Mechanism of valproic acid-induced hepatotoxicity in alpers syndrome using an induced pluripotent stem cell model

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Valproic acid (VPA) is a widely used antiepileptic drug to treat epilepsy and psychiatric disorders, but potentially causes idiosyncratic liver injury. Alpers-Huttenlocher syndrome (AHS), a neurogenetic disorder caused by mitochondrial DNA polymerase γ (POLG) mutations, has close correlation with fatal VPA hepatotoxicity. However, the mechanisms of this clinical mystery remain unknown. Here, we established an induced pluripotent stem cell (iPSC) toxicity model to explore the mechanism behind the high risk of VPA-induced liver injury in AHS. By this model, we demonstrated that AHS iPSCs-hepatocytes are more sensitive to VPA-induced mitochondrial-dependent apoptosis than controls. Furthermore, superoxide flashes, new spontaneous bursts of superoxide generation triggered by transient openings of the mitochondrial permeability transition pore (mPTP), occur more frequently in AHS iPSCs-hepatocytes. The mPTP inhibitor, cyclosporine A, is able to rescue VPA-induced apoptotic sensitivity. In addition, carnitine and N-acetylcysteine (NAC), which has been used to treat VPA-induced liver injury, also rescue VPA-induced apoptotic sensitivity.

Keywords: AHS; VPA hepatotoxicity; iPSC; Superoxide flashes

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Alpers-Huttenlocher syndrome is a fatal neurogenetic disorder first described more than 80 years ago. It is an autosomal recessive, developmental mitochondrial DNA depletion syndrome caused by mutations in POLG, with a clinical tetrad which characterized by refractory seizures, episodic psychomotor regression, cortical blindness and liver disease [1]. POLG is the only known polymerase responsible for replication and repair of mtDNA. It has been identified nearly 150 POLG mutations in patients with mitochondrial diseases such as AHS [2]. Children with AHS are normal at birth and develop normally over the first few weeks to years of life, but develop the symptoms of disease incrementally between the ages of 1 month and 25 years of age [3,4], and then die with a degenerative course over 3 months to 12 years [1]. Clinical studies showed that apoptotic cells exist in livers and brains of AHS patients [5]. However, after that further work was not reported and the mechanisms of AHS were still poorly understood.
VPA is a widely used first-generation antiepileptic drug predominantly to treat epilepsy and psychiatric disorders [6]. However, VPA treatment often leads to liver failure and death of AHS patients [7, 8]. Over 1 in 37,000 subjects exposed to VPA develop idiosyncratic liver injury. In young children, the proportion was higher, with the risk reaching 1 in 500 subjects [9]. Puzzlingly, AHS patients extremely have higher risk of developing fatal VPA-induced hepatotoxicity. About 1/3 of AHS patients developed liver failure within 3 months of exposure to VPA, and finally died [10, 11]. Previous study showed that POLG mutation increases the risk of VPA-induced AHS hepatotoxicity [8], but the mechanism about an increasing risk of VPA-induced hepatotoxicity in AHS patients is still a mystery.

Although several animal models have been established to study mitochondrial diseases caused by POLG mutations [12-14], the clinical variability and complexity of AHS cannot be determined at the cellular level. Understanding the molecular mechanisms of AHS has been challenging due to the absence of in vitro hepatocytes diseases models. Recently, the discovery of induced pluripotent stem cells (iPSCs) by Yamanaka and Takahashi [15] offered an ideal tool to study hepatocyte diseases by providing patient-derived hepatocytes. iPSCs, generated from adult human dermal fibroblasts, stably display human ESCs-like characteristics- and can differentiate into cell types of the three germ layers in vitro [15]. To obtain patient-derived hepatocytes, skin fibroblasts from 2 reported AHS patients [16, 17] were reprogrammed to iPSCs and then differentiated into hepatocyte-like cells. We established the first iPSC toxicity model of VPA-induced hepatotoxicity in AHS to explore the mechanism and provide a wonderful drug screening platform.

It has been reported that apoptotic cells exist in livers of AHS patients. In our study, we found that AHS iPSCs-Hep are more sensitive to VPA-induced apoptosis than controls (iPSCs-Hep and H1 ESCs-Hep). This apoptosis is through a mitochondrial pathway because of increased cleaved caspase-9,-3 and release of cytochrome c. Cytochrome c release is a special symbol of mitochondrial apoptosis. Caspase-9 is a member of caspase family of cysteine proteases involved in mitochondrial apoptosis, and caspase-3 is a substrate of activated caspase-9 [18]. Soluble and inner-membrane-bound optic atrophy 1 (OPA1) oligomers keep cristae junctions tight in the inner membrane space (IMS) of mitochondria, which protect cells from apoptosis by preventing cytochrome c release [19]. We analyzed and found that OPA1 complex showed lower expression level in AHS iPSCs-Hep than in controls. In addition, soluble OPA1 was detectable in control hepatocytes but not in AHS iPSCs-Hep. Thus, we demonstrated that the lacking of soluble forms in OPA1 complex in AHS iPSCs-Hep would prevent the formation of the tight cristae junctions in which cytochrome c stores.

It has been reported that deficiencies of mitochondrial oxidative phosphorylation can generate reactive oxygen species (ROS), which may induce cell apoptosis [20]. Our AHS iPSCs-Hep exactly expressed the phenotypes of oxidative phosphorylation disorder, in addition to POLG expression decline, mtDNA depletion, cristae disorganization and ATP production reduction. We did not find significant differences in total cellular ROS between AHS iPSCs-Hep and controls. However, superoxide flashes, a mitochondrial signals for oxidative stress induced apoptosis, occurred more frequently in AHS iPSCs-Hep than in controls. Superoxide flashes exhibit randomness of time and space, all-or-none properties, and provide a vital source of superoxide production across different cell types. The superoxide flashes are caused by transient openings of the mitochondrial permeability transition pore (mPTP) [21]. The mPTP opening events were more frequent in AHS iPSCs-Hep. In addition, CsA, a mPTP-specific inhibitor, reduced the percentage of apoptotic cells in VPA-treated AHS iPSCs-Hep, which indicates that VPA-induced apoptosis in AHS iPSCs-Hep is dependent on mPTP opening.

Carnitine and NAC have been used to treat VPA induced liver toxicity [22-26]. Carnitine may increase proper mitochondrial β-oxidation of VPA, thereby limiting the peroxisomal ω-oxidation of VPA, reducing the toxic ω-oxidation products like 4-en-VPA [27]. On the other hand, NAC acts as a precursor of cysteine for glutathione (GSH) synthesis, which may be exhausted in mitochondria in VPA-induced hepatotoxicity [28]. Our studies further demonstrated that the VPA induced apoptotic sensitivity of AHS iPSCs-Hep can be rescued by carnitine or NAC. Our study suggested that the AHS iPSCs-Hep is an ideal model for drug screening.

In conclusion, we established the first iPSCs model of mitochondrial hepatopathies and revealed the pathological mechanisms of VPA-induced hepatotoxicity which is dependent on mPTP opening. Thus, inhibitors of mPTP may be considered as candidates for treatment of the VPA-induced hepatotoxicity in AHS. Some mPTP inhibitors could be used to treat several diseases, such as ischemia-reperfusion (I/R) and neurodegeneration [29]. Furthermore, active mitochondrial ion channels, such as the ATP-sensitive potassium channel (KATP) and the large-conductance Ca++-activated potassium channel (BK) could protect brain and heart from I/R injury by modulation of mPTP opening [30-32]. It gave us an inspiration that KATP and BK may play crucial role in liver injury, and may become important therapeutic targets in the future. In
addition, carnitine and NAC can also promising to treat VPA-induced hepatotoxicity according to our study.

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Conflict of Interest

All authors declare no conflict of interest.

References


