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Full paper

# Resveratrol-loaded nanoemulsion prevents cognitive decline after abdominal surgery in aged rats

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ABSTRACT

The maladaptive response of aged microglia to surgery and consequent neuroinflammation plays a key pathogenic role in postoperative cognitive dysfunction (POCD). Here, we assessed the preventive effect of resveratrol (RESV) for POCD in aged rats. The emulsified form of RESV (*e*-RESV) was selected to improve its oral and brain bioavailability. Animals were assigned to one of four groups: *e*-RESV (80 mg/kg) versus vehicle treatment by abdominal surgery versus isoflurane anesthesia alone (n = 8 in each group). The dose-dependent effects of *e*-RESV were also assessed in dose range of 0–60 mg/kg. Either vehicle or *e*-RESV was administered intragastrically 24 h before surgery. Seven days after procedure, cognitive function was evaluated using a novel object recognition test, followed by measurement of hippocampal pro-inflammatory cytokine levels. Our results showed that pre-treatment with *e*-RESV attenuated the surgery-induced cognitive impairment and related hippocampal neuroinflammation at 40 mg/kg or higher doses. Additionally, the *ex-vivo* experiments revealed that the preemptive *e*-RESV regimen reduced the hippocampal microglial immune reactivity to lipopolysaccharide. Furthermore, *e*-RESV induced neuroprotective benefits were inhibited by the concomitant administration of sirtinol, a specific SIRT1 inhibitor. Our findings imply the preventive potential of *e*-RESV for POCD *via* the SIRT1 signaling pathway.



## 1. Introduction

There is an emerging concern that surgery may trigger and accelerate the age-related cognitive impairment, which is referred as postoperative cognitive dysfunction (POCD).[1] The development of POCD has been reported to be associated with long-term disability and increased mortality.[2] Although some preventive strategies for POCD have been proposed in preclinical studies, as well as small exploratory clinical trials, no established interventions are currently available.[3,4] Specifically, neuroinflammation, *i.e.*, a maladaptive microglial activation and overproduction of cytokines, is a key in the pathogenesis of neurodegenerative processes including Alzheimer's disease (AD) and POCD.[1,5–8] Our recent study reported that the postoperative neuroinflammation may transit from acute to chronic in an age- and hippocampal-specific manner, resulting in the development of POCD.[9] Therefore, the acute neuroinflammation during the early postoperative period may be critical therapeutic target for POCD.

Resveratrol (RESV), a polyphenol present in red wine, is well-known to have beneficial biochemical properties, including anti-aging and anti-neuroinflammatory effects.[10–12] Indeed, a preclinical study reported the protective effects of 12 weeks oral RESV treatment on aging-induced cognitive impairment.[12] In addition, a clinical trial for AD showed that chronic oral treatment with RESV for 53 weeks reduces pro-neuroinflammatory factors in cerebrospinal fluid, improving cognitive function.[13,14] Differing from the long-term progression of normal aging and AD, POCD is a consequence of the acute neuroinflammatory response triggered by the surgical procedure.[9] Therefore, acute, high-dose regimen during perioperative period may be appropriate for POCD prevention. However, our preliminary

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study indicated that a preemptive injection with a single high-dose of free RESV, even at maximum administrable dose, failed to induce any neuro-cognitive protection in aged rats (Supplementary data 1). One plausible explanation for this may be due to the low bioavailability of RESV, particularly in the brain.[15] Alternatively, nanoemulsion based drug delivery systems has recently emerged as a promising strategy for overcome this issue, enhancing RESV solubility and improving permeation across the blood brain barrier.[16–20] Consistently, we hypothesized that nanoemulfised form of RESV can be effective for preventing the development of POCD.

RESV-induced neuroprotection is thought to be mainly mediated by allosteric activation of sirtuin-1 (silent mating type information regulation 2 homolog 1; SIRT1), a major gene associated with longevity.[10,21] SIRT1 is an evolutionarily conserved mammalian nicotinamide adenine dinucleotide-dependent protein deacetylase that is implicated in a wide range of aging-related diseases.[22] As SIRT1 is also reported to regulate microglial activity,[23] we further hypothesized that preoperative RESV treatment can act as an anti-neuroinflammatory agent *via* the SIRT1 pathway.

To test our hypothesis, we investigated the effects of a preoperative single dose of RESV-loaded nanoemulsion (emulsified RESV; *e*-RESV) on the development of POCD in an aged rat model of abdominal surgery. The effects of *e*-RESV on the microglial phenotype in the hippocampus were also assessed in *ex-vivo* preparations.

## 2. Materials and methods

### 2.1. Animals and experimental designs

All experiments were approved by the Institutional Animal Care and Use Committee of Kochi Medical School. Wistar male rats aged 19–22 months were purchased from Alfresa Shinohara Chemicals Corporation (Kochi, Japan). The animals were divided into three sets of experiments (Fig. 1). Experiment-1 assessed the effects of *e*-RESV on POCD using a 2 × 2 experimental design: *e*-RESV (maximum dose; 80 mg/kg) versus vehicle emulsion of *e*-RESV (*e*-vehicle) treatment by abdominal surgery versus anesthesia alone (n = 8 in each group). Experiment-2 was conducted to observe the dose-dependent effects of *e*-RESV (0, 2.0, 20, 40, or 60 mg/kg; n = 6 in each dose group). In Experiment-3, to perform the SIRT1-related antagonist experiment using a specific SIRT1 inhibitor, sirtinol, surgical animals were randomly assigned to four treatment groups (n = 8 in each group): dimethyl sulfoxide (DMSO, a vehicle of sirtinol) with *e*-vehicle treated, sirtinol (5.0 mg/kg) with *e*-vehicle

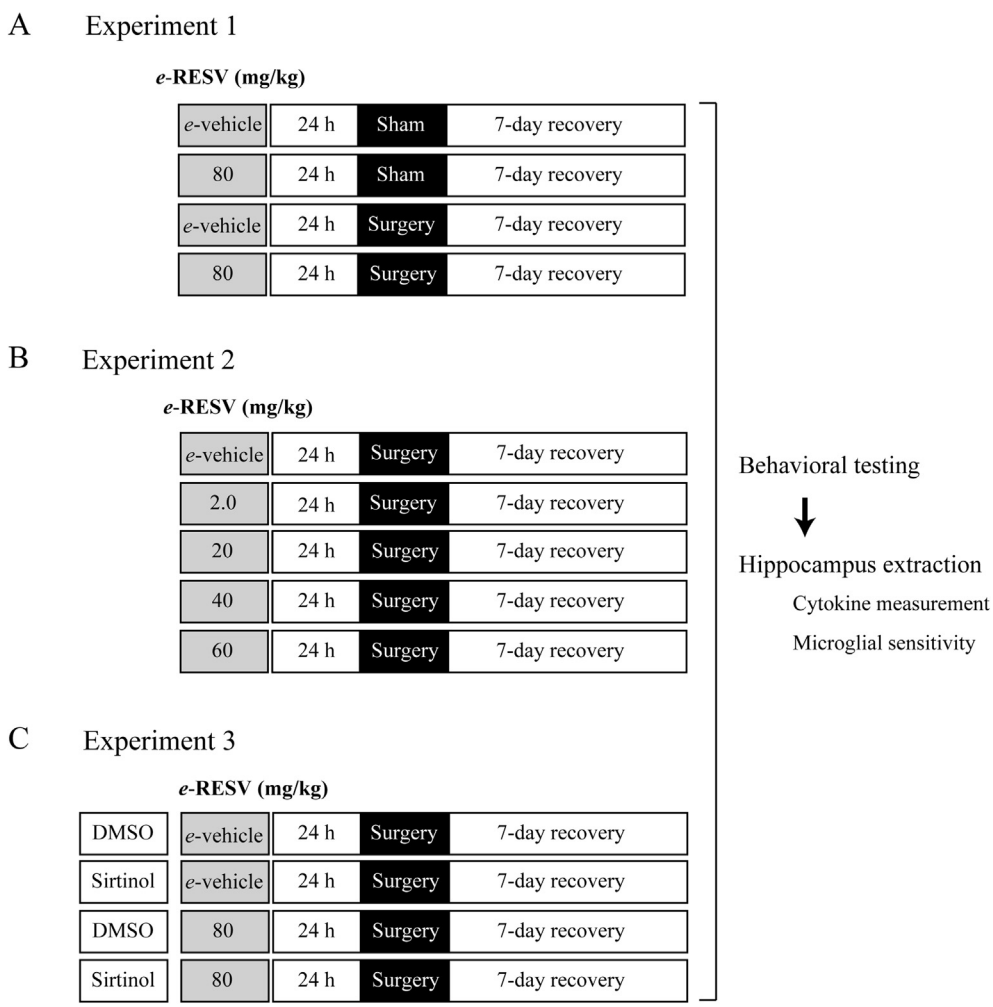


**Fig. 1. Schematic diagram of the three different experimental protocols.** (**A**) Experiment-1; Effects of emulsified resveratrol (*e*-RESV, 80 mg/kg) on postoperative cognitive impairment and related neuroinflammation (n = 8 in each group). (**B**) Experiment-2; Dose-dependent effects of *e*-RESV (0–60 mg/kg, n = 6 in each group). (**C**) Experiment-3; Antagonist study using sirtinol (n = 8 in each group).

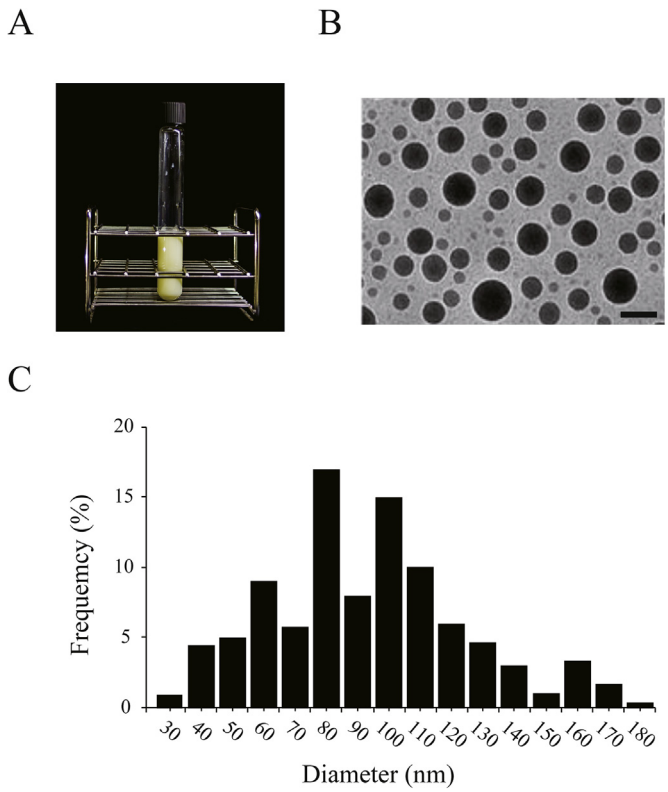


**Fig. 2. Emulisified resveratrol.** (**A**)Visual appearance. (**B**) Representative transmission electron microscope image. Nanoemusision droplets appear as dark micelles. Scale bar: 100 nm. (**C**) Size-distribution histogram analyzed by ImageJ counting over 300 emulisified resveratrol nanoparticles from a series of TEM images.

treated, DMSO with *e*-RESV (80 mg/kg) treated, and sirtinol (5.0 mg/kg) with *e*-RESV (80 mg/kg) treated.

### 2.2. Experimental model of abdominal surgery

Abdominal surgery was performed as *per* our previous study.[5] Briefly, laparotomy consisted in a 2.0-cm midline longitudinal incision into the peritoneum, small intestine exteriorization and manipulation for 3 min. During the inhalation period of 15 min, pulse rate, arterial oxygen saturation, and mean arterial blood pressure were measured noninvasively. Application of 0.2% ropivacaine (300 μl) was used for postoperative analgesia as our previous study.[24] Postoperative pain was rated by the rat grimace scale (RGS).[25] Locomotor activity was evaluated using the open-field test on postoperative day 7 based on the total accumulated counts of horizontal beam crosses for 60 min.

### 2.3. Preparation and administration of e-RESV

*e*-RESV was prepared by the low-energy emulsification technique based on previous study.[26] Briefly, two oils, orange oil and grape seed oil, were mixed together for 1 h, and RESV (TCI Development Co., Ltd, China) was then added to produce a final concentration of 0.5 g/ml. Following overnight stirring, a nonionic surfactant, polyoxyethylene sorbitan monooleate (Tween® 80, Sigma–Aldrich, St. Louis, MO), was added to the solution. After another hour of mixing, the resulting organic phase was added drop-wise to the aqueous phase (5.0 mM phosphate buffer, pH = 7), while stirred at 500 rpm for 10-min. The emulsion without RESV was used as the control *e*-vehicle. Either *e*-vehicle or *e*-RESV was administered intragastrically by gavage 24 h before surgery. Sirtinol (Sigma–Aldrich) was dissolved in DMSO (Sigma–Aldrich) based on the previous study,[27] and administered by an *i.p.* injection 24 h before surgery. To confirm *e*-RESV establishment, the emulsion was imaged with transmission electron microscopy (TEM). Briefly, particles were mounted on a Cu grid with a Formvar and carbon supporting film and stained with a 2% uranyl acetate solution. After drying, the stained samples were observed under a JEM-1400Plus electron microscope (JEOL, Japan). The nanoparticle size distribution was determined by analyzing the acquired images with the ImageJ software (version 1.51, National Institutes of Health, Bethesda, MD, USA).

### 2.4. Novel object recognition task

One week after surgery, cognitive function was assessed using a novel object recognition test, similar with our previous study.[5] On the testing day, during the familiarization phase, the animal was allowed to freely explore the open-field arena containing two identical objects for 5 min. After 1 h, in the testing phase, the rat was placed into the experimental chamber again with a new set of objects containing one identical and one novel for 5 min. Object

**Table 1**
Physiological parameters.

| Group | Mean arterial pressure (mmHg) | | Pulse rate (beats/min) | | Oxygen saturation (%) | | RGS | Total exploration time (seconds) |
|---|---|---|---|---|---|---|---|---|
| | Time 1 | Time 2 | Time 1 | Time 2 | Time 1 | Time 2 | | |
| **Experiment-1** | | | | | | | | |
| *e*-vehicle/sham | 98.5 ± 9.7 | 95.4 ± 10.0 | 369.2 ± 21.4 | 377.5 ± 26.7 | 97.5 ± 1.7 | 97.6 ± 1.5 | 0.11 ± 0.07 | 51.7 ± 8.2 |
| *e*-vehicle/surgery | 99.7 ± 10.4 | 97.2 ± 9.1 | 385.4 ± 25.0 | 385.3 ± 25.4 | 98.0 ± 1.1 | 98.2 ± 1.6 | 0.17 ± 0.02 | 48.7 ± 7.0 |
| *e*-RESV/sham | 101.0 ± 12.5 | 99.0 ± 10.6 | 370.1 ± 24.5 | 363.8 ± 30.6 | 98.1 ± 1.2 | 98.1 ± 1.4 | 0.12 ± 0.03 | 52.0 ± 6.8 |
| *e*-RESV/surgery | 103.6 ± 10.3 | 100.7 ± 9.4 | 381.6 ± 29.3 | 370.9 ± 28.8 | 98.4 ± 1.0 | 98.5 ± 1.5 | 0.21 ± 0.06 | 50.6 ± 9.1 |
| **Experiment-2** | | | | | | | | |
| *e*-vehicle | 96.4 ± 11.5 | 98.4 ± 7.7 | 375.2 ± 24.6 | 364.5 ± 25.4 | 97.2 ± 1.1 | 97.5 ± 0.9 | 0.14 ± 0.03 | 52.5 ± 10.4 |
| *e*-RESV 2 mg/kg | 95.2 ± 10.1 | 97.2 ± 8.0 | 387.5 ± 19.1 | 370.3 ± 29.6 | 97.6 ± 0.8 | 98.0 ± 0.9 | 0.19 ± 0.03 | 51.8 ± 8.0 |
| *e*-RESV 20 mg/kg | 101.7 ± 9.0 | 102.1 ± 8.8 | 370.3 ± 24.4 | 388.1 ± 27.1 | 98.1 ± 1.6 | 98.3 ± 1.7 | 0.15 ± 0.04 | 53.4 ± 7.1 |
| *e*-RESV 40 mg/kg | 95.3 ± 12.9 | 96.4 ± 10.6 | 381.0 ± 25.3 | 369.4 ± 30.2 | 98.0 ± 1.2 | 98.1 ± 1.1 | 0.14 ± 0.05 | 49.6 ± 6.6 |
| *e*-RESV 60 mg/kg | 94.6 ± 9.3 | 95.7 ± 8.7 | 370.6 ± 24.1 | 374.2 ± 25.0 | 98.4 ± 1.5 | 98.0 ± 1.4 | 0.20 ± 0.07 | 51.2 ± 7.8 |
| **Experiment-3** | | | | | | | | |
| DMSO/*e*-vehicle | 97.4 ± 8.5 | 99.5 ± 10.2 | 365.7 ± 19.6 | 367.4 ± 26.7 | 97.6 ± 1.5 | 97.8 ± 1.1 | 0.17 ± 0.05 | 56.7 ± 9.4 |
| Sirtinol/*e*-vehicle | 100.7 ± 11.3 | 97.2 ± 8.7 | 374.9 ± 25.0 | 371.5 ± 31.5 | 97.3 ± 1.0 | 98.0 ± 1.2 | 0.15 ± 0.03 | 53.0 ± 6.8 |
| DMSO/*e*-RESV | 93.1 ± 12.6 | 101.5 ± 7.9 | 371.0 ± 21.4 | 380.3 ± 25.1 | 97.9 ± 1.5 | 98.2 ± 1.0 | 0.18 ± 0.09 | 55.5 ± 7.0 |
| Sirtinol/*e*-RESV | 95.5 ± 9.0 | 96.2 ± 8.4 | 381.6 ± 25.8 | 375.0 ± 27.6 | 98.2 ± 1.7 | 98.0 ± 0.8 | 0.14 ± 0.05 | 51.4 ± 7.9 |

Each parameter was recorded at Time 1 – after induction of anesthesia, before procedure, and at Time 2 – immediately after procedure, before termination of anesthesia. RGS: rat grimace scale assessed 2 h after surgery. Total exploration time: total time spent exploring the two objects during the familiarization phase of novel object recognition test. Data were expressed as the mean ± standard deviation. Each group consisted of 8 animals.

exploration was defined as time spent sniffing the object with nose contact and/or within 1 cm. Recognition memory was expressed as the preference index; the ratio of time spent exploring an object during the familiarization phase or the novel object during the testing phase, over the total object exploration time.

### 2.5. Tissue collection and enzyme-linked immunosorbent assay

After behavior testing, animals were transcardially perfused, decapitated, and brains were harvested. The hippocampus was dissected, and then homogenized in ice-cold lysis buffer containing a protease inhibitor cocktail (P8340, Sigma–Aldrich). The hippocampal levels of interleukin-1β (IL-1β) and tumor necrosis factor-α (TNF-α) were analyzed using the ELISA kits for rat IL-1β (ER2IL1B, Thermo Scientific, USA) and TNF-α (438207, Biolegend, USA) according to the manufacturers' instructions. The data were normalized and expressed as pg TNF-α or IL-1β *per* mg tissue (pg/mg).

### 2.6. Isolation of hippocampal microglia

We further examined the effects of *e*-RESV on the pro-inflammatory phenotype of hippocampal microglia as previously described.[5] The hippocampus was digested with 0.1% trypsin and Dispase II (3.6 U/ml) for 1 h at 37 °C with shaking (100 strokes/min). The homogenates were centrifuged at 600 × g for 10 min at 4 °C. The pellets were re-suspended in 4 ml of 70% isotonic Percoll. The gradient was centrifuged at 2000 × g for 20 min. Microglial cells at the interface between the 70 and 37% Percoll layers were collected, and then plated at a density of $10^4$ cells/100 μl in DMEM containing 10% FBS. Before cell treatment, the medium was replaced with fresh serum-free medium followed by stimulation with lipopolysaccharide (LPS) at a concentration of 0.1, 1, 10, or 100 ng/ml, or media alone for 24 h at 37 °C in 5% $CO_2$. The IL-1β and TNF-α levels in the culture medium were measured by ELISA.

### 2.7. Statistical analysis

All data were expressed as the mean ± standard deviation (SD). For each dependent variable, group and/or other main effect(s) were tested with repeated measures ANOVA. Whenever ANOVA demonstrated significance, post hoc comparisons between the groups were performed in a pairwise manner by the Wilcoxon–Mann–Whitney test with Bonferroni correction. All data were analyzed using the statistical software SPSS (versions 11; SPSS Inc, Chicago, IL). Correlations between variables were analyzed by Pearson's correlation test. $P < 0.05$ was considered significant.

## 3. Results

The TEM images revealed that the *e*-RESV particles has a spherical morphology (Fig. 2B). The histogram shows a size range from 23 nm to 181 nm, and 62% of the total population was <100 nm (Fig. 2C). The mean particle diameter was 86.4 ± 12.7 nm (339 particles form 16 TEM images). All TEM images show almost an identical profile, indicating a high batch-to-batch reproducibility. In addition, no aggregations of *e*-RESV particles were visible under the optical microscope. These physicochemical features are largely consistent with a previous report.[26] No differences between groups in the physiological parameters during anesthesia and the RGS 2 h after surgery were observed (Table 1). In addition, the results of the open-field test conducted 6 days after surgery demonstrated no significant difference in any of the parameters measured within each treatment group (Supplementary data 2). Furthermore, the preemptive *e*-RESV regimen had no effect on blood coagulation parameters in aged rats (Supplementary data 3).

### 3.1. Novel object recognition performance

During the familiarization phase, there was neither intrinsic exploratory preference for either of the two objects nor significant difference in exploratory preference for the two objects in all groups. In addition, total exploration time did not differ within each treatment group (Table 1). These results implied that task motivation, curiosity, and ability during task performance were comparable among groups.

During the testing phase, the non-surgical control rats had a greater preference for the novel object compared with the familiar one (Fig. 3A, preference index: 77.3 ± 6.8%). The rats in the surgical group exhibited significantly impaired novel object recognition performance (Fig. 3A, preference index: 55.1 ± 8.2%, $p < 0.05$ *vs.* non-surgical control rats). However, pre-treatment with *e*-RESV

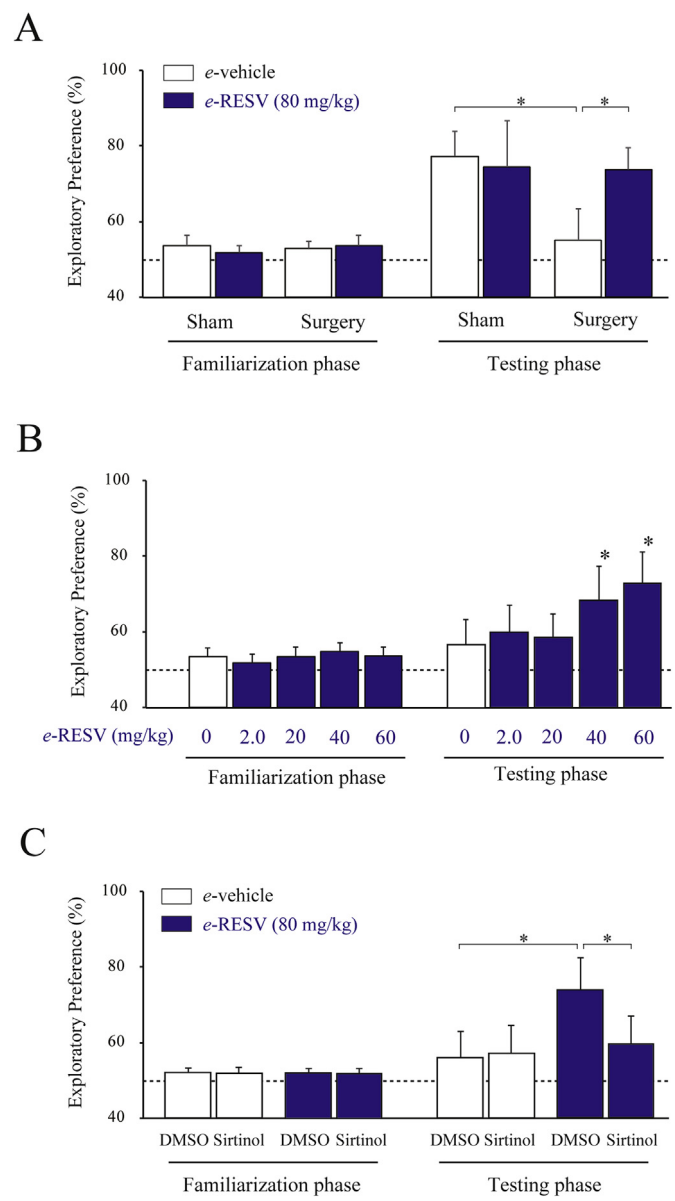


**Fig. 3. The results of the novel object recognition test.** The preference between two objects in the familiarization phase and testing phase of the novel object recognition test performed 7 days after sham or surgery is shown. Each vertical bar represents the mean ± SD. (**A**) Experiment-1, n = 8 in each group, $^*p < 0.05$. (**B**) Experiment-2, n = 6 in each group, $^*p < 0.05$ *vs.* *e*-RESV/0 mg/kg group. (**C**) Experiment-3, n = 8 in each group, $^*p < 0.05$.

significantly attenuated the surgery-induced cognitive impairment. In the dose-dependent experiment (Experiment-2), *e*-RESV inhibited surgery-induced memory deficit at 40 mg/kg or higher doses (Fig. 3B). Furthermore, in Experiment-3, although the sirtinol alone had no effect, it attenuated the effects of *e*-RESV (Fig. 3C). Neither *e*-RESV nor the surgical procedure had any significant influence on novel object recognition by adult rats (Supplementary data 4).

### 3.2. Levels of hippocampal cytokines

For the non-surgical rats, the average levels of hippocampal IL-1β and TNF-α were comparable between vehicle and *e*-RESV groups (Fig. 4A and Fig. 4B, respectively). However, for rats treated with the vehicle, levels of both cytokines were significantly higher in the surgery group than in the non-surgery group. These increases in both IL1-β and TNF-α were inhibited in the surgery with *e*-RESV group. The anti-neuroinflammatory effects of *e*-RESV were dose-dependent (Experiment-2; Fig. 4C and D) and blocked by sirtinol (Experiment-3; Fig. 4E and F). Taking all experimental groups in Experiment-1 together, novel object recognition performance in the testing phase was inversely correlated with the hippocampal levels of both IL-1β (Fig. 5A, n = 32; R2 = −0.692) and TNF-α (Fig. 5B, n = 32; R2 = −0.709). In another experiment using sentinel animals, plasma cytokine levels increased after surgery, whereas these increases returned to baseline levels within 3 days after surgery (Supplementary data 5). In addition, *e*-RESV had no effect on plasma cytokine levels after surgery.

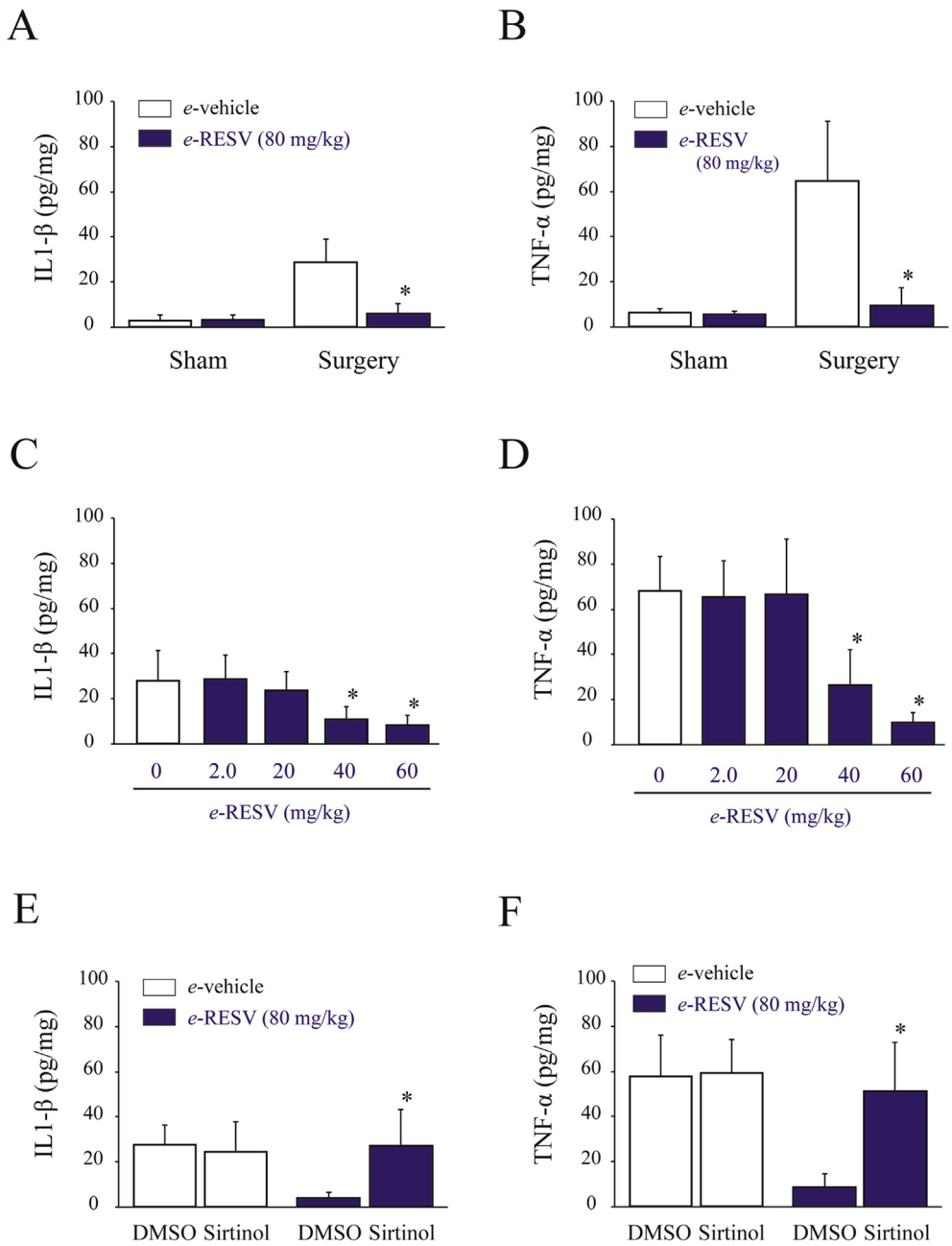


**Fig. 4. The levels of proinflammatory cytokines in the hippocampus.** The average levels of interleukin (IL)-1β and tumor necrosis factor (TNF)-α in each group in Experiment-1 (A and B, n = 8 in each group, $^*p < 0.05$), Experiment-2 (C and D, n = 6 in each group, $^*p < 0.05$ *vs.* *e*-RESV/0 mg/kg group), and Experiment-3 (E and F, n = 8 in each group, $^*p < 0.05$) are shown. Each vertical bar represents the mean ± SD.

### 3.3. Ex vivo microglial sensitivity to LPS

As the primary source for cytokines, microglia play as mediators of neuroinflammation.[28] Therefore, to investigate whether *e*-RESV influences microglial immunosensitivity, we measured the LPS sensitivity of hippocampal microglia isolated from animals in each group. In Experiment-1, there was no difference in baseline levels of IL-1β (Fig. 6A) and TNF-α (Fig. 6B) in all experimental groups. In microglia isolated from non-surgical groups, the LPS-induced increase in IL-1β was greatly attenuated in the *e*-RESV group compared with that in the vehicle group (Fig. 6A). On the other hand, the LPS-induced increase in IL-1β in the non-surgery with vehicle group was significantly exaggerated in the surgery with vehicle group. With respect to TNF-α levels, we found similar main effects among groups as those observed for IL-1β. Furthermore, the *e*-RESV-induced microglial anti-neuroinflammatory phenotype change was dose-dependent (Experiment-2; Fig. 6C and D), and attenuated by sirtinol (Experiment-3; Fig. 6E and F).

## 4. Discussion

In this study, we demonstrated that a prophylactic dose of *e*-RESV can prevent the development of cognitive deficits and related neuroinflammation in aged rats. The preoperative *e*-RESV regimen had no effect on surgery-induced systemic inflammatory responses, indicating that directly acts on the brain. Furthermore, our *ex-vivo* experiments confirmed that *in-vivo e*-RESV treatment leads to a reduction in microglial immune sensitivity. The neuroprotective benefits of *e*-RESV are almost completely inhibited by the concomitant administration of sirtinol, a specific SIRT1 inhibitor, suggesting involvement of the SIRT1 signaling pathway. These findings imply that a single preoperative dose of *e*-RESV provides effective perioperative neuro-cognitive tolerance, especially in vulnerable brains, *via* microglia-based anti-neuroinflammation.

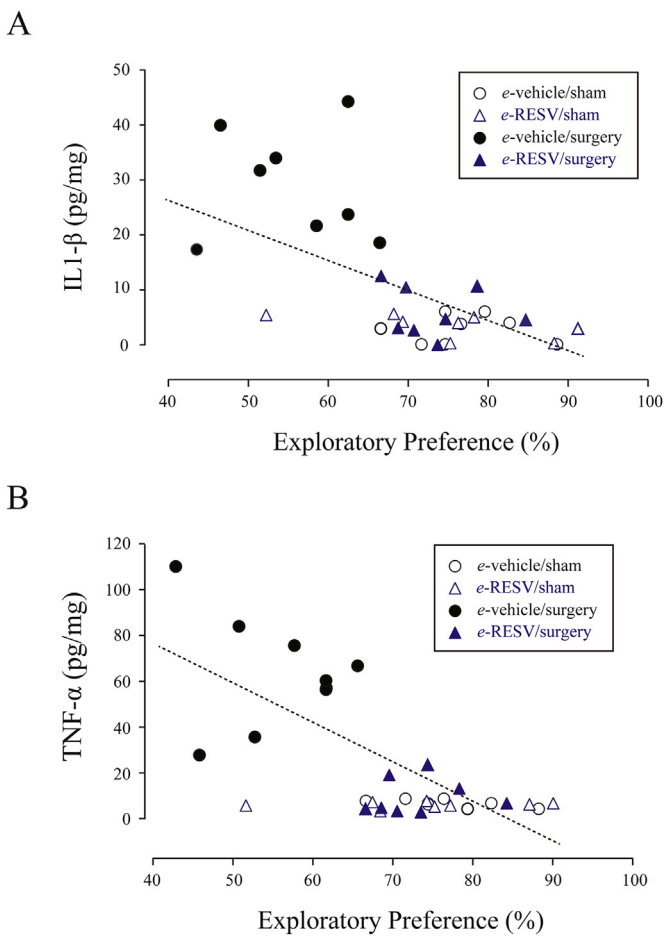


**Fig. 5. The relationship between the levels of proinflammatory cytokines and cognitive function after surgery**. Correlation of the levels of interleukin (IL)-1β (**A**) or tumor necrosis factor (TNF)-α (**B**) in the hippocampus of aged rats with the novel object recognition performance of each donor rat at the testing phase, demonstrating an inverse relationship. Data points are classified into four experimental groups in Experiment-1 (n = 8 in each group).

Although there is promising *in-vitro* biopharmacological data for RESV, its therapeutic use is limited due to low bioavailability.[29] Many strategies have been applied to overcome this issue, and the self-nanoemulsifying drug delivery system is currently attracting the most attention.[16] In practice, *e*-RESV has been reported to increase oral bioavailability and enhance *in-vivo* pharmacological efficiency.[17–19,30] Furthermore, our TEM analysis show that the majority of *e*-RESV nanoparticles achieved a sub-100 nm diameter (Fig. 2C), which is reported to be able to cross the blood–brain barrier.[29] Indeed, systematic injection regimen (*i.p.*) of RESV failed to induce neuro-cognitive protection (Supplementary data 1). Therefore, *e*-RESV may be an efficient nano-carrier that enhances both oral and brain bioavailability. In addition, the low-energy emulsification technique used in this study can be produced without specialized equipment, reducing production costs.[26] As such, *e*-RESV provides novel translational therapeutic options for neuroinflammation associated with the central nervous system.

Previous studies suggest that neuroinflammation plays a pivotal role in the underlying mechanism of POCD.[1,5–7] The surgery-induced peripheral inflammation may be transmitted to the brain through humoral and neural pathways, leading to microglial activation and neuroinflammatory response.[31] Moreover, we previously reported that aged microglia exaggerated pro-inflammatory cytokines release in response to immune stimulation, compared with young microglia.[5,28] This age-related phenotype change may explain why elderly patients are at a greater risk for POCD. Indeed, POCD did not develop in adult rats when they were subjected to our surgical model (Supplementary data 4). Therefore, the preemptive optimization of the age-related microglial pro-inflammatory phenotype may be beneficial for preventing the development of POCD. In this context, we provide the first preclinical evidence that *e*-RESV can be used as a mechanism-based preventive strategy against POCD, especially for elderly patients.

In the present study, the surgical procedure itself did not influence microglial reactivity in response to immune challenge with LPS (Fig. 6A). In contrast, the pre-treatment with *e*-RESV reduced the microglial immune sensitivity regardless surgical treatment in a SIRT1-dependent manner. Taken together, a single high-dose of *e*-RESV may induce microglial rejuvenation, making the vulnerable brain more tolerant against POCD development. Consistent with this, a previous study demonstrated that SIRT1 activation reduced inflammatory processes in mice microglia cell lines N9 and BV2.[23] Nevertheless, the preemptive effects of *e*-RESV may be transient in nature, and the accurate administration window remains unknown. However, the present findings indicate that our single dose regimen may be sufficient during the perioperative period.

Elderly patients are more susceptible to adverse drug reactions due to altered pharmacokinetics and pharmacodynamics.[32] RESV at therapeutic dose levels is not known to be toxic or cause significant adverse effects. Furthermore, some clinical trials presented a good safety profile of RESV in elderly patients with AD.[13,14] Accordingly, RESV may be well tolerated even in elderly patients. However, some *in-vitro* experiments have reported anticoagulant activity of RESV, suggesting an increased risk of hemorrhage during surgery.[33] Preemptive *e*-RESV may minimize this due to its rapid metabolism and

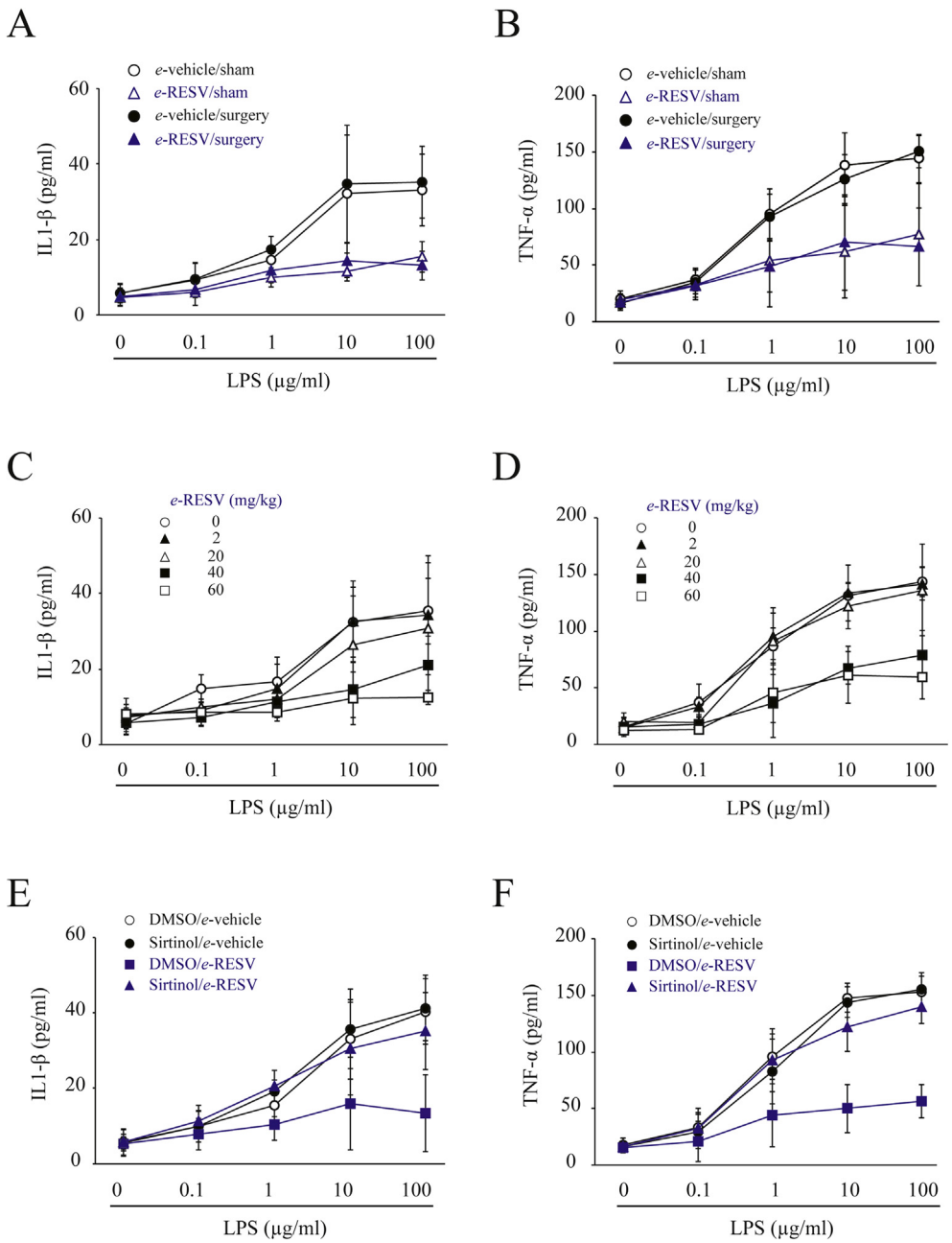


**Fig. 6. Concentration–response effects of *ex vivo* stimulation with lipopolysaccharide (LPS) on the production of interleukin (IL)-1β and tumor necrosis factor (TNF)-α in cultured microglia**. Hippocampal microglia were isolated from rats after the completion of cognitive testing in Experiment-1 (**A** and **B**, n = 8 in each group), Experiment-2 (**C** and **D**, n = 6 in each group), and Experiment-3 (**E** and **F**, n = 8 in each group). Cultured microglia were stimulated with 0.1, 1, 10, or 100 ng/ml, or media alone, and levels of IL-1β and TNF-α were measured from supernatants collected 24 h later. Each vertical bar represents the mean ± SD.

elimination from blood. Indeed, we found neither signs of bleeding nor changes in blood coagulation parameters in the *e*-RESV-treated surgical animals (Supplementary data 3). In addition, the *e*-RESV ingredients are widely used in cosmetics, food products, and pharmaceutical formulations. Nevertheless, the safety profile of *e*-RESV drug delivery system in human remains under investigated.

There are some limitations that should be addressed. First, we previously reported that neuroinflammation associated with POCD was found mainly in the hippocampus, and thus the present study focused on this brain region. However, *e*-RESV may interact with other brain regions. Second, since both TNF-α and IL-1β has been shown to play pivotal roles in neuroinflammation, we assessed these two cytokines. However, other molecules are reported to be constitutively expressed in activated microglia. Third, previous studies reported that nanoemulsion drug delivery system could increase the brain bioavailability of RESV,[18–20] while actual *in vivo* pharmacokinetic data of *e*-RESV is currently lacking. In addition, our TEM analysis showed a positive dark staining of *e*-RESV particles despite the standard negative staining method (Fig. 2A). Indeed, mixed results, *i.e.*, dark and light staining, has been reported using similar technique, suggesting that the staining outcomes may depend on each chemical condition.[34–36] Furthermore, we cannot rule out the possibility for the oil phase to crystallize and form solid particles, increasing contrast in TEM. If that's

the case, the *in vivo* pharmacokinetics of *e*-RESV may be affected.[37] Therefore, future pharmacology and preclinical studies are needed before our findings can be translated into clinical practice.

In the present study, the hippocampal neuroinflammation-associated POCD was attenuated by a preemptive dose of *e*-RESV, but not by the free form of RESV. The *e*-RESV drug delivery system may enhance the brain bioavailability, and reduce the microglial immune sensitivity *via* the SIRT1 pathway. As the safety profile of RESV has been well-established, our approach warrants further study with respect to the translational potential for POCD clinical management.

## Conflicts of interest

All authors have no financial or scientific conflict of interest regarding the research described in this manuscript.

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## Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.jphs.2018.08.006.

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