



Scientific Antioxidant And Anti-Inflammatory Models To Study Therapeutic Neuroprotective Interventions

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ABSTRACT

Oxidative stress and inflammation are among the causes of the majority of chronic illnesses, including neurological conditions. Neuroprotection, anti-inflammatory therapy, and antioxidant therapy are all closely connected. Due to the rising desire for innovative, distinctive chemicals, the neuroprotective properties of compounds, plant extracts, powders derived from medicinal plants are an exciting step forward in the hunt for effective protectors. Numerous studies indicate that finding substances with anti-inflammatory and antioxidant properties is of utmost importance in management of neurological disease. So, in this study we conducted a review to extract how antioxidant and anti-inflammatory assessment which can leads to development of neuroprotective agents by using various databases. Making neuroprotection therapy plans that precisely target neuroinflammatory and oxidative pathways has drawn a lot of attention. For a wide spectrum of neurological illnesses and traumas, a number of anti-inflammatory neuroprotective medications have been studied. Nevertheless, there is still a lot of enthusiasm in this field for creating neuroprotective techniques. This has led to greater consideration of the complement system as a possible therapeutic target for treating brain injury and neurodegenerative illnesses, as recent evidence indicates a key role for the complement system in these situations.

Keywords:

Anti-inflammatory, antioxidant, neuroprotective, assays, therapeutic efficacy

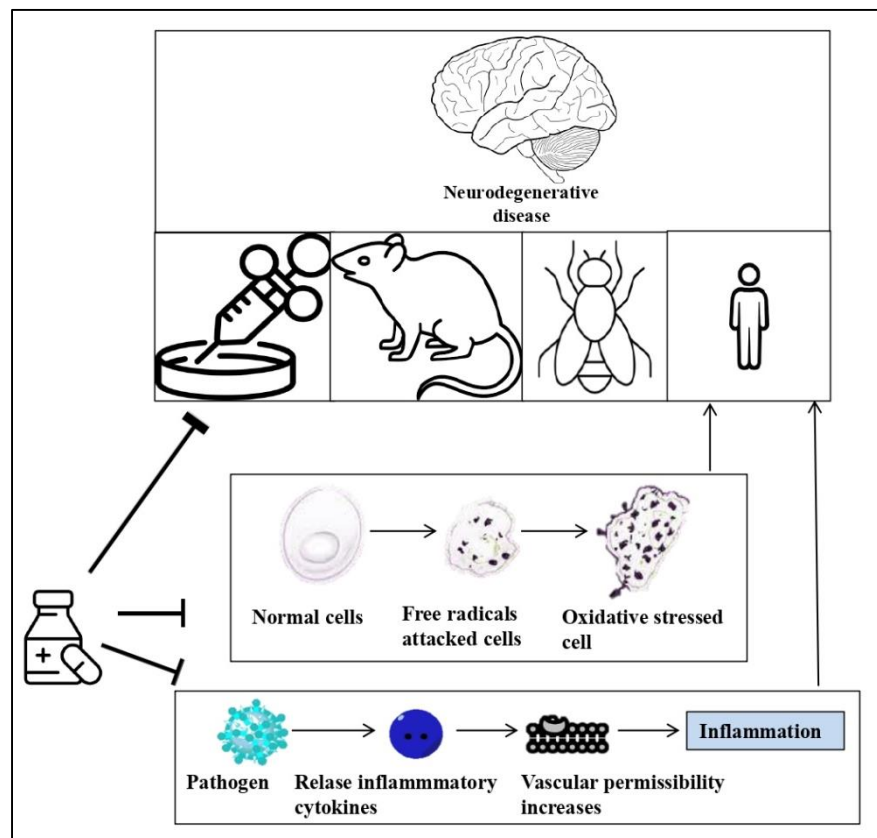
INTRODUCTION

The complex process of neuroinflammation is important in both pathological and homeostatic situations. Targeting brain inflammation has been viewed as a promising treatment in such situations since the neuroinflammatory process is a key factor in the course of many neurological disorders. Numerous compounds and their metabolites have been demonstrated to have anti-neuroinflammatory properties that can help prevent the start and progression of a number of neurological illnesses in recent research. The significance and

applicability of these compounds as neuroprotective agents will be discussed in this review, along with the molecular mechanisms by which they control neuroinflammation in the context of various brain diseases [1].

Oxidative stress, which is caused by excessive free radical production and stimulation of the inflammatory process, is a major contributor to neurodegenerative diseases [2]. The causes of most chronic diseases, including neurological diseases, include oxidative stress and inflammation [3]. Anti-inflammatory, antioxidant, and neuroprotection are all closely related (**Figure 1**)[4]. The neuroprotective activities of compounds, extract and other testing drugs are an exciting step forward in the search for efficient protectors due to the growing demand for novel, distinctive compounds [5]. Many studies suggest that the most crucial things is to find compounds with antioxidant and anti-inflammatory capabilities.

Figure 1: Showing relation between antioxidant, and anti-inflammatory activity and neuroprotection



Various types of tissue trigger inflammation as a biological reaction when they detect any foreign substance; the reaction's goals are to stop additional tissue harm and injury, clear and repair damaged tissue, and get rid of pathogenic substances. On the other hand, if inflammation persists for a long time, it develops into chronic inflammation and causes progressive deterioration. Glial cells, such as astrocytes and microglia, are present in Central nervous system (CNS) and act as the organ's immune system, protecting it from pathogens and preserving the health of neurons. Glial cells become activated as a result of tissue damage and systemic inflammation, which releases inflammatory mediators and causes both non-inflammatory diseases like Parkinson's disease, Alzheimer's disease, and Huntington's disease as well as inflammatory diseases like meningitis and multiple sclerosis in the brain [6].

Moreover inflammation or tissue damage can cause protein denaturation. Anti-inflammatory medications in general prevent thermally induced protein denaturation. The main cause of inflammatory conditions that lead to the production of auto-antigens, which might trigger the auto-immune response, can be protein denaturation. Thus, prevention of protein denaturation, which was non-steroidal anti-inflammatory drugs (NSAIDs)' primary mode of action before the discovery of their cyclooxygenase inhibitory effect, which will may be a key factor in NSAIDs' ability to reduce neurobiological pain [7,8].

So, taking all these facts in consideration, we had conducted a study to review and compile the possible involvement of antioxidant and anti-inflammatory activity in neuroprotection.

METHODOLOGY

A number of widely used databases, including SciFinder, Google Scholar, Google, MEDLINE, EMBASE, Scopus, PubMed, and Science Direct, were utilised to retrieve published papers (up until December 2022). We searched for and extracted published literature relating to antioxidant and anti-inflammatory role in neuroprotection using the keywords “antioxidant”, “neuroprotective”, “anti-inflammatory”, “Alzheimer”, “Huntington’s”, “assays” “neuro disease” and “Parkinson”. The language of searches was limited to English.

RESULTS

Role of antioxidant and anti-inflammatory in different neurobiological disease

Alzheimer’s disease

Alzheimer’s disease (most common neurodegenerative disease), is a persistent and irreversible pathological process. It is a complex disease, with chronic neuroinflammation and oxidative stress being major contributors for its onset and progression. Its distinctive loss of neuronal has been linked to the development of neurofibrillary tangles, which are primarily made of tau protein that has been hyperphosphorylated. The overactivity of GSK-3, a kinase involved in a number of pathogenic pathway including neuroinflammation, is linked to hyperphosphorylation of tau protein. Loss of neurons are also linked to the disruption in homeostasis of cytosolic Ca²⁺, which leads to apoptosis, the formation of free radicals, oxidative damage, and ultimately neuronal death. Researchers also studied new class of 4,7-dihydro-2H-pyrazolo[3-b]pyridines, which are multitargeted directed ligands with powerful antioxidant characteristics, has the ability to scavenge both nitrogen and oxygen radical species, as well as have anti-inflammatory properties. They can block calcium channels (L-type voltage dependent) and inhibit GSK-3, according to further characterization. On neurodegeneration in vitro models, novel compounds have also shown an intriguing neuroprotective characteristic. Lastly, testing drugs reverses the cell death brought on by hyperphosphorylation of tau in hippocampal slices by preventing the generation of reactive oxygen species. These medicines' multitarget profile is a novel therapeutic strategy that may be useful in the search for Alzheimer's disease treatments [9].

Traumatic Brain Injury (TBI) and Spinal Cord Injury

TBI term used to describe pathological circumstances including changes in brain function brought on by direct external force, such as a head blow. Widespread injuries to axonal and lesions that result in multiple CNS regions may cause symptoms of anomalies in cognitive, motor, behavioural, and affective domains. But memory-related cognitive impairment continues to be the most frequent and persistent aberration linked to TBI [10]. A variety of metabolic dysregulations, such as excitotoxicity, ischemia, calcium, and mitochondrial dysregulations, follow mainly damage of neuronal tissue in TBI and ultimately result in the secondary neuronal damage (inflammation-mediated). Because of this, it makes sense to incorporate anti-inflammatory therapies into the neuroprotective strategy of TBI therapy, either by using pharmaceutical medications to reduce the overactive microglia (or astrocytes) [11] or through alternative methods like cell replacement therapies [12], which have not yet been clinically validated as effective. The neuroinflammation strategy for treating TBIs should be understood as focusing on persistent inflammatory manifestations while maintaining healthy microglial function.

Cerebral Ischemia

The greatest pathogenic condition connected to cerebral ischemia is probably the cerebrovascular disease (stroke), which results from a disruption of blood flow (ischemia) or haemorrhage. Through the focal cerebral ischemia model induced by temporary middle cerebral artery occlusion (MCAO) in mice, study investigated the neuroprotective effect of Ursolic acid. The infarct size was significantly reduced after receiving 130 mg/kg (i.p.) of ursolic acid, and the level of the LPO marker, and MDA in the blood was low 24 hours after the stroke. In the latter case, both at the mRNA and protein levels, the antioxidant effect was accompanied by an increase in HO-1 nuclear and Nrf2 expression. However, it was discovered that Ursolic acid decreased the level of the Nrf2 cytoplasmic protein in the ischemic brain 24 hours after MCAO. Treatment with Ursolic acid, a potent anti-inflammatory, also reduced the level of NF- κ B and TLR4 expression after stroke in mice, both at the protein and mRNA levels. The Nrf2 signalling pathway is at the forefront of the proposed mechanism of action for Ursolic acid because it does not extend its protective effect in this mouse group and Nrf2/animals were more susceptible to brain injury (infarct size and oxidative and inflammatory scores) in the MCAO paradigm. As a result, the effect of ursolic acid is consistent with the Nrf2-antioxidant response element signalling pathway's established role in oxidative stress [13,14].

Assays used to study antioxidant activity

Ferric reducing antioxidant power (FRAP) assay

The freshly made FRAP reagent (2 mL) was mixed with various concentrations of plant extract or standards (10–500 g/mL). The samples were maintained at 37°C in a dark environment for 30 min, and absorbance at 593 nm was measured. For the calibration, new working solutions of ferrous sulphate were utilised. The ability of samples to decrease the amount of ferric ions was measured using linear calibration curve [15].

Total antioxidant capacity assay (TAC)

The TAC assay was performed in accordance with the published procedure, in which the assay mixture comprising 300 L of the testing drugs and 3000 L of phosphomolybdate reagent was maintained at 95°C for 1.5 hours. After 5 minutes of spinning at 3000 rpm, the supernatant solution's would be cooled and absorbance was measured at 765 nm. The EC50 values were given together with the TAC of the testing drugs and tested standards [16].

Phosphomolybdenum assay (PM)

By adopting the standard technique for the PM assay, total antioxidant activity can be calculated. A such reference was ascorbic acid. Each test tube included 3 ml of water (distilled) and 1 ml of the ammonium molybdate reagent before the testing formulations were added in various amounts. For 90 minutes, the tubes were incubated in water bath at 95°C. These tubes were incubated, cooled at the room temperature for 20–30 minutes, and the reaction mixture's absorbance was measured at 695 nm [17].

2,2-diphenyl-2-picrylhydrazyl radical scavenging assay(DPPH)

With testing formulations made in accordance with the procedure, the DPPH radical scavenging assay can be performed. The test samples were combined with 100 μ l of DPPH solution produced in ethanol (60 mol/l) in a variety of concentrations. The mixture was incubated for one and a half hours at the room temperature that too in the dark. And then the subsequent absorbance was measured at 517 nm. The standard utilised was ascorbic acid. The following equation of DPPH scavenging activity (%) was used to determine the DPPH scavenging activity of each sample[18].

Assays used to study anti-inflammatory activity

Hydrogen peroxide scavenging assay

Ascorbic acid served as the standard reference for testing the in vitro hydrogen peroxide (H₂O₂) scavenging activity. 0.6 ml of 4 mM H₂O₂ and phosphate buffer (0.5 ml) at pH 6.4 were combined with 0.5 ml of testing drug. Using phosphate buffer as a blank, the absorbance was then measured right away at 230 nm. Utilising the formula of % inhibition, the percentage inhibition was obtained. [19].

Proinflammatory cytokines

Tumour necrosis factor (TNF)- α , pro-inflammatory properties are widely known. Numerous inflammatory and autoimmune disorders, including Crohn's disease, rheumatoid arthritis, multiple sclerosis, and uveitis, have been linked to this cytokine. (TNF)- α targeting therapy plans have been used to combat inflammatory immunological processes. To determine the concentrations of TNF- α , IL-6, IL-1 β , IL-8 and MCP-1 cytokines, both in MCM, and in supernatants of the differently treated microspheroids, enzyme-linked immunosorbent assays (ELISA) can be performed according to manufacturers' instructions [20].

CONCLUSION

Worldwide, neurological illnesses have a significant role in both death and disability. The pathophysiology of wounds and disease processes includes a series of actions that frequently involve immune system molecules and cells interacting with central nervous system cells and structures. Because of this, there has been a lot of interest in creating treatment strategies for neuroprotection that specifically target neuroinflammatory and oxidative pathways. Numerous anti-inflammatory and antioxidant neuroprotective drugs have been researched for a range of neurological disorders and traumas. However, there is still a lot of interest in this area in developing neuroprotective methods. In light of this, recent evidence indicating a crucial function for the complement system in brain injury and neurodegenerative disorders is leading to increased discussion of the complement system as a promising therapeutic target for treating these conditions.

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