Journal of
Molecular Pathology

MDPI

Review

DRP1 Regulation as a Potential Target in Hypoxia-Induced Cerebral Pathology

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Abstract: The following review considers current concepts concerning the characteristics of DRP1-related mitochondrial division in brain cells during hypoxic-ischemic pathology. The functional role of DRP1 in neurons and astroglia in cerebral ischemia conditions was analyzed. We discuss the potential for regulating DRP1 activity through the selective inhibitor of mitochondrial fission, mdivi-1. The article also presents data on DRP1 involvement in astro- and microglia-mediated intercellular mitochondrial transport. Understanding of the molecular mechanisms responsible for mitochondrial fission during hypoxic-ischemic exposure will allow us to consider DRP1 as an effective therapeutic target for treating conditions with a hypoxic component.

Keywords: brain; ischemia; mitochondrial fission; DRP1; mdivi-1; neuroprotection

Citation: Fedorova, E.N.; Egorova, A.V.; Voronkov, D.N.; Mudzhiri, N.M.; Baranich, T.I.; Glinkina, V.V.; Krapivkin, A.I.; Mamedov, I.S.; Sukhorukov, V.S. DRP1 Regulation as a Potential Target in Hypoxia-Induced Cerebral Pathology. *J. Mol. Pathol.* **2023**, *4*, 333–348. https://doi.org/10.3390/jmp4040027

Academic Editor: Pasquale Pisapia

Received: 16 October 2023
Revised: 4 December 2023
Accepted: 7 December 2023
Published: 9 December 2023

1. Introduction

Hypoxia is a condition in which the body or specific tissues and organs experience a lack of oxygen due to external factors or unfavorable internal conditions. It is a significant factor in the development of various diseases and can be classified into several types based on its underlying causes including circulatory, anemic, histotoxic hypoxia, etc. Ischemia is a special case of circulatory hypoxia, in which blood supply to a certain area of tissue or organ is partially or completely stopped. Regardless of the cause of cerebral hypoxia, the outcome invariably involves the development of a series of pathological biochemical changes referred to as the "ischemic cascade". This, in turn, results in damage to nervous tissue and the death of brain neurons through necrosis or apoptosis, continual disruptions of neuronal plasticity, inhibition of the activity of adaptive transcription factors, and, as a consequence, disturbances in the expression of protective proteins [1]. The initial stage in initiating the hypoxic/ischemic cascade is a decrease in the level of oxygen supplied to the brain, resulting in a macroergic compound deficiency.

The mechanisms underlying brain damage in hypoxia are actively studied, but much remains unclear. It is evident that mitochondria, which undergo several morphofunctional changes, play an active role in adapting to such effects. The increase in the cell's energy demands leads to intensified mitochondrial dynamism, which involves the organelle biogenesis, fission, fusion, movement and mitophagy [2,3]. However, despite researchers' growing interest, the molecular mechanisms that regulate mitochondrial dynamics remain poorly understood. Recently, the view that mitochondrial fission is an early molecular event that induces apoptosis in various cell types, such as neurons, gained popularity [4].

However, recent studies suggest that traditional notions regarding the compensatory nature of mitochondrial proliferation in various tissues cannot be discounted [5]. Thus, in particular, it is important for maintaining neuronal function, including dendrite and dendritic spine development, neurotransmitter release, long-term potentialization induction, and mobilization of the reserve pool of neurotransmitter vesicles in the synapse [6–8].

Numerous studies demonstrate excessive activation of mitochondrial fission in cerebral ischemia [9], which allows us to consider mitochondrial dynamics as a potential target for neuroprotective effects, in particular through the inhibition of DRP1 protein. It is known that among the developed DRP1 blockers, MitoQ currently has passed the second phase of clinical trials [10], showing its efficacy in many diseases with a hypoxic component, thus emphasizing the relevance of targeting DRP1 in the development of modern therapeutic strategies.

Therefore, the present review discusses the role of DRP1-mediated mechanisms of mitochondrial dynamics regulation, examining their characteristics in neural tissue under brain hypoxia and their potential as a therapeutic target.

2. Role of DRP1 in the Regulation of Mitochondrial Dynamics

Mitochondrial fission is one of the modes of mitochondrial biogenesis that occurs in a stepwise, energy-dependent process. The activation of outer mitochondrial membrane (OMM) fission is facilitated by DRP1 (dynamin-related protein), a large cytoplasmic GTPase belonging to the dynamin family, which is involved in the formation of membrane vesicles during endocytosis. DRP1-mediated mitochondrial fission enables organelle proliferation and also plays an important role in mitochondrial quality control and regulated apoptosis [11].

It is assumed that the selection of DRP1-dependent fission variant is regulated by adaptor proteins—Mff, Fis1, MiD49 and MiD51, with Mff (mitochondrial fission factor) and Mids (mitochondrial dynamics protein) proteins providing equatorial fission and mitochondrial proliferation, while Fis1 (Fission 1) protein regulates their fragmentation [12].

One of the conditions for the initiation of mitochondrial fission with Mff involvement is their interaction with the tubules of the endoplasmic reticulum (ER), which is necessary to reduce the average diameter of mitochondria [13]. Thus, the regulation of actin assembly, required for mitochondrial pre-compression prior to Drp1 recruitment, binding of Drp1 to adaptor proteins, and its oligomerization, is mediated by ER-associated inverted formin 2 (INF2), which interacts with formin-binding Spire1C that is fixed in mitochondria [14]. The DRP1 protein oligomers undergo subsequent GTP-dependent self-assembly, resulting in the formation of a ring-shaped structure surrounding the mitochondrion. The compression of the mitochondrion eventually leads to its splitting, fueled by GTP energy.

In addition, it should be noted that, besides the traditional ER-mediated mechanism of DRP1 participation in mitochondrial fission, there are active ongoing discussions regarding the role of other cellular structures—elements of the cytoskeleton, vesicles of the Golgi apparatus [15,16]—in ensuring physiological and pathological, in particular, ischemia-induced, mitochondrial fission.

For example, actin polymerization on mitochondria has been shown to regulate Drp1 recruitment to the mitochondria's outer membrane. In addition, proteins involved in the regulation of the actin cytoskeleton, such as Arp2/3, cofilin, cortactin and Septin 2, gelsolin, and INF2 have been shown to be involved in the regulation of mitochondrial fission [17]. Two types of actin polymerization in response to stress factors have been identified [18]. The first response mechanism involves actin assembly around mitochondria due to damage caused by mitochondrial depolarization; this process is independent of Drp1 and plays a role in mitophagy. The second response mechanism is the calcium-induced formation of actin filaments throughout the cytosol, which leads to increased recruitment of Drp1 to the outer mitochondrial membrane. The involvement of these mechanisms of actin stress regulation in the control of mitochondrial dynamics in neural tissue under hypoxia requires further investigation.

Binding of DRP1 to Mid49 and Mid51 which are distributed on the MOM, also promotes the formation of mitochondrial fission complexes. It has been shown that, in ischemia, the interaction of DRP1 with Fis1 is a process of "loop binding followed by dissociation" [19]. At the same time, the role of Mid51 is still being debated. It is highly likely that the ambiguous results regarding its involvement in DRP1-dependent fission are due to the ability of Mid51 to bind adenosine diphosphate [20], thus determining the strong dependence of DRP1-Mid51 interaction on the microenvironment. It should also be noted that the relationship between Drp1, MFF and Mids is not limited exclusively to ligand–receptor interaction, but also includes numerous reciprocal regulatory influences [21,22], the mechanisms of which may be related to post-transcriptional modifications of Drp1 under ischemia and hypoxia.

In contrast to equatorial fission driven by the interaction of DRP1 with Mff and Mids, Fis1 enables peripheral fragmentation and is considered to be a major adaptor of DRP1 during stress-induced fission because its binding to DRP1 occurs before the mitophagy, which is necessary for the separation and subsequent recycling of damaged mitochondrial sites to lysosomes [23]. Recent research [24] has shown that the mitochondrial Mid51/Fis1 complex regulates the reorganization of the lysosomal network, and its inhibition disrupts lysosome detachment, leading to dissociation of mitochondrial and lysosomal dynamics processes, significantly disrupting cellular homeostasis. It is also worth mentioning that the presence of Fis1 effects that are unrelated to its direct interaction with DRP1 and characterized by its ability to block mitochondrial fusion by inhibiting the GTPase activity of the fusion proteins Mfn1, Mfn2, and OPA1 casts doubt on viewing Fis1 as a DRP1 receptor [25]. However, a recent study [26] showed a direct interaction between Fis1 and DRP1, and the authors considered Fis1 as a switch from homeostatic to mitophagic division.

Studies of DRP1 regulation have demonstrated that its oligomerization is controlled by post-translational modification of the protein through phosphorylation, sumoylation, S-nitrosylation, O-GlcNAcylation and ubiquitination [27], with the phosphorylation and ubiquitination processes being the most extensively researched. The main phosphorylation sites in human Drp1 isoform 1 are Ser616 and Ser637, equivalent to S600/S579 in isoform 3 and to S643/S622 in isoform 1 in mouse Drp1 [28]. Other functionally important phosphorylation sites include Ser40, Ser44, Ser585, Ser412, Ser684 [10,28]. Phosphorylation at Ser616 causes activation and subsequent translocation of Drp1 into mitochondria [10]. In contrast, phosphorylation of DRP1 at Ser637 by protein kinase A promotes its retention in the cytoplasm, thereby inhibiting fission and protecting mitochondria from autophagic degradation [29]. Even though Ser637 phosphorylation by PKA is associated with reduced Drp1 GTPase activity, the effect of other kinases is controversial and depends on a variety of conditions [10], for example, on the duration of hypoxia–ischemia exposure. A recent study has shown that phosphorylation of murine Drp1 at S600 (equivalent to human S637) acted as an upstream event for S579 (equivalent to human S616) phosphorylation, leading to mitochondrial fragmentation [28]. However, calcium-dependent calcium phosphatase, calcineurin, which is activated in response to increased cytoplasmic calcium, by dephosphorylating Drp1 at Ser 600 (equivalent to human Ser 637), enhances the translocation of Drp1 into mitochondria, promoting their subsequent fission [30]. Moreover, increased calcium levels stimulate calmodulin-dependent protein kinase (CAMK) alpha, which, by phosphorylating Drp1 at Ser 600 (equivalent to human Ser 637), increases its affinity to Fis1, also initiating the process of mitochondrial fission [31].

DRP1 ubiquitination is associated with the proteins PINK1 and PARKIN, which belong to the ubiquitin–proteosomal system (UPS) that mediates mitophagy [32]. Expressed on the OMM of old or damaged mitochondria, PINK1 is recognized and ubiquitinated by the Parkin protein. After ubiquitination PINK1 binds to the adaptor protein p62, which interacts with the key regulator of macroautophagy LC3, PINK1 is transported to the autophagosome and utilized during mitophagy [33]. Ubiquitination of DRP1 by the aforementioned proteins leads to its proteosome-dependent degradation and subsequent inactivation, thus preventing mitochondrial fission.

It should be mentioned that within the framework of this review we tried to discuss in more detail those mechanisms of DRP1 regulation, which will be further examined in the context of the significance of DRP1-dependent mitochondrial fission in neurons and glial cells under hypoxia–ischemia conditions (Figure 1).

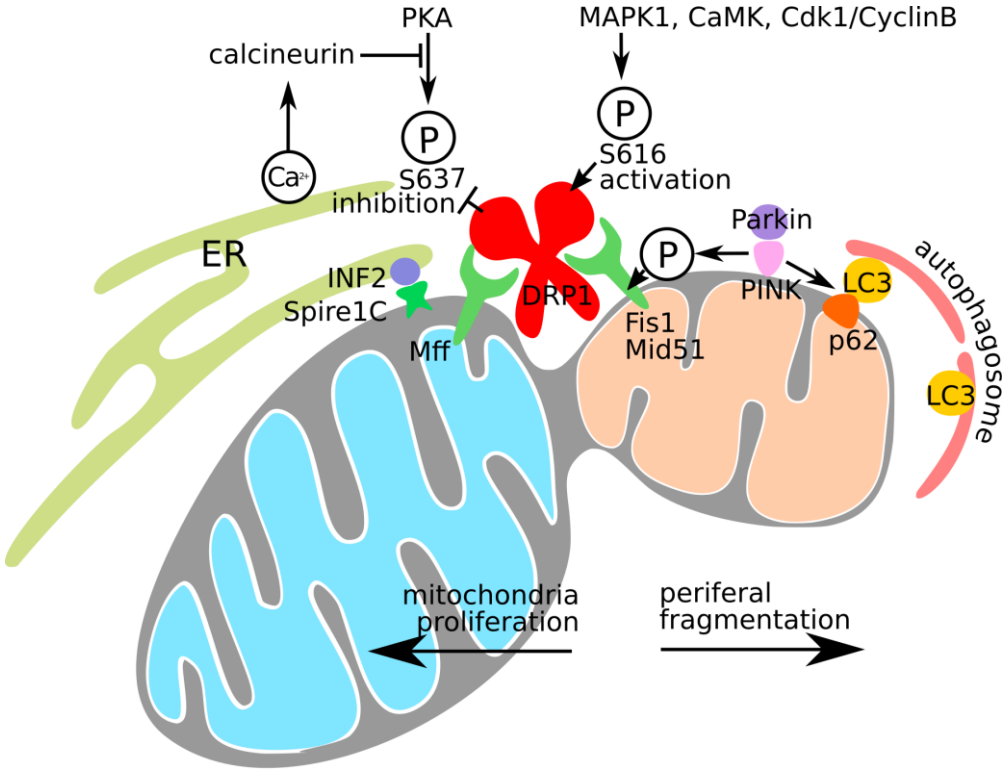

Figure 1. Interaction of key proteins involved in regulation of mitochondrial fission. CaMK—calmodulin-dependent protein kinase; Cdk1/CyclinB—Cyclin-dependent kinase 1/Cyclin B complex, maturation promoting factor; Drp1—GTPase Dynamin related protein 1; LC3—autophagosomal adaptor protein; MAPK—mitogen associated protein kinase 1; Parkin—E3 ubiquitin ligase Parkin; PINK—PTEN-induced kinase 1; PKA—protein kinase A; Spire1C—Spire Type Actin Nucleation Factor 1, actin binding protein; Adaptor complexes proteins: Fis1, Mff, Mid51, INF2, p62. ER—endoplasmic reticulum. Arrows with circled P—phosphorylation, S-number—serine-residue sites of phosphorylation. Inhibition is indicated by a bar at the end of the interaction line. Activation is indicated by arrow.

3. Mechanisms of DRP1-Dependent Mitochondrial Fission Regulation under Ischemia and Hypoxia Conditions in Neurons

Recent experimental studies demonstrate disturbances in the mitochondrial dynamics of neurons under ischemia specifically an increase in mitochondrial fission and a decrease in mitochondrial fusion, while there still is a pressing need to investigate the signaling pathways that regulate mitochondrial morphology and functional preservation under hypoxia–ischemia conditions.

In the research by Flippo et al. [34], the authors studied the role of AKAP/PKA-mediated regulation of mitochondrial fission in mice with focal ischemia. AKAP1 is an anchoring protein capable of binding protein kinase A (PKA) regulatory subunits and directing the enzyme to different subcellular compartments [35]. The AKAP1/PKA signaling complex regulates DRP1 activity through AKAP1-mediated anchoring of PKA to the OMM [36] followed by phosphorylation of DRP1 by PKA at serine 637, which results in DRP1 retention in the cytoplasm, thus inhibiting mitochondrial fission [29]. Mice with AKAP1 deletion exhibited high sensitivity to ischemic damage, corresponding to a larger ischemic focus volume and increased neurological deficit severity. Transmission electron

microscopy results showed that loss of AKAP1 leads to a decrease in mitochondrial area of hippocampal neurons and an increase in the number of mitochondrial contacts with the ER, suggesting AKAP1's potential protective effect in preventing the development of mitochondrial Ca^{2+} overload. Western blotting analysis indicated a significant increase in DRP1 levels in forebrain mitochondrial fractions from AKAP1-deficient mice, while DRP1 phosphorylation at serine 637 was reduced.

The study by Zhang et al. [37] demonstrates the relationship between the rearrangement of mitochondrial dynamics and the activation of mitogen-activated protein kinase (MAPK) p38 in cerebral ischemia. P38 MAPK is a stress-inducible kinase that regulates phosphorylation of intracellular enzymes, transcription factors, and cytosol proteins that are involved in cell survival and cell death, apoptosis and autophagy, inflammatory responses, and synaptic plasticity. Inhibition of p38 MAPK in rats with middle cerebral artery occlusion (tMCAO) reduced mitochondrial fission and mitophagy, contributing to a reduction in focal size and neurological deficit severity. Subsequent experiments [38] revealed that DRP1 is a substrate for p38 MARK, which, by phosphorylating DRP1 at Ser 616, activates the fission process, followed by mitochondrial dysfunction and neuronal death.

The balance between fusion and fission proteins is essential, among other things, to ensure mitophagy, which plays a crucial role in the pathogenesis of ischemic effects. Insufficient utilization of damaged mitochondria or excessive degradation of functionally active mitochondria leads to impaired mitochondrial homeostasis, neuronal damage and death [33]. The involvement of DRP1 in mitophagy is attributed to its capacity to separate impaired regions of mitochondria that are further subjected to cleavage, thereby preventing the accumulation of damage and the development of mitochondrial dysfunction [23].

The study of DRP1 protein level under hypoxia showed that DRP1 is regulated by Parkin protein [39]. In mouse Neuro2a (N2a) neuroblastoma cells subjected to oxygen-glucose deprivation (OGD) and reperfusion insult, intense mitochondrial fragmentation was observed, consistent with increased DRP1 expression and correlating decreased level of Parkin. Parkin knockdown enhanced DRP1 expression, and Parkin overexpression induced DRP1 degradation, reduced mitochondrial dysfunction by increasing cytochrome c-oxidase and ATP synthase activity, and decreased hypoxia-induced neuronal apoptosis. Thus, DRP1 acted as a downstream effector of Parkin, activating its proteasome-dependent degradation, which in turn identifies the Parkin-DRP1 pathway as a potential target for treating diseases caused by ischemia-reperfusion injury.

It was previously noted that the protein PINK1 (PTEN-induced kinase 1) is also involved in mitochondrial dynamics regulation and has a neuroprotective role in ischemia [40]. This study demonstrated that knockout of the PINK1 gene increased neuronal sensitivity to ischemic injury, while PINK1 overexpression inhibited DRP1 translation, reducing DRP1 translocation from the cytosol to the mitochondrial surface and, consequently, reducing OGD-induced mitochondrial fragmentation. The involvement of PINK1 in the regulation of mitochondrial fusion–division processes was further confirmed by administering the DRP1 inhibitor mdivi-1 (mitochondrial division inhibitor 1). This resulted in the elimination of the negative effects of PINK1 blockade, reducing neuronal death and restoring ATP production levels after OGD.

Another study [41], conducted in vivo in a tMCAO model and in vitro with OGD/R insult, found that ischemia-induced translocation of DRP1 to mitochondria accelerated p62-mediated autophagosome formation after ischemia-reperfusion. However, DRP1-induced ROS (reactive oxygen species) accumulation impaired the transformation of autophagosomes into autophagolysosomes by blocking autophagic flux through the RIP1/RIP3 pathway. In turn, undegraded autophagosomes were secreted as exosomes, triggering an inflammatory cascade with further mitochondrial damage, massive ROS production, and impaired autophagosomal degradation, thus establishing a vicious cycle that exacerbates the condition after ischemia-reperfusion insult. Due to increased translocation of DRP1 into mitochondria resulting in the accumulation of p62-labeled autophagosomes, indicating

a shift from mitochondria-associated p62 state to its cytosolic form, the authors hypothesized a likely competitive binding of DRP1 and p62 to a receptor protein on the outer mitochondrial membrane surface.

Investigation of the role of DRP1 in mitophagy also revealed that the temporary decrease in mitochondrial membrane potential (MMP) at the mitochondrial fission site was due to the interaction of DRP1 with the mitochondrial zinc transporter Zip1, which induces influx of zinc ions from the cytosol into the mitochondrial matrix [42]. Inhibition of the DRP1-Zip1 system led to the accumulation of damaged mitochondria, impairing their function by decreasing ATP synthesis and increasing ROS production. At the same time, excessive zinc entry into the mitochondrial matrix causes neuronal cell death under hypoxic/ischemic insult. Qi Z et al. [43] also showed that zinc overload leads to a disruption of the blood–brain barrier due to massive ROS generation in ischemic rat endotheliocytes both in vitro and in vivo. According to the Western blotting data, zinc accumulation in the mitochondrial matrix was accompanied by an increase in the ROS level and also correlated with the increase in the DRP1 level.

4. Response of DRP1-Mediated Processes in Astroglia to Hypoxia

There is an increased interest in the study of the mechanisms of neuro-glial, primarily, neuron–astrocyte interactions in the ischemic brain damage pathogenesis [44]. Astrocytes are recognized for their crucial role in the reorganization and repair of nervous tissue during ischemia. Nevertheless, molecular and cellular mechanisms of astrocytic dysfunction, and consequently the regulation of neuronal survival during ischemic exposure, remain poorly studied.

A number of works demonstrate the involvement of DRP1 in mitochondrial network remodeling in astrocytes in response to damaging effects [45,46]. Thus, Hoekstra et al. [47] showed that DRP1 knockout alters mitochondrial morphology and localization, increasing the length and area of the organelles, and decreasing their density in astrocyte processes. In turn, disruption of mitochondrial distribution in astroglia increased neuronal sensitivity to glutamate-mediated excitotoxicity, a cell death mechanism associated with excessive accumulation of glutamate in the synaptic gap with subsequent overexcitation of glutamate receptors, their further depolarization, massive calcium influx, activation of Ca^{2+}-dependent enzymes and development of mitochondrial dysfunction [48]. The increase in intracellular Ca^{2+} in response to extracellular glutamate was attributed to compromised intracellular Ca^{2+} buffering by abnormally distributed astrocytic mitochondria, indicating an important role of astrocytic DRP1 in neuronal survival [47].

In the study by Quintana DD et al. [49], the authors evaluated the effects of 3 h hypoxia followed by 10 h reoxygenation on the mitochondrial competence of primary rat astrocyte cultures. Hypoxia-induced metabolic stress led to a shift in fusion–division processes and reorganization of the entire mitochondrial network in astrocytes. Hypoxia increased the number of small mitochondria, which was accompanied with the dephosphorylation of DRP1 at serine 637, as measured by Western blotting. The researchers believe that such a rearrangement of mitochondria is a protective response to hypoxia, necessary both to increase energy production and to facilitate mitophagy during the reoxygenation phase.

The research by Halder A et al. [50] describes a sequence of events that lead to the rearrangement of mitochondrial dynamics in astroglia under hypoxia following the activation of TLR4 and TNFR1 receptors on the astrocyte surface. Astrocytes, acting as resident immunocompetent cells of the brain, are known to act like antigen-presenting cells (APC) that activate links of innate and acquired immunity under pathological influences [51]. Hypoxia-induced immunity has been shown to be mediated by astrocyte activation of TLR4 (Toll-like receptor) and TNFR1 (tumor necrosis factor receptor 1), the primary mediators of the innate immune response in the CNS [52]. In the previously mentioned work by Halder et al. [50] the C6 astrocyte culture was subjected to OGD conditions and the researchers assessed the mediators of innate immunity, markers of hypoxia, and mitochondrial dynamics regulator proteins. The authors found a predominance of mitochondrial fission over fusion processes,

which corresponded to a decrease in Mfn-1 expression alongside the increase in DRP1 levels. In addition, there was a decrease in mitochondrial membrane potential (MMP) and increase in apoptosis. Activation of TLR4 and TNFR1 under OGD increased the expression of the phos-65 subunit of the transcription factor NF-κB.

Previously, it was observed that, by penetrating into the nucleus, phos-65 can induce apoptosis [53] and alter the activity of PGC-1α, a key regulator of mitochondrial biogenesis [54]. In turn, PGC-1α is known to control the transcription of fusion-promoting Mfn1 genes [55], while the regulation of DRP1-driven fission remains the subject of active discussions. Double-staining experiments performed by Halder et al. showed colocalization of phos-65 NF-κB and PGC-1α in astrocytes after OGD, with an initial increase in PGC-1α 6 h after OGD, subsequently accompanied by a decrease in PGC-1α at both mRNA and protein levels, thus indicating a possible reciprocal relationship between phos-65 NF-κB and PGC-1α that leads to decreased fusion and increased mitochondrial fission [50]. Interestingly, a similar mechanism controlling mitochondrial dynamics and subsequent remodeling of the mitochondrial network was demonstrated in dopaminergic neurons in the work of Dabrowska A. et al. [56]. Using the ChIP method, the authors showed PGC-1a binding to the DRP1 promoter, thus determining its direct influence on the regulation of DRP1 expression. It was shown that moderate activation of PGC-1a increases DRP1 expression, maintaining the fusion–fission balance required for optimal biogenesis; on the other hand, overactivation of PGC-1a decreases DRP1, while simultaneously increasing MFN2 transcription.

5. DRP1 and Mitochondrial Transfer Activated under Ischemia Conditions

It is known that one of the neuroprotective effects of astrocytes is their participation in the so-called "horizontal transport" of mitochondria through astrocytic microvesicles, which is necessary, among other things, for the functioning of neurons under energy-deficient conditions. Astrocytes respond to various stimuli induced by ischemia/hypoxia, in particular to elevated levels of extracellular glutamate [57], by increasing the expression of CD38—ADP-ribosyl cyclase—and consequently increasing the production of cADPH and Ca^{2+} release from the ER (Figure 2). The elevation of Ca^{2+} may lead to the Drp1 activation by Ca-dependent kinases [58]. Mitochondrial vesicles (MDVs) contain mitochondrial material and whole mitochondria, and are formed and transported with the participation of Miro1 and Rab7 proteins [59–61]. MDV formation started from the generation of thin membrane protrusions by Miro1/2 and was followed by DRP1-dependent scission. Finally, MDVs can be secreted by astrocytes via multivesicular bodies (MVBs) as components of extracellular vesicles (EV). Thus, Drp1 appears to be essential for intercellular mitochondrial transport, but the relationship between Drp1 activation, the level of mitochondrial fragmentation, and the intensity of mitochondrial exchange between astrocytes and neurons remains to be established. Other mechanisms of mitochondrial transport are nanotube tunneling (TNT) and the secretion of undeveloped mitochondria, although the latter is poorly understood. TNTs are involved in the transport of mitochondria from astrocytes to neurons [62]. Participation in TNT formation is shown for Ca-binding protein S100A4 which interacts with the RAGE (receptor for advanced glycation end products) receptor. In addition, Miro1 and Connexin 43 have been identified as key players in mitochondrial transfer via TNT. Mitochondrial transfer from astrocytes to neurons has been shown to increase neuronal survival, restore neuronal mitochondrial membrane potential, increase ATP levels, normalize neuronal calcium dynamics, and increase dendrite length in vitro [62–64]. On the other hand, mitochondria and mitochondria-derived proteins in extracellular vesicles can stimulate the production of inflammatory responses and influence immune regulation [65].

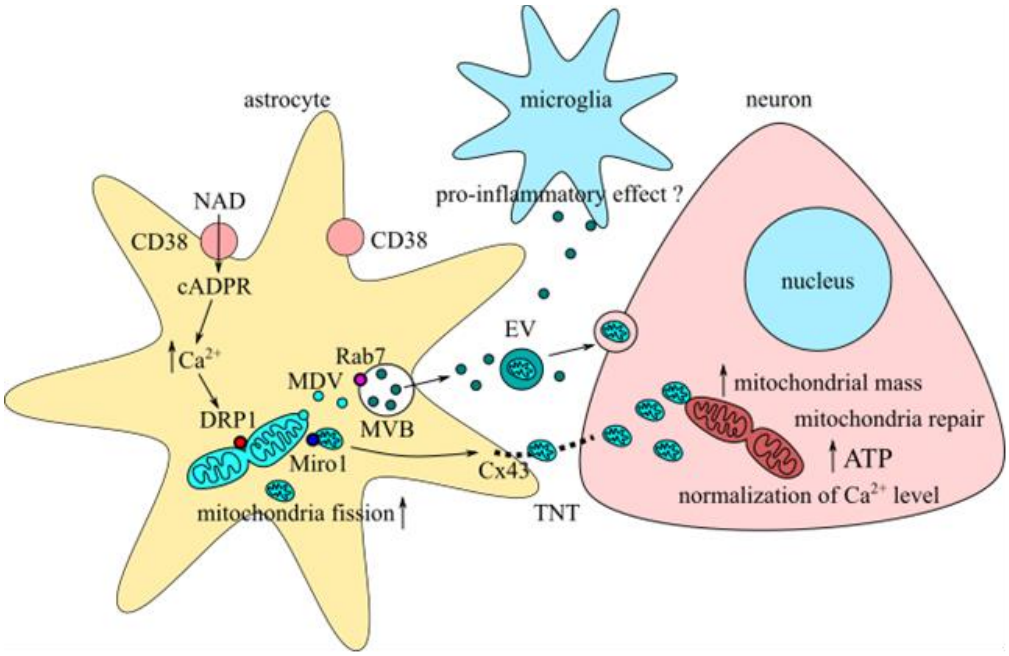

Figure 2. Proposed mechanism of mitochondrial transfer from astrocytes and its potential effects on neurons. NAD—Nicotinamide adenine dinucleotide; CD38—Cluster of differentiation 38 protein, with ADP-ribosyl cyclase activity; cADPR—Cyclic ADP Ribose; Drp1—GTPase Dynamin related protein 1; Miro1—Mitochondrial Rho GTPase 1; Rab7—Ras-related protein Rab-7, GTP-ase; Cx43—connexin 43 (GJA1), MDV—mitochondria derived vesicles; MVB—multivesicular bodies; EV—extracellular vesicles; TNT—tunneling nanotubules.

In the study by Liu et al. [66], inhibition of DRP1/Fis1-mediated mitochondrial fission in proinflammatory type A1 astrocytes reduced mitochondrial transport from astroglia to neurons in both in vitro and in vivo models of ischemia. Using an exosome-mediated protein antigen delivery system, exosomes carrying heptapeptide P110, which selectively blocks the DRP1/Fis1 interaction, were transported into type A1 astroglial cells. Under ischemic conditions, astrocytes were found to secrete pathological mitochondria, which are then captured and fused with neuronal mitochondria, exacerbating neuronal mitochondrial damage by decreasing their mitochondrial membrane potential, decreasing ATP synthesis, increasing ROS production, and increasing cytochrome c loss. Heptapeptide administration reduced mitochondrial dysfunction in astrocytes and promoted the transfer of functionally active mitochondria to nearby damaged neurons, increasing their viability. The results of immunofluorescence staining also showed an increase in the number of NeuN-positive cells in the penumbra zone, a perifocal zone localizing around the primary focus of necrosis, and a decrease in the number of astrocytes expressing the C3 complement component, a known marker of reactive glia [67].

The study of microglia-mediated mitochondrial transfer demonstrated similar results. In their other work, Liu et al. [68] showed that the activation of proinflammatory phenotype M1 microglia in rats with middle cerebral artery occlusion (tMCAO) was accompanied by prominent mitochondrial dysfunction and characterized by increased expression of fission adaptor proteins MFF, Fis1, Mid49 and Mid51, which are required for DRP1 recruitment. M1-type microglia secreted damaged and fragmented mitochondria with reduced membrane potential (MMR), impaired ATP synthesis and increased ROS production. The pathologically altered mitochondria were taken up by neurons, where they fused with the neuronal mitochondria, and contributed to the progression of ischemia-induced neuronal death through activation of mitochondrial-mediated apoptosis. An DRP1examination of mitochondrial morphology revealed that, after co-incubation of M1 microglia (Mito/M1-BV2) with SH-SY5Y neurons after OGD, neuronal mitochondria exhibited vacuolization,

cristae disintegration, and compromised mitochondrial membrane integrity. Interestingly, transplantation of mitochondria derived from activated M1 microglia into the ischemic zone significantly exacerbated tMCAO-induced damage, increasing infarct area and neurologic deficit severity.

The study by Lu et al. [69] also found negative consequences of the release of damaged mitochondria from activated microglia under high-altitude hypoxia conditions. Namely, treatment of astrocytes with medium from hypoxia-exposed microglia increased DRP1 activation, AQP4 expression, and increased mRNA levels of IL-6, TNF-α, and IL-1β in astrocytes. Meanwhile, mdivi-1 administration decreased the release of pathologically altered mitochondria from microglia and attenuated the edema severity. Taken together, all these data indicate that mitochondrial transfer mediated by microglia and astroglia may be a potential promising target for the development of effective neuroprotective strategies; however, its clinical application requires a more detailed study of the mechanisms of mitochondrial transfer regulation and possible side effects associated with its therapeutic application.

6. Regulation of DRP1 by Selective Inhibition of Mitochondrial Fission

The current management strategy for patients with ischemic stroke using thrombolytic therapy is known to possess several limitations, attributed to its narrow therapeutic window [70] and high risk of hemorrhagic complications [71]. In this regard, discovering novel therapeutic targets and the development of effective pharmacological agents is an imperative concern of modern medicine.

One of the promising areas in the treatment of ischemic stroke is related to the molecular mechanisms that regulate mitochondrial dynamics. Over the last two decades, several strategies have been proposed to inhibit mitochondrial fission, both through pharmacologic agents—mdivi-1 [72], P110 [73], NOS3 [74]—and using novel techniques such as photobiomodulation therapy [75] and mitochondrial transplantation [76]. Among pharmacological agents, one of the most selective DRP1 blockers is mdivi-1, which has shown efficacy in many neurological diseases, including brain injury [77] ischemia [72,78], intracranial hemorrhage [79] and neurodegeneration [80]. Since mdivi-1 is the most common and frequently used DRP1 inhibitor in experimental studies, we will review the mechanisms of its neuroprotective effects on various pathogenetic aspects of ischemic injury.

To date, most studies examining the neuroprotective effects of mdivi-1 have identified its anti-apoptotic effect. One such study by Tian Y et al. [81] showed that pretreatment of rat PC12 neuronal cell culture with mdivi reduced ROS formation, cytochrome c release and loss of mitochondrial membrane potential after hypoxia.

A study by Li et al. [82] shows that the administration of mdivi-1 attenuated cardiac arrest-induced ischemic brain damage by blocking cytochrome c and AIF (apoptosis-inducing factor)-dependent apoptosis pathways. In another study [83], it was also shown that, in a model of cardiac arrest, the effect of mdivi-1 application is comparable to the application of therapeutic hypothermia, and leads to a reduction in the manifestations of mitochondrial dysfunction related to the production of ROS, ATP synthesis and maintenance of mitochondrial membrane potential. In the study by Yu et al. [84], mouse hippocampus ischemia-reperfusion injury after liver transplantation was attributed to activation of DRP1 translocation to mitochondria as well as increased calcineurin expression. Administration of mdivi-1 and calcineurin inhibitor FK506 prevented the development of cognitive impairment, thus indicating a significant role of mitochondrial dynamics in the development of neurological disorders in ischemia-reperfusion injury.

Numerous studies demonstrate the involvement of mdivi-1 in the regulation of glutamate excitotoxicity. For example, pretreatment of neuronal culture with mdivi blocked glutamate-induced DRP1 recruitment to mitochondria [85], and also reduced mitochondrial calcium uptake by neurons both in the ischemia and the reoxygenation phases [81].

Ruiz et al. also showed [86] that the reduction of excitotoxic neuronal damage may also be due to a DRP1-independent mdivi-1 effect related to its ability to reversibly inhibit the activity of mitochondrial enzyme complex I, thereby modulating the mitochondrial ROS production [87]. In addition, the authors discovered the ability of mdivi-1 to reduce early LDH release and inhibit the activation of calpain, a Ca^{2+}-inducible cysteine protease that plays an important role in triggering neuronal death during ischemic and excitotoxic injury [88].

In their further work, Ruiz et al. [89] continued to study the neuroprotective effects of mdivi-1 in glial cells during excitotoxicity. Similarly to neurons, glutamate receptor activation-induced massive Ca^{2+} ion influx in oligodendrocytes during ischemia leads to their massive death in the gray and white matter of the CNS [90]. The authors found that the administration of mdivi-1 reduced mitochondrial fragmentation only when it was subtoxic to the oligodendroglial ionotropic glutamate receptors AMRAR, conversely exacerbating its damage under conditions of excitotoxicity. In their opinion, such differences in the effects of mdivi-1 are due to the differential effects of mdivi-1 on NMDA-mediated excitotoxicity and the lack of effects of mdivi-1 on AMPA-mediated calcium overload [86].

In addition, mdivi-1 induced depolarization of the oligodendrocyte mitochondrial membrane, caused depletion of calcium in ER and increased ROS production, thus increasing the sensitivity of oligodendroglia to disorders of calcium homeostasis and leading to cell death. Mdivi-1-regulated inhibition of mitochondrial fission observed during subtoxic activation is believed to be due to its ability to block the activity of mitochondrial respiratory chain complex I (ETC) [87], which leads to a mitochondrial membrane potential (MMP) decrease and a reduction in calcium overload. In turn, the reduction of cytoplasmic calcium decreases calcineurin activation and subsequent translocation of DRP1 to mitochondria. Therefore, the presence of mdivi-1 effects unrelated to the selective inhibition of DRP1, on the one hand, expands its cytoprotective capabilities, but, on the other hand, requires further differentiated study of its effects on neurons and glia.

Massive release of ATP from neurons and glial cells during ischemia is known to cause effects similar to the increase in extracellular glutamate levels in the synaptic gap [91]. By binding to purinergic receptors, ATP is able to increase Ca^{2+} ion entry into neurons and glial cells and facilitate glutamate release by astrocytes [92]. In the study by Cui M et al. [93], inhibition of DRP1 by mdivi-1 resulted in the increased expression of ectonucleoside triphosphate phosphohydrolase CD39 in astrocytes with subsequent activation of ATP hydrolysis and increased levels of extracellular adenosine, which, acting mainly through adenosine A1 receptors, decreased Ca^{2+} influx and release of excitotoxic glutamate, and also had a powerful vasodilating effect and stimulated angiogenesis in the ischemia zone.

In a recent study by Lu et al. [69], it was demonstrated that mdivi-1 administration can also reduce the severity of edema induced by exposure to high-altitude hypoxia. Participating in the formation of the blood–brain barrier, astroglial cells are recognized to play a fundamental role in regulating its permeability through the expression of aquaporin protein AQP4, which is situated in the astrocytic end-feet [94]. In their work, Lu et al. showed that mdivi-1 reduced mitochondrial fragmentation in astrocytes and microglia, inhibited the NF-κB signaling pathway, and decreased AQP4 synthesis, thus proving the involvement of mitochondrial dynamics dysregulation in the development of brain edema following simulated high altitude exposure [69]. The effects of mdivi-1 are summarized below (Table 1).

Therefore, the neuroprotective effects of mdivi-1 in ischemia are due to its antiapoptotic action, its ability to reduce glutamate excitotoxicity, calcium overload, and prevent the development of oxidative stress, as well as to reduce the severity of brain edema that develops after hypoxic exposure. However, the presence of mdivi-1 effects not related to the selective DRP1 inhibition, along with its adverse effect on oligodendrocytes, may limit the therapeutic potential of mdivi-1, and requires a differentiated approach to assess the effects of mdivi-1 on neurons and glial cells. Moreover, the involvement of DRP1 in

mitochondrial biogenesis and cell proliferation may also limit the use of mdivi-1 because of its potential effect on poorly differentiated cells.

Table 1. DRP1-dependent and -independent ischemia-activated effects of mdivi-1.

Object	Damage Model	Changes in Mitochondrial Morphology and Function	Effects of Mdivi-1	References
PC12 cells Hippocampal neurons	OGD/R Ischemia-reperfusion injury	↓ fragmentation, ↓ ROS, ↑ ATP, ↓ cytochrome c release, MMP maintenance	Reduction of neuronal death by blocking mitochondrial apoptotic pathways	[81–84]
Hippocampal neurons HT-22	Glutamate-induced damage	↓ fragmentation, preservation of MMP loss	Reduced neuronal death due to decreased glutamate excitotoxicity	[85]
PC 12 cells	OGD/R	↓ ROS, ↓ release of cytochrome c, preservation of MMP loss, ↓ mitochondrial Ca^{2+} uptake	↓ Apoptosis due to inhibition of mitochondrial Ca^{2+} uptake from ER cisterns	[81]
Primary culture of cortical neurons	Excitotoxicity induced by NMDA stimulation	↓ fragmentation, Inhibition of the activity of the respiratory chain complex 1 activity	Reduction of neuronal death by blocking complex 1, ↓ Ca^{2+} and ↓ calpain activation	[86]
Primary culture of oligodendrogliocytes	Glutamate-induced damage caused by AMPAR stimulation	(1) subtoxic activation of AMPAR: ↓ fragmentation, inhibition of respiratory chain complex 1 activity leading to ↓ MMP and ↓ Ca^{2+} levels	Protection of oligodendroglia from glutamate-induced cell death	[89]
		(2) excitotoxic activation of AMPAR: mitochondrial swelling, MMP depolarization, ER Ca^{2+} depletion, and increased mitochondrial ROS production	Increased oxidative stress and activation of apoptosis of oligodendrogliocytes	[89]
Primary culture of astrocytes	OGD	-	Increased expression of CD 39 through the cAMP/PKA/CREB signaling pathway, leading to an increased adenosine levels due to activation of ATP hydrolysis and a decrease in ischemic death of astrocytes	[93]
Primary culture of astrocytes and microglia	Hypobaric hypoxia	↓ fragmentation	Inhibition of ROS/NF-kB signaling pathway, with subsequent reduction in AQP4 levels and cerebral edema	[69]

↓: decrease; ↑: increase.

All this confirms the necessity of the further development of more selective molecules that target DRP1. At present, a number of new DRP1 inhibitors, such as Driptor [95], Dynasore [96], and P110 [97], have been developed that have demonstrated their protective properties in hypoxic–ischemic injuries. However, most of these drugs have been investigated in a cardiac ischemia model, which requires further investigation of their effects in neural tissue, given the severe metabolic differences between neurons and glial cells.

We also hypothesize that drugs that block protein interactions with DRP1 are likely to be safer and more effective. For example, a peptide-like inhibitor P110, which does not

affect DRP1 activity, holds great promise for its subsequent clinical translation. While there are currently no data on its side effects, the drug has already demonstrated its protective properties in several ischemia models [66,97].

7. Conclusions

Mitochondrial fission plays a key role in the development of pathophysiologic and pathomorphological changes in the brain during cerebral hypoxia–ischemia. Mitochondrial dynamics during ischemic exposure have their specific regulatory mechanisms, which should be taken into account when devising approaches for neuroprotection and neuroregeneration. Evidently, the impact of DRP1 activity regulation on mitochondrial dynamics is complex and should take into account its role in both mitochondrial proliferation and fragmentation with subsequent mitophagy. Such a directed regulation of DRP1 is possible by controlling its binding to adaptor proteins, including Mff and Fis1. Furthermore, the involvement of DRP1 in cell proliferation processes likely requires short-term and targeted inhibition of neuronal DRP1 to prevent the development of side effects in growing and renewing cell populations, and to prevent the accumulation of damage due to reduced mitophagy. The presence of the neuroprotective mechanisms of DRP1 inhibition in astroglia, as well as the involvement of DRP1 in providing astro- and microglia-mediated mitochondrial transfer, expands our views on the possibilities of mitochondrial dynamics modulation under ischemia and requires its further more detailed investigation.

The presence of neuroprotective mechanisms of DRP1 inhibition in astroglia, as well as the participation of DRP1 in providing astro- and microglia-mediated mitochondrial transfer, expands the notion of existing possibilities of mitochondrial dynamics modulation under ischemia. Speaking of clinical significance, the involvement of DRP1 protein in mitochondrial vesicle formation is extremely interesting and promising in terms of developing new therapeutic strategies. Recent studies have demonstrated approaches to isolating small mitochondrial microvesicles in biological fluids with subsequent quantitative and qualitative analysis of their composition, which makes them a valuable fraction of liquid biopsy material. Microvesicles carrying damaged mitochondrial components can act both as early diagnostic biomarkers and as factors determining the prognosis and management of patients with diseases caused by mitochondrial dysfunction. A determination of the exact mechanisms controlling the assembly and formation of microvesicles requires further studies and additional research.

Author Contributions: Conceptualization, D.N.V., A.V.E., E.N.F. and V.S.S.; methodology, E.N.F. and T.I.B.; writing—original draft preparation, E.N.F., A.V.E., D.N.V. and T.I.B.; writing—review and editing, E.N.F., A.V.E., N.M.M., A.I.K. and I.S.M.; visualization, D.N.V.; supervision, V.S.S. and V.V.G.; project administration, V.S.S. and V.V.G. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the state funding for basic research, project name "Fundamental aspects of neuroplasticity within a model of translational neuroscience", State registration No. 122041800164-3.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Rybnikova, E.; Samoilov, M. Current insights into the molecular mechanisms of hypoxic pre- and postconditioning using hypobaric hypoxia. *Front. Neurosci.* **2015**, *9*, 388. [CrossRef] [PubMed]
2. Whitley, B.N.; Engelhart, E.A.; Hoppins, S. Mitochondrial dynamics and their potential as a therapeutic target. *Mitochondrion* **2019**, *49*, 269–283. [CrossRef]
3. Yapa, N.M.B.; Lisnyak, V.; Reljic, B.; Ryan, M.T. Mitochondrial dynamics in health and disease. *FEBS Lett.* **2021**, *595*, 1184–1204. [CrossRef]
4. Onishi, M.; Yamano, K.; Sato, M.; Matsuda, N.; Okamoto, K. Molecular mechanisms and physiological functions of mitophagy. *EMBO J.* **2021**, *40*, e104705. [CrossRef]

5. Sukhorukov, V.S. Mitochondrial proliferation as adaptation mechanism in various diseases. In *Adaptation Biology and Medicine: Health Potentials*; Lukyanova, L., Takeda, N., Singal, P.K., Eds.; Narossa Publishing House: New Delhi, India, 2007; Volume 5, pp. 25–42.
6. Princz, A.; Kounakis, K.; Tavernarakis, N. Mitochondrial contributions to neuronal development and function. *Biol. Chem.* **2018**, *399*, 723–739. [CrossRef] [PubMed]
7. Sprenger, H.G.; Langer, T. The Good and the Bad of Mitochondrial Breakups. *Trends Cell Biol.* **2019**, *29*, 888–900. [CrossRef] [PubMed]
8. Singh, M.; Denny, H.; Smith, C.; Granados, J.; Renden, R. Presynaptic loss of dynamin-related protein 1 impairs synaptic vesicle release and recycling at the mouse calyx of Held. *J. Physiol.* **2018**, *596*, 6263–6287. [CrossRef]
9. Vongsfak, J.; Pratchayasakul, W.; Apaijai, N.; Vaniyapong, T.; Chattipakorn, N.; Chattipakorn, S.C. The Alterations in Mitochondrial Dynamics Following Cerebral Ischemia/Reperfusion Injury. *Antioxidants* **2021**, *10*, 1384. [CrossRef] [PubMed]
10. Hao, S.; Huang, H.; Ma, R.Y.; Zeng, X.; Duan, C.Y. Multifaceted functions of Drp1 in hypoxia/ischemia-induced mitochondrial quality imbalance: From regulatory mechanism to targeted therapeutic strategy. *Mil. Med. Res.* **2023**, *10*, 46. [CrossRef] [PubMed]
11. Giacomello, M.; Pyakurel, A.; Glytsou, C.; Scorrano, L. The cell biology of mitochondrial membrane dynamics. *Nat. Rev. Mol. Cell Biol.* **2020**, *21*, 204–224. [CrossRef]
12. Chiu, Y.H.; Lin, S.A.; Kuo, C.H.; Li, C.J. Molecular Machinery and Pathophysiology of Mitochondrial Dynamics. *Front. Cell Dev. Biol.* **2021**, *9*, 743892. [CrossRef]
13. Sukhorukov, V.S.; Voronkova, A.S.; Baranich, T.I.; Gofman, A.A.; Brydun, A.V.; Knyazeva, L.A.; Glinkina, V.V. Molecular Mechanisms of Interactions between Mitochondria and the Endoplasmic Reticulum: A New Look at How Important Cell Functions are Supported. *Mol. Biol.* **2022**, *56*, 69–82. [CrossRef]
14. Manor, U.; Bartholomew, S.; Golani, G.; Christenson, E.; Kozlov, M.; Higgs, H.; Spudich, J.; Lippincott-Schwartz, J. A mitochondria-anchored isoform of the actin-nucleating spire protein regulates mitochondrial division. *eLife* **2015**, *4*, e08828. [CrossRef] [PubMed]
15. Pagliuso, A.; Cossart, P.; Stavru, F. The ever-growing complexity of the mitochondrial fission machinery. *Cell. Mol. Life Sci. CMLS* **2018**, *75*, 355–374. [CrossRef] [PubMed]
16. Rasmussen, M.L.; Robertson, G.L.; Gama, V. Break on through: Golgi-Derived Vesicles Aid in Mitochondrial Fission. *Cell Metab.* **2020**, *31*, 1047–1049. [CrossRef]
17. Wang, S.; Tan, J.; Miao, Y.; Zhang, Q. Mitochondrial Dynamics, Mitophagy, and Mitochondria-Endoplasmic Reticulum Contact Sites Crosstalk under Hypoxia. *Front. Cell Dev. Biol.* **2022**, *10*, 848214. [CrossRef] [PubMed]
18. Chakrabarti, R.; Fung, T.S.; Kang, T.; Elonkirjo, P.W.; Suomalainen, A.; Usherwood, E.J.; Higgs, H.N. Mitochondrial dysfunction triggers actin polymerization necessary for rapid glycolytic activation. *J. Cell Biol.* **2022**, *221*, e202201160. [CrossRef]
19. Kalia, R.; Wang, R.Y.; Yusuf, A.; Thomas, P.V.; Agard, D.A.; Shaw, J.M.; Frost, A. Structural basis of mitochondrial receptor binding and constriction by DRP1. *Nature* **2018**, *558*, 401–405. [CrossRef]
20. Palmer, C.S.; Elgass, K.D.; Parton, R.G.; Osellame, L.D.; Stojanovski, D.; Ryan, M.T. Adaptor proteins MiD49 and MiD51 can act independently of Mff and Fis1 in Drp1 recruitment and are specific for mitochondrial fission. *J. Biol. Chem.* **2013**, *288*, 27584–27593. [CrossRef]
21. Yu, R.; Jin, S.B.; Ankarcrona, M.; Lendahl, U.; Nistér, M.; Zhao, J. The Molecular Assembly State of Drp1 Controls its Association with the Mitochondrial Recruitment Receptors Mff and MIEF1/2. *Front. Cell Dev. Biol.* **2021**, *9*, 706687. [CrossRef]
22. Yu, R.; Liu, T.; Jin, S.B.; Ankarcrona, M.; Lendahl, U.; Nistér, M.; Zhao, J. MIEF1/2 orchestrate mitochondrial dynamics through direct engagement with both the fission and fusion machineries. *BMC Biol.* **2021**, *19*, 229. [CrossRef] [PubMed]
23. Kleele, T.; Rey, T.; Winter, J.; Zaganelli, S.; Mahecic, D.; Perreten Lambert, H.; Ruberto, F.P.; Nemir, M.; Wai, T.; Pedrazzini, T.; et al. Distinct fission signatures predict mitochondrial degradation or biogenesis. *Nature* **2021**, *593*, 435–439. [CrossRef] [PubMed]
24. Wong, Y.C.; Kim, S.; Cisneros, J.; Molakal, C.G.; Song, P.; Lubbe, S.J.; Krainc, D. Mid51/Fis1 mitochondrial oligomerization complex drives lysosomal untethering and network dynamics. *J. Cell Biol.* **2022**, *221*, e202206140. [CrossRef]
25. Yu, R.; Jin, S.B.; Lendahl, U.; Nistér, M.; Zhao, J. Human Fis1 regulates mitochondrial dynamics through inhibition of the fusion machinery. *EMBO J.* **2019**, *38*, e99748. [CrossRef] [PubMed]
26. Nolden, K.A.; Harwig, M.C.; Hill, R.B. Human Fis1 directly interacts with Drp1 in an evolutionarily conserved manner to promote mitochondrial fission. *J. Biol. Chem.* **2023**, *299*, 105380. [CrossRef] [PubMed]
27. Green, A.; Hossain, T.; Eckmann, D.M. Mitochondrial dynamics involves molecular and mechanical events in motility, fusion and fission. *Front. Cell Dev. Biol.* **2022**, *10*, 1010232. [CrossRef]
28. Valera-Alberni, M.; Joffraud, M.; Miro-Blanch, J.; Capellades, J.; Junza, A.; Dayon, L.; Núñez Galindo, A.; Sanchez-Garcia, J.L.; Valsesia, A.; Cercillieux, A.; et al. Crosstalk between Drp1 phosphorylation sites during mitochondrial remodeling and their impact on metabolic adaptation. *Cell Rep.* **2021**, *36*, 109565. [CrossRef]
29. Cribbs, J.T.; Strack, S. Reversible phosphorylation of Drp1 by cyclic AMP-dependent protein kinase and calcineurin regulates mitochondrial fission and cell death. *EMBO Rep.* **2007**, *8*, 939–944. [CrossRef] [PubMed]
30. Cereghetti, G.M.; Stangherlin, A.; Martins de Brito, O.; Chang, C.R.; Blackstone, C.; Bernardi, P.; Scorrano, L. Dephosphorylation by calcineurin regulates translocation of Drp1 to mitochondria. *Proc. Natl. Acad. Sci. USA* **2008**, *105*, 15803–15808. [CrossRef]
31. Portz, P.; Lee, M.K. Changes in Drp1 Function and Mitochondrial Morphology Are Associated with the α-Synuclein Pathology in a Transgenic Mouse Model of Parkinson's Disease. *Cells* **2021**, *10*, 885. [CrossRef]

32. Sulkshane, P.; Ram, J.; Thakur, A.; Reis, N.; Kleifeld, O.; Glickman, M.H. Ubiquitination and receptor-mediated mitophagy converge to eliminate oxidation-damaged mitochondria during hypoxia. *Redox Biol.* **2021**, *45*, 102047. [CrossRef]
33. Lugovaya, A.V.; Emanuel, V.; Ivanov, A.; Artemova, A.; Semenova, E.; Semenova, V. Current views on the role of autophagy in the pathogenesis of acute ischemic stroke. *Patol. Fiziol. I Eksperimental'Naya Ter.* **2022**, *66*, 80–90. (In Russian) [CrossRef]
34. Flippo, K.H.; Gnanasekaran, A.; Perkins, G.A.; Ajmal, A.; Merrill, R.A.; Dickey, A.S.; Taylor, S.S.; McKnight, G.S.; Chauhan, A.K.; Usachev, Y.M.; et al. AKAP1 Protects from Cerebral Ischemic Stroke by Inhibiting Drp1-Dependent Mitochondrial Fission. *J. Neurosci. Off. J. Soc. Neurosci.* **2018**, *38*, 8233–8242. [CrossRef] [PubMed]
35. Carlson, C.R.; Ruppelt, A.; Taskén, K. A kinase anchoring protein (AKAP) interaction and dimerization of the RIalpha and RIbeta regulatory subunits of protein kinase a in vivo by the yeast two hybrid system. *J. Mol. Biol.* **2003**, *327*, 609–618. [CrossRef]
36. Merrill, R.A.; Strack, S. Mitochondria: A kinase anchoring protein 1, a signaling platform for mitochondrial form and function. *Int. J. Biochem. Cell Biol.* **2014**, *48*, 92–96. [CrossRef]
37. Zhang, X.M.; Zhang, L.; Wang, G.; Niu, W.; He, Z.; Ding, L.; Jia, J. Suppression of mitochondrial fission in experimental cerebral ischemia: The potential neuroprotective target of p38 MAPK inhibition. *Neurochem. Int.* **2015**, *90*, 1–8. [CrossRef] [PubMed]
38. Gui, C.; Ren, Y.; Chen, J.; Wu, X.; Mao, K.; Li, H.; Yu, H.; Zou, F.; Li, W. p38 MAPK-DRP1 signaling is involved in mitochondrial dysfunction and cell death in mutant A53T α-synuclein model of Parkinson's disease. *Toxicol. Appl. Pharmacol.* **2020**, *388*, 114874. [CrossRef]
39. Tang, J.; Hu, Z.; Tan, J.; Yang, S.; Zeng, L. Parkin Protects against Oxygen-Glucose Deprivation/Reperfusion Insult by Promoting Drp1 Degradation. *Oxidative Med. Cell. Longev.* **2016**, *2016*, 8474303. [CrossRef]
40. Zhao, Y.; Chen, F.; Chen, S.; Liu, X.; Cui, M.; Dong, Q. The Parkinson's disease-associated gene PINK1 protects neurons from ischemic damage by decreasing mitochondrial translocation of the fission promoter Drp1. *J. Neurochem.* **2013**, *127*, 711–722. [CrossRef]
41. Zeng, X.; Zhang, Y.D.; Ma, R.Y.; Chen, Y.J.; Xiang, X.M.; Hou, D.Y.; Li, X.H.; Huang, H.; Li, T.; Duan, C.Y. Activated Drp1 regulates p62-mediated autophagic flux and aggravates inflammation in cerebral ischemia-reperfusion via the ROS-RIP1/RIP3-exosome axis. *Mil. Med. Res.* **2022**, *9*, 25. [CrossRef]
42. Cho, H.M.; Sun, W. The coordinated regulation of mitochondrial structure and function by Drp1 for mitochondrial quality surveillance. *BMB Rep.* **2019**, *52*, 109–110. [CrossRef]
43. Qi, Z.; Shi, W.; Zhao, Y.; Ji, X.; Liu, K.J. Zinc accumulation in mitochondria promotes ischemia-induced BBB disruption through Drp1-dependent mitochondria fission. *Toxicol. Appl. Pharmacol.* **2019**, *377*, 114601. [CrossRef]
44. Alia, C.; Cangi, D.; Massa, V.; Salluzzo, M.; Vignozzi, L.; Caleo, M.; Spalletti, C. Cell-to-Cell Interactions Mediating Functional Recovery after Stroke. *Cells* **2021**, *10*, 3050. [CrossRef] [PubMed]
45. Jackson, J.G.; Robinson, M.B. Regulation of mitochondrial dynamics in astrocytes: Mechanisms, consequences, and unknowns. *Glia* **2018**, *66*, 1213–1234. [CrossRef] [PubMed]
46. Huan, Y.; Hao, G.; Shi, Z.; Liang, Y.; Dong, Y.; Quan, H. The role of dynamin-related protein 1 in cerebral ischemia/hypoxia injury. *Biomed. Pharmacother.* **2023**, *165*, 115247. [CrossRef] [PubMed]
47. Hoekstra, J.G.; Cook, T.J.; Stewart, T.; Mattison, H.; Dreisbach, M.T.; Hoffer, Z.S.; Zhang, J. Astrocytic dynamin-like protein 1 regulates neuronal protection against excitotoxicity in Parkinson disease. *Am. J. Pathol.* **2015**, *185*, 536–549. [CrossRef] [PubMed]
48. Shen, Z.; Xiang, M.; Chen, C.; Ding, F.; Wang, Y.; Shang, C.; Xin, L.; Zhang, Y.; Cui, X. Glutamate excitotoxicity: Potential therapeutic target for ischemic stroke. *Biomed. Pharmacother.* **2022**, *151*, 113125. [CrossRef] [PubMed]
49. Quintana, D.D.; Garcia, J.A.; Sarkar, S.N.; Jun, S.; Engler-Chiurazzi, E.B.; Russell, A.E.; Cavendish, J.Z.; Simpkins, J.W. Hypoxia-reoxygenation of primary astrocytes results in a redistribution of mitochondrial size and mitophagy. *Mitochondrion* **2019**, *47*, 244–255. [CrossRef] [PubMed]
50. Halder, A.; Yadav, K.; Aggarwal, A.; Singhal, N.; Sandhir, R. Activation of TNFR1 and TLR4 following oxygen glucose deprivation promotes mitochondrial fission in C6 astroglial cells. *Cell. Signal.* **2020**, *75*, 109714. [CrossRef]
51. Sutter, P.A.; Crocker, S.J. Glia as antigen-presenting cells in the central nervous system. *Curr. Opin. Neurobiol.* **2022**, *77*, 102646. [CrossRef]
52. Song, T.T.; Bi, Y.H.; Gao, Y.Q.; Huang, R.; Hao, K.; Xu, G.; Tang, J.W.; Ma, Z.Q.; Kong, F.P.; Coote, J.H.; et al. Systemic pro-inflammatory response facilitates the development of cerebral edema during short hypoxia. *J. Neuroinflamm.* **2016**, *13*, 63. [CrossRef]
53. Khandelwal, N.; Simpson, J.; Taylor, G.; Rafique, S.; Whitehouse, A.; Hiscox, J.; Stark, L.A. Nucleolar NF-κB/RelA mediates apoptosis by causing cytoplasmic relocalization of nucleophosmin. *Cell Death Differ.* **2011**, *18*, 1889–1903. [CrossRef]
54. Alvarez-Guardia, D.; Palomer, X.; Coll, T.; Davidson, M.M.; Chan, T.O.; Feldman, A.M.; Laguna, J.C.; Vázquez-Carrera, M. The p65 subunit of NF-kappaB binds to PGC-1alpha, linking inflammation and metabolic disturbances in cardiac cells. *Cardiovasc. Res.* **2010**, *87*, 449–458. [CrossRef]
55. Tilokani, L.; Nagashima, S.; Paupe, V.; Prudent, J. Mitochondrial dynamics: Overview of molecular mechanisms. *Essays Biochem.* **2018**, *62*, 341–360. [CrossRef]
56. Dabrowska, A.; Venero, J.L.; Iwasawa, R.; Hankir, M.K.; Rahman, S.; Boobis, A.; Hajji, N. PGC-1α controls mitochondrial biogenesis and dynamics in lead-induced neurotoxicity. *Aging* **2015**, *7*, 629–647. [CrossRef]
57. Bruzzone, S.; Verderio, C.; Schenk, U.; Fedele, E.; Zocchi, E.; Matteoli, M.; De Flora, A. Glutamate-mediated overexpression of CD38 in astrocytes cultured with neurones. *J. Neurochem.* **2004**, *89*, 264–272. [CrossRef]

58. Lai, Y.S.; Chang, C.C.; Chen, Y.Y.; Nguyen, T.M.H.; Xu, J.; Chen, Y.C.; Chang, Y.F.; Wang, C.Y.; Chen, P.S.; Lin, S.C.; et al. Optogenetically engineered Ca^{2+} oscillation-mediated DRP1 activation promotes mitochondrial fission and cell death. *J. Cell Sci.* **2023**, *136*, jcs260819. [CrossRef]
59. Liang, W.; Sagar, S.; Ravindran, R.; Najor, R.H.; Quiles, J.M.; Chi, L.; Diao, R.Y.; Woodall, B.P.; Leon, L.J.; Zumaya, E.; et al. Mitochondria are secreted in extracellular vesicles when lysosomal function is impaired. *Nat. Commun.* **2023**, *14*, 5031. [CrossRef]
60. König, T.; Nolte, H.; Aaltonen, M.J.; Tatsuta, T.; Krols, M.; Stroh, T.; Langer, T.; McBride, H.M. MIROs and DRP1 drive mitochondrial-derived vesicle biogenesis and promote quality control. *Nat. Cell Biol.* **2021**, *23*, 1271–1286. [CrossRef]
61. Geng, Z.; Guan, S.; Wang, S.; Yu, Z.; Liu, T.; Du, S.; Zhu, C. Intercellular mitochondrial transfer in the brain, a new perspective for targeted treatment of central nervous system diseases. *CNS Neurosci. Ther.* **2023**, *29*, 3121–3135. [CrossRef] [PubMed]
62. Fairley, L.H.; Grimm, A.; Eckert, A. Mitochondria Transfer in Brain Injury and Disease. *Cells* **2022**, *11*, 3603. [CrossRef]
63. English, K.; Shepherd, A.; Uzor, N.E.; Trinh, R.; Kavelaars, A.; Heijnen, C.J. Astrocytes rescue neuronal health after cisplatin treatment through mitochondrial transfer. *Acta Neuropathol. Commun.* **2020**, *8*, 36. [CrossRef]
64. Zhou, Z.; Dai, W.; Liu, T.; Shi, M.; Wei, Y.; Chen, L.; Xie, Y. Transfer of massive mitochondria from astrocytes reduce propofol neurotoxicity. *Neurosci. Lett.* **2023**, *818*, 137542. [CrossRef]
65. She, Z.; Xie, M.; Hun, M.; Abdirahman, A.S.; Li, C.; Wu, F.; Luo, S.; Wan, W.; Wen, C.; Tian, J. Immunoregulatory Effects of Mitochondria Transferred by Extracellular Vesicles. *Front. Immunol.* **2021**, *11*, 628576. [CrossRef]
66. Liu, W.; Su, C.; Qi, Y.; Liang, J.; Zhao, L.; Shi, Y. Brain-targeted heptapeptide-loaded exosomes attenuated ischemia-reperfusion injury by promoting the transfer of healthy mitochondria from astrocytes to neurons. *J. Nanobiotechnol.* **2022**, *20*, 242. [CrossRef] [PubMed]
67. Lawrence, J.M.; Schardien, K.; Wigdahl, B.; Nonnemacher, M.R. Roles of neuropathology-associated reactive astrocytes: A systematic review. *Acta Neuropathol. Commun.* **2023**, *11*, 42. [CrossRef] [PubMed]
68. Liu, W.; Qi, Z.; Li, W.; Liang, J.; Zhao, L.; Shi, Y. M1 Microglia Induced Neuronal Injury on Ischemic Stroke via Mitochondrial Crosstalk between Microglia and Neurons. *Oxidative Med. Cell. Longev.* **2022**, *2022*, 4335272. [CrossRef] [PubMed]
69. Lu, Y.; Chang, P.; Ding, W.; Bian, J.; Wang, D.; Wang, X.; Luo, Q.; Wu, X.; Zhu, L. Pharmacological inhibition of mitochondrial division attenuates simulated high-altitude exposure-induced cerebral edema in mice: Involvement of inhibition of the NF-κB signaling pathway in glial cells. *Eur. J. Pharmacol.* **2022**, *929*, 175137. [CrossRef] [PubMed]
70. Powers, W.J.; Rabinstein, A.A.; Ackerson, T.; Adeoye, O.M.; Bambakidis, N.C.; Becker, K.; Biller, J.; Brown, M.; Demaerschalk, B.M.; Hoh, B.; et al. 2018 Guidelines for the Early Management of Patients with Acute Ischemic Stroke: A Guideline for Healthcare Professionals from the American Heart Association/American Stroke Association. *Stroke* **2018**, *49*, e46–e110. [CrossRef]
71. Yaghi, S.; Willey, J.Z.; Cucchiara, B.; Goldstein, J.N.; Gonzales, N.R.; Khatri, P.; Kim, L.J.; Mayer, S.A.; Sheth, K.N.; Schwamm, L.H.; et al. Treatment and Outcome of Hemorrhagic Transformation after Intravenous Alteplase in Acute Ischemic Stroke: A Scientific Statement for Healthcare Professionals from the American Heart Association/American Stroke Association. *Stroke* **2017**, *48*, e343–e361. [CrossRef] [PubMed]
72. Nhu, N.T.; Li, Q.; Liu, Y.; Xu, J.; Xiao, S.Y.; Lee, S.D. Effects of Mdivi-1 on Neural Mitochondrial Dysfunction and Mitochondria-Mediated Apoptosis in Ischemia-Reperfusion Injury after Stroke: A Systematic Review of Preclinical Studies. *Front. Mol. Neurosci.* **2021**, *14*, 778569. [CrossRef] [PubMed]
73. Qi, X.; Qvit, N.; Su, Y.C.; Mochly-Rosen, D. A novel Drp1 inhibitor diminishes aberrant mitochondrial fission and neurotoxicity. *J. Cell Sci.* **2013**, *126*, 789–802. [CrossRef] [PubMed]
74. Bastian, C.; Zaleski, J.; Stahon, K.; Parr, B.; McCray, A.; Day, J.; Brunet, S.; Baltan, S. NOS3 Inhibition Confers Post-Ischemic Protection to Young and Aging White Matter Integrity by Conserving Mitochondrial Dynamics and Miro-2 Levels. *J. Neurosci. Off. J. Soc. Neurosci.* **2018**, *38*, 6247–6266. [CrossRef] [PubMed]
75. Tucker, L.D.; Lu, Y.; Dong, Y.; Yang, L.; Li, Y.; Zhao, N.; Zhang, Q. Photobiomodulation Therapy Attenuates Hypoxic-Ischemic Injury in a Neonatal Rat Model. *J. Mol. Neurosci.* **2018**, *65*, 514–526. [CrossRef] [PubMed]
76. Chang, C.Y.; Liang, M.Z.; Chen, L. Current progress of mitochondrial transplantation that promotes neuronal regeneration. *Transl. Neurodegener.* **2019**, *8*, 17. [CrossRef] [PubMed]
77. Wu, Q.; Gao, C.; Wang, H.; Zhang, X.; Li, Q.; Gu, Z.; Shi, X.; Cui, Y.; Wang, T.; Chen, X.; et al. Mdivi-1 alleviates blood-brain barrier disruption and cell death in experimental traumatic brain injury by mitigating autophagy dysfunction and mitophagy activation. *Int. J. Biochem. Cell Biol.* **2018**, *94*, 44–55. [CrossRef]
78. Kumar, R.; Bukowski, M.J.; Wider, J.M.; Reynolds, C.A.; Calo, L.; Lepore, B.; Tousignant, R.; Jones, M.; Przyklenk, K.; Sanderson, T.H. Mitochondrial dynamics following global cerebral ischemia. *Mol. Cell. Neurosci.* **2016**, *76*, 68–75. [CrossRef]
79. Fan, L.F.; He, P.Y.; Peng, Y.C.; Du, Q.H.; Ma, Y.J.; Jin, J.X.; Xu, H.Z.; Li, J.R.; Wang, Z.J.; Cao, S.L.; et al. Mdivi-1 ameliorates early brain injury after subarachnoid hemorrhage via the suppression of inflammation-related blood-brain barrier disruption and endoplasmic reticulum stress-based apoptosis. *Free Radic. Biol. Med.* **2017**, *112*, 336–349. [CrossRef]
80. Bido, S.; Soria, F.N.; Fan, R.Z.; Bezard, E.; Tieu, K. Mitochondrial division inhibitor-1 is neuroprotective in the A53T-α-synuclein rat model of Parkinson's disease. *Sci. Rep.* **2017**, *7*, 7495. [CrossRef]
81. Tian, Y.; Li, B.; Shi, W.Z.; Chang, M.Z.; Zhang, G.J.; Di, Z.L.; Liu, Y. Dynamin-related protein 1 inhibitors protect against ischemic toxicity through attenuating mitochondrial Ca^{2+} uptake from endoplasmic reticulum store in PC12 cells. *Int. J. Mol. Sci.* **2014**, *15*, 3172–3185. [CrossRef] [PubMed]

82. Li, Y.; Wang, P.; Wei, J.; Fan, R.; Zuo, Y.; Shi, M.; Wu, H.; Zhou, M.; Lin, J.; Wu, M.; et al. Inhibition of Drp1 by Mdivi-1 attenuates cerebral ischemic injury via inhibition of the mitochondria-dependent apoptotic pathway after cardiac arrest. *Neuroscience* **2015**, *311*, 67–74. [CrossRef]
83. Wang, P.; Li, Y.; Yang, Z.; Yu, T.; Zheng, G.; Fang, X.; Huang, Z.; Jiang, L.; Tang, W. Inhibition of dynamin-related protein 1 has neuroprotective effect comparable with therapeutic hypothermia in a rat model of cardiac arrest. *Transl. Res. J. Lab. Clin. Med.* **2018**, *194*, 68–78. [CrossRef]
84. Yu, X.; Jia, L.; Yu, W.; Du, H. Dephosphorylation by calcineurin regulates translocation of dynamin-related protein 1 to mitochondria in hepatic ischemia reperfusion induced hippocampus injury in young mice. *Brain Res.* **2019**, *1711*, 68–76. [CrossRef] [PubMed]
85. Grohm, J.; Kim, S.W.; Mamrak, U.; Tobaben, S.; Cassidy-Stone, A.; Nunnari, J.; Plesnila, N.; Culmsee, C. Inhibition of Drp1 provides neuroprotection in vitro and in vivo. *Cell Death Differ.* **2012**, *19*, 1446–1458. [CrossRef]
86. Ruiz, A.; Alberdi, E.; Matute, C. Mitochondrial Division Inhibitor 1 (mdivi-1) Protects Neurons against Excitotoxicity through the Modulation of Mitochondrial Function and Intracellular Ca^{2+} Signaling. *Front. Mol. Neurosci.* **2018**, *11*, 3. [CrossRef] [PubMed]
87. Bordt, E.A.; Clerc, P.; Roelofs, B.A.; Saladino, A.J.; Tretter, L.; Adam-Vizi, V.; Cherok, E.; Khalil, A.; Yadava, N.; Ge, S.X.; et al. The Putative Drp1 Inhibitor mdivi-1 Is a Reversible Mitochondrial Complex I Inhibitor that Modulates Reactive Oxygen Species. *Dev. Cell* **2017**, *40*, 583–594.e6. [CrossRef]
88. Lai, T.W.; Zhang, S.; Wang, Y.T. Excitotoxicity and stroke: Identifying novel targets for neuroprotection. *Prog. Neurobiol.* **2014**, *115*, 157–188. [CrossRef]
89. Ruiz, A.; Quintela-López, T.; Sánchez-Gómez, M.V.; Gaminde-Blasco, A.; Alberdi, E.; Matute, C. Mitochondrial division inhibitor 1 disrupts oligodendrocyte Ca^{2+} homeostasis and mitochondrial function. *Glia* **2020**, *68*, 1743–1756. [CrossRef] [PubMed]
90. Matute, C. Glutamate and ATP signalling in white matter pathology. *J. Anat.* **2011**, *219*, 53–64. [CrossRef]
91. Kim, Y.; Davidson, J.O.; Green, C.R.; Nicholson, L.F.B.; O'Carroll, S.J.; Zhang, J. Connexins and Pannexins in cerebral ischemia. *Biochim. Biophys. Acta Biomembr.* **2018**, *1860*, 224–236. [CrossRef]
92. Eyo, U.B.; Miner, S.A.; Ahlers, K.E.; Wu, L.J.; Dailey, M.E. P2X7 receptor activation regulates microglial cell death during oxygen-glucose deprivation. *Neuropharmacology* **2013**, *73*, 311–319. [CrossRef] [PubMed]
93. Cui, M.; Ding, H.; Chen, F.; Zhao, Y.; Yang, Q.; Dong, Q. Mdivi-1 Protects Against Ischemic Brain Injury via Elevating Extracellular Adenosine in a cAMP/CREB-CD39-Dependent Manner. *Mol. Neurobiol.* **2016**, *53*, 240–253. [CrossRef] [PubMed]
94. Ikeshima-Kataoka, H. Neuroimmunological Implications of AQP4 in Astrocytes. *Int. J. Mol. Sci.* **2016**, *17*, 1306. [CrossRef] [PubMed]
95. Wu, D.; Dasgupta, A.; Chen, K.H.; Neuber-Hess, M.; Patel, J.; Hurst, T.E.; Mewburn, J.D.; Lima, P.D.A.; Alizadeh, E.; Martin, A.; et al. Identification of novel dynamin-related protein 1 (Drp1) GTPase inhibitors: Therapeutic potential of Drpitor1 and Drpitor1a in cancer and cardiac ischemia-reperfusion injury. *FASEB J. Off. Publ. Fed. Am. Soc. Exp. Biol.* **2020**, *34*, 1447–1464. [CrossRef]
96. Martinez-Carrasco, R.; Argüeso, P.; Fini, M.E. Dynasore protects ocular surface mucosal epithelia subjected to oxidative stress by maintaining UPR and calcium homeostasis. *Free Radic. Biol. Med.* **2020**, *160*, 57–66. [CrossRef] [PubMed]
97. Disatnik, M.H.; Ferreira, J.C.; Campos, J.C.; Gomes, K.S.; Dourado, P.M.; Qi, X.; Mochly-Rosen, D. Acute inhibition of excessive mitochondrial fission after myocardial infarction prevents long-term cardiac dysfunction. *J. Am. Heart Assoc.* **2013**, *2*, e000461. [CrossRef] [PubMed]