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18 kDa Translocator Protein in Mitochondria-Related Pathology: The Case of Traumatic Brain Injury

Nahum Rosenberg, Nasra Yasin, Leo Veenman, Orit Rosenberg, Abraham Weizman and Moshe Gavish

Abstract

Translocator protein (TSPO) takes part in mitochondrial adenine triphosphate (ATP) production and transport. Mitochondrial TSPO is a part of the apoptotic and cell necrotic mechanism. Ligands to TSPO, endogenous and synthetic, have different effects on metabolism and protein expression in human well-differentiated metabolically active cells. In general, most of the TSPO ligands affect the cellular function or metabolism in the same general direction, but different specific TSPO ligands have their own unique effects in human cells. Regulation of gene expression via the actions of TSPO ligands on the mitochondrial TSPO may form an essential mechanism for the regulation of cellular functions, especially during acute organ injury, such as acute brain damage. The exact mode of action of the specific TSPO ligands is not clear enough and should be further investigated. TSPO is a potential target for therapeutic efforts to mitigate secondary tissue injury caused by programmed cell death.

Keywords: TSPO, ligand, mitochondria, cell death

1. The structure, abundance and function of TSPO

The 18 kDa translocator protein (TSPO), previously known as a peripheral benzodiazepine receptor (PBR) [1], is a highly conserved protein with various life essential functions in eukaryotic and prokaryotic species [2]. The TSPO gene in humans is situated on the chromosome 22q13.3 [3]. The amino acid sequence of TSPO of human origin (169 amino acids): 1 mappwvpamg ftlapslgcf vgsrfvhgeg lrwyaglqkp swhpphwvlg pvwgtlysam 61 gygsylvwke

lggftekavv plglytgqla lnwawppiff garqmgwalv dllllvsgaaa 121 attvawyqvs plaarllypy lawlaftttl nycvwrdnhg wrggrrlpe [4].

TSPO takes part in mitochondrial ATP production and transport, and is located on cytoplasmic and nuclear membranes and on the outer membrane of mitochondria. TSPO is abundant in metabolically active cells in different organs, such as brain, kidney, and so forth. [2]. TSPO has been also found in other organs and generally is abundant in steroid-secreting tissues. Recently, it has been detected in high abundance in osteoblasts [5]. TSPO interacts with ligands to modulate various molecular cellular activities [5–9] by affecting cell death. TSPO is thought to be involved in mitochondrial cholesterol transport and related to cell death pathways (apoptosis and necrosis) as a functional part of the mitochondrial permeability transition pore (MPTP), along with additional related receptors and protein structures, for example, voltage-dependent anion channel (VDAC) and the adenine nucleotide translocase (ANT) [1, 2]. The existence of functional interconnection between TSPO and MPTP has been challenged recently in studies showing that the MPTP can induce apoptosis and cholesterol transport without the involvement of TSPO [10]. Thus, the exact mechanism of the TSPO involvement in cell death has not been determined yet, but its functional role in this process is strongly supported [1, 2, 6–8].

2. TSPO ligands

Ligands, either endogenous or synthetic, to TSPO, such as protoporphyrin IX (PPIX), PK 11195, Ro5–4864, FGIN-1-27, induce different effects on metabolism and protein expression in human well-differentiated metabolically active cells. For example, Ro5–4864, FGIN-1-27 and PPIX cause similar effects, for example, reducing cellular [^{18}F]-fluorodeoxyglucose ([^{18}F]-FDG) incorporation and parallel decrease in ATP generation [6–8]. The cellular effects of PK 11195 show protective attempts for cellular "detoxification" by increasing the cellular mitochondrial mass (**Figure 1**) [5].

In general, most of the TSPO ligands affect the cellular function or metabolism in the same general direction, but different specific TSPO ligands have their own unique effects in human cells. Regulation of gene expression via the actions of TSPO ligands on the mitochondrial TSPO may form an essential mechanism for the regulation of cellular functions.

The exact mode of action of the specific TSPO ligands is not clear enough and should be further investigated. Due to the evidence of the nonuniform response of cells to the different specific ligands, an attempt to elucidate the role of the TSPO in cellular metabolism and modulation of cell phenotype should be promoted.

3. TSPO role in organ pathology and injury

Changes in TSPO expression have been linked to several pathological conditions, including cancer, endocrine diseases, and neurological diseases [2]. For example, in the normal brain,
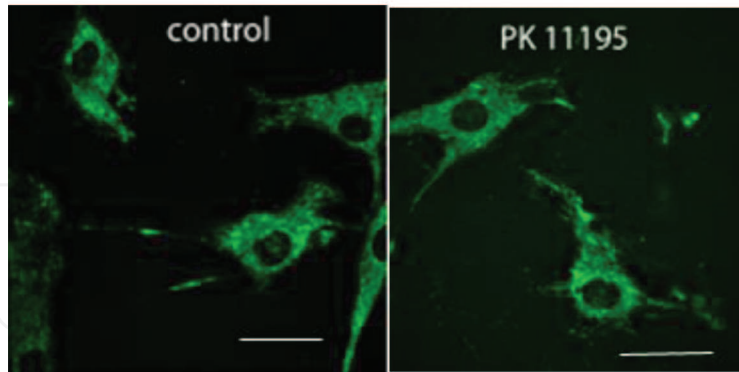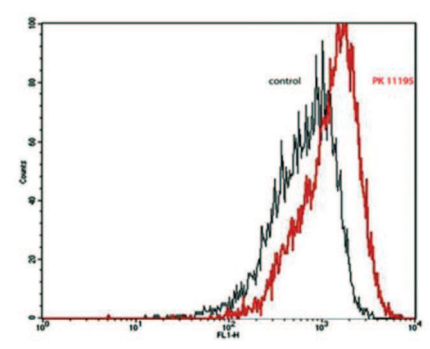
Figure 1. A: Microscopic image of cells stained by Mitotracker green stain (MTG). Strings of green stained mitochondria are apparent. Confocal microscopy, scale – 20 μ. B: Flow cytometry of cells stained by MTG. The histogram of the mitochondrial mass is shifted showing when exposed to PK 11195 (10^{-5} M) indicating on increase in the mitochondrial mass in comparison with the unexposed control.

overall TSPO expression is low, and TSPO is mainly found in glia and at very low levels in neurons [9]. But in the abnormal brain, TSPO is mainly expressed in glia, some hypertrophic astrocytes, infiltrating macrophages, and at low levels in neurons.

TSPO expression is upregulated in the injured brain and topographically localized in the inflamed areas. Additionally, in various neuropathologies, that is, gliomas, ischemia, viral encephalitis, neurodegenerative disorders (Parkinson's disease, Huntington's disease, Alzheimer's disease, and amyotrophic lateral sclerosis), local high expression of TSPO is evident [9–11, 12].

Mitochondria are the key regulators of cell survival and death. Mitochondria interact with numerous specific proteins, which are involved in genetic forms of neurodegenerative diseases [5, 9, 11]. When TSPO is a mitochondrial protein, it plays an important role in various cellular pathways related to brain damage and neurodegenerative disease [12].

The potential intracellular mechanisms related to TSPO include Ca^{++} release, ATP production, reactive oxygen species (ROS) generation, and cytochrome C release from the mitochondria in relation to programmed cell death [7, 13, 14].

4. TSPO role in cell death: the case of neural injury

Regulation of programmed cell death is not sufficient to establish tissue repair. In brain pathologies, when the TSPO is upregulated, there is a typical association with microglial activation and inflammation. High level of TSPO expression in glia, as well as the increased proliferation of microglia in gliosis, suggests that TSPO could serve as an index of the state and progression of traumatic brain injury (TBI) [3]. These studies suggest that TSPO expression measurement can be used as a biomarker of active brain disease. The precise etiology of differences in TSPO expression and its relation to injury to neural tissue, while TBI or in neurodegenerative conditions, have not been resolved yet [14].

It is possible that high TSPO local brain tissue concentration might be an etiologic factor in progressive tissue damage in TBI. This theory is based on the revealed TSPO role in the cholesterol transport via inner mitochondrial membrane that accelerates cell proliferation during reactive gliosis, which is an essential part in the pathogenesis following TBI [10].

Secondary injury has the most important effect in brain damage related to TBI. This process is caused predominantly by mitochondrial shutdown. Mitochondrial dysfunction progresses to apoptosis by the increase in oxidative stress due to depleted ATP synthesis. The final result is a local to the injured site brain tissue damage that is clinically expressed by a spectrum of cognitive or motoric impairments. Therefore, it might be logical to suggest that therapeutic efforts in TBI should address cellular pathways connected to TSPO. But there is no current therapeutic evidence that can support this hypothesis, and therefore this is a highly appealing area for further research. There is an ongoing research on novel methods to prevent, diagnose, and treat TBI focusing on maintaining mitochondrial function [15, 16].

TBI is still the leading cause of disability in young adults worldwide. Its major mechanisms are diffuse axonal injury, cerebral contusion, ischemic neurological damage, and intracranial hematomas. All these pathologies are associated to some extent with mitochondrial dysfunction [15].

Significant necrosis of the brain cortical tissue occurs rapidly upon experimental TBI. Exacerbation of the primary lesion occurs during the hours after the brain injury and leads consequently to significant neurological dysfunction. Cyclosporine A (CsA, an immunosuppressant) has shown a neuroprotective role in TBI. CsA inhibits the opening of the MPTP, which in turn maintains the mitochondrial membrane potential, and calcium homeostasis of mitochondria. These results demonstrate that MPTP modulation and mitochondrial homeostasis maintenance are related to the neuroprotective role of CsA. The CsA neuroprotective effect following brain injury indicated that pharmacological therapies can be designed to greatly affect the mitochondria and neurological outcome following a brain injury [15]. Since TSPO has been suggested to be functionally related to the MPTP, it might be involved in these cellular pathways and might be targeted in the future research of TBI treatment.

One of the cellular pathways following TBI at the cellular level is related to energetic shutdown, with subsequential interference with Ca^{2+} homeostasis. This process starts following initial brain injury by an "ischemia-like" pattern, after direct impact to the brain tissue. Additionally, this process causes impairment in the cerebral blood flow, with further local ischemic process. As the result of this initial impact, the cellular ATP synthesis decreases, and

mitochondrial membrane potential collapses. As a result, the nerve terminal membrane is depolarized, glutamate and aspartate (excitatory neurotransmitters) are released and voltage-dependent Ca^{2+} and Na^+ channels are activated. By the self-accelerating process, additional Ca^{2+} is recruited causing its pathologically increased concentration. The Ca^{2+} overload causes increased catabolic effect due to enhanced release of free radicals, with eventual cell death via cellular membrane damage [16].

ATP production via oxidative phosphorylation is the primary function of mitochondria, and Ca^{2+} is the characteristic stimulatory signal for the activation of numerous mitochondrial enzymes [16]. Mitochondria play a pivotal role in cell survival. Mitochondrial dysfunction is an early event in the CNS injury that progresses to cell necrosis.

Thus, the cell death, neural and glial, following TBI is the pathophysiological cause for overall brain damage. Cell death occurs by apoptosis, autophagy, and necrosis [17, 18]. A programmed cell necrosis (necroptosis) has a distinct and characteristic morphology due to autophagy [19]. Mitochondria are the source to cell death propagation, in addition to the extrinsic pathway and caspase-12-mediated endoplasmic reticulum (ER) apoptotic pathway. The extrinsic pathway is amplified by mitochondria and causes apoptosis. The BH3-only protein Bid links the extrinsic and mitochondrial apoptosis. The mitochondrial apoptosis can be caspase-dependent or independent. If caspase-dependent, it involves cytochrome C, otherwise apoptosis inducing factor (AIF) governs the caspase-independent pathway.

Therefore, it may be deduced, according to the abovementioned studies, that mitochondrial membrane, Ca^{2+} and reactive oxygen species (ROS) are shown to mediate synergistically the process of cell damage induced by TBI. Mitochondrial outer membrane permeabilization (MOMP) and mitochondrial permeability transition (MPT), which are part of apoptotic pathway, are the suggested mechanisms of the pores' formation in the mitochondrial membranes. An additional important factor, the Bcl-2 family, is regulatory in apoptosis and cell necrosis as well. There are also numerous other proapoptotic factors that might be interconnected with the mentioned pathways, but their role is not clear. These factors coordinate the apoptotic activity and related to mitochondria, ER, and lysosome.

The regulation of Ca^{2+} flux between mitochondria and ER is a control point during apoptosis, along with mitochondria-associated membranes. It is important to mention that the cathepsin proteases that are released from lysosome enhance the mitochondria-mediated cell death. These cellular events are related to secondary brain damage and other pathological processes involving programmed cell death.

Additional cellular pathways, not directly related to mitochondria, such as neuron cells death by excitotoxicity, due to elevated levels of synaptic neurotransmitters, also occur during TBI. Excitotoxicity is mediated by glutamate and involves several synaptic receptors, for example, AMPA, NMDA, voltage-dependent Ca^{2+} channels (VDCC) and metabotropic glutamate (mGlu) receptors [13, 20, 21]. The Na^+- and Cl^--dependent influx is associated with immediate cell swelling [22–25]. Extra-synaptic NMDA receptor activation can activate a cAMP response element binding (CREB) protein shut-off which, in turn, causes loss of $\Delta\Psi m$ and apoptosis [26]. Stress-activated protein kinases (SAPKs) are another class of signaling molecules that are part of NMDA receptor-dependent cell death [27].

Eventually, the mitochondrial membrane is one of the last barriers to cell death. Different receptors related to the mitochondrial membrane, including TSPO, have a crucial role in protection from or propagation to cell death. In the case of the secondary damage following TBI, the possibility of therapeutic targeting of this receptor has been raised [28].

The preservation of energy supply alone is not enough for mitochondria protection. The ongoing research on TSPO might also lead to the finding of protective pathways that will avoid the cell death cascades during the brain or other tissue injury. In the series of four papers, it was found that from all studied, only one type of synthetic TSPO ligand, PK 11195 (10^{-5} M), evolves a protective cellular response by elevating the cellular mitochondrial mass [6]. PK 11195 is unsuitable for clinical use because of its overall toxic effects *in vivo*, but it is clear that its interaction with TSPO evolves a protective anti-necrotic effect in cells. Therefore, TSPO is a good candidate for future research of mitigation of secondary tissue damage which is currently irreversible.

In conclusion, mitochondria play a pivotal role in the secondary cellular insult such as in the TBI and other types of tissue necrotic damage. The mitochondrial malfunction that occurs during such pathological effects results in cellular energy loss, with the activation of cell death pathways, which involve the interrelated actions of excitotoxicity, ROS, caspases, the Bcl-2 family and apoptosis inducing factor (AIF). In this process, the role of TSPO is still elusive but with a strong indication of a possible regulatory involvement [5]. With the clarification of the function and structure of TSPO on mitochondria, mitochondrial-targeted multipotential therapeutic strategies via TSPO might provide new hope for the treatment of cellular death, such as in TBI or in other acute injury states in other organs, with high energy demanding cellular content.

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