Proteasome inhibitor treatment in alcoholic liver disease

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Abstract

Oxidative stress, generated by chronic ethanol consumption, is a major cause of hepatotoxicity and liver injury. Increased production of oxygen-derived free radicals due to ethanol metabolism by CYP2E1 is principally located in the cytoplasm and in the mitochondria, which does not only injure liver cells, but also other vital organs, such as the heart and the brain. Therefore, there is a need for better treatment to enhance the antioxidant response elements. To date, there is no established treatment to attenuate high levels of oxidative stress in the liver of alcoholic patients. To block this oxidative stress, proteasome inhibitor treatment has been found to significantly enhance the antioxidant response elements of hepatocytes exposed to ethanol. Recent studies have shown in an experimental model of alcoholic liver disease that proteasome inhibitor treatment at low dose has cytotoxic effects against ethanol-induced oxidative stress and liver steatosis. The beneficial effects of proteasome inhibitor treatment against oxidative stress occurred because antioxidant response elements (glutathione peroxidase 2, superoxide dismutase 2, glutathione synthetase, glutathione reductase, and GCLC) were up-regulated when rats fed alcohol were treated with a low dose of PS-341 (Bortezomib, Velcade®). This is an important finding because proteasome inhibitor treatment up-regulated reactive oxygen species removal and glutathione recycling enzymes, while ethanol feeding alone down-regulated these antioxidant elements. For the first time, it was shown that proteasome inhibition by a highly specific and reversible inhibitor is different from the chronic ethanol feeding-induced proteasome inhibition. As previously shown by our group, chronic ethanol feeding causes a complex dysfunction in the ubiquitin proteasome pathway, which affects the proteasome system, as well as the ubiquitination system. The beneficial effects of proteasome inhibitor treatment in alcoholic liver disease are related to proteasome inhibitor reversibility and the rebound of proteasome activity 72 h post PS-341 administration.

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Key words: Alcoholic liver disease; Glutathione; Oxidative stress; Proteasome inhibitor treatment; Steatosis

INTRODUCTION

Excessive alcohol consumption is recognized worldwide as a leading cause of disease, disability, and death[10]. Alcoholic liver disease (ALD) is a collective term for the pathophysiological changes caused by chronic alcohol consumption. These changes include oxidative stress generation, liver steatosis and inflammatory response fibrosis, and cirrhosis.
The marked generation of oxidative stress associated with ethanol metabolism is one of the main liver injuries caused by chronic alcohol consumption. Oxidative stress causes dysfunctions in several cellular mechanisms, such as DNA repair and antioxidant systems. CYP2E1, which is up-regulated to metabolize ethanol[^2], in mitochondria[^3] and activated Kupffer cells[^4], generates free radicals through the oxidation of NADPH to NADP+, which induces hepatocyte necrosis and apoptosis[^4]. In addition, high levels of reactive oxygen species (ROS) promote lipid peroxidation and end-products formation, such as malondialdehyde and 4-hydroxynonenal. These aldehydes are highly interactive and form adducts by binding covalently to cellular proteins, thus forming antigenic adducts, which cause inflammation[^5].

Oxidant stress can be counterbalanced by the hepatocyte antioxidant defense, which induces both enzymatic and non-enzymatic mechanisms. One of the major mechanisms by which cells protect themselves against oxidative stress is the up-regulation of a wide range of antioxidant genes. Among the intracellular antioxidant molecules, reduced glutathione (GSH) is the most abundant intracellular non-protein thiol in cells. Glutathione is the first level of cellular antioxidative response, and is important for a variety of biological functions, including protection of cells from oxidative damage by free radicals, detoxification of xenobiotics, and membrane transport. By keeping the cellular environment in a reduced state, GSH is responsible for the removal of potentially toxic electrophiles and metals, thereby protecting cells from toxic oxygen products[^8]. Furthermore, GSH exhibits a large panel of actions in controlling gene expression, apoptosis mechanisms, and membrane transport[^9]. Therefore, cells tightly regulate the synthesis, utilization, and export of GSH. L-S,R-buthionine sulfoximine (BSO), a potent specific inhibitor of γ-glutamylcysteine synthetase, the rate-limiting enzyme in GSH biosynthesis, has been used to deplete intracellular GSH and reverse drug resistance in tumor cells[^10], showing that GSH is a chemoresistance factor in cancer cells. The second level of cellular antioxidative response is the gene expression up-regulation of antioxidative enzymes. Among the enzymatic antioxidant defenses are: (1) glutathione synthetase (GSS) and superoxide dismutases (SOD), which dismutates O₂⁻ into H₂O₂ and O₂; (2) catalase, which removes H₂O₂, generating H₂O and O₂; and (3) glutathione peroxidase (GPX) and glutathione reductase (GR), which, using the cofactor NADPH, decompose H₂O₂, while reducing glutathione[^11]. Alcohol has been shown to deplete GSH levels, particularly in the mitochondria. Mitochondria are usually characterized by high levels of GSH needed to eliminate the ROS generated during respiratory chain activity[^12], and can not synthesize GSH, but import it from the cytosol using a carrier protein embedded in the membrane surrounding the mitochondria. Alcohol has been reported to interfere with the function of this carrier protein, thereby leading to the depletion of mitochondrial GSH[^13].

**CURRENT TREATMENT FOR ALD**

Several pharmacotherapeutic studies have been undertaken to cure alcoholic hepatitis. The best known are the treatments that block tumor necrosis factor α and reduce inflammation (pentoxifylline, infliximab, etanercept[^14-16]). However, these treatments are associated with an increase of infections and death.

The antioxidant therapy included Vitamin E supplementation. However, the outcome of clinical trials did not show any improvement in patients with alcoholic hepatitis[^17]. In addition, it has been shown that alcohol not only increases the production of ROS, but it also inhibits the antioxidants defense. It has also been shown that antioxidant therapy alone, or in combination with corticosteroids, did not improve 6-mo survival in severe alcoholic hepatitis[^18]. The drug most widely used for alcoholism today is Disulfiram. Disulfiram, an inhibitor of aldehyde dehydrogenase, prevents acetaldehyde metabolism, and causes immediate and severe negative reactions to alcohol intake[^19]. Recently, it has been used as an anti-tumor drug because it has the characteristic of a proteasome inhibitor[^20,21]. New drugs and new treatments are thus needed since currently available treatments are not adequate. The latest studies using Disulfiram point to the potential of proteasome inhibitor treatment for ALD.

**PROTEASOME INHIBITOR TREATMENT FOR ALD**

Proteasome controls the degradation of cellular proteins and is closely implicated in signal transduction, development and cell cycle progression[^22], antigen processing and immune response[^23], and inflammation[^24]. Proteasome inhibition has already proved to be a novel and promising strategy for the treatment of cancer[^25-27]. Specifically, PS-341 (Bortezomib, Velcade[^28]), a boronic acid dipetide with selective activity as a proteasome inhibitor, has demonstrated clinical efficacy in patients with multiple myeloma[^29], and has been approved by the U.S. Food and Drug Administration[^30]. It is now under evaluation for its activity in a variety of other hematologic and solid malignancies[^30-33]. Proteasome is considered an antioxidant defense in the cell due to its activity of removing damaged and oxidized proteins. Numerous reports have demonstrated that proteasome inhibitors cause an accumulation of oxidatively damaged proteins, indicating that a large majority of oxidatively damaged proteins, both in the cytosol and the nucleus of mammalian cells, are removed by the 20S proteasome[^34,35]. However, it is also important to mention that proteasome inhibition is also an antioxidative defense, as it leads to an up-regulation in the gene expression of antioxidative enzymes. Although it is now well established that impairment of the ubiquitin proteasome pathway is implicated in the pathogenesis of ALD, a growing body of evidence shows that proteasome inhibitors provide protection against oxidative stress in the brain and in the heart[^36-39].
Ethanol ingestion appears to have diverse effects on 26S proteasome activity, and only related to the up-regulation of antioxidative enzyme expression[48], while proteasome inhibition by PS-341 activates the gene expression of GSH recycling enzymes[48]. These authors showed a significant increase in the gene expression of antioxidative enzymes, such as glutathione reductase (GR), glutathione synthetase (GS), glutathione peroxidase 2 (GPX2), and superoxide dismutase 2 (SOD2), when rats were treated with the proteasome inhibitor PS-341. Exposure to a non-toxic low dose of proteasome inhibitor induced an increase in the antioxidative defense, thus suppressing ROS production, and therefore protecting against oxidative stress-induced hepatotoxicity due to chronic ethanol feeding. The beneficial effects of proteasome inhibition are not only related to the up-regulation of antioxidative enzyme gene expression[49], but also to the up-regulation of heat shock proteins[48], which is believed to prevent protein misfolding and the formation of protein aggregates. Thus, it is now postulated that these cytoprotective qualities obtained by the inhibition of proteasome at non-toxic doses might be beneficial in the treatment of hepatocyte injury associated with ALD. In addition, PS-341 is a highly specific and reversible proteasome inhibitor that produces a recovery and even a rebound of proteasome activity to higher levels 48 to 72 h post-treatment[48]. At the same time that proteasome inhibitor treatment up-regulated the antioxidant response elements, it down-regulated SREBP1-c[48] and the lipogenic enzymes gene expression, thus significantly decreasing steatosis in the liver of rats fed ethanol chronically[48]. It has also been found that IkB was significantly stabilized by proteasome inhibitor treatment in the liver of rats fed ethanol for 1 mo, which reflected a significant decrease in nNuclear factor (NF)-kB activation and a decrease in the expression of inflammatory genes regulated by NF-kB[48].

Chronic ethanol exposure increases the production of pro-inflammatory cytokines and disrupts immune defenses, increasing susceptibility to and the severity of infections. Proteasome inhibitor treatment can modulate the chronic ethanol-induced impairment of immune response and its consequences on host defense against microbial pathogens and tissue injury. These discoveries strongly indicate that proteasome inhibitor treatment has great potential in alcoholic liver disease therapy.

CONCLUSION

PS-341 is currently used in humans as an antitumor drug[26], and numerous studies have shown that it also represents a potential drug treatment for alcoholic liver disease. Proteasome inhibitors are a promising treatment to reduce ROS production, to reduce liver steatosis, and to reduce the production of pro-inflammatory cytokines caused by chronic ethanol feeding.

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REFERENCES

1. Li TK. Quantifying the risk for alcohol-use and alcohol-attributable health disorders: present findings and future research needs. J Gastroenterol Hepatol 2008; 23 Suppl 1: S2-S8
2. Bardag-Gorce F, Yuan QX, Li J, French BA, Fang C, Ingelman-Sundberg M, French SW. The effect of ethanol-induced cytochrome p4502E1 on the inhibition of proteasome activity by alcohol. Biochem Biophys Res Commun 2000; 279: 23-29
3. Cahill A, Cunningham CC, Adachi M, Ishii H, Bailey SM, Fromenty B, Davies A. Effects of alcohol and oxidative stress on liver pathology: the role of the mitochondrion. Alcohol Clin Exp Res 2002; 26: 907-915
4. Thakur V, Pritchard MT, McMullen MR, Wang Q, Nagy LE. Chronic ethanol feeding increases activation of NADPH oxidase by lipopolysaccharide in rat Kupffer cells: role of increased reactive oxygen in LPS-stimulated ERK1/2 activation and TNF-alpha production. J Leukoc Biol 2006; 79: 1348-1356
5. Conde de la Rosa L, Schoemaker MH, Vrenken TE, Buit-Homan M, Havinga R, Jansen PL, Moshage H. Superoxide anions and hydrogen peroxide induce hepatocyte death by different mechanisms: involvement of JNK and ERK MAP kinases. J Hepatol 2006; 44: 918-929
6. Nieto N, Friedman SL, Cederbaum AI. Stimulation and proliferation of primary rat hepatic stellate cells by cytochrome P450 2E1-derived reactive oxygen species. Hepatology 2002; 35: 62-73
7. Heller JJ, Crowley JR, Hazen SL, Salvy DM, Wagner P, Pennathur S, Heinecke JW. P-hydroxyphenylacetaldehyde, an aldehyde generated by myeloperoxidase, modifies phospholipid amino groups of low density lipoprotein in human atherosclerotic intima. J Biol Chem 2000; 275: 9957-9962
8. Anderson ME. Glutathione: an overview of biosynthesis and modulation. Chem Biol Interact 1996; 111-112: 1-14
9. Hammond CL, Lee TK, Ballatori N. Novel roles for glutathione in gene expression, cell death, and membrane transport of organic solutes. J Hepatol 2001; 34: 946-954
10. Fojo T, Bates S. Strategies for reversing drug resistance. Oncogene 2003; 22: 7512-7523
11. Chang P, Cheng E, Brooke S, Sapolsky R. Marked differences in the efficacy of post-insult gene therapy with catalase versus glutathione peroxidase. Brain Res 2005; 1063: 27-31
12. Goy A, Younes A, McAulughlin P, Pro B, Romaguera JE, Hagemeister F, Fayad L, Dang NH, Samaniego F, Wang M, Brogli K, Samuels B, Gilles F, Sarris AH, Hart S, Trehu E, Schenkein D, Cabanillas F, Rodriguez AM. Phase II study of proteasome inhibitor bortezomib in relapsed or refractory B-cell non-Hodgkin’s lymphoma. J Clin Oncol 2005; 23: 667-675
13. Fernández-Checa JC, Kaplowitz N, García-Ruiz C, Colell A.
Bardag-Gorce F. Proteasome inhibitor and oxidative stress

Miranda M, Mari M, Ardele E, Morales A. GSH transport in mitochondria: defense against TNF-induced oxidative stress and alcohol-induced defect. Am J Physiol 1997; 273: G7-G17

Akriviades E, Botta R, Briggs W, Han S, Reynolds T, Shakil O. Pentoxifylline improves short-term survival in severe acute alcoholic hepatitis: a double-blind, placebo-controlled trial. Gastroenterology 2000; 119: 1637-1648

Naveau S, Chollet-Martin S, Dharancy S, Mathurin P, Jouet P, Piquet MA, Davion T, Oberti F, Brötz P, Emilie D. A double-blind randomized controlled trial of infliximab associated with prednisolone in acute alcoholic hepatitis. Hepatology 2004; 39: 1390-1397

Boetticher NC, Peine CJ, Kwo P, Abrams GA, Patel T, Aqel B, Boardman L, Gores GJ, Harmsen WS, McClain CJ, Kamath PS, Shah VH. A randomized, double-blind, placebo-controlled multicenter trial of etanercept in the treatment of alcoholic hepatitis. Gastroenterology 2008; 135: 1953-1960

Mezey E, Potter JR, Rennie-Tankersley L, Caballeria J, Pares A. A randomized placebo controlled trial of vitamin E for alcoholic hepatitis. J Hepatol 2004; 40: 40-46

Stewart S, Prince M, Bassendine M, Hudson M, James O, Jones D, Record C, Day CP. A randomized trial of antioxidant therapy alone or with corticosteroids in acute alcoholic hepatitis. J Hepatol 2007; 47: 277-283

Wright C, Moore RD. Disulfiram treatment of alcoholism. Am J Med 1990; 88: 647-655

Cvek B, Doverk Z. The value of proteasome inhibition in cancer. Can the old drug, disulfiram, have a bright new future as a novel proteasome inhibitor? Drug Discov Today 2008; 13: 716-722

Wickström M, Danielsson K, Rickardson L, Gullbo J, Nygren P, Isaksson A, Larsson R, Lövborg H. Pharmacological profiling of disulfiram using human tumor cell lines and human tumor cells from patients. Biochem Pharmacol 2007; 73: 25-33

Naujokat C, Berges C, Hölt A, Wieczorek H, Fuchs D, Ovens J, Miltz M, Sadeghi M, Opelz G, Daniel V. Proteasomal chymotrypsin-like peptidase activity is required for essential functions of human monocyte-derived dendritic cells. Immunology 2007; 120: 120-132

Kloetzl PM. The proteasome and MHC class I antigen processing. Biochim Biophys Acta 2004; 1695: 225-233

Viserkruna A, Joeris T, Seidel D, Kroesen A, Loddenkemper C, Zeitz M, Kaufmann SH, Schmidt-Ullrich R, Steinhoff M. Proteasome-mediated degradation of IkappaBalpha and processing of p105 in Crohn disease and ulcerative colitis. J Clin Invest 2006; 116: 3195-3203

Groll M, Huber R, Moroder L. The persisting challenge of selective and specific proteasome inhibition. J Pept Sci 2009; 15: 58-66

Gillardini A, Marmiroli P, Cavaletti G. Proteasome inhibition: a promising strategy for treating cancer, but what about neurotoxicity? Curr Med Chem 2008; 15: 3025-3035

Chauhan D, Bianchi G, Anderson KC. Targeting the UPS as therapy in multiple myeloma. BMC Biochem 2008; 9 Suppl 1: S1

Richardson PG, Barlogie B, Berenson J, Singhal S, Jagnanath S, Irwin D, Rajkumar SV, Skrlovic G, Alsania M, Alaxanion R, Siegel D, Orlowski RZ, Kuter D, Limentani SA, Lee S, Hideshima T, Esseltine DL, Kauffman M, Adams J, Schenken DP, Anderson KC. A phase 2 study of bortezomib in relapsed, refractory myeloma. N Engl J Med 2003; 348: 254-261

Dou QP, Goldfarb RH. Bortezomib (millennium pharmaceuticals). J Drugs 2002; 5: 828-834

Davis NB, Taber DA, Ansari RH, Ryan CW, George C, Vokes EE, Vogelzang NJ, Stadler WM. Phase II trial of PS-341 in patients with renal cell cancer: a University of Chicago phase II consortium study. J Clin Oncol 2004; 22: 115-119

Kondagunta GV, Drucker B, Schwartz L, Back J, Marion S, Russo P, Mazumdar M, Motzer RJ. Phase II trial of bortezomib for patients with advanced renal cell carcinoma. J Clin Oncol 2004; 22: 3720-3725

Shah MH, Young D, Kindler HL, Webb I, Kleiber B, Wright J, Grever M. Phase II study of the proteasome inhibitor bortezomib (PS-341) in patients with metastatic neuroendocrine tumors. Clin Cancer Res 2004; 10: 6111-6118

Papandreu CN, Daliani DD, Nix D, Yang H, Madden T, Wang X, Pien CS, Milikan RE, Tu SM, Pagliaro L, Kim J, Adams J, Elliott P, Esseltine D, Petrusch A, Dieringer P, Perez C, Logothetis CJ. Phase I trial of the proteasome inhibitor bortezomib in patients with advanced solid tumors with observations in androgen-independent prostate cancer. J Clin Oncol 2004; 22: 2108-2121

Jung T, Engels M, Kaiser B, Poppek D, Grune T. Intracellular distribution of oxidized proteins and proteasome in HT22 cells during oxidative stress. Free Radic Biol Med 2006; 40: 1303-1312

Kelly SM, Vanslyke JK, Musil LS. Regulation of ubiquitin-proteasome system mediated degradation by cytosolic stress. Mol Biol Cell 2007; 18: 4279-4291

Yamamoto N, Sawada H, Izumi Y, Kume T, Katsuki H, Shimohama S, Akaike A. Proteasome inhibition induces glutathione synthesis and protects cells from oxidative stress: relevance to Parkinson disease. J Biol Chem 2007; 282: 4346-4352

Williams AJ, Dave JR, Tortella FC. Neuroprotection with the proteasome inhibitor MLN519 in focal ischemic brain injury: relation to nuclear factor kappaB (NF-kappaB), inflammatory gene expression, and leukocyte infiltration. Neurochem Int 2006; 49: 106-112

Nencioni A, Grünbech F, Patrone F, Ballestero A, Brossart P. Proteasome inhibitors: antitumor effects and beyond. Leukemia 2007; 21: 30-36

Lorenz M, Wilck N, Meiners S, Ludwig A, Baumann G, Stangl K, Stangl V. Proteasome inhibition prevents experimentally-induced endothelial dysfunction. Life Sci 2009; 84: 929-934

Donohue TM, Cederbaum AI, French SW, Barve S, Gao B, Osna NA. Role of the proteasome in ethanol-induced liver pathology. Alcohol Clin Exp Res 2007; 31: 1446-1459

French SW. Intragastric ethanol infusion model for cellular and molecular studies of alcoholic liver disease. J Biomed Sci 2001; 8: 20-27

Preedy VR, Adachi J, Asano M, Koll M, Mantle D, Niemola E, Parkila S, Paike AG, Peters T, Rajendram R, Seitz H, Ueno Y, Worrall S. Free radicals in alcoholic myopathy: indices of damage and preventive studies. Free Radic Biol Med 2002; 32: 683-687

Gouillon Z, Lucas D, Li J, Haghjoo AL, French BA, Fu P, Fang C, Ingelman-Sundberg M, Donohue TM, French SW. Inhibition of ethanol-induced liver disease in the intragastric feeding rat model by clormethiazole. Proc Soc Exp Biol Med 2000; 224: 302-308

Bousquet-Dubouch MP, Nguyen S, Bouryssie D, Burlet-Schiltz O, French SW, Monsarrat B, Bardag-Gorce F. Chronic ethanol feeding affects proteasome-interacting proteins. Proteomics 2009; 9: 3609-3622

Oliva J, Dedes J, Li J, French SW, Bardag-Gorce F. Epigenetics of proteasome inhibition in the liver of rats fed ethanol chronically. World J Gastroenterol 2009; 15: 705-712

Zeng T, Zhang CL, Zhi ZY, Yu LH, Zhao XL, Xie QY, Diallyl trisulfide (DATS) effectively attenuated oxidative stress-mediated liver injury and hepatic mitochondrial dysfunction in acute ethanol-exposed mice. Toxicology 2008; 252: 86-91

Das SK, Vasudevan DM. Alcohol-induced oxidative stress. Life Sci 2007; 81: 177-187

Bardag-Gorce F, Oliva J, Lin A, Li J, French BA, French SW. Proteasome inhibitor up regulates liver antioxidative en-
zymes in rat model of alcoholic liver disease. Exp Mol Pathol 2011; 90: 123-130

49 Meiners S, Ludwig A, Lorenz M, Dreger H, Baumann G, Stangl V, Stangl K. Nontoxic proteasome inhibition activates a protective antioxidant defense response in endothelial cells. Free Radic Biol Med 2006; 40: 2232-2241

50 Bardag-Gorce F, Vu J, Nan L, Riley N, Li J, French SW. Proteasome inhibition induces cytokeratin accumulation in vivo. Exp Mol Pathol 2004; 76: 83-89

51 Oliva J, Lin A, Li J, French BA, French SW, Bardag-Gorce F. Proteasome inhibitor treatment reduces hepatic steatosis by decreasing lipogenic enzymes gene expression. The 33rd Annual RSA Scientific Meeting; 2010 Jun 26-30; San Antonio, Texas, USA. Alcoholism: Clinical Experimental Research, 2010: 87A, Abstract #308

52 Bardag-Gorce F, Oliva J, Li A, Li J, French SW. The beneficial effects of proteasome inhibitor treatment in alcoholic liver disease. FASEB J 2011; 25: 366.9

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