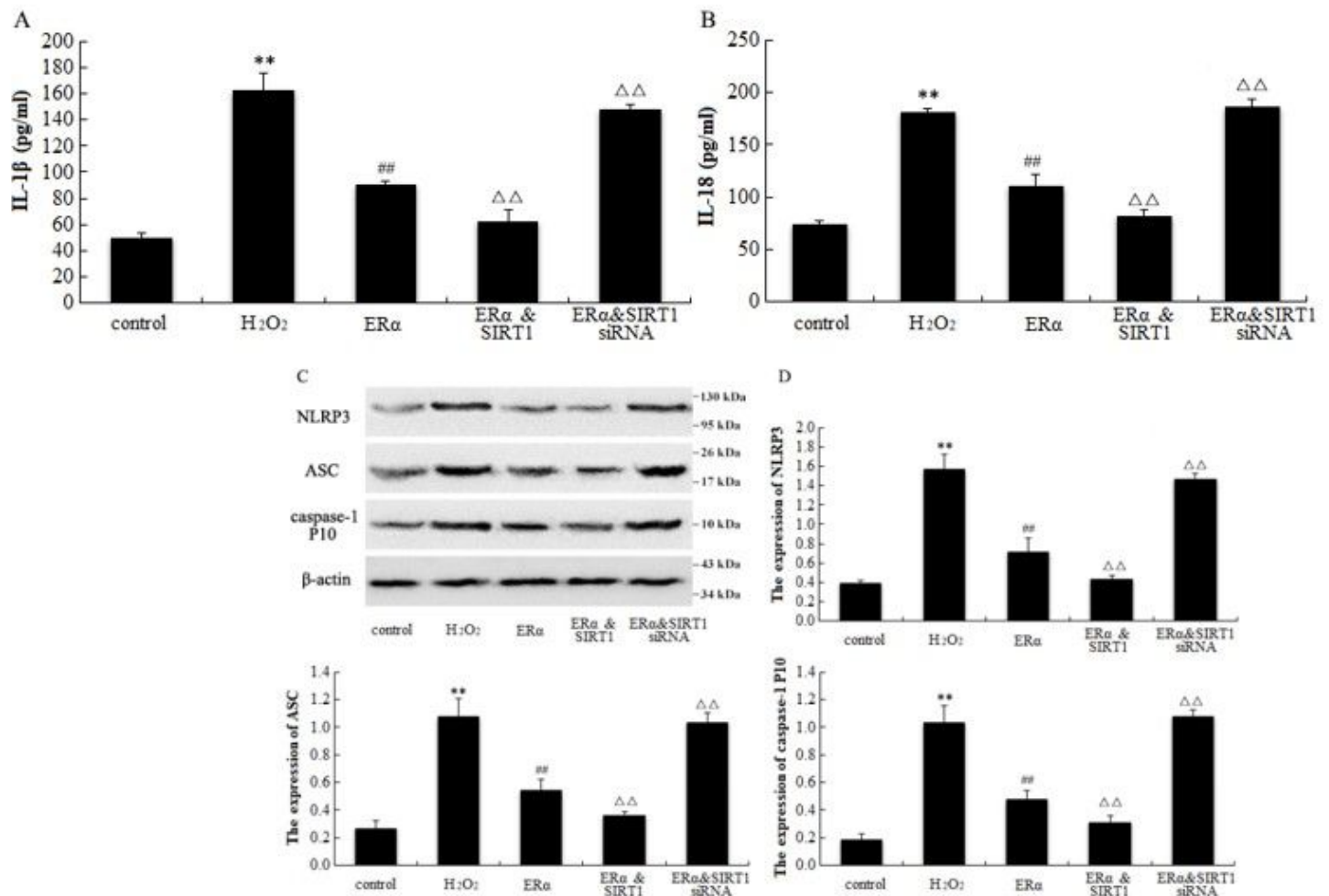
Figure 4

Quantification of the inflammatory cytokines IL-1β and IL-18 using ELISA (A and B). Western blot analysis of ASC, NLRP3, caspase-1 p10 expression in PC12 cells (C and D). The findings are of three individual assays and are mutually supportive. ERα: transfected with a wild-type Estrogen Receptor α (0.5µg) before H2O2 treatment (400µM for 12h), ERα&SIRT1: co-transfected a wild-type SIRT1 (0.5µg) with Estrogen Receptor α (0.5µg) before H2O2 treatment, ERα&SIRT1 siRNA: co-transfected the SIRT1 small interference RNA (0.5µg) with Estrogen Receptor α (0.5µg) before H2O2 treatment, H2O2: hydrogen peroxide. **P<0.01 vs sham group, ## P<0.01 vs H2O2 group, △△P<0.01 vs ERα group.
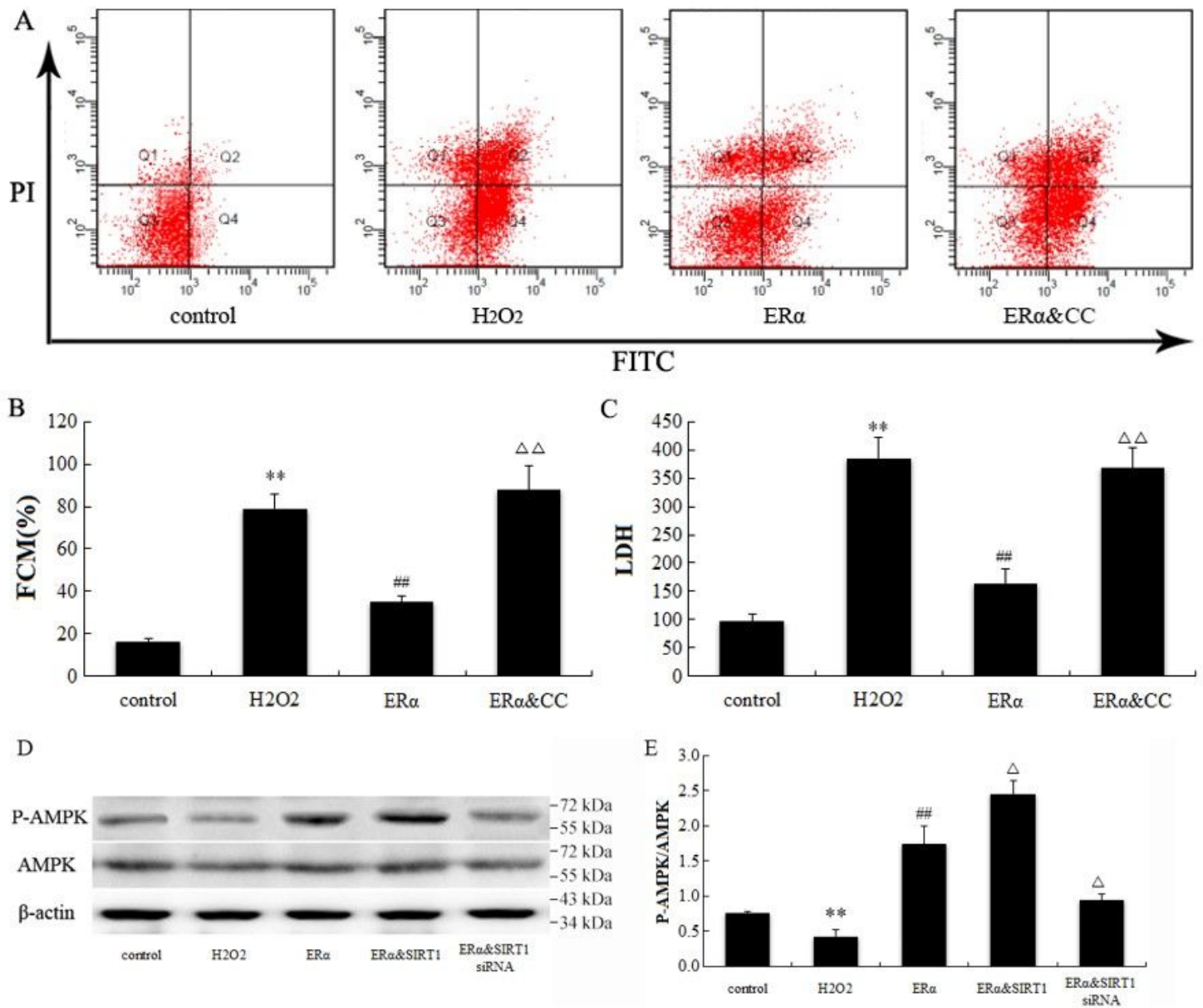
Figure 5

FCM assay utilised for observation of apoptotic PC12 cells (A and B), LDH production demonstrating LDH levels in culture media (C), Western blot analysis of PC12 cells for AMPK/P-AMPK expression levels (D and E). The findings are of three individual assays and are mutually supportive. ERα: transfected with a wild-type Estrogen Receptor α (0.5μg) before H2O2 treatment (400μM for 12h), ERα&CC: AMPK-inhibitor Compound C treatment and transfected with a wild-type Estrogen Receptor α (0.5μg) before H2O2 treatment, ERα&SIRT1: co-transfected a wild-type SIRT1 (0.5μg) with Estrogen Receptor α (0.5μg) before H2O2 treatment, ERα&SIRT1 siRNA: co-transfected the SIRT1 small interference RNA (0.5μg) with Estrogen Receptor α (0.5μg) before H2O2 treatment, H2O2: hydrogen peroxide. *P<0.05 vs sham group, ## P<0.01 vs H2O2 group, △P<0.05, △△P<0.01 vs ERα group.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- ARRIVEchecklist.xlsx