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REVIEW

Glutathione in Brain: Overview of Its Conformations, Functions, Biochemical Characteristics, Quantitation and Potential Therapeutic Role in Brain Disorders

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Abstract

Glutathione (GSH) is an important antioxidant found abundantly and synthesized intracellularly in the cytosol in a tightly regulated fashion. It has diverse physiological functions, including protection against reactive oxygen species and nitrogen species, antioxidant defense as well as maintenance of cellular thiol status. The human brain due to the high oxygen consumption is extremely susceptible to the generation of reactive oxygen species. GSH plays a paramount role in brain antioxidant defense, maintaining redox homeostasis. The depletion of brain GSH has also been observed from both autopsies as well as in vivo MRS studies with aging and varied neurological disorders (Alzheimer's disease, Parkinson's disease, etc.). Therefore, GSH enrichment using supplementation is a promising avenue in the therapeutic development for these neurological disorders. This review will enrich the information on the importance of GSH synthesis, metabolism, functions, compartmentation and inter-organ transport, structural conformations and its quantitation via different techniques. The transportation of GSH in the brain via different interventional routes and its potential role in the development of therapeutic strategies for various brain disorders is also addressed. Very recent study found significant improvement of behavioral deficits including cognitive decline, depressive-like behaviors, in APP (NL−G-F/NL−G-FG-) mice due to oral GSH administration. This animal model study put an emergent need to complete GSH supplementation trial in MCI and AD patients for cognitive improvement as proposed earlier.

Keywords Alzheimer's disease · Clinical interventions · Conformations · Glutathione · Antioxidant · Magnetic resonance spectroscopy · MEGA-PRESS · Quantitation

Introduction

Glutathione (GSH, γ-glutamyl-cysteinyl-glycine) is an intracellular linear tripeptide comprising of glutamic acid (Glu), cysteine (Cys) and glycine (Gly) amino acids present ubiquitously in all mammalian cells [1]. The presence of the sulfhydryl (SH) group of Cys moiety, renders GSH a potent antioxidant property by interacting with reactive oxygen species /reactive nitrogen species (ROS/RNS) [2, 3]. Upon reduction, GSH forms two molecules which dimerize by disulfide linkage to form oxidized glutathione disulfide (GSSG) [2]. The reduced (GSH) and oxidized disulfide form (GSSG) are interconvertible, with reduced GSH being present as the predominant form [4]. GSH acts in coordination with other redox-active compounds like nicotinamide adenosine diphosphate (NADPH) to regulate and maintain cellular redox status [5]. Glutathione's mechanism of action involves enzyme glutathione peroxidase (GPx) and glutathione reductase (GR), where GPx is responsible for the conversion of GSH to oxidized form GSSG and GR reduces GSSG back to GSH [6, 7]. The GSH–GSSG cycle inside the cell is primarily involved in the detoxification of hydrogen peroxide (H_2O_2) to water and oxygen. Additional functions of GSH

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involve (i) maintenance of antioxidant defense (ii) cellular redox status (iii) detoxification of xenobiotics (iv) Cys reservoir (v) maturation of iron–sulfur (Fe–S) cluster proteins (vi) storage and transport of nitric oxide (NO) [8–10].

The profound role of GSH in the brain as a detoxifying agent is critically important because of its higher vulnerability towards oxidative stress (OS), as it utilizes 20% of the O_2 consumed by the body whereas constitutes only 2% of body weight [11]. GSH is also involved in other cellular processes such as neuroinflammation and ferroptosis, which brings the attention of pharmacologists pertaining to medical interventions for therapeutic benefits. The depleted levels of GSH trigger ROS generation implicated in the cell death causing various neurological diseases like Alzheimer's disease (AD), Parkinson's disease (PD) and multiple sclerosis (MS) [12–16].

Various autopsy as well as in vivo studies indicated that GSH level varies across different brain regions and also with different neurological diseases [14, 17–21]. Several autopsy studies showed that the GSH levels were found to be decreased in the AD brain in comparison to the healthy controls (HC) [17, 18]. A post-mortem analysis of Mild cognitive impairment (MCI) brain samples showed a significant reduction in hippocampal (HP) GSH level [19]. To detect the brain GSH in vivo MEscher GArwood-Point RESolved Spectroscopy (MEGA-PRESS) technique is preferred due to several clinical applications [20–25]. Similarly from the in vivo MRS study, GSH levels were reported to be statistically significant in different brain regions like frontal cortex (FC) and HP among MCI and AD patients [14, 20]. The GSH exists in two conformations as extended and in the closed form [22, 23, 26]. Structural aspects of GSH have also clinically indicated from a very recently published in vivo study, which showed that closed GSH conformer was depleted in the anterior cingulate cortex (ACC) and posterior cingulate cortex (PCC) regions, therefore, suggested to be a potential biomarker for AD [21].

In order to replenish the brain antioxidant defense homeostasis, GSH or N-acetyl cysteine (NAC) as a supplement were reported to be beneficial in modulating GSH levels as suggested in different clinical trials [27–34]. The intervention of supplements to the brain were reported via different modes of delivery like intranasal, intravenous, sublingual and subcutaneous mode, in healthy as well as individuals suffering from neurological disorders [28, 31–33, 35–42].

Several studies were found which were focused on varied aspects of GSH such as structure, synthesis, functions, and its use in therapeutic practice. In order to accumulate all the information pertaining to GSH, we present this detailed review article with a focus on GSH biosynthesis, regulation, functions, and metabolism. The GSH in vivo detection and conformational states are also presented in this review. The compartmentalization, Inter-organ transfer and GSH transport to the brain via blood brain barrier (BBB) and its importance in various brain disorders are also discussed. GSH deficiency, therefore, plays a crucial role in aging as well as the pathogenesis of many neurological diseases. Based on these mechanisms and functions mentioned, some potential approaches for supplementation and its therapeutic benefits are also discussed. The review therefore also concentrates on various clinical supplementation studies with the intention to enrich the master antioxidant GSH in the brain. This review presents a critical discussion of the applications of GSH and approaches toward clinical intervention studies of various neurological diseases.

GSH-Biosynthesis, Regulation and Metabolism

GSH is found in the cytosol of all mammalian tissues in the range of 1 to 10 mM concentration [43]. GSH is synthesized using constituent amino acids i.e., Glu, Cys, and Gly in two steps where Glu and Cys combine to form γ-Glu-Cys in the presence of enzyme glutamate cysteine ligase (GCL, EC 6.3.2.2), which further combines with Gly in the presence of GS (Glutathione synthetase; EC 6.3.2.3) to synthesize GSH (Fig. 1) [4]. GCL enzyme is a heterodimer composed of two subunits i.e., catalytically active heavy subunit as GCLC (73 kDa), and light modifier subunit as GCLM (30 kDa). GCLC functions as a substrate-binding unit whereas the GCLM modulates the binding affinity of GCLC by altering the K_m value. GS is a homodimer (52 kDa) comprising of two identical subunits

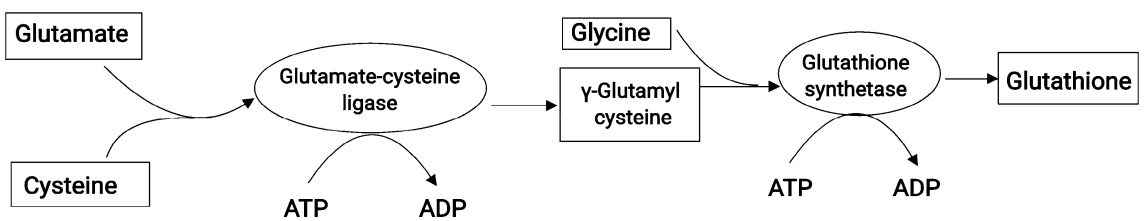

Fig. 1 Synthesis of GSH. GSH synthesis is a two-step process where Glu, Cys, and Gly are catalyzed in the presence of enzymes GCL and GS. The figure was designed using BioRender with information taken from existing literature [4]

[44]. GSH possesses a unique peptide bond formed by the γ-carboxyl group of N-terminal glutamate with the Cys residue. This specific peptide linkage protects GSH from being cleaved by most peptidases which hydrolyze at the α-carboxyl peptide bond of N-terminal amino acids. GSH is cleaved only at the outer surfaces of tissues containing γ-glutamyl transferase (γ-GT) whereas, it remains relatively stable inside the cell [45]. GSH is synthesized in all cell types with the maximum amount of GSH synthesized and exported from the liver. The source of substrates required for GSH synthesis varies between different cell types. Thus, alterations in the GSH plasma level may not reflect changes in GSH synthesis in a specific cell [46]. The whole blood (mainly red blood cells) may contribute approximately 10% of the complete GSH synthesis in humans [47, 48]. A decline in the amount of GCL and GS has been reported to result in the reduction of GSH levels in red blood cells of AD patients [49].

GSH synthesis regulation occurs (a) via non-allosteric feedback competitive inhibition where GSH (Ki = 2.3 mM) binds with glutamate thus, preventing glutamate from binding to Cys, (b) via the availability of its precursor L-cysteine [43, 50–53]. The availability of Cys is known to be affected by certain factors like diet and amino acid uptake. The hepatocellular level of Cys is regulated via electroneutral sodium/amino acid co-transporters (ASC) system and cysteine glutamate exchanger (X_c^-) as well as the trans-sulfuration pathway [52, 54]. Other major determinants in the regulation of the rate of GSH synthesis are enzymes GCL and GS. Changes in GCL subunits (GCLC and GCLM) occurring at the transcriptional and post-transcriptional level has been extensively studied [55–57]. The post-transcriptional regulation of GCLC involves mRNA stabilization/destabilization of certain signaling molecules (PI3K/AKT/p70S6K) activated by insulin [58]. Unlike GCL, regulation by enzyme GS has been unexplored and overlooked and requires further research to reach conclusive inference.

GSH acts as an antioxidant in two ways as (i) it directly reacts non-enzymatically with free radicals such as superoxide radical ($O_2^{\cdot -}$), NO, or hydroxyl ion (OH^-) [59–62] and (ii) indirectly functions as a reducing agent by donating an electron to H_2O_2 for its reduction to water and O_2 in the presence of GPx enzyme [63, 64]. GPx (EC 1.11.1.9) exists in two forms i.e., selenium-dependent and selenium independent. Four seleno-cysteine-containing isozymes of GPx reported to date are GPx 1, 2, 3 and 4 [65]. Now, GSH is reported to be extensively used as a co-substrate by GPx reducing H_2O_2 to water and O_2 molecules and conversion of lipid hydroperoxides (LOOH) into organic peroxides (ROOH) producing GSSG [7]. GSSG formed further follows two fate (i) gets reduced back to GSH by GR [6] (ii) excreted outside the cell. GR (EC 1.8.1.7) functions as dimeric disulfide oxidoreductase which acts by transferring an electron from NADPH to GSSG, thereby regenerating GSH [66].

Functions of GSH

The OS in the biological process occurs as a result of the excessive production of free radicals. Oxidative stressors such as ROS are derived from a series of univalent reduction of O_2 molecules produced as a result of aerobic respiration and substrate oxidation. ROS are involved in various biological processes such as cell growth, cell signaling, immune responses [67–69]. Excessive ROS production is known to damage the cellular system by oxidation of major biomolecules (lipids, proteins, and nucleic acids) [70–73]. Mitochondria being the most redox-active cell organelle produces mitochondrial ROS in the form of $O_2^{\cdot -}$, hydroxyl radical (·OH) and H_2O_2 [74–77].

Mitochondrial complex I (~ 1 MDa), comprising of 45 polypeptides in mammals, is the entry point of electrons from NADH into the electron transport chain (ETC). Electrons released from NADH are accepted by the flavin mononucleotide (FMN) cofactor, which then passes the electrons through a chain of Fe–S clusters to the ubiquinone (Q) reduction site. During these events, NADH is oxidized to NAD^+, FMN is reduced to $FMNH_2$ and Q is reduced to ubiquinol (QH_2) [78, 79]. Large amounts of $O_2^{\cdot -}$ (by univalent reduction of O_2) generation have been reported in isolated mitochondria via two modes of operations: mode 1, when NADH is abundantly present (high NADH/NAD^+ ratio) and mode 2, when there is a large proton-motive force (Δp) and reduced Q pool. Thus, the kinetics and thermodynamics factors favoring the interaction of potent one-electron donors with O_2 controls the mitochondrial ROS production [80]. The predominant site of $O_2^{\cdot -}$ production in both the modes is in complex I, thus making it a major source of $O_2^{\cdot -}$ production within the mitochondria [81–84]. The mechanism of $O_2^{\cdot -}$ production in two modes are different as the production of $O_2^{\cdot -}$ in mode 1 occurs during the reduction of FMN to $FMNH_2$ in the presence of abundant NADH whereas, the mode 2 involves $O_2^{\cdot -}$ production via reverse ETC where electrons are forced back from QH_2 to complex I [85]. Under some conditions, when the build-up of NADH and Q coincides with a large Δp, both modes operate simultaneously. Mitochondrial complex III (~ 240 kDa monomer) accepts electrons from Q pool and reduces cytochrome-c. The complex III has been regarded as a source of $O_2^{\cdot -}$ within mitochondria for a long period of time [86, 87]

The superoxide dismutase (SOD) neutralizes $O_2^{\cdot -}$ into H_2O_2 which follows three fates (i) it is reduced by Fe^{2+} (normally present in cells) which donates an electron to produce ·OH, OH^- and Fe^{3+} in Fenton's reaction. An ·OH is a highly reactive molecule with high oxidizing ability. It

oxidizes cellular components such as proteins, lipids and DNA by removal of an electron (associated with H atom) (ii) it combines with $O_2^{\cdot-}$ to generate OH^-, ·OH and O_2 in Haber Weiss's reaction, (iii) H_2O_2 is neutralized to H_2O and O_2 in a reaction catalyzed by inter-conversion of GSH to GSSG via GPx. The ROS production within the mitochondria results in oxidative damage to mitochondrial lipid membrane, proteins, DNA and ATP synthesizing ability. The mitochondrial ROS also causes derangement in the majority of cellular metabolic functions including the citric acid cycle, urea cycle, amino acid metabolism, haem synthesis, Fe–S center assembly and fatty acid oxidation. The mitochondrial oxidative damage may also result in the release of intermembrane space proteins such as cytochrome-c in cytosol leading to activation of the cellular apoptotic machinery. Consequently, mitochondrial oxidative damage contributes to a wide variety of pathologies [88]. The detailed mechanism of free radical generation and fate of H_2O_2 are illustrated in Fig. 2.

To maintain redox homeostasis, the cellular system possesses an antioxidant defense system as a counteractive mechanism which includes various antioxidants such as SOD, catalase, vitamin C, vitamin E, and GSH. Catalase is predominantly found in peroxisomes but its mitochondrial expression shows antioxidative effectiveness to be dependent upon the oxidant and the site of ROS production. The absence of catalase inside the mitochondria increases its vulnerability towards OS generation [90, 91]. GSH is mainly responsible for protection against ROS and RNS however other important functions include maintenance of redox status, detoxification of xenobiotics, regulation of cellular events including gene expression, DNA and protein synthesis, apoptosis, signal transduction, protein glutathionylation, cytokine production and immune response [8–10]. The specialized structural, biochemical and biophysical properties and its ability to synthesize independently determine its potential functions as a primary antioxidant [92, 93].

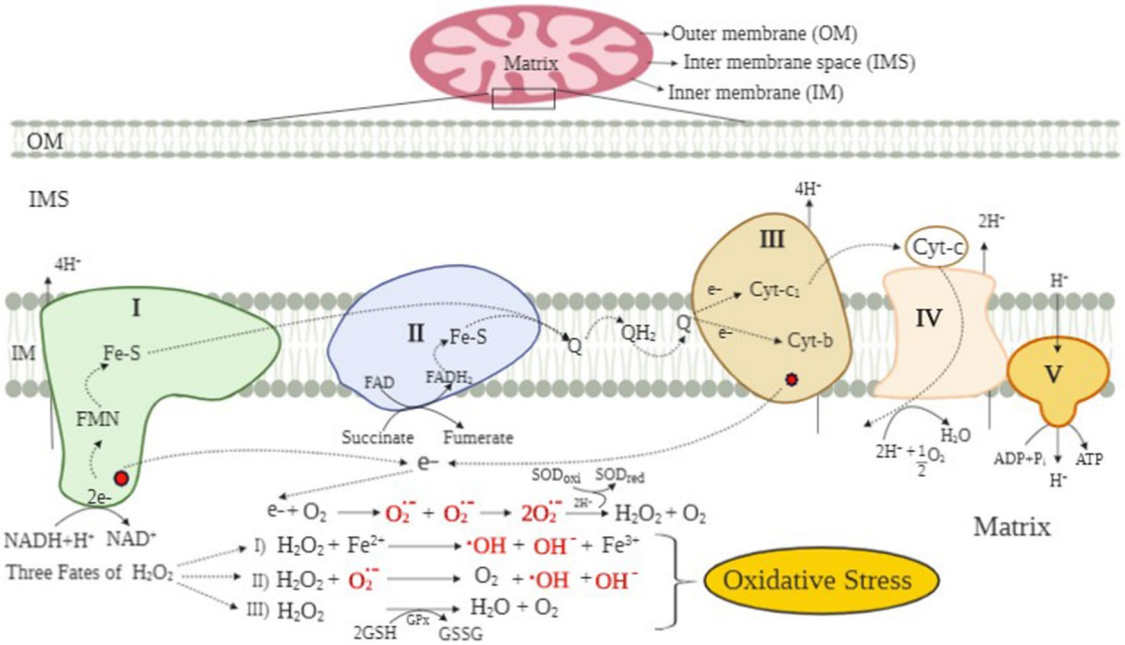

Fig. 2 Schematic view of ROS production and antioxidant defense system in mitochondria. NADH generated by the citric acid cycle inside the cell serves as an electron donor. During electron transport chain (ETC), electrons are passed through mitochondrial complexes "down the line". Two electrons removed from NADH are accepted by an FMN cofactor in Complex I. During this phase, NADH+ H^+ is oxidized to NAD^+ by reducing FMN to $FMNH_2$. The electrons from $FMNH_2$ are passed further through Fe–S clusters to Q reduction site where Q is reduced to QH_2. Complex II catalyzes the oxidation of succinate to fumarate and electrons gained during this reaction are accepted by FAD and passed further via a chain of Fe–S clusters to Q. During these chains of events FAD is reduced to $FADH_2$ and Q is reduced to QH_2. Complex III accepts electrons from QH_2 which are transferred one by one in a complex process called the Q-cycle onto another electron carrier, cytochrome-c. Reduced cytochrome-c further gets oxidized by complex IV i.e. cytochrome-c oxidase to reduce oxygen to water. The proton-motive force (Δp) generated in the form of H^+ ions finally leads to ATP synthesis via complex V i.e. ATP synthase, thereby completing the electron transport chain. However, some electrons can escape the respiratory chain (complex I and complex III processes) and combine with O_2 to form $O_2^{\cdot-}$. The superoxide dismutase (SOD) present inside mitochondria reduces $O_2^{\cdot-}$ to H_2O_2 which follows three fates where, (i) it is reduced by Fe^{2+} to produce ·OH, OH^- and Fe^{3+} in Fenton's reaction. (ii) it combines with $O_2^{\cdot-}$ to generate OH^-, ·OH, and O_2 in Haber Weiss's reaction, (iii) it is converted to H_2O and O_2, catalyzed by inter-conversion of GSH to GSSG via GPx. The figure was designed using BioRender with information taken from existing literature [64, 74, 76, 77, 88, 89]

GSH donates an electron to form two glutathione-thiyl radicals (GS*), which further combine to form GSSG. The antioxidant function of GSH involves catalytic oxidation of thiol group of its Cys moiety in the presence of GPx to produce GSSG which in turn is reduced to GSH in the presence of GR. The detoxification of H_2O_2 to water and O_2 molecules is catalyzed by the conversion of GSH to GSSG via GPx [64, 94]. NO produced from L-arginine by nitric oxide synthase (NOS), is a reactive gaseous free radical known to react with $O_2^{\cdot-}$ and cause nitrosative stress inside the cell by forming nitroxyl radicals (HNO), nitric oxides (NO_2, N_2O_4, N_2O_3), peroxynitrite ($ONOO^-$) and S-nitrosothiols (RSNO) [95]. These nitric oxide radicals generated are neutralized to S-nitroso glutathione (GSNO) by GSH thereby reducing nitrosative stress [96, 97]. GSH is also involved in the conversion of LOOH into lipid hydroxides (LOH), protein oxidant (P-SOH) into glutathionylated protein, and also helps in DNA repair (Fig. 3) [10, 94, 98, 99]. GSH also aids in the storage of Cys molecules as reactive Cys undergoes rapid autoxidation to form Cystine. Cystine produces toxic O_2 radicals, therefore GSH act as a carrier and scavenger for the transport of Cys molecule [100].

Studies have also reported that GSH is primarily responsible for detoxification of xenobiotics, either directly by conjugating or acting as a substrate in conjugation reactions. Total antioxidant capacity levels were decreased on chronic exposure to xenobiotics like alcohol, explicating the fact that chronic exposure to xenobiotics leads to lipid peroxidation, depletion of cytosolic GSH stores and mitochondrial damage [101]. The transcriptional control involved behind the detoxification of xenobiotics is nuclear factor erythroid 2-related factor 2 (Nrf2)/Kelch like ECH associated protein 1 (Keap1) system [102]. GSH, being involved in detoxification could be an effective therapeutic strategy to decrease the toxic effects of xenobiotics inside the body.

GSH plays another important function, where it is involved in the maintenance of metal homeostasis. The redox-active metals like iron, copper, and chromium, undergo redox cycling and cause OS directly via Fenton's reaction. Redox-inactive metals such as lead, cadmium, mercury, cobalt, and arsenic exhaust cell's SH reserves and are indirectly involved in ROS formation [103, 104]. Redox-active metals cause cellular damage via production of ROS i.e., ·OH, $O_2^{\cdot-}$ and H_2O_2 (Fig. 2). The mechanism of redox-active metal-induced oxidative damage is extensively studied whereas the mechanism of redox inactive metals remains elusive and feasibly involves increased lipid peroxidation, alteration in thiol status and DNA damage [105, 106]. Metal induced toxicity and interactions with cellular aggregates have also been associated with neurodegenerative disorders such as AD [107, 108]. The role of GSH in the neutralization of redox-active metal like copper and iron is well documented where GSH is believed to be responsible for their mobilization, transport, and delivery to specific target molecules [109, 110]. The involvement of GSH in the maturation of Fe–S cluster proteins is also reported [111, 112].

GSH is also known to be involved in the maintenance of optimal cytokine levels. Pro-inflammatory cytokines i.e., tumor necrosis factor-α (TNF-α), Interleukin-1β (IL-1β) and IL-6 gene expression were increased due to GSH depletion, which further led to check the expression of anti-inflammatory cytokines i.e., IL-10. The levels of IL-10 remained unaltered elucidating the fact that GSH depletion leads to stimulation of pro-inflammatory cytokines and not anti-inflammatory cytokines [113, 114]. This GSH homeostasis alteration is resulted due to upregulation in nuclear factor kappa light chain enhancer of activated B cells (NFκβ) and c-Jun N-terminal kinase (JNK) signaling pathway which could be the plausible apoptotic pathway towards neuronal cell death [115, 116].

The balance of the GSH/GSSG ratio is crucial for the maintenance of redox status and cell survival [117, 118]. Starvation induced OS resulted in decreased GSH and shift towards more oxidizing conditions which further potentiated autophagy [119]. GSH reduction was observed in response to the treatment of tetrahydrobiopterin (BH4; an OS inducer)

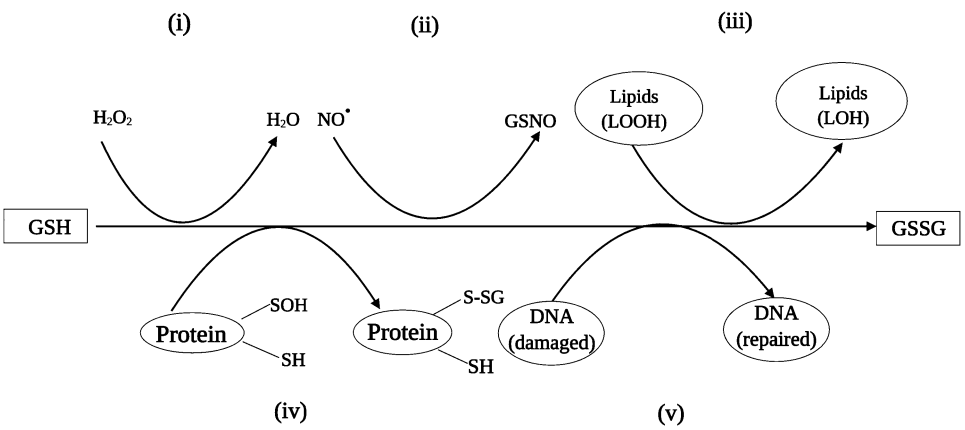

Fig. 3 Involvement of GSH in cellular detoxification. GSH conversion to GSSH releases an H^+ moiety which is utilized for neutralization of oxidants such as (i) H_2O_2, (ii) NO·, (iii) LOOH and (iv) P-SOH into their less toxic forms i.e. H_2O, GSNO, LOH, PS-SG respectively, (v) GSH is also involved in nucleic acid repair. The figure was designed using BioRender with information taken from existing literature [10, 94, 96–99]

via activation of $p38^{MAPK}$/p53 signaling cascade of apoptosis [120]. GSH depletion due to increased OS resulted in GPx4 inhibition that potentiated iron-induced cell death (ferroptosis) [121–123]. Overall the above-reported evidences suggest the involvement of GSH in various cell death pathways i.e. autophagy, apoptosis, and ferroptosis. Autophagy and apoptosis involve common inducing conditions (starvation) with autophagy underlying the first cell response (survival) before proceeding to apoptosis. GSH function in cellular detoxification is illustrated in Fig. 3.

Compartmentalization and Inter-Organ Transport of GSH

GSH pool is compartmentalized in different intracellular organelles like cytoplasm, mitochondria, endoplasmic reticulum (ER), and nucleus. GSH synthesized in the cytosol, is transported and distributed for its utilization in different organelles where it is involved in the regulation of cellular redox status. The reduced form of GSH is prominent in all the organelles except ER. In ER, GSSG serves as an oxidizing equivalent for the production of nascent polypeptides conformation by intramolecular disulfide bond formation between Cys residues [124–127]. In the nucleus, GSH plays a crucial role in maintaining the redox status of sulphydryl groups of proteins involved in DNA synthesis and repair. In addition, GSH also reduces ribonucleotides to give deoxyribonucleotides [125]. Due to the fact that mitochondria have a pivotal role in programmed cell death (apoptosis) and are the site of extensive ROS production, it constitutes 10–15% of total cellular GSH [128]. The inner mitochondrial membrane is rich in a specific phospholipid, cardiolipin which provides stability and fluidity to the membrane. Cardiolipin is associated with cytochrome-c at the inner mitochondrial membrane. The mitochondrial GSH (mGSH) protects cardiolipin from oxidative damage thus prevents the inner membrane from destabilization and dissociation of cytochrome-c. ROS generation results in an increase in internal membrane permeability for calcium ions which triggers apoptosis [45]. Thus, mGSH plays an important role here by preventing apoptosis which is triggered by the release of cytochrome-c from the inner mitochondrial membrane. Hence, reduction in mGSH has been associated with various diseases such as diabetes mellitus, liver cirrhosis, neurological diseases like AD and PD [17, 129–131].

GSH is secreted in high concentrations which are translocated from hepatocytes into bile canalicular spaces and plasma [132, 133]. It is hydrolyzed into its constituent amino acids (i.e. Glu, Cys and Gly) in bile spaces where it is captured by hepatocytes to resynthesize GSH. The plasma GSH level is relatively low (22–27 μM), apparently due to its fast utilization by tissues having γ-GT enzyme located on the external surface of some tissues [134]. The highest amount of γ-GT activity is found in the kidney thus, kidney is the major organ for the extraction of GSH from plasma. The GSH translocation to extracellular spaces is an important factor for cellular GSH turnover. GSH from plasma is extracted by kidneys where it is hydrolyzed into its constituent amino acids by membranous enzymes (γ-GT) and peptidases. Renal tubular cells also secrete GSH into the tubular lumen where it is hydrolyzed into its constituent amino acids. These amino acids are taken up by renal tubular cells to resynthesize GSH. These constituent amino acids are also transferred to plasma from where they are translocated to the liver and small intestine through Na-dependent amino acid transporters for GSH synthesis. The GSH synthesized in intestinal epithelial cells is degraded and the constituents amino acids are taken up by the liver to restore GSH [135, 136]. While in the brain, the GSH is translocated from the plasma via the BBB [137]. The concentration of GSH varies in different tissues. Liver and small intestine contain highest concentration of GSH in body ranging from 1 to 6 mM [43], followed by kidney (2–5 mM) [138], brain (2–3 mM) [139], blood (~ 1 mM) [140, 141], and lung epithelial cells (0.42 mM) [142]. The combination of secretion, hydrolysis, and restoration of GSH and translocation of its constituent amino acids constitutes (i) Hepato-renal cycle (ii) Entero-hepatic cycle (iii) Intra-renal cycle and (iv) Intra-hepatic cycle, as illustrated in Fig. 4.

GSH in the Brain

Biosynthesis

The brain constitutes only 2% of the bodyweight but utilizes 20% of the O_2 used by the whole body. Due to the high O_2 utilization and poor antioxidant status, the brain is highly susceptible to OS [11]. Amongst the major central nervous system (CNS) cells, the concentration of GSH is highest in astrocytes followed by neurons [143–146]. GSH is synthesized as de novo in astrocyte using its constituent amino acids Glu, Cys and Gly. The GSH synthesized in an astrocyte is transported to extracellular space via multiple drug resistance protein 1 (MRP1) where it is hydrolyzed into CysGly and γ-GluX moieties by enzyme γ-GT [147–149]. The CysGly moiety is cleaved into Cys and Gly via neuronal ectopeptidase aminopeptidase which is transported to neuron via excitatory amino acid carrier 1 (EAAC1) and glycine transporter (GlyT2) respectively [149–155]. The Cys and Gly combine with Glu (provided by glutamate-glutamine cycle operating between astrocytes and neurons) to synthesize GSH [156] (Fig. 5). Due to the major role played in the synthesis and transport of GSH precursors, astrocyte act as a neuroprotector against OS and neurodegeneration [157].

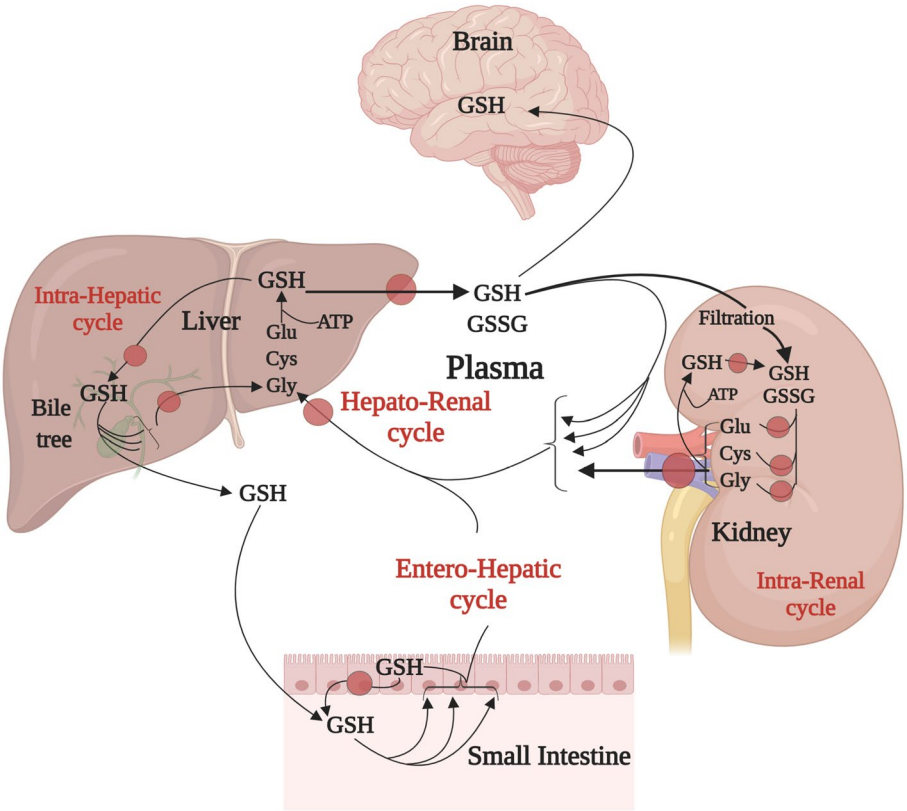

Fig. 4 Inter-organ GSH transport. GSH is secreted from hepatocytes into empty spaces and bile canalicular spaces. This GSH is degraded in kidneys by membranous γ-GT and other peptidases. GSH from the liver is also transferred to the small intestine where it is hydrolyzed to its constituent amino acids on the brush border membrane. The resulting amino acids are taken up by intestinal epithelial cells by Na dependent transporters and transferred to plasma from where they are transferred back to the liver for its regeneration. GSH is translocated from plasma to the brain via the BBB. The figure was designed using BioRender with information taken from existing literature [136]

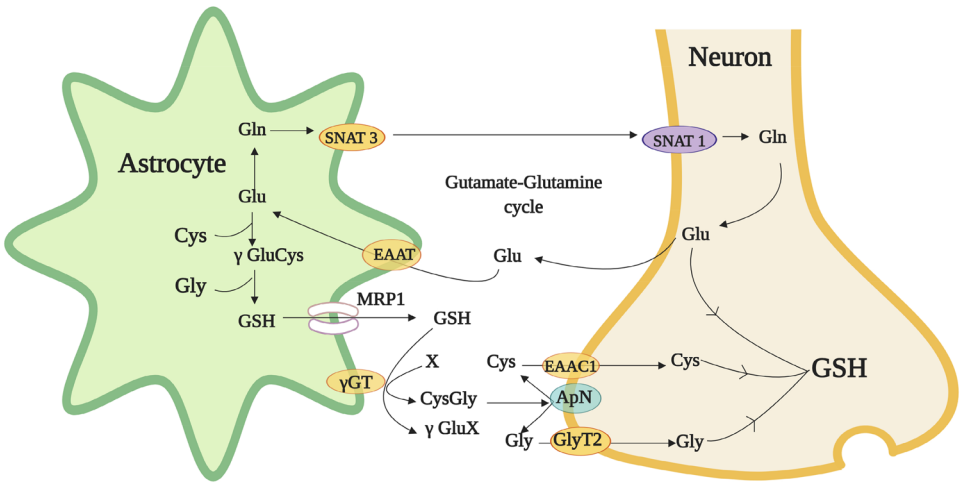

Fig. 5 GSH synthesis in CNS. GSH is exported from the astrocyte through MRP1 and is catabolized by the ectozyme γ-GT to provide CysGly and γ-GluX. The resulting amino acids are further catabolized by aminopeptidase into Cys and Gly and are taken up by the neuron via EAAC1 and GlyT2 respectively. In the neuron, Cys and Gly are combined with Glu (provided by glutamate-glutamine cycle) to synthesize GSH. The figure was designed using BioRender with information taken from existing literature [149, 153]

Route and Transport Across BBB

BBB formed by the blood vascular endothelial cell lines the cerebral microvessels and is required to control the brain microenvironment by separating the blood from the brain. Various molecules are transported through BBB via different routes [158, 159]. Paracellular transport through tight junctions is responsible for the transport of simple water-soluble molecules, while other lipid-soluble substances like steroids and alcohol are transported passively via diffusion. Other molecules such as amino acids and glucose are transported via solute carrier-mediated transport based on concentration gradient with the use of ATP as an energy source. GSH is a tripeptide known to cross the BBB through Na-dependent GSH transporter, which belongs to the solute carrier family [137]. Other routes like receptor-mediated transcytosis, adsorptive-mediated transcytosis are responsible for the transport of macromolecules and charged molecules with characteristic receptor-ligand interaction and electrostatic properties respectively (Fig. 6) [160]. The efflux transporters are also present for the extrusion of drugs outside the brain [161].

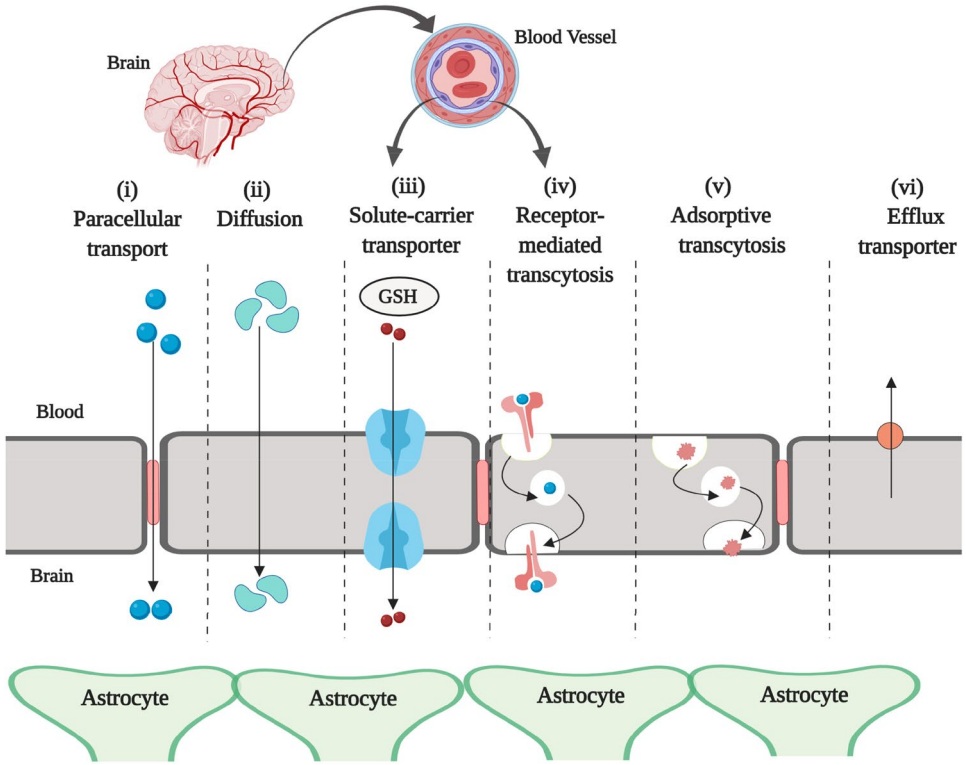

Fig. 6 Routes of transport across the blood brain barrier (BBB): (i) Several simple water molecules pass the BBB through paracellular transport (ii) Certain other compounds like lipid molecules pass passively via diffusion. (iii) Solute carrier transporters are involved in energy mediated active transport of certain molecules like glucose, amino acids, and nucleosides. (iv) Receptor-mediated transcytosis requires the receptor binding of ligand and can transport various molecules like peptides and proteins across the BBB. (v) Adsorptive-mediated transcytosis involves the transfer of charged molecules like cationic peptide to cross the barrier via electrostatic interactions. (vi) Efflux transporter is responsible for the extrusion of drugs out of the brain. The figure was designed using BioRender with information taken from existing literature [158]

Based on the previous studies on GSH uptake by endothelial cells in rat and bovine brain led to experiments of GSH uptake in human cerebrovascular endothelial cells and fetal human embryonic cells. Na dependent GSH transporters are reported to be localized in the lumen of endothelial cells [162–164]. This Na-dependent GSH transporter was shown to be bidirectional. Uptake of GSH by astrocytes was also checked which elucidated the presence of Na dependent GSH transporters in both astrocytes and brain endothelial cells. Once it reaches to astrocytes, GSH takes its path until it reaches neuron via carrier-mediated transporters (Fig. 7) [137].

Biophysical, Chemical and Conformational Characteristics of GSH

The physical and chemical properties of GSH are enlisted as—molecular weight 307 g/mol, intermediate molecular flexibility with nine rotatable bonds, the lipophilicity of—6.4; and molecular topological surface area of 160 Å [93]. Nuclear magnetic resonance (NMR) and molecular dynamic studies have reported that GSH exists in two interconvertible conformations "extended" and "folded" (closed) forms, though in aqueous solution it remains typically in "extended" form [26]. Under physiological conditions, the conformational distribution and hydrogen-bonding network plays a crucial role in GSH functioning. Due to the presence of Cys molecule, the GSH is considered to be very sensitive to the surrounding environment. The analysis of NMR spectra obtained in the inert environment reported that GSH exists mainly in closed conformation which is identified by the presence of Cys H_β peak at 2.79 ppm while the GSH also exists as extended conformation, identified by the presence of Cys H_β peak at 2.95 ppm [22, 23, 26]. Very recently, for the first time, study reported alterations of in vivo GSH closed conformers among normal, MCI and AD brain using the MEGA-PRESS pulse sequence [21] (Fig. 8).

Brain GSH Quantification

Differences in GSH content between brain regions may reflect variations in the availability of GSH for vital cellular

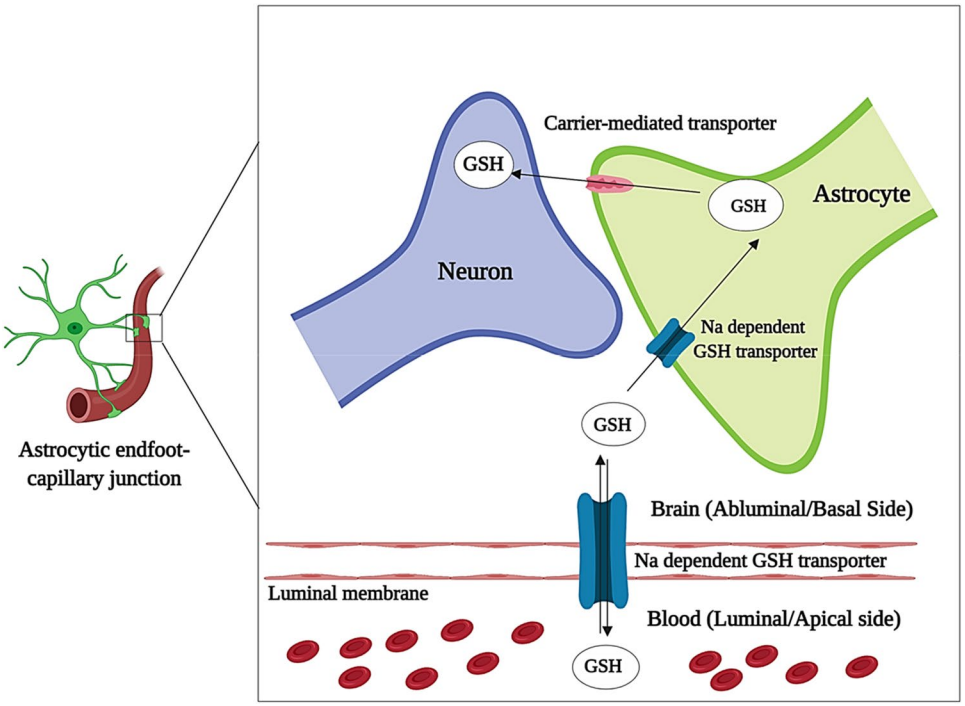

Fig. 7 GSH transport across the blood brain barrier (BBB): GSH uptake from the blood (luminal side) to the brain (abluminal side) is mediated via Na dependent GSH transporter localized at the luminal membrane. This GSH from abluminal side en-route astrocyte via Na dependent GSH transporter, which is effluxed through carrier-mediated transporter to neuron. The figure was designed using BioRender with information taken from existing literature [137]

functions. The highest concentration of GSH is observed in the cortex followed by HP, cerebellum, striatum and substantia nigra (SN) [168, 169]. GSH metabolism-related disorders in the brain are highly correlated with an increased level of OS due to ROS generation [170]. Thus, to develop a clear understanding, accurate estimation of GSH content in the brain is necessary with clinical significance. GSH measurement in autopsy brain was performed by various methods such as high-performance liquid chromatography with UV detection and spectroscopic technique for evaluation of the oxidative product of 5,5′-Dithio-bis (2-nitrobenzoic acid) [171, 172]. However, the in vivo detection of GSH in the human brain is performed by proton magnetic resonance spectroscopy (^{1}H MRS).

Autopsy Studies

GSH levels in the cingulate cortex region of autopsy brain samples (N = 10) were found to be decreased in the AD brain in comparison to the HC, whereas no significant changes in GSH levels were observed in the PD brain [17]. In another study, prefrontal cortex regions obtained from autopsy brain tissues of non-cognitively impaired (N = 10), MCI (N = 8), mild/moderate AD (N = 4), and late-stage AD (N = 9) patients were assessed for GSH levels and significantly decreased GSH levels were reported in post mitochondrial supernatant, mitochondria, and synaptosomal fractions in MCI, AD and late AD samples in comparison to controls [18]. A study investigated GSH in HP (N = 6), from post-mortem autopsy brain of MCI patients, showed that GSH was significantly decreased in HP in comparison to HC samples [19].

In Vivo Studies Using MRS

In order to detect the GSH in vivo in various brain regions, special pulse sequence MEGA-PRESS is considered as a selective and confirmatory method of choice [14, 23, 32, 173]. This technique has several clinical applications, as it is useful in understanding various neurological disease processes [20–25]. The multi-centric study from our laboratory confirmed the existence of two GSH conformers (extended and closed) as illustrated in (Fig. 9a) [23]. Additionally, a very recent study from our laboratory also for the first time showed the clinical importance of GSH and reported that the closed GSH conformer is depleted in ACC and PCC regions and therefore suggested to be a potential biomarker for AD [21]. In our earlier in vivo MRS study, the GSH levels were reported to be statistically significant in the right and left FC region among AD female and male (F/M-7/7) patients as compared to healthy young female and male (F/M-20/25) controls respectively [20]. Another study conducted to detect GSH concentrations in different brain regions like the HP (right and left regions) suggested that MCI (N = 22) and AD (N = 21) patients had significantly decreased GSH levels when compared to healthy old control (N = 21) [14] (Fig. 9b).

Apart from neurodegenerative diseases, in vivo detection was also proven to be beneficial in epilepsy, schizophrenia, and MS [24, 25, 174]. A study conducted with

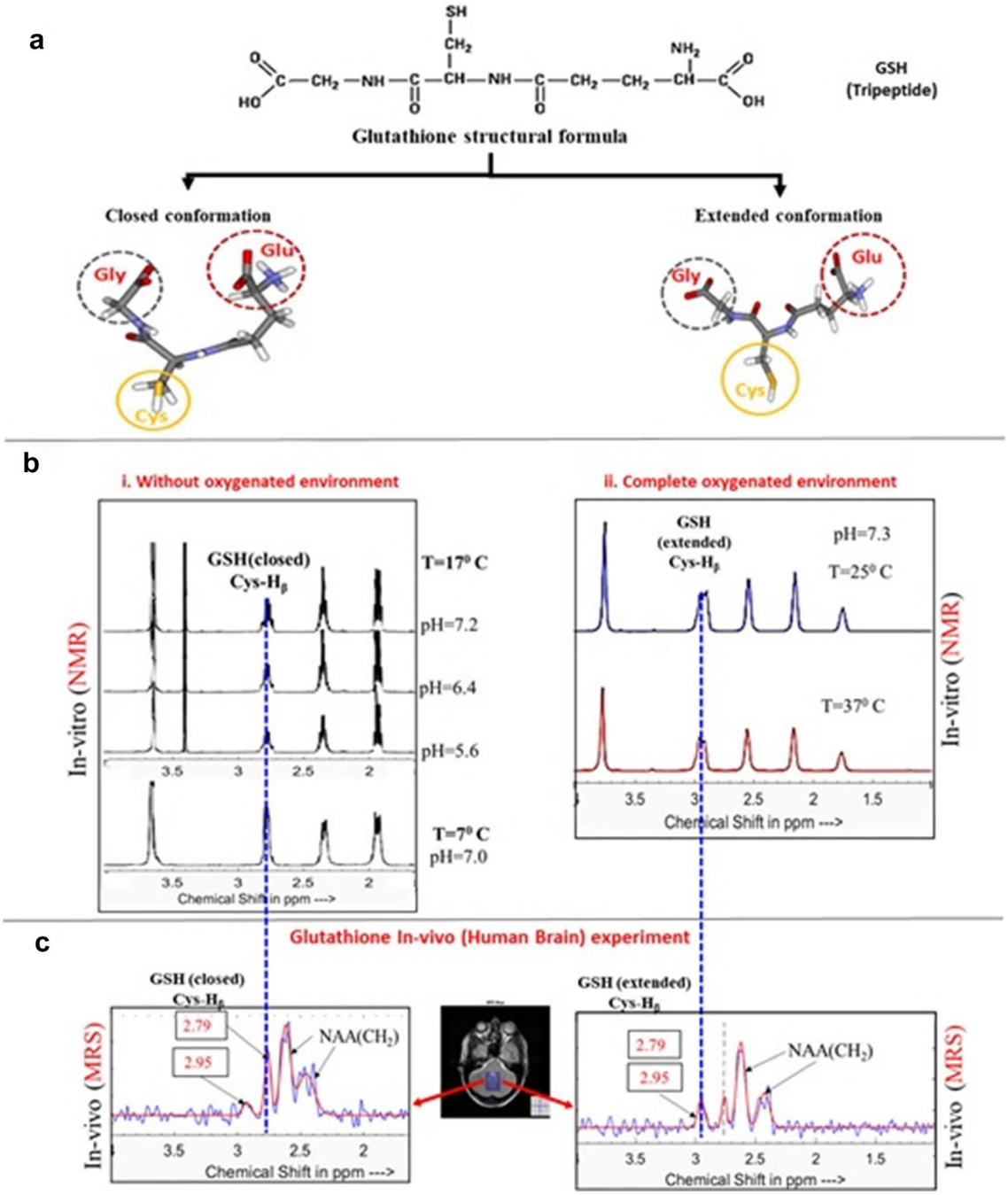

Fig. 8 Structure of GSH and its conformations: **a** GSH structure and its two confirmations i.e., closed and extended. **b** NMR experiment results depicting the Cys-H_β peak of GSH at 2.79 ppm for closed and 2.95 ppm for extended conformer. NMR spectra of GSH within two different environments (i) without oxygenated environment and (ii) completely oxygenated environment. **c** In vivo (human brain) MRS data were processed using in-house developed MATLAB based MRS signal processing and analysis toolbox, KALPANA. [165]. Necessary permissions were taken to reproduce GSH structure and the NMR spectra from the publishers—Elsevier [14], IOS Press [23] FEBS [166], Springer [26] and Frontiers [167]

the objective to measure GSH level in the parieto-occipital region in epilepsy patients showed that GSH/water ratio was significantly reduced as compared to HC [174, 175]. GSH levels quantified in the posterior medial FC of patients with schizophrenia (N = 20) showed a significant negative correlation between GSH levels and scale for assessment of negative symptoms total scores

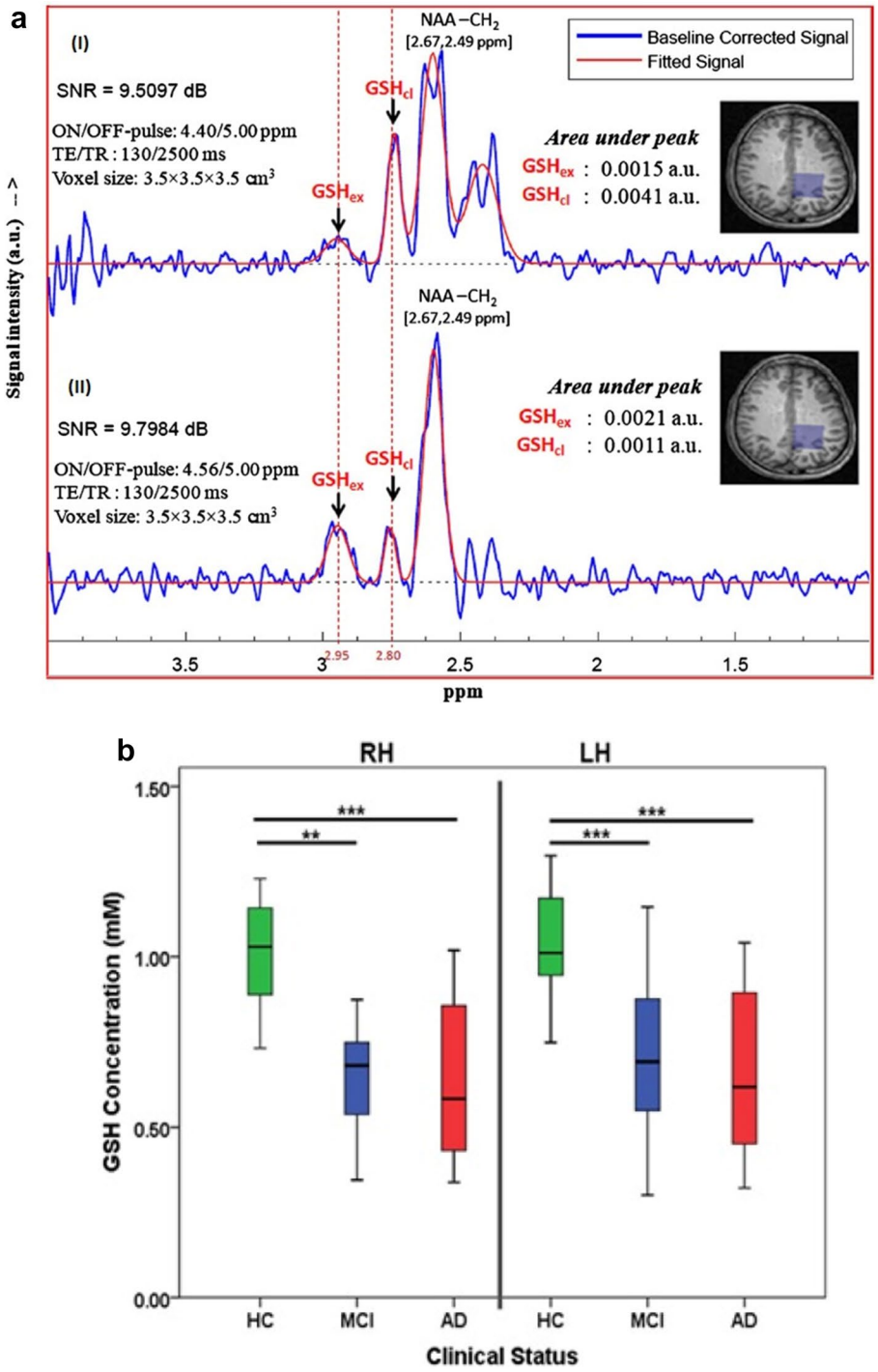

Fig. 9 GSH detection via MRS. **a** Quantitation of GSH peaks using MRS from healthy subjects (voxel size = 3.5 × 3.5 × 3.5 cm^3 on the left parietal cortex). The MEGA-ON and MEGA-OFF excitation pulse positions are (I) 4.40 ppm and 5.00 ppm, (II) 4.56 ppm and 5.00 ppm, respectively. These were processed using in-house developed MATLAB based MRS signal processing and analysis toolbox, KALPANA [165] to get Cys H_β peak of extended (2.95 ppm) and closed form (2.80 ppm) of GSH. The figure reproduced with permission from IOS Press [23]. **b** Box plot representation of GSH concentrations in the right hippocampus (RH) and left hippocampus (LH) regions among HC (green), MCI (blue) and AD (red) subjects. A significant reduction was observed in the RH and LH regions of AD and MCI patients in comparison with HC. All significant values were set at $p < 0.05$ (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$). Necessary permissions were taken to reproduce the figure from the publisher—Elsevier [14] (Color figure online)

($r = -0.68$) and negative symptom subscore on Brief Psychiatric Rating Scale ($r = -0.60$) [24]. Similar studies for GSH detection were conducted in the grey matter region in MS patients where a significant decrease in GSH concentration (2.4 ± 0.5 mM) was observed as compared to control (3.3 ± 0.1 mM). However, no significant difference in white matter was observed [25].

Supplementation for GSH Enrichment

To maintain the disturbed antioxidant defense homeostasis in the brain, certain antioxidants such as GSH, vitamin C, vitamin E, and NAC were provided as a supplement to healthy as well as patients with various neurological disorders [27–30, 34, 39, 176–181]. However, it has been reported that vitamin C and E are dependent on GSH for

their regeneration [92]. Therefore, GSH and its precursors like NAC could be the potential therapeutic strategy in the form of supplements. NAC is a precursor to the amino acid L-cysteine and consequently the antioxidant GSH. NAC is also reported to be effective in crossing the BBB [182, 183]. The GSH level is replenished intracellularly by the intervention of Cys prodrug—NAC therefore is considered as a well-tolerated antidote for Cys/GSH deficiency [184]. The NAC promotes GSH biosynthesis, scavenges ROS, and upholds detoxification consequently effective in treating disease-associated OS [34, 178, 180, 185]. The reduced GSH levels were also responsible for increased OS in various neurodegenerative disorders like AD, PD, and ALS which further led to cellular death as reported in several studies [116, 129, 186, 187]. To prevent this pathology, exogenous GSH was delivered and the progression of disease was evaluated [31–33, 35]. This delivery of GSH or NAC for therapeutic application is accomplished via different modes of delivery which are discussed further in detail.

Modes of Delivery for GSH and NAC Supplementation to the Brain

The modes of GSH or NAC delivery to the brain are similar to any other drug such as oral, intravenous, intranasal, sublingual and intramuscular. Orally administered drugs are the most common which undergo the first-pass metabolism i.e., drugs are first absorbed in the gut, reach the liver via the hepatic portal vein and then enter the systemic circulation. The intestinal wall and liver consist of enzyme γ-GT which metabolizes GSH into its constituent amino acids thereby decreasing the systemic bioavailability of drugs [181]. There are different studies conducted with oral GSH supplementation in different doses and conditions [27, 29, 181, 188–191]. Similarly, the oral formulation for NAC was also evaluated [30, 34, 178–180]. While the intravenously administered drug is directly provided in the veins to reach the bloodstream [192]. Likewise, the administration of intranasal drug travels through fast axonal transport along with the perinuclear space surrounding the nerve cell via trigeminal and olfactory nerves to reach into the cerebrospinal fluid (CSF)/brain interstitial fluid through a transcellular pathway. This mode of transfer allows the direct transfer of drugs from an intranasal cavity to the brain by circumventing the BBB [193, 194]. Sublingually administered drug is absorbed in oral mucosa and reaches directly to the bloodstream through the ventral surface of the tongue [195]. Similarly, the intramuscularly administered drug is injected into the muscles from where it reaches the blood [196]. Several studies of GSH, as well as NAC supplementation in healthy as well as various disorders such as PD, amyotrophic lateral sclerosis (ALS), MS and autism spectrum disorder (ASD) through intranasal, intravenous, sublingual and subcutaneous mode, were also reported [28, 31–33, 35–42].

Clinical Trials Involving GSH and NAC Supplementation in Healthy Individuals

The supplementation of GSH or NAC was found to be beneficial among HC as evidenced in several clinical trials conducted with different outcome measures [27–29, 42, 181, 197]. A pilot randomized trial with oral liposomal GSH (500 and 1000 mg for 1 month) in HC (N = 12) has been reported to be effective in elevating GSH levels and improving the immune function and OS [29]. A trial conducted to determine the long-term effectiveness of oral GSH (250 and 1000 mg/day, 6 months) on body stores in healthy adults (N = 54), showed increased GSH levels in the blood after 1, 3 and 6 months vs baseline [27]. Another study conducted to compare the bioavailability, effect on OS markers and the safety of a sublingual form of GSH with two commonly used dietary supplements, NAC and oral GSH, where sublingual GSH (450 mg), Oral (450 mg) and NAC (200 mg) were provided for 3 weeks to 20 normal volunteers. Significant superiority of the sublingual form of GSH over the oral form, both in terms of bioavailability and positive effects on OS was observed [28]. In a randomized, double-blind, placebo-controlled clinical trial investigating effects of oral GSH supplementation (500 mg, for 4 weeks) on biomarkers of systemic OS in human volunteers (N = 40), no significant changes in total GSH and measures of GSH/GSSG ratio were observed [181].

Evidence of GSH and NAC Supplementation Trials in Various Neurological Disorders

Disturbance in the level of GSH or associated enzymes (GPx) are observed in different neurological disorders such as AD [14, 198], PD [15, 199], MS [200, 201], ASD [202], and ALS [203]. Therefore, providing GSH or NAC as a supplement reported being beneficial in modulating the brain GSH level as suggested in different clinical trials [30–32, 39, 42, 180, 197, 204, 205]. Different clinical trials involving GSH or NAC as an intervention are discussed further for AD, PD, MS, ASD and ALS diseases.

Alzheimer's and Parkinson's Disease

AD is a neurodegenerative disorder characterized by progressive memory loss, disorientation and pathological markers (senile plaques and neurofibrillary tangles) [206]. Similarly, PD is also a neurodegenerative disorder characterized by the loss of dopaminergic neurons affecting mobility and muscle control [207]. The increased OS and GSH reduction have been well found to be associated with these

neurodegenerative disorders [208, 209]. Reduced levels of GSH was observed in regions susceptible to AD and PD like the HP, FC and SN [14, 15, 198, 199]. The study was performed to evaluate the intranasal GSH uptake in PD where 15 participants were involved in self-administration of GSH and results were observed after different time-periods. A significant increase in GSH concentration was observed after 45 min of GSH administration. This result demonstrates the plausible role of GSH in the therapeutics of PD [32]. To the best of our knowledge, no clinical trial was found to assess brain GSH levels in AD patients with the intervention of GSH.

Several trials with NAC supplements were reported in AD and PD patients. A double-blinded, multi-site, phase II study was conducted in 106 AD individuals randomized to oral nutraceutical formulation (folate, alpha-tocopherol, B12, S-adenosyl methionine, NAC, acetyl-L-carnitine) or placebo for 3 or 6 months. The active control showed significant improvement in cognitive performance and the Dementia Rating Scale [34]. Similarly, a placebo-controlled trial of 34 MCI patients, 600 mg of oral NAC (along with folate, alpha-tocopherol, vitamin B12, S-adenosyl methionine, and acetyl-L-carnitine) for 6 months was associated with improvement in dementia rating scale and preservation of executive function [40]. Another clinical trial specifically testing NAC in probable AD (N = 43) reported that oral NAC (50 mg/kg/day) failed to significantly improve MMSE scores in 6 months duration [179]. In another open-label 4-week prospective study with an oral NAC in PD (N = 5) and HC (N = 3), where brain GSH in the occipital cortex was measured using ^{1}H MRS (3 and 7 T) before and after 28 days of 6000 mg NAC/day. Although peripheral antioxidant measures (catalase and GSH/GSSG) increased significantly, no significant increases in brain GSH were observed for the healthy and PD groups [180]. Another study with 3 PD, 3 Gaucher disease (GD) patients and 3 HC subjects observed that a single intravenous dose of NAC resulted in an increase of the blood GSH/GSSG ratio which was followed by an increase in brain GSH concentrations in all those subjects. No conclusive outcome was inferred due to the small sample size and study duration [39].

Multiple Sclerosis

MS is a chronic autoimmune, inflammatory neurological disease of CNS attacking myelinated axons (white matter) [210]. Enzymes like GPx and selenium were reported to be reduced in MS patients [200, 201]. Therefore, supplementation with selenium salts is being tested in a patient along with GSH, to check whether selenium treatment helps in the treatment of pathology. In a study, 18 MS patients were provided with 6 tablets of selenium (equivalent to 6 mg) for five weeks and GPx levels were monitored and quantified. GPx levels were increased significantly in the treatment group (10.4 ± 4.5 micro katal per gram (μkat/g) protein) as compared to control group (3.97 ± 2.09 μkat/g protein) [31].

Autism Spectrum Disorder

ASD is a set of neurodevelopmental disorders characterized by behavioral deficits and non-verbal interactions such as reduced eye contact, facial expression, and body gestures [211]. Children with autism were reported to be diagnosed with lower plasma GSH levels, therefore, the study was designed to check whether GSH supplementation increases the plasma GSH levels in autistic children [202]. The study was an eight-week, open-label trial using oral lipoceutical GSH (N = 13) or trans-dermal GSH (N = 13) in ASD children of 3 to 13 years of age. The oral treatment group showed significant increases in plasma reduced GSH, but not whole-blood GSH levels post supplementation [33].

Similarly, in a 12-week, double-blind, randomized, placebo-controlled study, autistic children (N = 33) were initiated 900 mg oral NAC daily for 4 weeks, then 900 mg twice daily for 4 weeks and 900 mg three times daily for 4 weeks. Significant improvements on the Aberrant Behavior Checklist irritability subscale, Repetitive Behavior Scale-Revised stereotypies measure, and Social Responsiveness Scale mannerisms scores. While no significant improvement in other subscales were reported [178]. Another 12-week randomized, double-blind, placebo-controlled trial of oral NAC with the target dose of 60 mg/kg/day in three divided doses in ASD youth (N = 31) found no statistically significant difference in Clinical Global Impression—Improvement scale but the GSH level in blood was significantly higher in the NAC group ($p < 0.05$). However, no significant difference in the GSH/GSSG ratio was observed [30].

Amyotrophic Lateral Sclerosis

ALS is a fatal motor neuron disorder characterized by progressive loss of the upper and lower motor neurons at the spinal or bulbar level [212]. Impairment of the antioxidant defense system could be the potential reason for the etiology, supported by the studies of the lowered activity of enzymes like GPx in blood and CSF of ALS patients [203]. Due to the reported observations of the lowering of antioxidant enzymes, GSH supplementation could be beneficial for the treatment of disease. A pilot trial of reduced GSH was conducted in 32 ALS patients for 6 months, where 600 mg dose of GSH was provided intramuscularly each day. No significant difference was observed between the treated ALS and control groups after GSH treatment [35].

Similar to GSH, a randomized, double-blind, placebo-controlled clinical trial to assess the effect of treatment with NAC was also conducted in ALS patients. A dose of 50 mg/

kg per day subcutaneously for 12 months was provided and observed that NAC did not result in a major increase in 12-month survival or a reduction in disease progression [41].

Conclusion

GSH being a master antioxidant displays a remarkable metabolic and regulatory versatility. GSH plays a crucial role in various cellular processes including cell signaling, balance of thiol redox status and detoxification of xenobiotic. Moreover, studies so far elucidate the fact that dysregulation of GSH homeostasis due to increased OS, is involved in various neurological disorders like AD, PD, MS, autism, and ALS, etc. In this review article, we have addressed how GSH acts as a master antioxidant by casing all aspects like function, metabolism, compartmentalization, transport, and synthesis of tripeptide inside the cellular system. We also discussed GSH in vivo detection using advanced spectroscopy techniques. Delineating the therapeutic effectiveness of GSH and NAC as a supplement through various clinical trials will augment the understanding of the treatment of various neurological diseases. Upcoming GSH supplementation studies will be an important step towards clinical neuroscience research which in turn will be beneficial for a wide range of patients suffering from neurological illnesses.

Future Perspectives

GSH is a master antioxidant as it has a beneficial impact on substantially all organs of the living body including the brain. To the best of our knowledge, no phase III randomized controlled trial is existing for MCI or AD patients, wherein brain GSH level is monitored non-invasively with the GSH supplementation. Recent mouse model studies have concluded that behavioral deficits including cognitive decline, depressive-like behaviors, and anxiety-related behaviors observed in APP (NL−G-F/NL−G-FG-) mice were significantly improved by oral GSH administration [213]. This study has validated our ongoing work [14, 20, 21] and hypothesis that oral GSH supplementation [204] will like to have huge impact for possible cognitive improvement in MCI and AD patients subject to verification through an urgent clinical trail.

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Author Contributions Dr. PKM (Professor) conceptualized the research idea and was involved in writing the manuscript, figure preparation, critically reviewing and finalization of the manuscript. Ms. DD (R&D Engineer), Dr. KM (Research Associate) and Mr. RM (Clinical Coordinator) contributed equally to the literature search, critically reviewing the literature, writing and reviewing the manuscript as well as designing the figures.

Compliance with Ethical Standards

Conflict of interest All the authors agree with the content of the article and declare no conflict of interest (personal, academic or financial) associated with this study.

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