Acute Liver Cell Damage in Patients With Anorexia Nervosa: A Possible Role of Starvation-Induced Hepatocyte Autophagy

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Background & Aims: **Acute liver insufficiency is a rare complication of anorexia nervosa. The mechanisms for this complication are unclear. The aim of this study was to describe patient characteristics and clarify the mechanisms involved.** ***Methods:*** **Liver specimens from 12 patients (median age, 24 years; median body mass index, 11.3 kg/m^2), with a prothrombin index <50% and/or an International Normalized Ratio >1.7 and anorexia nervosa as the only cause for acute liver injury were analyzed. A detailed pathologic examination was performed, including under electron microscopy.** ***Results:*** **Liver cell glycogen depletion was a constant finding. There was a contrast between the increase in serum alanine aminotransferase (56 times normal on average; 1,904 IU/L) and the absence of significant hepatocyte necrosis on histology. Centrilobular changes (trabecular atrophy and/or sinusoidal fibrosis) were observed in 6 patients. There were rare or no (<5%) terminal deoxynucleotidyl transferase-mediated dUTP nick-end labeling–positive hepatocytes, suggesting that apoptosis was not the primary mechanism. Hepatocytes from 4 patients showed numerous autophagosomes, a morphologic hallmark of autophagy, on electron microscopy. In contrast, the mitochondria, endoplasmic reticulum, and nuclei were normal in most cells. These features were absent in 11 control patients. The outcome was favorable in all patients, with a rapid return to normal liver function.** ***Conclusions:*** **Anorexia nervosa with extremely poor nutritional status should be added to the list of conditions causing acute liver insufficiency. Our findings show that starvation-induced autophagy in the human liver may be involved in liver cell death during anorexia nervosa, even though other mechanisms of liver cell damage could also play a role.**

Anorexia nervosa is a relatively common eating disorder; the lifetime risk among women is estimated to be 0.3%–1%.[1] Mild changes in liver tests are frequent during anorexia nervosa, with an increase in serum aminotransferase levels in up to 60% of patients.[2] Thus, the 2006 practice guidelines of the American Psychiatric Association recommend systematic assessment of aminotransferase and alkaline phosphatase levels in these patients.[1] Occasional observations of marked increases in aminotransferase levels have been reported.[3–10] Acute liver insufficiency with a significant decrease in coagulation factors appears to be a more uncommon complication.[11] The finding of an inverse correlation between body mass index (BMI) and aminotransferase levels suggests that starvation alone could cause some liver cell damage.[7] However, the mechanisms of liver injury during anorexia nervosa remain unclear. In particular, there are no objective data concerning possible cellular changes during this condition.

In this article, we report a series of 12 consecutive patients presenting with acute liver insufficiency and severe anorexia nervosa as the only cause of liver injury. Different pathways of cell death were investigated to clarify the mechanisms involved. Macroautophagy has been shown to play a pivotal role in type 2 (also called *autophagic*) programmed cell death in response to starvation in yeast and animals.[12] Owing to the severe undernutrition in patients with anorexia nervosa, we hypothesized that autophagy could be a possible cause of liver cell damage, leading to significantly impaired liver function.

Patients and Methods

Study Population

From January 1995 to January 2008, 486 patients were admitted to our tertiary care centre with a diagnosis of early acute liver insufficiency, characterized by a decrease in prothrombin index <50% of normal and/or International Normalized Ratio (INR) >1.7 and no evidence of prior liver disease.[13] None of these patients had

Abbreviations used in this paper: Atg, AuTophaGy-related; BMI, body mass index; GRP, glucose-related protein; INR, International Normalized Ratio; PAS, Periodic-acid-Schiff; TUNEL, terminal deoxynucleotidyl transferase-mediated dUTP nick-end labeling.

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encephalopathy at admission. Twelve of these 486 patients had anorexia nervosa as the only identifiable cause of acute liver disease.

The diagnosis of anorexia nervosa was based on the criteria of the *Diagnostic and Statistical Manual of Mental Disorders*, 4th edition.[14] These criteria include refusal to maintain body weight at or above a minimally normal weight for age and height, intense fear of gaining weight or becoming fat even though underweight, disturbance in the way the person's body weight or shape is experienced, undue influence of body weight or shape on self-evaluation, or denial of the severity of the existing low body weight, and amenorrhea.

Patient Management

All patients were given *N*-acetylcysteine intravenously until the prothrombin index exceeded 50% of normal (INR >1.7). Glucose (5%–15%) was infused depending on the glucose concentration at admission. Patients received enteral supplementation starting from 0 (4 patients) to 500 kcal/day until the prothrombin index normalized. Thereafter, calories were gradually increased. Hypokalemia, hypoglycemia, hypophosphoremia, and hypomagnesemia were corrected as needed.

Ten patients underwent a detailed hemodynamic assessment by transthoracic echocardiography and/or right heart catheterization via a Swan-Ganz catheter. Liver biopsy was performed within 1–9 days (median, 2) after peak aspartate aminotransferase (AST) owing to the severity of liver damage and uncertainties concerning the cause. One patient underwent ultrasound-guided percutaneous biopsy and 11 patients underwent transjugular biopsy.

Histology and Immunostaining

Tissue specimens were fixed in formalin and paraffin embedded. We cut 5-mm-thick sections from paraffin blocks and stained them with hematoxylin and eosin, Mallory trichrome, Picrosirius red, Perls, and Periodic-acid-Schiff (PAS). Frozen sections were available in 10 patients and stained with Oil-Red O to evaluate steatosis. The following variables were analyzed on histology: fibrosis, lobular inflammation, centrilobular necrosis or atrophy of hepatocyte, hepatocytic swelling and clarification, steatosis (Oil Red O), PAS staining evaluating glycogen deposition, and ceroid pigments. Apoptotic cells were identified by the terminal deoxynucleotidyl transferase-mediated dUTP nick-end labeling (TUNEL) assay, using the Apop Tag Peroxydase Kit S7101 (Chemicon International, Temecula, CA).

Because of the extremely poor nutritional status and major alteration of liver cell function in these patients, hepatocytes were analyzed for proteins involved in autophagy. Genetic studies on yeast have identified 30 AuTophaGy-related (*Atg*) genes required for the formation of autophagosomes.[15] Most of the *Atg* genes are conserved in higher eukaryotes. Immunohistochemical analyses were performed in 9 patients with anorexia nervosa using the ATG5 (AuTophaGy-related 5) antibody (Abgent, San Diego, CA; 1:100 dilution). ATG5 immunostaining identifies autophagy protein 5-like, which is a key protein in autophagosome formation, constitutively expressed in all cells.[16–18]

Because the accumulation of newly synthesized unfolded proteins resulting in endoplasmic reticulum stress can trigger autophagy under certain conditions,[19] evidence of endoplasmic reticulum stress was investigated. KDEL is a sequence present at the carboxy-terminus of soluble endoplasmic reticulum resident proteins: glucose-related protein (GRP) 78 (also known as BIP) and GRP 94. BIP is a key regulator of endoplasmic reticulum function. Immunohistochemical analyses were performed in the hepatocytes of 9 patients using the KDEL antibody (Calbiochem, Darmstadt, Germany) at a 1:2,000 dilution according to the manufacturer's instructions.

Electron Microscopy

Part of the liver specimen from 4 patients in the study population was fixed in a 2% solution of glutaraldehyde buffered with 0.2 mmol/L cacodylate and post fixed in osmium tetroxide before embedding in epoxy resin for electron microscopy. Ultrathin sections stained with uranyl acetate and lead citrate were examined with an electron microscope Jeol 10 10 (Tokyo, Japan).

Controls

Ten patients with histologically normal livers were used as controls for immunohistochemical analysis. Details on these patients are given in Supplementary Table 1 (available online at www.gastrojournal.org). Eleven patients were used as controls for electron microscopy. Five of these 11 patients had histologically normal livers and 6 patients had various causes of liver cell damage characterized by necrosis and/or apoptosis. Details on this group are given in Supplementary Table 2. Analyses were performed by investigators who were unaware of patient groups. The study conformed to the ethical guidelines of the 1975 Declaration of Helsinki.

Results

Patient Characteristics

As shown in Table 1, most patients were young females, with a median BMI of 11.3. BMI at admission corresponded in all cases to the lowest BMI the patients had ever had. Six of 12 patients had severe hypoglycemia and coma at presentation. Concomitant medications are listed in Table 1. None of the patients had any symptoms suggesting underlying chronic liver disease.

Detailed results of all diagnostic tests are presented in Supplementary Table 3. Briefly, none of the patients had markers of infection for hepatitis A, B, or C viruses, significant autoantibody titers, alcohol abuse, diabetes, or hyperlipidemia. None of the patients had ingested paracetamol