Case Report

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Autophagy-enhancing drug carbamazepine diminishes hepatocellular death in fibrinogen storage disease

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Abstract

Fibrinogen storage disease (FSD) is a rare autosomal-dominant hereditary disorder characterized by hypofibrinogenemia and accumulation of fibrinogen aggregates within the hepatocellular endoplasmatic reticulum (ER). Some FSD patients present with elevated amino-transferases and fibrosis/cirrhosis similar to alpha-1-antitrypsin deficiency (ATD), also an ER storage disease. Pharmacological stimulation of autophagy has been shown to mediate clearance of protein aggregates and halt progression of liver fibrosis in *in vivo* models of ATD. Our aim was to evaluate the presence of autophagy and a possible response to autophagy-enhancing therapy in patients with FSD.

Hepatic fibrosis was assessed by transient elastography in 2 newly identified FSD families with fibrinogen Aguadilla and Brescia mutations, encompassing 8 affected members. Available liver biopsies were assessed for autophagy. Two patients, who had had elevated alanine amino-transaminase levels (2–5 above upper limit of normal), were treated with the autophagy enhancer carbamazepine (CBZ).

Transient elastography did not show evidence of significant fibrosis in any affected family members. Quantitative electron microscopy of one patient showed a 5.15-fold increase of late stage autophagocytic vacuoles compared to control livers. CBZ at low anticonvulsive treatment levels led to rapid normalization of alanine-aminotransferase and decrease of caspase-cleaved and uncleaved cytokeratin-18 fragments (M30 and M65). These effects reversed after discontinuation of treatment.

Response to CBZ may be mediated by pharmacologically enhanced autophagy resulting in reduction of aggregate-related toxicity in FSD. These results suggest clinical applicability of pharmacological stimulation of autophagy in FSD, but potentially also in other related disorders.

Keywords: Fibrinogen storage disease; Fibrinogen; Cirrhosis; Elasticity imaging techniques; Autophagy.

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Abbreviations: FSD, fibrinogen storage disease; ER, endoplasmatic reticulum; FGC, fibrinogen gamma chain; H&E, hematoxylin/eosin; TEM, transmission electron microscopy; ATD, alpha-1-antitrypsin deficiency; CBZ, carbamazepine; IQR, interquartile range; ALT, serum alanine aminotransferase; AST, aspartate aminotransferase; INR, international normalized ratio; OM, original magnification; EVG, Elastic Van Gieson; n.t., not tested.

Introduction

Fibrinogen storage disease (FSD) is a rare autosomal-dominant hereditary disorder characterized by hypofibrinogenemia and accumulation of fibrinogen precursor proteins within the hepatocellular endoplasmatic reticulum (ER). Four mutations within the fibrinogen gamma chain (*FGC*) gene that cause protein aggregation, ER retention, and consequent hypofibrinogenemia in heterozygous individuals have been identified to date: fibrinogen Brescia (γ284 Gly→Arg), fibrinogen Aguadilla (γ375 Arg→Trp), fibrinogen AI DuPont (γ314 Thr→Pro) and fibrinogen Angers (γdel346-350) [1,2]. Aggregated fibrinogen is visible as small faintly eosinophilic coarse globules on hematoxylin/eosin (H&E) stained sections. Using transmission electron microscopy (TEM), a diagnostic "fingerprint-like" pattern can be seen within the ER. To date 26 individuals have been described (Supplementary data). Patients show variable severity in liver disease ranging from an inert carrier state to cirrhosis (Supplementary data).

Macroautophagy (referred to hereafter as autophagy) is a highly conserved and regulated cellular clearance mechanism [3]. Non-selective autophagy mediates metabolic adaption to different nutritional conditions and maintains cellular homeostasis. Selective autophagy clears aggregated and misfolded proteins as well as damaged organelles via cytosolic sequestration and subsequent lysosomal degradation.

In murine models of hepatic alpha-1-antitrypsin deficiency (ATD), autophagy-enhancing drugs carbamazepine (CBZ) and rapamycin reduced the hepatocellular burden of aggregated protein as well as liver fibrosis [4,5].

FSD and ATD share a similar pathogenesis: the accumulation of aggregated misfolded proteins within the endoplasmatic reticulum and development of progressive fibrotic liver disease [6]. In a yeast model of FSD, autophagy contributes to endoplasmatic reticulum clearance of fibrinogen Aguadilla gamma chain [7]. Here we characterize two newly identified FSD families and evaluate the effects of pharmacological stimulation of autophagy by CBZ in affected members with elevated serum amino-transferases.

Patients and methods

Patients

Probands of both families, a 6-year old girl and a 5-year old boy, were referred to a tertiary institution for investigation of persistently elevated serum aminotransferases. Diagnostic liver biopsies were performed, which led to diagnoses of FSD. Tissue for paraffin sections was formalin-fixed, paraffin-processed and stained using routine procedures and stains. For quantification of autophagy, control liver tissue was obtained from 3 children with normal/nearly normal diagnostic liver biopsies and an 8-month old infant, who underwent liver transplantation for severe ATD (Supplementary data). Tissue for TEM was fixed in glutaraldehyde (patient F2 III-1, control patients 1-3, control ATD patient) or deparaffinized (patient F1 III-4) and processed using standard osmium tetroxide treatment, epon® resin embedding and uranyl acetate/lead citrate staining.

Genetic testing

Genetic testing was performed for subtyping of FSD in the probands and their families. Informed consent according to the German Genetic Diagnosis Act was obtained on all members tested. Genomic DNA was isolated from EDTA blood samples. Exons 8 and 9 of the fibrinogen gamma gene were amplified and both strands were sequenced by conventional dideoxy Sanger sequencing using a GenomeLab GeXP capillary sequencer (Beckman Coulter, Krefeld, Germany). Oligonucleotide primers used for amplification and sequencing were: exon 8 forward: 5′- AGGGTC AGCATGTGATGGTT -3′; and reverse: 5′- TCCACTTCCAGTTTCAAAGAA -3′, and exon 9 forward: 5′- ACTGGCAATGCACTTCGTAA -3′; and reverse: 5′- AAAAAGGAAGA AACTTTCAGAGAA -3′.

Transient elastography

Liver stiffness was measured by transient elastography (FibroScan®, Echosens, France) using the XL probe for F1 I-2, the M probe for all other adults, and the S probe (S2 setting) for children. Ten consecutive measurements were performed on each patient and the median and interquartile ranges (IQR) were calculated. Liver stiffness measurements of <6 kPa (⩽18 years) and <7 kPa (>18 years) were regarded as normal (Supplementary data).

Carbamazepine treatment

Initiation of CBZ treatment was approved by the institutional ethics committee of Hannover Medical School and informed consent was obtained from parents of treated patients. Daily doses of CBZ were increased incrementally from 2×20 mg to maintenance doses of 2×150 mg (14.2 mg kg^{-1} d^{-1}, F2 II-1) and 2×200 mg (15.8 mg kg^{-1} d^{-1}, F1 III-4). Duration of CBZ treatment was 219 days for F1 III-4 and 208 days for F2 II-1.

Cytokeratin-18 detection

For quantification of uncleaved and caspase-cleaved cytokeratin-18 within patients' sera, the M30 Aptosense and M65 ELISAs (Peviva, Bromma, Sweden) were used as described [8]. Sera from 9 healthy children (5.7–8.8 years, mean age 6.4 years) were used as control group.

Case report

Clinical history of probands

The proband of family 1 (F1 III-4), a 6-year old Caucasian girl, was found to have persistently elevated serum alanine aminotransferase (ALT) levels as well as prolonged prothrombin time on routine blood tests. The medical history was unremarkable. The proband of family 2 (F2 III-1), a 5-year old Caucasian boy, presented with repeated and prolonged episodes of epistaxis. Investigations for exclusion of a coagulopathy revealed hypofibrinogenemia and elevated ALT levels. Serologies for autoimmune hepatitis or hepatotrophic viruses were negative in both patients and none of them received any medication.

Diagnostic liver biopsies

The liver biopsies of both probands showed hepatocytes with granular cytoplasm and focal coarse irregular globules (Fig. 1A). F1 III-4's biopsy showed scattered apoptotic hepatocytes, mitoses, and necroinflammatory foci in low density (Fig. 1B and C). There was mild nodular regenerative hyperplasia and minimal collagenous expansion of portal tracts (Fig. 1D).

Transmission electron microscopy

In both patients, material examined by TEM showed aggregates of curved tubular "fingerprint-like" structures within the distended ER (Fig. 1E). Poor preservation of membranous structures in the biopsy of F1 III-4 due to re-embedding of paraffin-processed tissue rendered this sample unsuitable for detection of

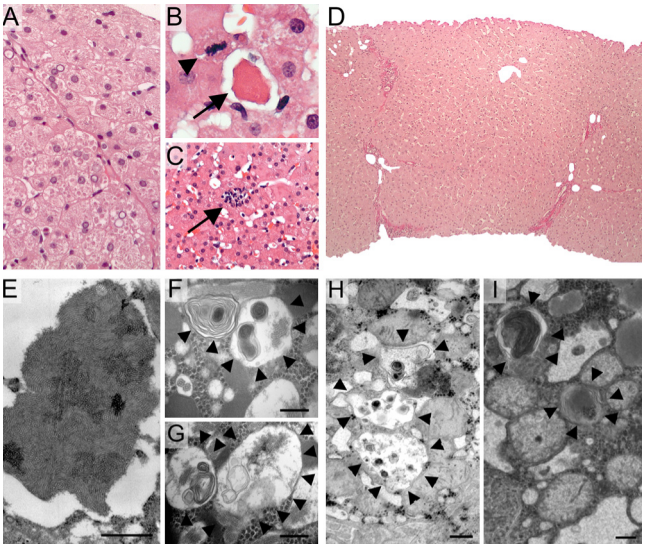

Fig. 1. Histology and TEM of liver biopsies. (A) Liver biopsy of F2 III-1 showing coarse inclusions, H&E original magnification (OM) 200×; (B) apoptosis (arrow), mitosis (arrowhead), F1 III-4, H&E OM 400×; (C) necroinflammatory activity (arrow), F1 III-4, H&E OM 100×; (D) focal periportal fibrosis, F1 III-4, Elastic Van Gieson (EVG) OM 100×; (E) aggregates show densely packed curved tubules diagnostic of FSD (F1 III-4); (F, G) late stage autophagocytic vacuoles in FSD containing aggregates as cargo (F2 III-1); (H) rare autophagocytic vacuole in a control liver (control patient 1); (I) autophagocytic vacuoles in an explanted liver of a 8-month old infant with severe neonatal ATD. Bars = 500 nm.