BUTYRATE REDUCES VALPROATE-INDUCED HEPATOTOXICITY, OXIDATIVE STRESS AND MITOCHONDRIAL DYSFUNCTION IN HEPG2 CELL LINE

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Sodium valproate (VPA), widely used as antiepileptic drug, is potentially hepatotoxic causing reversible or fatal liver injury [1] especially in patients with mitochondrial diseases or with POLG1 gene mutations [2].

VPA-induced hepatic injury appear to be dose- and time-dependent; commonly it causes delayed hepatic damage, but it also occurs at an early stage and at therapeutic doses [1,3]. The mechanism responsible for VPA hepatotoxicity is not completely defined, even if it has been demonstrated the involvement of reactive metabolites of VPA and oxidative stress caused by mitochondrial oxidative phosphorylation, ATP depletion and impairment of ROS detoxification system [2,4]. ROS generation along with mitochondrial damage and intracellular glutathione depletion, are the most important indicators of hepatotoxicity in primary human hepatocyte cultures [5]. Recent reports suggest drug-induced oxidative stress also significantly correlate with drug-induced liver injury (DILI) risk. Developing new protective therapeutic approaches able to limit VPA-induced liver damage can lead to the reduction of drug-toxicity.

Recently, we demonstrated that butyrate, a short-chain fatty acid produced by intestinal bacterial fermentation of dietary fibers, modulates hepatic mitochondrial function, efficiency and dynamic in obese and steatotic mice reducing hepatic oxidative stress [6].

The aim of this in vitro study was to evaluate the effect of sodium butyrate (BuNa) in limiting VPA-induced citotoxicity and the associated oxidative and mitochondrial damage in HepG2 cell line.

First, the modification of cell viability induced by high concentration of VPA (2 mM) in presence or not of BuNa (0,5-1 mM) after 24, 48 and 72 h has been evaluated. Fresh medium containing BuNa and/or VPA was added on the cells every 24 h. Confirming previous data [5], VPA significantly reduced cell viability only after 72 h (25% mortality vs untreated cells). BuNa concentrations showed a protective effect reducing cell mortality (-15% mortality vs VPA-stimulated cells). Consistently, we chose 48 hours as the time selected for the subsequent evaluation of cell VPA toxicity, to avoid alterations of viability. Cells were seeded in P6 well plates (10-6 cells/well) and pre-treated for 1 h with BuNa before VPA addition for 48 h. Then, cells were washed with PBS and cell lysates obtained for further analysis.

Butyrate (1 mM) significantly restored the protein expression of MnSOD-2, an anti-oxidant enzyme which clears reactive oxygen species (ROS). Consistently, BuNa dampened the VPA-induced increase of ROS production and malondyaldeide (MDA), as marker of lipid peroxidation.

We have also evaluated butyrate effect on VPA-induced mitochondrial dysfunction in terms of respiration, efficiency and dynamics. Mitochondria were isolated as previously described [7], and

mitochondrial bioenergetics were assessed using a high-throughput analyzer, Seahorse. Mitochondrial state 3 respiration, evaluated using succinate or palmytoil-carnitine as substrate and ADP, was altered by VPA stimulation, while BuNa counteracted VPA toxic effect, increasing oxygen consumption rate. Moreover, mitochondrial H2O2 yield release and ROS production were determined; BuNa limited H2O2 and ROS amount induced by VPA. Accordingly, VPA-stimulated cells showed a lower aconitase and SOD activity measured spectrophotometrically, that was increased by BuNa.

In conclusion, we demonstrated the efficacy of butyrate in reducing hepatotoxicity of VPA and subsequent mitochondrial oxidative stress in HepG2 cells, at a high concentration comparable to that considered toxic in clinical monitoring. Our data suggest butyrate potential role as therapeutic strategy to limit VPA-induced hepatotoxicity, a limiting adverse drug reaction following its chronic administration.

References

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