Redox Biology 3 (2014) 29–39

Contents lists available at ScienceDirect

Redox Biology

journal homepage: www.elsevier.com/locate/redox

Review Article

# Ethanol-induced oxidant stress modulates hepatic autophagy and proteasome activity

Terrence M. Donohue, Jr. [a,b,c,d,e,*], Paul G. Thomes [a,b]

[a] Research Service (151), VA-Nebraska-Western Iowa Health Care System, Omaha, NE 68105, USA
[b] Department of Internal Medicine, College of Medicine, USA
[c] Department of Biochemistry and Molecular Biology, College of Medicine, USA
[d] Department of Pathology and Microbiology, College of Medicine, USA
[e] The Center for Environmental Health and Toxicology, College of Public Health, University of Nebraska Medical Center, Omaha, NE, 68198, USA

ARTICLE INFO

*Article history:*
Received 12 September 2014
Received in revised form
27 October 2014
Accepted 28 October 2014
Available online 31 October 2014

*Keywords:*
Autophagy
Ubiquitin proteasome
Ethanol
proteopathy
Steatosis

ABSTRACT

In this review, we describe research findings on the effects of alcohol exposure on two major catabolic systems in liver cells: the ubiquitin–proteasome system (UPS) and autophagy. These hydrolytic systems are not unique to liver cells; they exist in all eukaryotic tissues and cells. However, because the liver is the principal site of ethanol metabolism, it sustains the greatest damage from heavy drinking. Thus, the focus of this review is to specifically describe how ethanol oxidation modulates the activities of the UPS and autophagy and the mechanisms by which these changes contribute to the pathogenesis of alcohol-induced liver injury. Here, we describe the history and the importance of cellular hydrolytic systems, followed by a description of each catabolic pathway and the differential modulation of each by ethanol exposure. Overall, the evidence for an involvement of these catabolic systems in the pathogenesis of alcoholic liver disease is quite strong. It underscores their importance, not only as effective means of cellular recycling and eventual energy generation, but also as essential components of cellular defense.



**Contents**

Introduction . . . 30
Importance of intracellular catabolic systems . . . 30
Alcoholic liver disease (ALD) . . . 30
Ethanol and intracellular catabolic systems . . . 30
Basal macroautophagy in eukaryotic cells . . . 30
Autophagy in cultured cells after ethanol exposure . . . 31
Evidence that ethanol decreases lipophagy . . . 33
Ethanol and autophagy in vivo . . . 33
The ubiquitin–proteasome system . . . 33
Ethanol consumption and the UPS . . . 34
Ethanol exposure and the 20S proteasome . . . 34
Proteasome activity and alcoholic-induced steatosis . . . 35
Summary and future work . . . 36
Disclaimer . . . 36
Conflicts of interest . . . 36
Acknowledgments . . . 36
References . . . 37

* Correspondence to: Liver Study Unit, Research Service (151), Omaha Veterans Affairs Medical Center, 4101 Woolworth Avenue, Omaha, NE 68105, USA.
*E-mail address:* tdonohue@unmc.edu (T.M. Donohue, Jr.).

http://dx.doi.org/10.1016/j.redox.2014.10.006

## Introduction

### *Importance of intracellular catabolic systems*

During the 20th century, the biomedical literature emphasized cellular anabolic processes, including, DNA replication, RNA transcription, protein synthesis, and the assembly of complex lipids and carbohydrates. In contrast, scientific interest in macromolecular catabolism was low, partly because of the erroneous notion that once such macromolecules are synthesized, they become permanent, irreplaceable cellular fixtures. Other scientists believed that one or more cellular degradation systems existed but it/they had minor physiological importance. In the late 1930s and early 1940s, definitive isotope studies by Schoenheimer and colleagues [1] demonstrated that cellular constituents are dynamic, as they continuously undergo breakdown and replenishment. This discovery prompted more research effort into catabolic systems. In the 1950s and 1960s, groundbreaking work by De Duve, using subcellular fractions from rat liver, revealed that lysosomes are distinct cellular organelles, containing acid hydrolases that catalyze the breakdown of all macromolecular forms [2–4]. De Duve and colleagues also found that liver cells use lysosomes to digest their own contents, a process he named autophagy or "self eating" [5,6]. Numerous studies of protein catabolism in the late 1960s and early 1970s, reported that the *in vivo* half-lives of individual proteins are essentially constant but are quite distinct from each other, ranging widely from several minutes to several days. From each protein's half-life, one can estimate its synthesis rate, which is balanced with its rate of degradation [7]. Investigators examined the reasons for such diverse catabolic rates. They revealed that the primary sequence, particularly a protein's $NH_2$-terminal amino acid, its native conformation, and its size all strongly influence its rate of degradation [8,9]. These investigations laid the groundwork that led to the discovery in the late 1970s and early 1980s, of the soluble, proteolytic pathway now known as the ubiquitin–proteasome system (UPS) [10,11]. The UPS is now considered the principal proteolytic pathway in all eukaryotic cells. While the discoveries of lysosomes, autophagy and the UPS were independent events that, like other major biomedical discoveries, were first met with skepticism by other scientists, their impact has been far-reaching. It is now clear that disturbances of autophagy or the UPS are directly linked to the causes, exacerbation, and even the alleviation of disease. In fact, both catabolic pathways have become therapeutic targets. Liver disease that is caused by the hereditary disorder, alpha-1-antitrypsin (α-1AT) deficiency, in which the mutated form of α-1-AT accumulates and aggregates in liver cells, is ablated in animal models after treatment with the anti-seizure drug, carbamazepine, which reportedly accelerates autophagy [12]. Others report that *in vivo* treatment with a gene vector that expresses the transcription factor EB (TFEB), an important regulator of autophagy and lysosome biogenesis [13], also activates the autophagic pathway to enhance α-1AT removal. The proteasome inhibitor, bortezomib (Velcade®) is used with other anti-cancer drugs as an effective treatment for the hematological malignancy, multiple myeloma [14–16]. In the absence of disease, autophagy and the UPS maintain normal cell function by degrading larger molecules to smaller ones, which are further broken down to generate ATP. Both catabolic pathways are cytoprotective because they remove damaged proteins and dysfunctional organelles thereby preventing interference with normal cell function. While autophagy and the UPS occupy distinct cellular locations, they exhibit overlap in function by degrading some of the same protein substrates [17–19]. For example, when proteins aggregate, they become less recognizable substrates for and resistant to proteolysis by the proteasome. Such aggregates are more readily degraded by autophagy [20], probably because the acidic interior of the lysosome (~pH 4.7) [21] denatures such proteins for eventual digestion by the diverse array of proteases (cathepsins) that reside in that organelle. However, should the functions of the UPS and autophagy falter simultaneously, the potential for pathology increases significantly. In the liver, chronic, heavy alcohol consumption impedes both pathways. Such disturbances are linked to the pathogenesis of alcohol-induced liver injury.

### *Alcoholic liver disease (ALD)*

Alcoholic beverages have been used and abused for centuries. It is likely that liver injury caused by heavy drinking is one of the oldest liver ailments known to humans [22]. Because the liver is the principal site of ethanol oxidation, it sustains the greatest injury after alcohol abuse. The severity of ALD ranges from steatosis (fatty liver) to decompensated cirrhosis. Despite many decades of investigation into the causes of and the treatments for ALD, the disease still remains difficult to manage in the clinic [22]. Current standards of care that include abstinence, nutrition therapy and corticosteroid treatment have had marginal success [23,24]. This is partly because patients who present with alcoholic hepatitis have end-stage or near end-stage liver disease after years of heavy drinking.

Hepatic ethanol oxidation proceeds by two major pathways: alcohol dehydrogenase (ADH), which resides in the cytosol and cytochrome P450 2E1 (CYP2E1), which is a component of the endoplasmic reticulum (a.k.a. the microsome fraction). Both enzymes oxidize ethanol to generate acetaldehyde. CYP2E1 is unique because it also catalyzes the oxidation of other compounds that are chemically and functionally distinct from ethanol. These include the industrial solvent, carbon tetrachloride [25], the antipyretic, acetaminophen [25,26], and the anesthetic, halothane [27]. Furthermore, heavy ethanol consumption consistently increases the hepatic content of the CYP2E1 apoenzyme. A major mechanism for its induction is that ethanol stabilizes CYP2E1 by protecting it from degradation by the proteasome [28]. From a toxicological standpoint, CYP2E1 induction is very important when one considers the dangers of heavy drinking combined with simultaneous exposure to any or all of the aforementioned substrates. For example, when hepatic CYP2E1 enzyme levels increase after excessive alcohol consumption, the hepatotoxicity of acetaminophen is intensified because the drug is more rapidly converted to its toxic metabolite, *N*-acetyl-*p*-benzoquinone imine (NAPQI) by elevated levels of CYP2E1.

Here, we document research on how ethanol-elicited oxidant stress, generated mostly by ethanol oxidation, affects the UPS and autophagy during alcohol-induced liver injury. It is clear that both catabolic pathways are vital for maintaining a healthy liver. Evidence strongly indicates that disruption of autophagy and UPS by oxidants derived from ethanol metabolism contribute significantly to the development of steatosis and proteopathy that occur during the course of ALD pathogenesis. Here, we describe both catabolic systems and the changes that occur in each after ethanol exposure.

## Ethanol and intracellular catabolic systems

### *Basal macroautophagy in eukaryotic cells*

During macroautophagy (i.e. autophagy), all macromolecules (proteins, nucleic acids, complex carbohydrates and triglycerides) and organelles (dysfunctional organelles, including damaged mitochondria) are degraded to smaller molecules (e.g. amino acids and glucose) to generate usable energy, and to eliminate potentially toxic cellular waste [29]. In the liver, enhanced autophagy is generally regarded as cytoprotective [30]. Autophagy in liver is