ABSTRACT

Introduction and Aims: Nonalcoholic fatty liver disease and steatohepatitis (NAFLD/NASH) if untreated lead to liver fibrosis, cirrhosis and cancer. Due to the absence of specific pharmacological NASH management, we revolved several molecular targets and compounds potentially influencing glucose, lipid and energy metabolism such as a mammalian target of rapamycin (mTORC1/C2) inhibitor - Ku-0063794 (KU), a sterol regulatory element-binding protein (SREBP1/2) inhibitor - fatostatin (FAT), and a galaninergic system modulator - celastrol (CEL). The purpose of the research was to explore the possible pharmacological benefits of KU, FAT, and CEL in nutritional preclinical models of NAFLD/NASH.

Methods and Results: *In vitro*, KU, FAT, and CEL pre-treatments alleviated palmitic acid-induced lipotoxicity in primary hepatocyte cultures. *In vivo,* the pharmacokinetics of KU was studied first, with higher bioavailability noted after intraperitoneal (i.p.) than after oral administration. For all pharmacodynamics studies, male C57BL/6 mice were fed a high-fat western-type diet (WD) with fructose and glucose (FG) in drinking water for 8-16 weeks to induce NAFLD/NASH and subsequently individual substances were administered to them despite the continued WD/FG diet. KU (5 mg/kg i.p. daily for 3 weeks or 3 times a week for 16 weeks) reduced hepatotoxicity, oxidative stress, liver triglycerides, and TNF-α mRNA but did not fully reverse NASH histopathological progression. FAT (15 mg/kg i.p. every 2-3 days for 4 weeks) and CEL (200 μg/kg i.p. each second day for 4 weeks) decreased glycemia, body, fat tissue and liver weights, serum liver enzymes, cholesterol, liver steatosis, NAFLD activity scores, and some lipogenic and inflammatory genes. However, FAT produced systemic inflammation (as evidenced by increased serum TNF-α) and eczematous symptoms on the skin. On the other hand, CEL up-regulated *Ppargc1α* and *Galr2* genes and reduced lipid peroxidation in the liver without any noticed toxicity.

Conclusion: KU, FAT, and CEL each provided some benefits in alleviating NAFLD-to-NASH progression. KU had limited success, while FAT was highly effective in suppressing metabolic anomalies and histopathological changes in the liver but exhibited skin toxicity. CEL was sufficiently effective and safe, making it as a promising candidate for advanced pharmacological testing in the treatment of NASH and other metabolic dysfunction-related diseases.

Keywords: NAFLD, NASH, Ku-0063794, fatostatin, celastrol, *in vivo, in vitro*