Explore the Advancements in Research on Pyroptosis of Microglial Cells in Acute Carbon Monoxide Poisoning

Mengyang Li[1], Jing Yang[1], Liuxu Wang[1], Haiyu Xue[1], Xiaoqing Zhao[1], Yaoyue Wang[1], Taojie Huang[1], Mingyao Jiang[1], Xuanyan Ren[1], Yuru Xia[1], Mingxuan Gao[1], Xuyan Deng[1], Yang Yuan[1], Si Liu[1], Yixuan He[2], Siyu Zhang[1,*]

[1]North Henan Medical University, Xinxiang 453500, China

[2]Henan Medical University, Xinxiang 453003, China

*Corresponding email: shiyu15937135743@163.com

Abstract

Carbon monoxide (CO), a pervasive environmental toxicant, has emerged as one of the leading causes of fatal poisoning. In the short term, inhalation of excessive amounts of this gas can lead to a systemic illness, primarily affecting the central nervous system, known as acute carbon monoxide poisoning (ACMP). Despite numerous hypotheses proposed by scholars - such as the apoptosis and autophagy theories, ischemia-reperfusion injury and free radical hypotheses, and the excitatory amino acid hypothesis - the precise mechanisms of action remain inadequately elucidated. Consequently, the primary aim of this study is to outline recent research into focal areas and elucidate the critical role of microglial pyroptosis in the molecular mechanisms underlying ACMP. It is anticipated that this will provide a theoretical foundation for a deeper understanding of the pathological mechanisms of ACMP and the exploration of novel therapeutic drug targets.

Keywords

Acute carbon monoxide poisoning, Apoptosis and autophagy, Microglial cells, Pyroptosis

Introduction

Carbon monoxide (CO) is one of the most common toxic gases associated with fatal poisoning, with elevated concentrations capable of causing varying degrees of toxicity and damaging multiple human physiological systems [1]. An atmospheric CO concentration exceeding 200 ppm, if inhaled for more than two hours, can lead to ACMP [2]. The global incidence of ACMP is significant, with the United States alone reporting over 500 fatalities annually due to this condition. Such poisoning can induce pathophysiological changes, including cerebral neuroinflammation and tissue edema within hours, which may impair learning and memory abilities. In severe cases, it can lead to delayed encephalopathy after acute carbon monoxide poisoning (DEACMP) [3]. DEACMP is the most severe and common sequela of acute carbon monoxide poisoning, with many individuals experiencing mild neuropsychiatric deficits, including personality changes and cognitive impairments [4]. The prevalence of mild cognitive impairment is notably elevated, with a significant proportion of affected individuals continuing to exhibit neuropsychiatric symptoms despite undergoing hyperbaric oxygen therapy [5]. Furthermore, CO demonstrates an exceptional affinity for hemoglobin, approximately 200 times greater than that of oxygen [6]. As a result, the presence of CO impedes hemoglobin's ability to transport oxygen efficiently, culminating in tissue hypoxia. This condition can lead to myocardial injury, arrhythmia, and, ultimately, cardiac failure [7]. To date, numerous scholars have investigated the mechanisms underlying acute carbon monoxide poisoning internationally. However, the precise pathogenesis of this condition remains inadequately elucidated. Current hypotheses encompass apoptosis and autophagy, reperfusion injury, the Free Radical Theory, the excitatory amino acid hypothesis, and the burgeoning theory of pyroptosis [8-10]. The burgeoning theory of pyroptosis in cellular biology posits that inflammation plays a pivotal role in the brain damage caused by carbon monoxide poisoning [11]. Upon undergoing pyroptotic cell death, microglial cells release a wide array of inflammatory cytokines, indicating that the attenuation

of inflammation could potentially alleviate neurological impairments and decrease mortality rates in affected individuals [12]. Examining the relationship between microglial pyroptosis and acute carbon monoxide poisoning will enhance our comprehension of pathogenesis, thereby offering valuable insights for clinical prevention and therapeutic strategies.

The clinical manifestations of acute carbon monoxide poisoning

Acute carbon monoxide poisoning is associated with non-specific symptoms that can be categorized into mild, moderate, and severe intoxication. Mild poisoning is characterized by brief exposure and carboxyhemoglobin (COHb) levels ranging from 10% to 20% in the blood. It manifests early symptoms such as headaches, myalgia, dizziness, nausea, and may occasionally lead to transient syncope. Mental clarity is generally maintained, and symptoms typically resolve quickly upon exposure to fresh air and removal from the toxic environment, usually without any lasting effects. Moderate poisoning, associated with a slightly longer exposure duration, involves COHb levels of 30% to 40% and may present symptoms such as collapse and coma, in addition to those observed in mild poisoning. The skin and mucous membranes often display the distinctive cherry-red coloration indicative of carbon monoxide poisoning. Timely intervention and resuscitation can swiftly restore consciousness in patients, with full recovery typically occurring within several days, often without any residual sequelae. In cases of severe CO poisoning, characterized by prolonged exposure, delayed detection, excessive inhalation of coal gas, or brief exposure to high concentrations of CO, COHb levels in the bloodstream can exceed 50%. This condition may result in the loss of all reflexes, urinary and fecal incontinence, hypotension, tachypnea, profound coma, and potentially progression to shock. Generally, the duration of the coma correlates with the severity of the symptoms. Long-term sequelae may include cognitive impairments such as dementia, reduced comprehension ability, memory decline, and limb paralysis.

Clinical investigations have revealed that patients experiencing acute CO poisoning who are exposed to CO for periods exceeding 4.8 hours face a substantially increased risk of developing DEACMP [13]. Corresponding studies suggest that CO exposure for durations surpassing 6 hours may further elevate the risk of DEACMP, as indicated by an odds ratio (OR) of 1.28 with a 95% confidence interval (CI) of (0.09, 2.90). This increased risk is likely due to the prolonged exposure to CO, which results in elevated (COHb) levels in the bloodstream, thereby intensifying cerebral ischemia and hypoxia.

The theories of apoptosis and autophagy

The role of Apoptotic Factors

Apoptosis is a genetically regulated, autonomous, and orderly process of cell death that is essential for maintaining homeostasis. It is characterized by its proactive nature, involving the activation, expression, and modulation of a series of genes. This process is not indicative of self-injury under pathological conditions; rather, it represents an active adaptation to optimize survival in each environment. Jurič and colleagues hypothesized that CO poisoning triggers endogenous apoptosis through the caspase-9 pathway and exogenous apoptosis via caspase-8, leading to apoptosis in neuronal cells. Following CO poisoning, numerous neurons and astrocytes undergo apoptosis, resulting in the necrosis of neural cells, which disrupts brain tissue architecture and leads to the development of DEACMP. Astrocytes play a crucial role in regulating synaptic transmission, maintaining baseline neuronal metabolism, mitigating oxidative stress, and promoting neuronal viability and growth [14]. The substantial damage to glial cells compromises neurotransmission and triggers degenerative alterations within the brain. Through cellular experimentation, the study conclusively demonstrated a significant reduction in the number of astrocytes when cultured in a medium containing CO. Following CO exposure, the activation of caspase-3 and the subsequent apoptosis in hippocampal neurons highlight the critical involvement of caspase-3 in the pathogenesis of CO-induced brain injury.

The changes of HO-1 and Apoptosis

The study demonstrates that ten days following CO poisoning, there is a sustained upregulation of heme oxygenase-1 (HO-1) expression within the hippocampus, particularly in regions vulnerable to hypoxia, such as the CA1 and dentate gyrus (DG) areas [15]. These regions notably exhibit significant neuronal necrosis. Upon administration of the heme oxygenase inducer, hemin, there is an intensified expression of HO-1 in the

hippocampus, which correlates with an exacerbation of hippocampal injury and an increased number of necrotic neurons. This finding implies that the prolonged overexpression of HO-1 in brain tissue following CO poisoning may contribute to pathogenesis. Furthermore, Zhao's investigation into the alterations of HO-1 expression in relation to apoptosis in DEACMP revealed that the changes in HO-1 expression are consistent with the variations and progression of apoptosis-related proteins (Bax, Bcl-2) and cellular apoptosis.

The mechanism of autophagy

Under typical physiological conditions, when cellular function is compromised due to factors such as nutritional deficiencies, stress, or hypoxia, the body mobilizes energy and essential substrates to facilitate cellular repair, thereby exerting a protective effect on the damaged cells. Nevertheless, excessive autophagy may be detrimental to cellular integrity [16]. Research indicates that autophagy, like necrosis and apoptosis, serves as a mechanism of programmed cell death [17]. One such pathway involves oxidative stress: Carbon monoxide exposure results in the excessive generation of reactive oxygen species (ROS) within cells, which can inflict damage on cellular components, including lipids, proteins, and DNA (deoxyribonucleic acid) [18]. Oxidative stress functions as a catalyst for autophagy activation, serving as a protective mechanism to remove damaged organelles and proteins, thereby preventing the spread of cellular damage caused by the excessive production of ROS. This activation is mediated through three distinct pathways: the upregulation of BNIP-3 expression via HIF-2α, activation, the transcriptional induction of LC3 and Atg5 through PKR-like endoplasmic reticulum kinase (PERK) activation, and the induction of p62 protein expression via NRF2 activation. These pathways collectively stimulate the expression of the Atg1 homolog, ULK1, through the action of adenosine monophosphate-activated protein kinase (AMPK), resulting in the dissociation of the AMPK-ULK1 complex. This process subsequently inhibits the activation of serine/threonine protein kinases and deactivates the mTOR-C1 signaling pathway, ultimately leading to the initiation of autophagy [19,20]. Consequently, in cases of carbon monoxide poisoning, cerebral cells can activate autophagy through multiple pathways.

Reperfusion Injury

Reperfusion injury is a phenomenon characterized by tissue damage that occurs despite the restoration of blood supply, particularly under conditions such as post-ischemic reperfusion. In the context of CO poisoning, tissue hypoxia can lead to re-injury upon the re-establishment of blood flow. Following CO poisoning, cells may produce excessive ROS, which have the potential to damage cellular membranes, proteins, and DNA. The re-oxygenation of hypoxic tissues can result in an increased generation of oxygen free radicals, thereby exacerbating reperfusion injury. During reperfusion, there is a marked increase in ROS production mediated by NADPH oxidases. These ROS instigate lipid peroxidation and a reduction in reduced glutathione (GSH) levels, ultimately leading to cell death [21]. The potential mechanisms underlying the elevated ROS levels associated with cellular perfusion-reperfusion injury include the binding of CO to cystathionine beta-synthase (CBS), which inhibits the production of hydrogen sulfide (H2S) and consequently reduces its ROS-scavenging effects. Furthermore, the interaction of carbon monoxide (CO) with myoglobin (Mb) results in an increase in carboxymyoglobin (COMb), which hinders Mb's capacity to clear nitric oxide (NO), thereby causing elevated NO levels (pathway A→D1→D2→J) [22]. This elevation in NO suppresses the oxygen-sensing function of prolyl hydroxylase domain (PHD) proteins, leading to tissue hypoxia (pathway A→D1→D2→J) [23]. Additionally, NO inhibits cystathionine β-synthase (CBS), reducing hydrogen sulfide (H2S) production and diminishing its ROS scavenging ability. The reperfusion process may activate inflammatory cells, such as neutrophils and monocytes, which can release inflammatory mediators, resulting in cellular and tissue damage. During reperfusion, there may be a sudden increase in intracellular calcium ion concentration, leading to calcium overload [24]. This surge can activate a cascade of intracellular enzymes, thereby initiating cellular injury.

The Free Radical Theory

When carbon monoxide poisoning induces severe hypoxia in brain tissue, there is an escalation of electron leakage within the cellular respiratory chain, leading to the generation of numerous reactive oxygen species (ROS). These free radicals initiate lipid peroxidation

within brain tissue, thereby compromising the integrity of the myelin sheath surrounding nerve fibers and neuronal cells [25]. Additionally, ROS can trigger inflammatory responses and disrupt vascular tone, further exacerbating damage to brain tissue [26]. The primary components of various biomembranes, unsaturated fatty acids, are particularly susceptible to lipid peroxidation by ROS, which adversely affects membrane fluidity and permeability. This process results in edema and necrosis in neuronal cells, thereby aggravating brain tissue injury [27]. Simultaneously, dysfunction of the mitochondrial membrane can inhibit the activity of cytochrome C and reduced coenzyme II oxidase, leading to a decrease in the production of reduced coenzyme II and subsequent depletion of cellular energy [28]. Conversely, increased activity of xanthine oxidase enhances peroxidative reactions, culminating in apoptosis. The accumulation of necrotic neuronal cells contributes to the degeneration of brain tissue, manifesting as DEACMP [29].

Excitatory Amino Acid Toxicity Theory

The role of the Excitatory Amino Acid Toxicity Theory in CO poisoning is to explain how CO causes neuronal damage and cell death by affecting the metabolism and functions of excitatory amino acids (such as glutamate and aspartate) in the central nervous system.

CO poisoning may cause neuronal damage and trigger the abnormal release of excitatory amino acids within cells. Under normal circumstances, these amino acids act as neurotransmitters in synaptic transmission, but when in excess, they can be toxic to neurons. Some studies have found that after acute CO poisoning, the concentration of excitatory amino acids increases significantly, especially glutamate [30]. The main targets of glutamate are located on the postsynaptic membrane receptors. Activation of N-methyl-D-aspartate (NMDA) and α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors: Excitatory amino acids lead to an increase in intracellular calcium ions by activating NMDA receptors and AMPA receptors [31]. These receptors play a crucial role in neuronal excitability and synaptic transmission. The activation of NMDA and AMPA receptors cause the calcium channels on the cell membrane to open, allowing a large influx of calcium ions and leading to an overload of intracellular calcium ion concentration [32]. Calcium ions overload can activate a series of intracellular enzymes, including the activation of caspases, resulting in cell damage.

New Pyroptosis Theory

The background of the proposal of the Pyroptosis Theory

In the context of cerebral ischemia and hypoxia, exogenous CO demonstrates a greater affinity for hemoglobin than oxygen, promoting the formation of COHb upon its entry into the bloodstream via the alveoli [33]. This interaction reduces the number of hemoglobin molecules available for oxygen binding, thereby decreasing the blood's oxygen-carrying capacity and impairing oxygen delivery to tissues, ultimately leading to tissue hypoxia. Additionally, intracellular CO can inhibit glycolytic pathways in erythrocytes, resulting in reduced synthesis of 2,3-diphosphoglycerate (2,3-DPG). This inhibition further diminishes hemoglobin's oxygen-carrying efficiency, exacerbating systemic hypoxia. In response to hypoxic conditions, cells transition from oxidative phosphorylation to anaerobic glycolysis, which results in energy depletion and a subsequent deceleration of metabolic processes [34].

Cerebral injury and neuroinflammation following acute CO poisoning

Due to the central nervous system's limited ability to compensate for hypoxia, cerebral edema can develop rapidly, often within 2 to 4 hours following acute CO poisoning and may reach peak severity within 24 to 48 hours, potentially persisting for several days. The clinical manifestations in patients vary according to the concentration of COHb in the blood. In healthy individuals, COHb levels typically range from 5% to 10%, with endogenous carbon monoxide contributing approximately 0.4% to 0.7%. In cases of mild carbon monoxide poisoning, COHb levels may exceed 10%, leading to symptoms such as headache and dizziness. Moderate poisoning is characterized by COHb levels exceeding 30%, resulting in a coma with potential responsiveness to painful stimuli, albeit with diminished reflexes. Severe poisoning, indicated by COHb levels exceeding 50%, can lead to deep coma and even shock [35]. In 2014, Tsai conducted a study on the effects of CO poisoning on the brain, focusing primarily on its long-term impacts on cerebral perfusion and neural function. The study revealed that severe acute CO poisoning could result in cerebral hypoxic-ischemic

injury [36]. However, the mechanisms underlying ischemia and hypoxia are insufficient to fully account for all clinical symptoms. Recent research has indicated that following ischemic and hypoxic injury, microglia rapidly undergo inflammatory activation. This activation extends to surrounding microglia and other types of glial cells through the release of numerous pro-inflammatory factors, culminating in persistent neuroinflammatory injury. This process may be a critical factor contributing to acute carbon monoxide poisoning [37].

The key cells in the Theory of Pyroptosis

Microglia (MG) are pivotal cells in the neuroinflammatory response and serve as crucial immune components within the brain. Under normal physiological conditions, they constitute approximately 10% of the total brain cell population, ranking as the third most prevalent type of immune cell in the brain. Microglia are distributed throughout the central nervous system, exhibiting regional variations in their distribution across the brain. They are most densely concentrated in the hippocampal formation, olfactory bulb, telencephalon, basal ganglia, and substantia nigra. Recent research has highlighted the role of microglial inflammatory activation and the subsequent inflammatory response as significant factors in the early and sustained damage to the nervous system associated with hypoxic-ischemic brain damage (HIBD). The excessive activation of microglia can initiate a cascade effect, recruit additional inflammatory cells and release inflammatory mediators, thereby exacerbating ischemic stroke damage [38]. P2Y12, along with other purinergic substances such as ATP, is implicated in the microglial activation process [39].

Recent studies indicate that the activation of caspase-1 in microglia serves a protective function in cerebral ischemic brain injury. M1-type microglia (MG) exert immunological effects through the secretion of tumor necrosis factor-α (TNF-α), interleukin-12 (IL-12), interleukin-23 (IL-23), along with various chemokines and inflammatory mediators, to eradicate pathogens, tumors, and damaged cells [40]. The pro-inflammatory cytokines and free radicals released by activated microglia initiate or exacerbate neuronal damage responses. Conversely, neuronal damage activates microglia, thereby establishing a pathological cycle. Microglia-mediated inflammation is currently the primary focus in the defense against stroke and traumatic brain injury. Consequently, the inflammatory response mediated by microglia may significantly contribute to the pathogenesis of hypoxic-ischemic encephalopathy. Therefore, strategically modulating microglial activation pharmacologically at different stages of cerebral ischemia and hypoxia could help mitigate neuronal damage. This approach involves regulating cytokine secretion and maintaining an appropriate inflammatory response. This may be an effective preventive and therapeutic measure for preventing and treating the degenerative changes of neuronal function after cerebral ischemia.

The discovery of the Theory of Pyroptosis

Pyroptosis, also known as inflammatory cell death, is a form of programmed cell death mediated by Gasdermin proteins. It is characterized by the rupture of the cell membrane and the rapid release of cellular contents. It depends on Caspase-1 and is accompanied by an inflammatory response. Pyroptosis is an important innate immune response of the body and plays a crucial role in fighting against infections. The origin of pyroptosis Xu et al. first reported Caspase-1-dependent cell death in the study of macrophages from mice infected with Gram-negative Shigella. Zychlinsky found in their research that Shigella flexneri could induce programmed cell death in the infected host macrophages, but it was initially considered to be apoptosis [41]. The study of macrophages infected with Salmonella also confirmed the existence of Caspase-1-dependent programmed cell death, accompanied by the release of many pro-inflammatory factors [42]. Cookson first proposed the concept of pyroptosis. Currently, the specific pathogenesis of carbon monoxide poisoning is unknown [43]. Previous studies have shown that ischemia and hypoxia, oxidative stress, apoptosis and necrosis, demyelination of white matter, abnormal neurotransmitter metabolism, synaptic remodeling, etc. may be its main mechanisms. In the previously discussed mechanisms, taking apoptosis as an example, apoptotic cells display distinct morphological changes: cell shrinkage, reduced volume, a round or oval shape, dense cytoplasm, and purple nuclear fragments. These apoptotic bodies are rapidly cleared by phagocytically active cells after their formation. During this process, the cellular contents are wrapped by the plasma membrane and will not be released into the interstitial tissue. Therefore, the apoptosis process does not generate an

inflammatory response [44]. And the inflammatory response is an extremely important manifestation of nerve cell death. Previous studies could not verify this phenomenon. Therefore, pyroptosis has become a breakthrough for this phenomenon.

The mechanism of the Theory of Pyroptosis

Pyroptosis is primarily mediated through two distinct pathways: the classical and non-classical pathways. Despite differences in their mechanistic processes, both pathways necessitate the activation of specific proteins within the Caspase family and are mediated by the inflammasome. The classical pyroptotic pathway is predominantly dependent on the activation of Caspase-1. According to Zhou et al., when host cells are invaded by exogenous or endogenous microorganisms, the transmembrane protein toll-like receptor 4 (TLR4) on the cell membrane detects this stimulus and subsequently activates the nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) pathway. This activation induces the conversion of inactive inflammatory cytokines (IL-1β, IL-18) into their active forms and promotes the expression of inflammasomes. Subsequently, the Caspase-1 enzyme is activated, which then cleaves members of the Gasdermin protein family, enabling them to form pores in the cell membrane. The formation of these pores facilitates the rapid release of cellular content, ultimately resulting in pyroptosis [45]. The Toll-like receptor (TLR) and nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) signaling pathways are integral to the process of pyroptosis. The TLR pathway facilitates the synthesis and release of cytokines, while the NF-κB pathway enhances the release of pro-inflammatory factors. Furthermore, research conducted by Chang et al. on the TLR4/NF-κB signaling pathway's role in brain injury among premature infants revealed that activation of these pathways promotes the release of pro-inflammatory factors, thereby instigating an inflammatory response [46].

Pathways and mechanisms of Non-classical Pyroptosis

The Non-classical Pyroptosis pathway diverges from the classical pathway and can be categorized into two distinct types. The first type is mediated by Caspase-4/5/11, which directly recognizes and is activated by bacterial Lipopolysaccharide (LPS) [47]. The second type involves Caspase-3/8 and is associated with tumor necrosis factor-alpha (TNF-α). In this pathway, TNF-α can induce Caspase-3 to cleave gasdermin D (GSDMD), resulting in pore formation on the plasma membrane and the conversion of apoptosis into pyroptosis. Additionally, when Caspase-3/8 interacts with TNF-α, it activates the Caspase-8 complex, which subsequently cleaves GSDMD. Despite the distinct mechanisms underlying these pathways, both ultimately culminate in pyroptosis. In summary, pyroptosis is a multifaceted process involving diverse signaling pathways and molecular mechanisms. A comprehensive exploration of the mechanisms underlying pyroptosis offers novel insights and directions for developing improved therapeutic strategies for pyroptosis-related diseases. Additionally, it enhances the prevention and diagnosis of acute carbon monoxide poisoning and facilitates the research and development of new clinical drugs.

Conclusion

This study is a conceptual paper focusing on Chinese medical tourists seeking fertility treatment in Malaysia. It conducts an in-depth analysis of the intrinsic connections between cultural proximity, service quality (measured through SERVQUAL dimensions), and revisit intention. This research constructs and demonstrates the theoretical mechanism by which cultural proximity influences service quality and thereby affects revisit intention. It thereby addresses a gap in the fertility tourism literature, which has traditionally overlooked service quality as a mediating variable.

From a theoretical perspective, most existing studies on revisit intention in medical tourism take subjective satisfaction as the core mediating variable. This study breaks through this limitation by adopting the SERVQUAL dimensions (tangibles, reliability, responsiveness, assurance, and empathy) as the objective carriers for quantifying service quality. It clarifies how cultural proximity, such as shared language, recognition of practices like "confinement" (postnatal care rituals), and support from the Chinese community in Malaysia, translates into tourists' revisit intention. This translation occurs through specific service dimensions. In doing so, it establishes a clear theoretical framework and provides operable variable measurement pathways for subsequent empirical research in this field.

In terms of practical implications, the synergistic effect

of cultural proximity and high-quality services holds significant value for the Malaysian fertility medical tourism sector. Cultural proximity can reduce cross-cultural perceived risks and communication costs for Chinese tourists. This enables medical institutions to quickly gain tourists' trust and develop a differentiated competitive advantage, distinct from Western medical destinations such as those in Europe and the United States. Enhancing service quality based on the SERVQUAL dimensions can directly optimize tourists' medical experience. For instance, medical institutions can improve Chinese-language service procedures (responsiveness), deploy medical teams familiar with Chinese culture (empathy), and upgrade medical facilities that meet Chinese-style needs (tangibles). Such measures can significantly enhance tourists' sense of belonging and satisfaction, thereby increasing tourist retention rates and word-of-mouth communication effects. The combination of cultural proximity and high-quality services helps the Malaysian fertility medical sector attract more Chinese clients, alleviating issues in China's domestic assisted reproductive technology (ART) sector, such as uneven resource distribution and limited success rates. Meanwhile, it promotes the development of Malaysia's medical tourism industry toward the "culturally adaptive" niche, facilitating the sustainable development of this sector.

It should be noted that this study is currently in the conceptual research phase. Future research will enter the empirical verification phase. Specifically, it plans to collect sample data from Chinese tourists who have received fertility treatment in Malaysia through questionnaires and use structural equation modeling (SEM) to verify the theoretical hypotheses proposed in this study. This will further examine the causal relationships and the strength of the mediating effect among cultural proximity, each dimension of service quality, and revisit intention. The results of subsequent empirical research will provide data support for the improvement of the theoretical model and offer more targeted practical suggestions for Malaysian fertility medical institutions, industry associations, and policymakers. This will help this sector better meet the needs of the Chinese market and achieve long-term and stable development.

Funding

This work was not supported by any funds.

Acknowledgements

The authors would like to show sincere thanks to those techniques who have contributed to this research.

Conflicts of Interest

The authors declare no conflict of interest.

References

[1] Savioli, G., Gri, N., Ceresa, I. F., Piccioni, A., Zanza, C., Longhitano, Y., Candura, S. M. (2024) Carbon monoxide poisoning: from occupational health to emergency medicine. *Journal of Clinical Medicine*, 13(9), 2466.

[2] Dent, M. R., Rose, J. J., Tejero, J., Gladwin, M. T. (2024) Carbon monoxide poisoning: from microbes to therapeutics. *Annual Review of Medicine*, 75(1), 337-351.

[3] Huang, Y. Q., Peng, Z. R., Huang, F. L., Yang, A. L. (2020) Mechanism of delayed encephalopathy after acute carbon monoxide poisoning. *Neural Regeneration Research*, 15(12), 2286-2295.

[4] Wang, R., Li, K., Wang, Z., Wang, Y., Zhang, H. (2024) Changes of nuclear factor Kappa-B pathway activity in hippocampus after acute carbon monoxide poisoning and its role in nerve cell injury. *Molecular Neurobiology*, 61(8), 5206-5215.

[5] Jiang, W., Zhao, Z., Wu, Q., Wang, L., Zhou, L., Li, D., Tan, Y. (2021) Study on brain structure network of patients with delayed encephalopathy after carbon monoxide poisoning: based on diffusion tensor imaging. *La Radiologia Medica*, 126(1), 133-141.

[6] Xu, Q., Rose, J. J., Chen, X., Wang, L., DeMartino, A. W., Dent, M. R., Gladwin, M. T. (2022) Cell-free and alkylated hemoproteins improve survival in mouse models of carbon monoxide poisoning. *JCI Insight*, 7(21), e153296.

[7] Jiang, M., Yu, C. H., Xu, Z., Qin, Z. (2024) Binding of carbon monoxide to hemoglobin in an oxygen environment: force field development for molecular dynamics. *Journal of Chemical Theory and Computation*, 20(10), 4229-4238.

[8] Trujillo-Rangel, W. Á., García-Valdés, L., Méndez-del Villar, M., Castañeda-Arellano, R., Totsuka-Sutto, S. E., García-Benavides, L. (2022)

Therapeutic targets for regulating oxidative damage induced by ischemia-reperfusion injury: a study from a pharmacological perspective. *Oxidative Medicine and Cellular Longevity*, 2022(1), 8624318.

[9] Coburn, R. F. (2022) Carbon monoxide (CO), nitric oxide, and hydrogen sulfide signaling during acute CO poisoning. *Frontiers in Pharmacology*, 12, 830241.

[10] Ai, Y., Meng, Y., Yan, B., Zhou, Q., Wang, X. (2024) The biochemical pathways of apoptotic, necroptotic, pyroptotic, and ferroptotic cell death. *Molecular Cell*, 84(1), 170-179.

[11] Angelova, P. R., Myers, I., Abramov, A. Y. (2023) Carbon monoxide neurotoxicity is triggered by oxidative stress induced by ROS production from three distinct cellular sources. *Redox Biology*, 60, 102598.

[12] Newton, K., Strasser, A., Chan, F. K.-M., Condon, S. M., Darding, M., Dillon, C. P., Drag, M., Fulda, S., Ganz, M., Hornung, V., Ichijo, H., Kearney, C. J., Kelly, G. L., Kist, M., Komiyama, T., Koonin, E. V., Korsmeyer, S. J., Kraft, C., Lamkanfi, M., Green, D. R. (2024) Cell death. *Cell*, 187(2), 235-256.

[13] Zhang, Y., Lu, Q., Jia, J., Li, S., Li, Z., Wu, H., Zhang, B., Wu, Y. (2021) Multicenter retrospective analysis of the risk factors for delayed neurological sequelae after acute carbon monoxide poisoning. *The American Journal of Emergency Medicine*, 46, 165-169.

[14] Li, N., Meng, X. E., Li, H., Fan, D. F., Pan, S. Y. (2018) Efficacy of combined glucocorticoid and hyperbaric oxygen therapy against delayed encephalopathy after carbon monoxide poisoning, and its effect on expression of immune-associated cytokines. *Tropical Journal of Pharmaceutical Research*, 17(6), 1177-1183.

[15] Warenits, A.-M., Mäger, J., Sterz, F., Ettl, F., Haugk, M., Schriefl, C., Clodi, C., Weihs, W., Högler, S., Scherer, T., Fischer, H. (2020) Motor cortex and hippocampus display decreased heme oxygenase activity 2 weeks after ventricular fibrillation cardiac arrest in rats. *Frontiers in Medicine*, 7, 254.

[16] Wang, S., Long, H., Hou, L., Feng, B., Ma, Z., Wu, Y., Zeng, Y., Cai, J., Zhang, D. W., Zhao, W. (2021) Autophagy and mitochondrial homeostasis during infection: a double-edged sword. *Frontiers in Cell and Developmental Biology*, 9, 738932.

[17] LIU, P., TIAN, W.S. (2019) The study of pathogenesis of delayed encephalopathy after acute carbon monoxide poisoning. *Inner Mongolia Medical Journal*, 51(9), 1043-1046.

[18] Alva, R., Mirza, M., Baiton, A., Lazuran, L., Samokysh, L., Bobinski, A., Cowan, A. (2022) Oxygen toxicity: cellular mechanisms in normobaric hyperoxia. *Cell Biology and Toxicology*, 39(1), 111-143.

[19] Wang, M., Tan, J., Miao, Y., Zhang, Q. (2019) Role of oxidative stress in urine albumin overload-induced autophagy in renal proximal tubular epithelial cells. *Chinese Journal of Geriatrics*, 312-316.

[20] Virág, L., Robaszkiewicz, A., Rodriguez-Vargas, J. M., Oliver, F. J. (2019) Self-defense of macrophages against oxidative injury: Fighting for their own survival. *Redox Biology*, 26,101261.

[21] Su, L.J., Zhang, J.H., Gomez, H., Murugan, R., Hong, X., Xu, D., Jiang, F., Peng, Z.-Y. (2019) Reactive oxygen species-induced lipid peroxidation in apoptosis, autophagy, and ferroptosis. *Oxidative Medicine and Cellular Longevity*, 2019, 5080843.

[22] Fuschillo, S., De Felice, C., Balzano, G., Gaudiosi, C. (2020) Nitric oxide and hydrogen sulfide: a nice pair in the respiratory system. *Current Medicinal Chemistry*, 27(42), 7136-7148.

[23] Palmieri, E., Raineri, D., Padroni, C., Hirsch, E. (2021) Nitric oxide mediates direct restriction of pyruvate dehydrogenase complex via generation of nitroxyl during macrophage polarization. *The FASEB Journal*, 35(1), 03596.

[24] Wang, R., Wang, M., He, S., Sun, G., Sun, X. (2020) Targeting calcium homeostasis in myocardial ischemia/reperfusion injury: an overview of regulatory mechanisms and therapeutic reagents. *Frontiers in Pharmacology*, 11, 872.

[25] Angelova, P. R., Abramov, A. Y. (2021) Sources and triggers of oxidative damage in neurodegeneration. *Free Radical Biology and Medicine*, 173, 52-63.

[26] Candelario. Jalil, E., Dijkhuizen, R. M., Magnus, T. (2022) Neuroinflammation, stroke, blood-brain

barrier dysfunction, and imaging modalities. *Stroke*, 53(5), 1473-1486.

[27] Sadzak, A., Milićević, B., Ivošević, T., Jurašin, D. D. (2020) The structural integrity of the model lipid membrane during induced lipid peroxidation: the role of flavonols in the inhibition of lipid peroxidation. *Antioxidants*, 9(5), 430.

[28] Jones, C. L., Stevens, B. M., D'Alessandro, A., Culp-Hill, R., Reisz, J. A., Pei, S., Gustafson, A., Khan, N., DeGregori, J., Pollyea, D. A. (2019) Cysteine depletion targets leukemia stem cells through inhibition of electron transport complex II. *Blood*, 134(4), 389-394.

[29] Granger, D. N., Kvietys, P. R. (2015) Reperfusion injury and reactive oxygen species: the evolution of a concept. *Redox Biology*, 6, 524-551.

[30] MacMullin, P., Hodgson, N., Damar, U. (2020) Increase in seizure susceptibility after repetitive concussion results from oxidative stress, parvalbumin-positive interneuron dysfunction and biphasic increases in glutamate/GABA ratio. *Cerebral Cortex*, 30(12), 6108-6120.

[31] Malyala, S., Zhang, Y., Strubbe, J. O., Bazil, J. N. (2019) Calcium phosphate precipitation inhibits mitochondrial energy metabolism. *PLOS Computational Biology*, 15(1), e1006719.

[32] Stewart, A. F. R., Chen, H.-H. (2022) N-methyl-D-aspartate receptor functions altered by neuronal PTP1B activation in Alzheimer's disease and schizophrenia models. *Neural Regeneration Research*, 17(10), 2208-2210.

[33] Viscomi, C., Zeviani, M. (2019) Breathe: Your mitochondria will do the rest… if they are healthy! *Cell Metabolism*, 30(4), 628-629.

[34] Figueiredo-Pereira, C., Dias-Pedroso, D., Soares, N. L., Vieira, H. L. A. (2020) CO-mediated cytoprotection is dependent on cell metabolism modulation. *Redox Biology*, 32, 101470.

[35] Rose, J. J., Wang, L., Xu, Q., McTiernan, C. F., Shiva, S., Tejero, J., Gladwin, M. T. (2017) Carbon monoxide poisoning: pathogenesis, management, and future directions of therapy. *American Journal of Respiratory and Critical Care Medicine*, 195(5), 596-606.

[36] Shields, D. C., Haque, A., Banik, N. L. (2020) Neuroinflammatory responses of microglia in central nervous system trauma. *Journal of Cerebral Blood Flow & Metabolism*, 40(1), 25-33.

[37] Zhang, S. (2019) Microglial activation after ischaemic stroke. *Stroke and Vascular Neurology*, 4(2), 71-74.

[38] Yu, T., Zhang, X., Shi, H., Tian, J., Sun, L., Hu, P., Sun, H., Zhou, X. (2019) P2Y12 regulates microglia activation and excitatory synaptic transmission in spinal lamina II neurons during neuropathic pain in rodents. *Cell Death & Disease*, 10(3), 165.

[39] Palizgir, M. T., Akhtari, M., Shahram, F., Davatchi, F. (2018) Curcumin reduces the expression of interleukin 1β and the production of interleukin 6 and tumor necrosis factor alpha by M1 macrophages from patients with Behcet's disease. *Immunopharmacology and Immunotoxicology*, 40(4), 297-302.

[40] Nirmala, J. G., Lopus, M. (2020) Cell death mechanisms in eukaryotes. *Cell Biology and Toxicology*, 36(2), 145-164.

[41] Ashida, H., Suzuki, T., Sasakawa, C. (2021) Shigella infection and host cell death: a double-edged sword for the host and pathogen survival. *Current Opinion in Microbiology*, 59, 1-7.

[42] Hos, N. J., Ganesan, R., Gutiérrez, S., Hos, D., Klimek, J., Abdullah, Z., Robinson, N. (2017) Type I interferon enhances necroptosis of Salmonella Typhimurium-infected macrophages by impairing antioxidative stress responses. *Journal of Cell Biology*, 216(12), 4107-4121.

[43] Ma, D., Yang, B., Guan, B., Song, L., Liu, Q., Fan, Y., Xu, H. (2021) A bibliometric analysis of pyroptosis from 2001 to 2021. *Frontiers in Immunology*, 12, 731933.

[44] Zhou, Y. S., Yu, L. H., Dong, L. (2023) Molecular mechanisms of cellular pyroptosis and its trophic regulation. *Journal of Animal Nutrition*, 35(12), 7648-7657.

[45] Chang, L., Wang, X. H., Zhang, Y., Wang, H., Li, M. (2019) Progress in the TLR4/NF-κB signaling pathway in brain injury in preterm infants. *The World's Latest Medical Information Abstract*, 19(35), 131-132.

[46] Wright, S. S., Vasudevan, S. O., Rathinam, V. A. (2022) Mechanisms and consequences of

noncanonical inflammasome-mediated pyroptosis. *Journal of Molecular Biology*, 434(4), 167245.

[47] Gram, A. M., Booty, L. M., Bryant, C. E. (2019) Chopping GSDMD: Caspase-8 has joined the team of pyroptosis-mediating caspases. *The EMBO Journal*, 38(10), e102065.