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Targeting Hypoxia-Inducible Factor 1-Alpha with 2-Methoxyestradiol as a Potential Treatment for Sepsis-Associated Encephalopathy: A Systematic Review

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Abstract

Hypoxia-inducible factor 1-alpha (HIF-1α) is a critical biomarker in sepsis, mediating cellular responses to hypoxia, regulating immune cell survival, and promoting inflammation. Sepsis-associated encephalopathy (SAE) is a severe complication characterized by cognitive dysfunction and increased mortality, driven by cytokine-induced blood-brain barrier (BBB) disruption, neuroinflammation, microglial activation, impaired cerebral perfusion, and oxidative stress. This systematic review investigates the therapeutic potential of directly inhibiting HIF-1α with 2-methoxyestradiol (2ME2) to mitigate SAE pathogenesis, focusing on evidence from rat models of neuroinflammation, and explores the translational relevance for human treatment. A systematic literature search was conducted using Covidence software across PubMed, Embase, and Scopus. 11 studies were included in the final analysis.

2ME2 was evaluated across three rat models: cerebral ischemia (n = 6), traumatic brain injury (n = 2), and subarachnoid hemorrhage (n = 3). In all models, 2ME2 administration resulted in significant reductions in HIF-1α expression, neuronal cell death, BBB permeability, and cerebral edema, alongside improved neurological function. The most effective outcomes were observed with 2.5-16 mg/kg dosages. Lower doses showed limited efficacy, while higher doses were associated with increased mortality.

Preclinical evidence supports 2ME2 as a promising therapeutic candidate for SAE, potentially exerting neuroprotective effects by reducing pro-inflammatory cytokine release, microglial activation, and BBB disruption. Furthermore, 2ME2 may have broader applicability in treating traumatic brain injury and intracranial hemorrhage. Nonetheless, further studies are warranted to evaluate its safety and efficacy in both animal models of sepsis and future human clinical trials.

1. Introduction

Sepsis is a life-threatening condition caused by a dysregulated host response to infection and is estimated to account for approximately 20% of all global deaths [1]. Despite advances in critical care, it remains a major focus of research due to its high morbidity and mortality. A common yet underrecognized complication of sepsis is sepsis-associated encephalopathy (SAE)—a clinical diagnosis made by exclusion, characterized by acute alterations in mental status in the absence of overt central nervous system (CNS) infection [2]. The prevalence of SAE varies, with one retrospective study reporting its presence in 53% of 2,513 ICU patients with sepsis [3], while other sources estimate rates as high as 71% [4], particularly among older patients [5]. SAE has been independently associated with higher mortality, greater ICU resource utilization, and longer hospital stays [6]. Additionally, both animal and human studies increasingly demonstrate that SAE may lead to long-term cognitive and psychological impairments [7, 8, 9].

A key factor implicated in SAE pathogenesis is hypoxia-inducible factor 1-alpha (HIF-1α) [10]. In the presence of lipopolysaccharide (LPS), HIF-1α upregulates the production of pro-inflammatory cytokines such as TNF-α, IL-1β, and IL-6, as well as blood–brain barrier (BBB) permeability-inducing factors including vascular endothelial growth factor (VEGF), matrix metalloproteinases (MMPs), and aquaporin channels (AQPs) [11]. This disruption of the BBB facilitates the infiltration of peripheral

immune cells, cytokines, and reactive oxygen species (ROS) into the brain parenchyma, triggering microglial activation and neuroinflammation [12].

Resultant damage to the CA1 and CA3 regions of the hippocampus is believed to contribute to the cognitive deficits observed in SAE patients [13]. Clinically, HIF-1α has been validated as a biomarker for sepsis severity and prognosis [14]. Although HIF-1α inhibitors have demonstrated efficacy in mitigating SAE-related pathology and improving outcomes in animal models, they have yet to be evaluated in human clinical trials for sepsis [15, 16, 17]. One promising candidate is 2-methoxyestradiol (2ME2), an endogenous estrogen metabolite and potent HIF-1α inhibitor. In a 2010 phase I trial for advanced solid malignancies, 2ME2 was found to be generally well tolerated [18].

Mechanistically, 2ME2 binds to the colchicine site on tubulin, disrupting microtubule function and inhibiting the intracellular transport necessary for HIF-1α stabilization and accumulation [19, 20]. Given its dual action—both on microtubules and HIF-1α—along with a favorable safety profile, 2ME2 is a potential therapeutic candidate for SAE. However, a gap remains in research regarding its application in SAE-specific models. This systematic review aims to evaluate the neuroprotective and anti-inflammatory mechanisms of 2ME2 in animal models of neuroinflammation that share pathophysiological overlap with SAE. The ultimate goal is to assess its potential as an adjunctive therapy for mitigating SAE progression and improving clinical outcomes.

2. METHODS

2.1. Protocol and Guidance

This systematic review was conducted in accordance with the *Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) 2020* guidelines [21]. The study protocol was registered in the PROSPERO database (Registration ID: CRD420250641546).

2.2. Eligibility Criteria

Studies were included based on the PICOS framework (Participants, Interventions, Comparators, Outcomes, and Study Design) [22]:

- **Participants:** Animal models with experimentally induced neuroinflammatory conditions associated with elevated HIF-1α expression.
- **Interventions:** Neurological injury models including ischemia, traumatic brain injury (TBI), and subarachnoid hemorrhage (SAH).
- **Comparators:** Placebo, pharmaceutical co-treatments, or no treatment.
- **Primary Outcome:** HIF-1α expression following 2ME2 treatment.
- **Secondary Outcomes:** Measures related to neurological dysfunction aligned with SAE pathogenesis
- **Study Design:** Peer-reviewed experimental studies published and mortality rates post-treatment.in English.

The following types of publications were excluded: systematic reviews, meta-analyses, literature reviews, editorials, commentaries, case studies, observational studies, conference abstracts, and articles published in languages other than English.

2.3. Search Strategy

A comprehensive literature search was performed using Covidence software to identify relevant experimental studies investigating the effects of 2ME2 on HIF-1α inhibition in rodent models of neuroinflammation. Three electronic databases were searched: PubMed, Embase, and Scopus. The search strategy used the following terms: "(Hypoxia-Inducible Factor-1-alpha) AND ((neurological) OR (cerebral)) AND ((Outcome) OR (treatment) OR (therapy))."

2.4. Study Selection

Studies were screened and selected in two phases using predefined inclusion and exclusion criteria. In the first phase, titles and abstracts were reviewed. In the second phase, full-text screening was conducted. At each stage, two independent reviewers assessed each study and classified it as *"include," "exclude,"* or *"maybe."* Studies were only advanced to the next stage with mutual agreement. Discrepancies were resolved through group discussion among all four reviewers until consensus was reached.

2.5. Data Extraction

Data were extracted independently by two reviewers using a standardized Google Sheets template. Information was collected from text,

tables, graphs, and figures. After initial extraction, a cross-review was performed by assigning each reviewer to validate another's data for accuracy and completeness. The first author provided oversight to ensure consistency and prompt resolution of discrepancies. If essential data were missing, the corresponding study author was contacted via email and text, with a 24-hour response window. Extracted data included:

- Study design and sample size
- Type of neurological injury model
- 2ME2 dosage and administration route
- Timing of intervention
- Reported outcomes related to HIF-1α levels, neurological recovery, injury severity, and therapeutic efficacy

2.6. Quality Assessment

The quality of each included study was assessed using the *CAMARADES* checklist, which evaluates 10 criteria:

1. Publication in a peer-reviewed journal
2. Temperature control
3. Random allocation to treatment/control
4. Blinded model induction
5. Blinded outcome assessment
6. Use of an anesthetic without known neuroprotective properties
7. Use of an appropriate animal model
8. Sample size calculation
9. Compliance with animal welfare regulations
10. Declaration of potential conflicts of interest

Two reviewers independently scored each study (1 = criterion met, 0 = not met), tallied scores, and resolved disagreements through discussion. Unresolved discrepancies were adjudicated by the first author.

2.7. Risk of Bias Assessment

Risk of bias was evaluated using the *SYRCLE Risk of Bias* tool. Studies were assessed across multiple domains, including selection, performance, detection, attrition, and reporting biases. Each criterion was scored as:

- 1 = criterion met
- 0 = criterion not met

Each study was independently assessed by two reviewers within each group, and discrepancies were resolved through discussion. The first author made final decisions when consensus could not be reached.

3. RESULTS

3.1. Study Selection and Characteristics

The *PRISMA 2020* study selection process is outlined in Figure 1. After duplicates were removed, the search yielded 744 articles, of which 17 studies were selected for full-text review following title and abstract screening. Six studies were excluded—one due to retraction, four due to wrong intervention, and one due to incorrect study design—resulting in 11 studies included in the final analysis.

Table 1 summarizes the characteristics of the included studies, which were published between 2007 and 2022. All were randomized controlled trials (RCTs) involving in vivo rodent models of neuroinflammation, including ischemia (n = 6), SAH (n = 3), and TBI (n = 2). Animal models used included Sprague-Dawley rats, male C57/BL6 mice, and male C57B1/6N mice.

2ME2 was administered either intraperitoneally or intravenously (via femoral or tail vein), using dimethyl sulfoxide (DMSO) or isotonic saline as solvents. Doses ranged from 1 mg/kg to 150 mg/kg, and administration occurred up to three hours post-injury. Neurological damage was assessed through outcomes including BBB permeability, neuronal cell death, infarct/contusion volumes, cerebral edema, and behavioral assessments. 10 studies measured cerebral cell death, 8 studies assessed BBB permeability or contributing factors, 5 studies evaluated cerebral edema, 6 studies assessed neurological behavior, and 4 studies reported mortality rates.

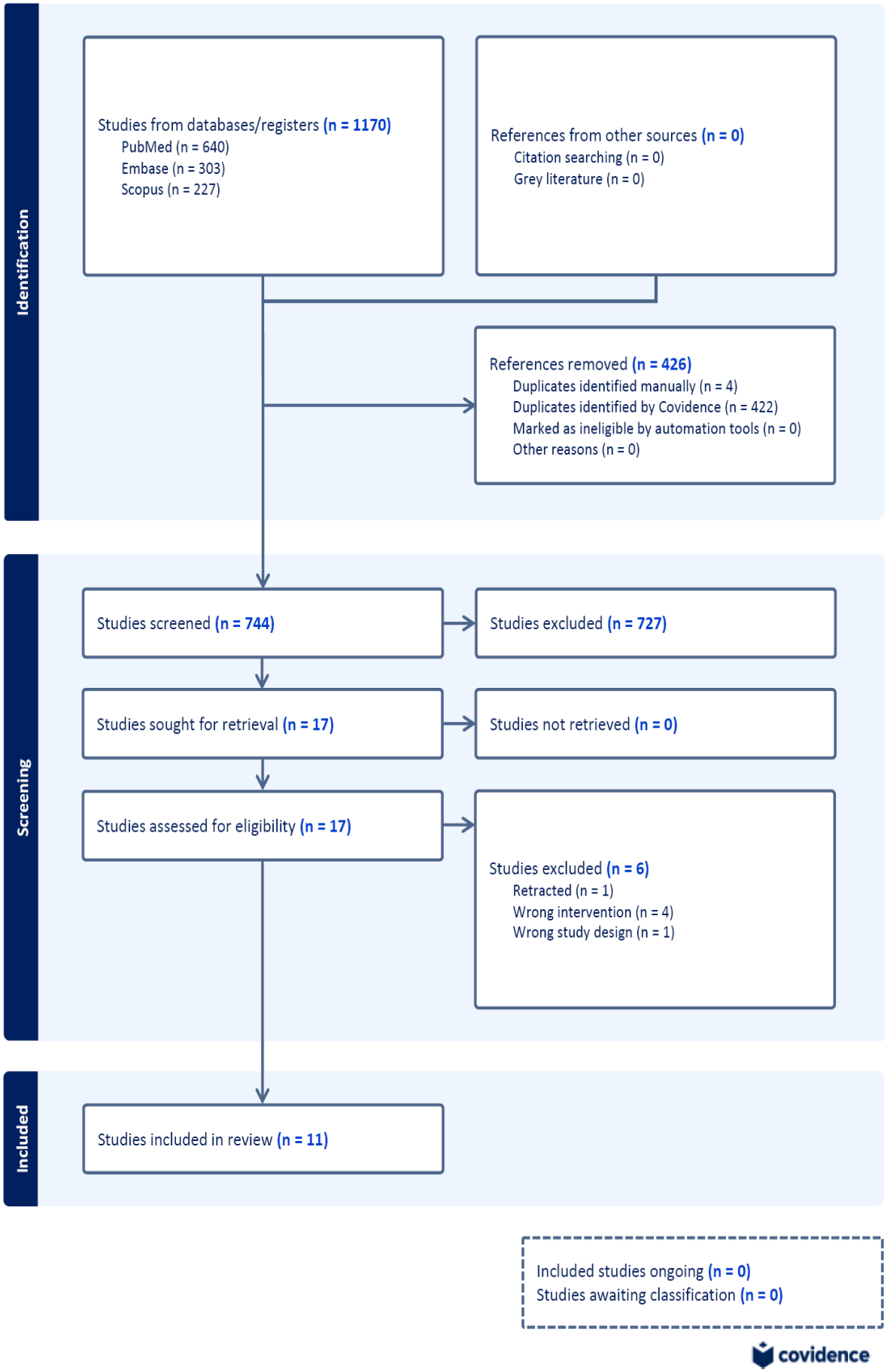

Figure 1. *PRISMA 2020 flow diagram of the systematic review.*

Table 1. *Summary Table of Included Studies.*

Study ID (First Author, Year)	Animal Species	Model of Injury	2ME2 Dosage, Delivery Method, and Timing	Treatment Group	Comparator	2ME2 Effect on Neurological Outcomes and Mortality*	2ME2 Effect on SAE Mediators*
Luo C, 2017 [23]	Male C57/BL 6 mice	tMCAO	1 μM IP 30 min after tMCAO	tMCAO + DEX + 2ME2	tMCAO + DEX	Increased infarct volumes	Decreased Bcl-2
Chen W, 2008 [24]	7-day-old postnatal Sprague-Dawley rats	Modified Rice-Vannucci model of hypoxic-ischemic (HI) injury (Rice et al., 1981)	100 μL of 1.5, 15, and 150 mg/kg IP 5 min after HI; 15 mg/kg 3 h after HI	HI + 2ME2	HI	Dose-dependent decrease in infarct volumes at 15 mg/kg and 150 mg/kg given at 5 min, but not 3 h after HI; 15 mg/kg 2ME2 given 5 min after HI decreased neuronal cell death in CA1 and CA3 hippocampal regions; 6/10 rats in 150 mg/kg group died; Decreased penetration of IgG into brain parenchyma; Decreased brain water	Decreased VEGF
Chen C, 2007 [25]0	Male Sprague-Dawley rats	MCAO	2 mL of 5 mg/kg IP 1 h after reperfusion (3 hours after MCAO)	MCAO + 2ME2 + DMSO	MCAO + DMSO	Decreased infarct volumes; Decreased Evans blue dye extravasation of BBB; Improved neurological deficits; Decreased 7-day mortality	Decreased VEGF, BNIP3, and cleaved caspase-3
Chen C, 2010 [26]	Male Sprague-Dawley rats	Modified MCAO (Kawamura et al., 1991)	5 mg/kg and 15 mg/kg IP of 2ME2 at reperfusion (90 min after MCAO)	MCAO + 50% dextrose + 2ME2	MCAO + 50% dextrose	Both dosages decreased infarct volumes at 24 and 72 h after reperfusion; Both dosages improved neurological deficits	Decreased VEGF, MMP-2, and MMP-9
Wang Y, 2018 [27]	Rats (unspecified)	Ischemia-reperfusion (I/R) injury (Longa et al., 1989; Belayev et al., 1996)	2 mL of 5 mg/kg IP at same time of ischemia	I/R + 2ME2	I/R	Improved neurological function score; Decreased cerebral infarction ratio; Decreased TUNEL-positive cells	Decreased Bcl-2 and Bax
Higashida T, 2011 [28]	Male Sprague-Dawley rats	tMCAO	16 mg/kg IP at same time of MCAO	tMCAO + 2ME2	tMCAO	Decreased brain water content at 1 and 24 h after MCAO	Decreased AQP-4 and AQP-9
Xiong A, 2022 [29]	Adult male Sprague-Dawley rats	Controlled cortical impact (CCI) injury model of TBI	2.5 mg/kg IV via tail vein within 30-60 min after TBI	TBI + 2ME2 + NS	TBI + NS	21 days post-TBI: Decreased brain water content; Improved neurological functioning via Morris water maze tests; Increased hippocampal CA1 NeuN-positive neurons	Decreased AQP-4, MMP-9, VEGF, and pTau/Tau ratio; Increased occludin, claudin-5, MAP2, SYN
Yan J, 2006 [30]	Male Sprague-Dawley rats	SAH (Bederson et al., 1995 with modifications by Gules et al., 2002 and Kusaka et al., 2004)	5 mg/kg IP 1 h after SAH	SAH + 2ME2 + DMSO	SAH + DMSO	Decreased neurological deficits at 12, 36, and 48 h after SAH (D609 only decreased at 36 and 48 h); Decreased TUNEL staining in endothelial and smooth muscle cells; Decreased mortality	Decreased VEGF and BNIP3
Wu C, 2013 [31	Male Sprague-Dawley rats	Endovascular perforation SAH model	2 mL of 5 mk/kg IP 1 h after SAH	SAH + 2ME2 + DMSO	SAH + DMSO	Improved neurologic scores; Reduced brain water content; Decreased EB dye extravasation of BBB; Decreased TUNEL-	Decreased BNIP3 and VEGF

		(Bederson et al. 1995) with slight modifications				positive cells in ipsilateral basal cortex; Non-significant decrease in mortality	
Hu Q, 2022 [32]	Male Sprague-Dawley rats	Endovascular puncture model of SAH (Xie et al., 2018)	5 mg/kg IP 1 h after SAH	SAH + 2ME2 + DMSO	SAH + DMSO	24 h after SAH: Improved neurological functions; Reduced TUNEL staining in ipsilateral basal cortex; Decreased brain water content; Decreased Evans blue dye extravasation of BBB; Non-significant decrease in mortality	Decreased microglial activation; Decreased IL-1β, IL-6, TNF-α, MMP-9, and VEGF; Increased ZO-1 and occludin
Schaible E, 2014 [33]	Male C57B1/6N mice	CCI injury model of TBI	10 mg/kg and 20 mg/kg IP 30 min after TBI	TBI + 2ME2 + DMSO	TBI + DMSO	24 h after SAH: Only significant reduction in contusion volumes with 20 mg/kg ; Decreased TUNEL-positive cells in the lesioned cortical hemisphere only with 20 mg/kg	Decreased BNIP3 and TNF- α; Non-significant decreases in IL-1β, IL-6, and VEGF

** = All reported results are statistically significant ($p < 0.05$ or less) unless otherwise noted.*

3.2. Risk of Bias and Quality of Evidence

The risk of bias is depicted in Figure 2. Six studies were deemed at low risk of bias, and five at unclear risk. None were rated as high risk.

Quality of evidence is reported in Table 2. The mean quality score across included studies was 6.58 (SD: 1.24), with scores ranging from 5 to 9.

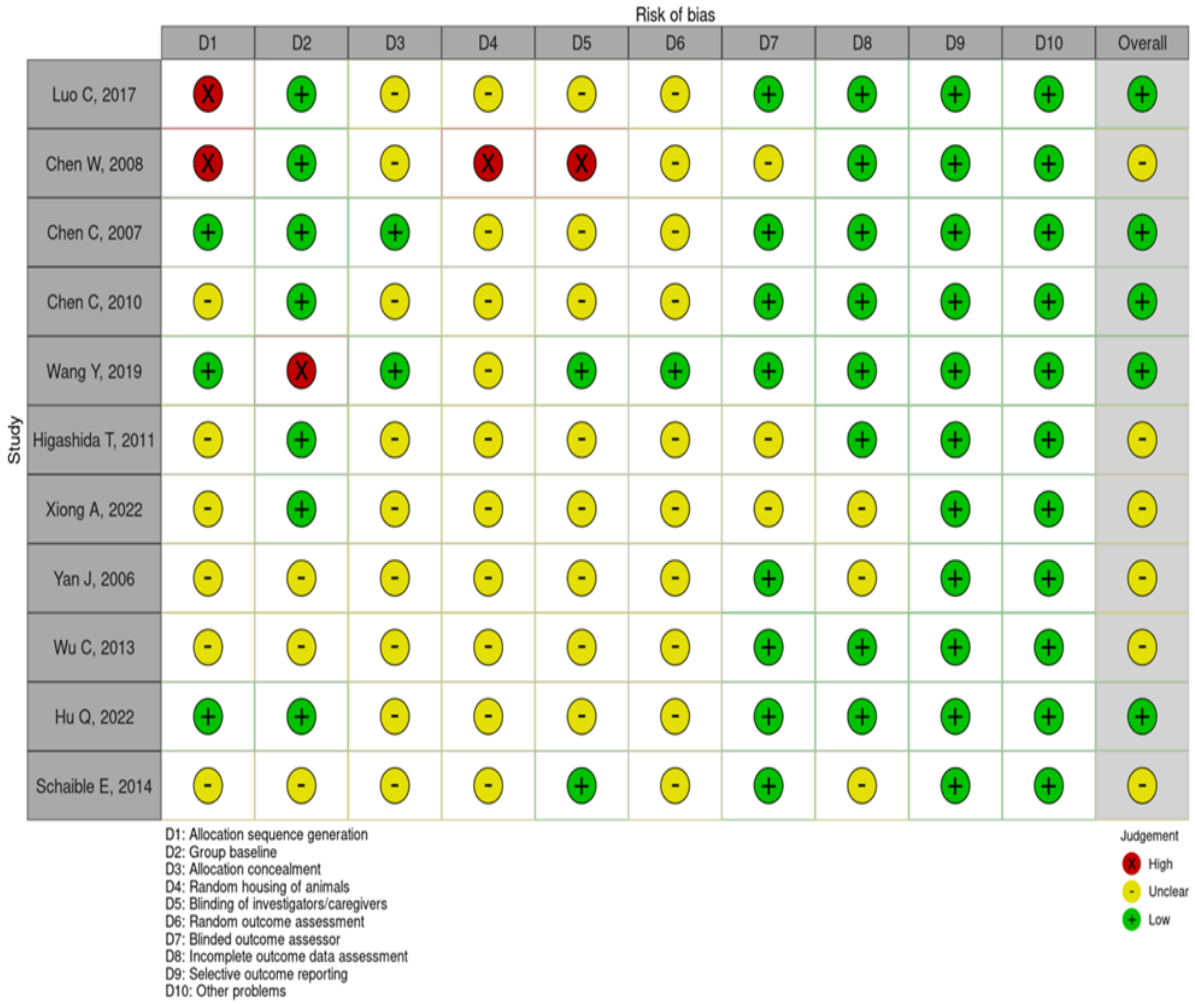

Figure 2. *Risk of bias of included studies using the SYRCLE Risk of Bias tool. This figure was generated using the Risk-of-bias VISualization (robvis) tool [46].*

Table 2. *CAMARADES checklist for quality of evidence of included studies.*

Study ID (First Author, Year)	Peer-Reviewed Journal	Temperature Control	Randomized	Blinded Model	Blinded Assessment of Outcome	Anesthetic without Significant Neuroprotective Activity	Appropriate Animal Model	Sample Size Calculation	Compliance with Animal Welfare Regulations	Statement of Conflicts of Interest	Final Score
Luo C, 2017 [23]	1	1	0	1	1	0	1	1	1	1	8
Chen W, 2008 [24]	1	1	0	0	0	0	1	1	1	0	5
Chen C, 2007 [25]	1	1	1	9	1	0	1	1	1	0	7
Chen C, 2010 [26]	1	1	0	0	1	0	1	1	1	0	6
Wang Y, 2018 [27]	1	1	1	0	0	0	1	1	0	1	6
Higashida T, 2011 [28]	1	1	0	0	0	0	1	1	1	0	5
Xiong A, 2022 [29]	1	0	1	0	0	0	1	0	1	1	5
Yan J, 2006 [30]	1	1	1	0	1	0	1	1	1	0	7
Wu C, 2013 [31]	1	1	1	0	1	0	1	1	1	0	7
Hu Q, 2022 [32]	1	1	1	0	1	0	1	0	1	1	7
Schaible E, 2014 [33]	1	1	1	1	1	0	1	1	1	1	9

3.3. Primary Outcome: HIF-1α Expression

All eleven studies reported elevated HIF-1α levels following neurological injury. Treatment with 2ME2 led to a significant reduction in HIF-1α expression. In addition, 2ME2 decreased HIF-1α levels more effectively than tricyclodecan-9-yl-xanthogenate (D609) (12.93 ± 3.91 vs. 21.67 ± 2.50) and slightly more than YC-1, another known HIF-1α inhibitor.

3.4. Secondary Outcomes

3.4.1. Cerebral Cell Death

All ten studies measuring cell death reported significant reductions following 2ME2 treatment. Five studies showed decreased TUNEL staining (indicating reduced DNA fragmentation) in the ipsilateral cortex. Five studies reported significant reductions in infarct volumes. Conversely, one study reported increased infarct volume when 2ME2 was co-administered with dexmedetomidine (DEX). Three studies observed reduced levels of B-cell leukemia/lymphoma 2 (BCL-2), BCL2/adenovirus E1B interacting protein 3 (BNIP3), BCL-2 associated X (Bax), or cleaved caspase-3, key markers of apoptosis. Two additional studies demonstrated the preservation of hippocampal neurons, specifically the CA1 and CA3 regions. One study using Nissl staining reported increased numbers of surviving neurons in the penumbral zone. One study found significantly decreased contusion volume following TBI and 2ME2 treatment. Dose and timing appeared to influence the efficacy of 2ME2. For instance, Chen et al. (2008) found reduced infarct volumes at 15 mg/kg (18 ± 2%) and 150 mg/kg (10 ± 3%), but not at 1.5 mg/kg (30 ± 1%). The 15 mg/kg dose of 2ME2 was used for the rest of the experiment and was found to be effective when administered five minutes after HI, but not three hours. Similarly, Chen et al. (2010) reported dose-dependent improvements based on the time of administration post-injury. Throughout the rest of the studies that did not evaluate dose-dependency, successful amelioration of neurological deficits was reported across 2ME2 dosages ranging from 2.5 - 16 mg/kg.

3.4.2. Blood–Brain Barrier (BBB) Permeability

All four studies that directly measured BBB permeability found it significantly improved after 2ME2 treatment. Indicators included reduced IgG infiltration and diminished Evans Blue dye extravasation (up to 60% reduction), supporting the restoration of BBB integrity. Eight studies examined BBB-related molecular factors, seven of which revealed significant

downregulation of VEGF, MMP-2, MMP-9, AQP-4, or AQP-9. Schaible et al. (2014) did not note a significant decrease in VEGF gene expression in a TBI model. Furthermore, 2ME2 mitigated the disruption of tight junction proteins ZO-1, claudin-5, synaptophysin (SYN), and occludin.

3.5. Cerebral Edema

All five studies evaluating edema observed significant reductions in the ipsilateral hemisphere following 2ME2 administration.

3.6. Inflammatory Markers and Microglial Activation

One study showed that 2ME2 significantly reduced levels of IL-1β, IL-6, TNF-α, and microglial activation within 24 hours post-treatment. However, one study found that IL-1β and IL-6 gene expression were not significantly reduced 24 hours after TBI.

3.7. Neurological Deficits

All five studies evaluating neurological function observed significant improvement in 2ME2-treated groups. Performance gains were noted in the Garcia et al. scoring system (motor and sensory assessment), Morris water maze (reduced search time and escape latency, and modified Garcia scoring system.

3.8. Mortality

Among the four studies measuring mortality, two found significantly reduced mortality in the 2ME2 groups (Cheng et al. (2007): 11.11% (2ME2) vs. 25% (control); Yan et al. (2006): 14.2% (2ME2) vs. 40% (control)), while two studies reported non-significant reductions (Wu et al. (2013): 25.00% vs. 35.00%; Hu et al. (2022): 25.00% vs. 31.42%). Chen et al. (2008) did not perform a statistical analysis on mortality but noted that 6 of 10 rats treated with 150 mg/kg 2ME2 did not survive.

4. Discussion

This systematic review of eleven preclinical studies using rodent models of ischemia, SAH, and TBI demonstrates that administration of 2ME2 following neurological insult significantly reduces HIF-1α levels in the ipsilateral cerebral hemisphere. Additionally, several downstream effectors of HIF-1α—including VEGF and MMPs—were also significantly attenuated. These findings suggest that postconditioning with 2ME2 exerts a neuroprotective role in the cerebrum across diverse models of brain injury through HIF-1α inhibition.

Importantly, 2ME2 consistently reduced cerebral cell death through gross measurement and analysis of apoptotic markers, including neuronal loss in the hippocampal CA1 and CA3 regions—areas crucial for memory and learning and often implicated in SAE-associated cognitive deficits. Although one study noted increased infarct volumes, it was only when 2ME2 was administered alongside DEX, a highly selective α_2-adrenoceptor agonist that has demonstrated contradicting results as a potential HIF-1α inducer and inhibitor [34, 35]. These histological findings were supported by improvements in neurological behavior and gross measurements of infarct and contusion volumes across various models, including improved performance on the Garcia scoring system and Morris water maze [25, 29, 31, 32]. The neuroprotection in these regions raises the possibility that 2ME2 not only mitigates acute neuronal injury but may also preserve long-term cognitive functioning in SAE.

One of the most compelling findings was 2ME2's ability to preserve BBB integrity. This review demonstrated that 2ME2 treatment was significantly associated with reduced penetration of IgG and Evans Blue dye into the parenchyma, alongside decreased cerebral edema and microglial activation. On a molecular level, 2ME2 down regulated VEGF, aquaporins (AQP-4 and AQP-9), and MMPs, while upregulating tight junction proteins such as ZO-1, claudin-5, SYN, and occludin. Since BBB disruption is a key driver of peripheral immune cell extravasation and subsequent neuroinflammation in SAE, this mechanism is particularly critical. Restoration of BBB integrity could effectively interrupt the vicious cycle of cytokine entry and sustained neural injury.

Despite 2ME2's promising anti-inflammatory effects through downregulation of pro-inflammatory cytokine production from HIF-1α inhibition, quantified cerebral cytokine levels following 2ME2 treatment were inconsistent. Hu et al. (2022) reported significantly decreased IL-1β, IL-6, and TNF-α, while Schaible et al. (2014) noted non-significant decreases in IL-1β and IL-6 gene expression [32, 33]. However, 2ME2's efficacy cannot be solely evaluated based on these findings. Its concurrent ability to reduce BBB extravasation may itself limit cytokine ingress into the cerebrum and influence measured

concentrations. These results limit definitive conclusions about 2ME2's anti-inflammatory efficacy and thus, future studies in SAE models are warranted to model this interaction more precisely. Another key insight from the data is the dose and timing-dependent nature of 2ME2's neuroprotective efficacy. Across several studies, moderate doses (2.5-16 mg/kg) resulted in significant reductions in infarct volumes, neuronal cell death, and cerebral edema. However, Chen et al. (2008) found that sub-therapeutic (1.5 mg/kg) and high (150 mg/kg) doses were ineffective or associated with increased mortality, respectively. Although 2ME2 has shown an acceptable safety profile in oncological Phase I and II clinical trials—most commonly causing anemia, fatigue, liver enzyme elevations, and gastrointestinal symptoms—these contradictions suggest a need for precise dose optimization in future clinical applications, particularly in patients with acute neurological injury [36, 37, 38, 24]. In addition, 2ME2's efficacy varied when administered from onset up to three hours after the neurological insult. Chen et al. (2008) found a significant decrease in infarct volumes when 15 mg/kg of 2ME2 was given five minutes after ischemic injury, but not at three hours [24]. Conversely, Chen et al. (2007) observed significant decreases in infarct volumes when 5 mg/kg of 2ME2 was administered three hours after ischemia [25]. These results suggest that the timing of 2ME2 administration following a neuroinflammatory insult may also be a critical factor in determining its efficacy and safety.

4.1. Comparison with Other Studies

Our findings align with previous research examining HIF-1α inhibition in SAE and related conditions. For example, Zhao et al. (2022) demonstrated that echinomycin, another HIF-1α inhibitor, reduced TNF-α and IL-6 levels in the hippocampus and reversed cognitive deficits in a murine model of SAE [39]. Similarly, remifentanil was shown to alleviate LPS-induced neuroinflammation in rats via HIF-1α suppression [40]. The question of whether HIF-1α should be inhibited or upregulated also remains context-dependent. While downregulation of HIF-1α appears protective when administered post-injury, several studies have shown that preconditioning with 2ME2 may worsen neurological injury. One study by Zhang et al. (2014) found that rats pretreated with 2ME2 or YC-1 exhibited increased infarct volumes and worse neurological scores after MCAO [41]. Complementing this finding, other studies have suggested that preconditioning with HIF-1α induction (e.g., hypoxia) can be protective, potentially due to the induction of downstream targets such as VEGF, erythropoietin (EPO), GLUT-1, GLUT-3, and nitric oxide [42, 43].

These molecules enhance angiogenesis, oxygen delivery, glucose metabolism, and cerebral blood flow, which may prepare the brain to better tolerate subsequent injury [44]. This duality reinforces the idea that timing and context are critical determinants of whether HIF-1α modulation will be beneficial or harmful. Thus, we propose that the timing of HIF-1α modulation is pivotal. During the initial injury, downregulating HIF-1α may suppress harmful inflammation and apoptosis. In contrast, prior activation of HIF-1α and its downstream targets may offer preconditioning benefits. Further research is needed to elucidate optimal timing and context for therapeutic intervention.

4.2. Study Limitations

Despite promising results, several limitations should be acknowledged. The first is the absence of SAE-specific models in the reviewed literature. Although the included studies used models that share pathophysiological overlaps with SAE, none incorporated a septic trigger or systemic inflammation. Furthermore, the heterogeneity among these animal models prevented the performance of a meta-analysis. Comparisons across studies were further limited by variations in 2ME2 delivery (i.e., intraperitoneal vs. intravenous) and dosage regimens. 2ME2's pleiotropic nature also complicates its therapeutic interpretation. While its primary mechanism involves HIF-1α inhibition via microtubule depolymerization, 2ME2 also weakly interacts with estrogen receptors and is metabolized via cytochrome P450 enzymes to estrogenic compounds, which may contribute to its anti-inflammatory effects [45]. Thus, future studies should evaluate potential sex-specific responses to treatment, particularly since most reviewed studies used male rodents. Few studies provided long-term data, making it difficult to determine whether 2ME2 confers sustained neuroprotection. Finally, while this review focused on neuroinflammatory models with relevance to SAE, none of the included studies specifically modeled SAE, which limits direct applicability.

4.3. Implications for Clinical Practice and Research

These findings have important implications for the future treatment of SAE. While current sepsis management focuses on infection control and supportive care, targeted strategies to address neuroinflammation are lacking. This review highlights 2ME2 as a promising adjunctive therapy due to its HIF–1α–mediated neuroprotective effects and favorable safety profile in humans. However, before clinical translation, further research is needed in SAE-specific animal models to optimize timing and dosing, evaluate long-term effects, clarify delivery mechanisms, and investigate potential sex- and age-related differences in treatment responses. Such studies will be critical in determining whether 2ME2 can become a viable therapeutic agent for SAE and other forms of sepsis-related brain injury. If validated in such models, 2ME2 could be evaluated in early-phase clinical trials targeting septic ICU patients with elevated HIF–1α levels.

5. Conclusion

Current treatment strategies for sepsis-associated encephalopathy (SAE) remain limited, primarily addressing infection control and systemic support, with few interventions targeting the underlying neuroinflammatory processes. This systematic review highlights the therapeutic potential of 2-methoxyestradiol (2ME2), which consistently reduced HIF-1α expression, preserved blood–brain barrier integrity, mitigated cerebral edema and infarct size, improved neurological outcomes, and, in some models, reduced mortality. The observed benefits of 2ME2 appear to be dose- and timing-dependent, with moderate doses administered post-injury producing the most favorable results. These findings support 2ME2 as a promising adjunctive therapy for SAE. However, further research using SAE-specific models is essential to evaluate its translational relevance, optimal dosing strategies, and safety profile in both preclinical and clinical settings.

5.1. Artificial Intelligence (AI) Authoring Tools

ChatGPT was used in certain sections of this manuscript to refine and assist with professional and scientific diction.

References

[1] Rudd KE, Johnson SC, Agesa KM, et al. Global, regional, and National Sepsis Incidence and mortality, 1990–2017: Analysis for the Global Burden of Disease Study. *The Lancet*. 2020; 395(10219):200-211. doi: https://doi.org/10.1016/S0140-6736(19)32989-7

[2] Te G, Gb Y. Sepsis-associated Encephalopathy. Nature reviews. Neurology. Published October 1, 2012. https://pubmed.ncbi.nlm.nih.gov/22986430/

[3] Sonneville R, de Montmollin E, Poujade J, et al. Potentially modifiable factors contributing to sepsis-associated encephalopathy. *Intensive Care Medicine*. 2017; 43(8):1075-1084. doi: https://doi.org/10.1007/s00134-017-4807-z

[4] Piva S, Bertoni M, Gitti N, Rasulo FA, Latronico N. Neurological complications of sepsis. *Current Opinion in Critical Care*. 2023; 29(2):75-84. doi:https://doi.org/10.1097/MCC.0000000000001022

[5] Chen J, Shi X, Diao M, et al. A retrospective study of sepsis-associated encephalopathy: epidemiology, clinical features and adverse outcomes. *BMC Emergency Medicine*. 2020; 20:77. doi:https://doi.org/10.1186/s12873-020-00374-3

[6] Semmler A, Frisch C, Debeir T, et al. Long-term cognitive impairment, neuronal loss and reduced cortical cholinergic innervation after recovery from sepsis in a rodent model. *Experimental Neurology*. 2007; 204(2):733-740. doi:https://doi.org/10.1016/j.expneurol.2007.01.003

[7] Peters van Ton AM, Meijer-van Leijsen EMC, Bergkamp MI, et al. Risk of Dementia and Structural Brain Changes Following Nonneurological Infections During 9-Year Follow-Up*. *Critical Care Medicine*. 2021; 50(4):554-564. doi:https://doi.org/10.1097/ccm.0000000000005313

[8] Sonneville R, Benghanem S, Jeantin L, et al. The spectrum of sepsis-associated encephalopathy: a clinical perspective. *Critical Care*. 2023; 27(1). doi:https://doi.org/10.1186/s13054-023-04655-8

[9] Zhao L, Song Y, Zhang Y, et al. HIF-1α/BNIP3L induced cognitive deficits in a mouse model of sepsis-associated encephalopathy. *Frontiers in immunology*. 2022; 13: 1095427. doi:https://doi.org/10.3389/fimmu.2022.1095427

[10] Frede S, Stockmann C, Freitag P, Fandrey J. Bacterial lipopolysaccharide induces HIF-1 activation in human monocytes via p44/42 MAPK and NF-κB. *Biochemical Journal*. 2006; 396(3):517-527. doi:https://doi.org/10.1042/bj20051839

[11] Takata F, Nakagawa S, Matsumoto J, Dohgu S. Blood-Brain Barrier Dysfunction Amplifies the Development of Neuroinflammation: Understanding of Cellular Events in Brain Microvascular Endothelial Cells for Prevention and Treatment of BBB Dysfunction. *Frontiers in Cellular Neuroscience*. 2021; 15. doi:https://doi.org/10.3389/fncel.2021.661838

[12] Huang C, Shen Y, Zhang F, et al. Sepsis-associated encephalopathy: the potential role of microglia and microglia-derived exosomes. *J Neuroinflammation.* 2022; 19(1):25. doi:10.1186/s12974-022-02598-5.

[13] Jing G, Zuo J, Fang Q, et al. Erbin protects against sepsis-associated encephalopathy by attenuating microglia pyroptosis via IRE1α/Xbp1s-Ca^{2+} axis. *J Neuroinflammation.* 2022;19(1):237. doi:10.1186/s12974-022-025 98-5.

[14] Ruan H, Li Y, Zhang Q, et al. IDENTIFICATION AND CLINICAL VALIDATION OF HYPOXIA-INDUCIBLE FACTOR 1α PROTEIN AS THE POTENTIAL BIOMARKER IN PATIENTS WITH SEPSIS. *Shock*. 2023; 59(6):855-863. doi:https://doi.org/10.1097/shk.0000000000002122

[15] Wang Y, Ni P, Zhuang D, et al. Early hyperbaric oxygen therapy through regulating the HIF-1α signaling pathway attenuates Neuro inflammation and behavioral deficits in a mouse model of Sepsis-associated encephalopathy. *Journal of neuroimmunology*. 2024; 391: 578367. doi:https://doi.org/10.1016/j.jneuroim.2024.578367

[16] Zhang N, Ma Y, Li Y, et al. Paeonol prevents sepsis-associated encephalopathy via regulating the HIF1A pathway in microglia. *International Immunopharmacology*. 2024; 143: 113287. doi:https://doi.org/10.1016/j.intimp.2024.113287

[17] Guan S, Sun L, Wang X, Huang X, Luo T. Propofol inhibits neuroinflammation and metabolic reprogramming in microglia in vitro and in vivo. *Frontiers in Pharmacology*. 2023; 14:1161810. doi:https://doi.org/10.3389/fphar.2023.1161810

[18] Tevaarwerk AJ, Holen KD, Alberti D, et al. Phase I Trial of 2-Methoxyestradiol NanoCrystal Dispersion in Advanced Solid Malignancies. *Clinical Cancer Research*. 2009; 15(4):1460-1465. doi:https://doi.org/10.1158/1078-0432.ccr-08-1599

[19] Mabjeesh NJ, Escuin D, LaVallee TM, et al. 2ME2 inhibits tumor growth and angiogenesis by disrupting microtubules and dysregulating HIF. *Cancer Cell*. 2003; 3(4):363-375. doi:https://doi.org/10.1016/s1535-6108 (03)00077-1

[20] Becker CM, Rohwer N, Funakoshi T, et al. 2-Methoxyestradiol Inhibits Hypoxia-Inducible Factor-1α and Suppresses Growth of Lesions in a Mouse Model of Endometriosis. *The American Journal of Pathology*. 2008; 172(2):534-544. doi:https://doi.org/10.2353/ajpath.2008.061244

[21] Page MJ, Moher D, Bossuyt PM, et al. *PRISMA 2020 explanation and elaboration: updated guidance and exemplars for reporting systematic reviews*. *BMJ.* 2021; 372:n160. doi:10.1136/bmj.n160.

[22] Page MJ, Moher D, Bossuyt PM, et al. PRISMA 2020 Explanation and elaboration: Updated Guidance and Exemplars for Reporting Systematic Reviews. *British Medical Journal*. 2021; 372(160). doi:https://doi.org/10.1136/bmj.n160

[23] Luo C, Ouyang M-W, Fang Y-Y, et al. Dexmedetomidine protects mouse brain from ischemia-reperfusion injury via inhibiting neuronal autophagy through up-regulating HIF-1α. *Front Cell Neurosci.* 2017; 11:197. doi:10.3389/fncel.2017.00197.

[24] Chen W, Jadhav V, Tang J, Zhang JH. HIF-1α inhibition ameliorates neonatal brain injury in a rat pup hypoxic–ischemic model. *Neurobiology of Disease*. 2008; 31(3):433-441. doi:https://doi.org/10.1016/j.nbd.2008.05.020

[25] Chen C, Hu Q, Yan J, et al. Multiple effects of 2ME2 and D609 on the cortical expression of HIF-1α and apoptotic genes in a middle cerebral artery occlusion-induced focal ischemia rat model. *Journal of Neurochemistry*. 2007;102(6):1831-1841. doi:https://doi.org/10.1111/j.1471-4159.2007.04652.x

[26] Chen C, Ostrowski RP, Zhou C, Tang J, Zhang JH. Suppression of hypoxia-inducible factor-1α and its downstream genes reduces acute hyperglycemia-enhanced hemorrhagic transformation in a rat model of cerebral ischemia. *Journal of Neuroscience Research*. 2010; 88(9):2046-2055. doi:https://doi.org/10.1002/jnr.22361

[27] Wang Y, Tang Y, Yang M, Huang X. Dexmedetomidine alleviates cerebral ischemia-reperfusion injury in rats via inhibition of hypoxia-inducible factor-1α. *Journal of Cellular Biochemistry*. 2018;120(5):7834-7844. doi:https://doi.org/10.1002/jcb.28058

[28] Higashida T, Peng C, Li J, et al. Hypoxia-Inducible Factor-1α Contributes to Brain Edema after Stroke by Regulating Aquaporins and Glycerol Distribution in Brain. *Current Neurovascular Research*. 2011;8(1):44-51. doi:https://doi.org/10.2174/156720211794520251

[29] Xiong A, Li J, Xiong R, et al. Inhibition of HIF-1α-AQP4 axis ameliorates brain edema and neurological functional deficits in a rat controlled cortical injury (CCI) model. *Scientific Reports*. 2022; 12(1):2701. doi:https://doi.org/10. 1038/s41598-022-06773-9

[30] Yan J, Chen C, Lei J, et al. 2-methoxyestradiol reduces cerebral vasospasm after 48 hours of experimental subarachnoid hemorrhage in rats. *Experimental Neurology*. 2006;202(2):348-356.doi:https://doi.org/10.1016/j.expneurol.2006.06.009

[31] Wu C, Hu Q, Chen J, et al. Inhibiting HIF-1α by 2ME2 ameliorates early brain injury after experimental subarachnoid hemorrhage in

rats. *Biochemical and Biophysical Research Communications*. 2013; 437(3): 469-474. doi:https://doi.org/10.1016/j.bbrc.2013.06.107

[32] Hu Q, Du Q, Yu W, Dong X. 2-Methoxyestradiol Alleviates Neuroinflammation and Brain Edema in Early Brain Injury after Subarachnoid Hemorrhage in Rats. *Frontiers in Cellular Neuroscience*. 2022; 16. doi:https://doi.org/10.3389/fncel.2022.869546

[33] Schaible EV, Windschügl J, Wiesia Bobkiewicz, et al. 2-Methoxyestradiol confers neuroprotection and inhibits a maladaptive HIF-1α response after traumatic brain injury in mice. *Journal of neurochemistry*. 2014; 129(6):940-954. doi:https://doi.org/10.1111/jnc.12708

[34] Shi J, Yu T, Song K, et al. Dexmedetomidine ameliorates endotoxin-induced acute lung injury in vivo and in vitro by preserving mitochondrial dynamic equilibrium through the HIF-1a/HO-1 signaling pathway. *Redox Biology*. 2021; 41:101954-101954. doi:https://doi.org/10.1016/j.redox.2021.101954

[35] Meng Q, Guo P, Jiang Z, Bo L, Bian J. Dexmedetomidine inhibits LPS-induced proinflammatory responses via suppressing HIF1α-dependent glycolysis in macrophages. *Aging*. 2020; 12(10):9534-9548. doi:https://doi.org/10.18632/aging.103226

[36] Tevaarwerk AJ, Holen KD, Alberti D, et al. Phase I Trial of 2-Methoxyestradiol NanoCrystal Dispersion in Advanced Solid Malignancies. *Clinical Cancer Research*. 2009; 15(4):1460-1465. doi:https://doi.org/10.1158/1078-0432.ccr-08-1599

[37] Harrison MR, Hahn NM, Pili R, et al. A phase II study of 2-methoxyestradiol (2ME2) NanoCrystal® dispersion (NCD) in patients with taxane-refractory, metastatic castrate-resistant prostate cancer (CRPC). *Investigational New Drugs*. 2010; 29(6): 1465-1474. doi:https://doi.org/10.1007/s10637-010-9455-x

[38] Lacy M, Richardson P, Gertz M, et al. Novel therapy with 2-methoxyestradiol (2ME2) for the treatment of relapsed and plateau phase multiple myeloma. *Journal of Clinical Oncology*. 2007; 25(18_suppl):8108-8108. doi:https://doi.org/10.1200/jco.2007.25.18_suppl.8108

[39] Zhao L, Song Y, Zhang Y, et al. HIF-1α/BNIP3L induced cognitive deficits in a mouse model of sepsis-associated encephalopathy. *Frontiers in immunology*. 2022; 13: 1095427. doi:https://doi.org/10. 3389/fimmu.2022.1095427

[40] Özcan MS, Aşcı H, Karabacak P, Özden ES, İmeci OB, Özmen Ö. Remifentanil Ameliorates Lipopolysaccharide-Induced Neuroinflammation by Regulating the Phosphatidylinositol 3-Kinase/Serine–Threonine Protein Kinase/Hypoxia-Inducible Factor 1 Alpha Pathway. *Pharmacology Research & Perspectives*. 2025; 13(1). doi:https://doi.org/10.1002/prp2.70071

[41] Zhang Z, Yan J, Taheri S, Ke Jian Liu, Shi H. Hypoxia-inducible factor 1 contributes to N-acetylcysteine's protection in stroke. *Free Radical Biology and Medicine*. 2014; 68:8-21. doi:https://doi.org/10.1016/j.freeradbiomed.2013.11.007

[42] Guan S, Sun L, Wang X, Huang X, Luo T. Propofol inhibits neuroinflammation and metabolic reprogramming in microglia in vitro and in vivo. *Frontiers in Pharmacology*. 2023; 14:1161810. doi:https://doi.org/10.3389/fphar.2023.1161810

[43] Wang Y, Ni P, Zhuang D, et al. Early hyperbaric oxygen therapy through regulating the HIF-1α signaling pathway attenuates Neuroinflammation and behavioral deficits in a mouse model of Sepsis-associated encephalopathy. *Journal of neuroimmunology*. 2024; 391:578367. doi:https://doi.org/10.1016/j.jneuroim.2024.578367

[44] Chen H, Ma D, Yue F, et al. The Potential Role of Hypoxia-Inducible Factor-1 in the Progression and Therapy of Central Nervous System Diseases. *Current Neuropharmacology*. 2022; 20(9):1651-1666. doi:https://doi.org/10.2174/1570159x19666210729123137

[45] Mooberry SL. Mechanism of action of 2-methoxyestradiol: new developments. *Drug Resistance Updates*. 2003;6(6):355-361. doi:https://doi.org/10.1016/j.drup.2003.10.001

[46] McGuinness, LA, Higgins, JPT. Risk-of-bias VISualization (robvis): An R package and Shiny web app for visualizing risk-of-bias assessments. Res Syn Meth. 2020; 1- 7. https://doi.org/10.1002/jrsm.1411

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