

## Chronic ethanol exposure closes the door to vitamin C in pancreatic acinar cells. Focus on “Uptake of ascorbic acid by pancreatic acinar cells is negatively impacted by chronic alcohol exposure”

Alexander L. Ticho<sup>3</sup> and Waddah A. Alrefai<sup>1,2</sup>

<sup>1</sup>Jesse Brown VA Medical Center, Chicago, Illinois; <sup>2</sup>Division of Gastroenterology and Hepatology, Department of Medicine, University of Illinois at Chicago, Chicago, Illinois; and <sup>3</sup>Department of Physiology and Biophysics, University of Illinois at Chicago, Chicago, Illinois

PANCREATITIS IS AN INFLAMMATORY DISEASE that causes significant health care burden worldwide. Treatment of pancreatitis is severely limited by a lack of understanding of the molecular mechanisms underlying the pathophysiology (2). Recent evidence has implicated oxidative stress within pancreatic acinar cells (PACs) in the pathogenesis of this disease (8). Ascorbic acid (AA) is a crucial antioxidant in PACs, where it is present in relatively high concentrations under normal physiological conditions (5). A decrease in the availability of AA may occur in disorders such as chronic alcoholism, although the underlying mechanisms are not fully understood.

Chronic ethanol exposure is a major risk factor for both acute and chronic pancreatitis (2). Interestingly, both alcohol use and acute pancreatitis are associated with decreased plasma concentration of AA (4, 6). Furthermore, vitamin C supplementation has been shown to be moderately effective in treatment of acute and chronic pancreatitis (1). However, it is unclear how ethanol affects AA homeostasis in the pancreas and whether this disruption contributes to the development of pancreatitis. Thus, identifying the molecular mechanisms by which ethanol alters AA homeostasis in pancreatic acinar cells is of critical importance. One important component of AA homeostasis is the transport process that mediates AA uptake in PACs. In this issue of *American Journal of Physiology-Cell Physiology*, Subramanian et al. (7) identified a novel mechanism by which alcohol disrupts AA homeostasis through impairing the uptake of AA in PACs. The in vitro and in vivo studies presented in this article provide strong evidence that chronic exposure to ethanol negatively regulates expression of the sodium-dependent vitamin C transporter-2 (SVCT-2) through transcriptional repression.

The SVCT (*Slc23*) family of transmembrane transport proteins mediate the cellular uptake of vitamin C. SVCT-1 is expressed primarily in epithelial cells, while SVCT-2 is the predominant isoform in other tissues (7). Consistent with reports that suggest SVCT-2 is more functionally important than SVCT-1 in the pancreas (3), Subramanian et al. established that SVCT-2 was the predominant vitamin C transporter in human and mouse PACs. SVCT-2 mRNA and hnRNA expression was markedly higher than SVCT-1 in primary human PACs and 266-6 cells, a mouse PAC cancer line.

The authors used both in vitro and in vivo models of chronic ethanol exposure to investigate the effects of alcohol on AA uptake. Interestingly, when 266-6 cells were exposed to etha-

nol for 4 days, a decrease in [<sup>14</sup>C]ascorbic acid uptake was observed relative to control-treated cells, with a concomitant decrease in SVCT-2 mRNA and protein levels. Similarly, isolated PACs from mice fed the Lieber-DeCarli liquid ethanol diet for 1 month displayed impaired [<sup>14</sup>C]ascorbic acid uptake relative to mice fed a liquid diet without ethanol. PACs from ethanol-fed mice also displayed decreased SVCT-2 mRNA and protein expression. This comprehensive approach and the use of an established in vivo model for chronic alcohol consumption further enhanced the relevance of the studies. It is important to note that exposure to ethanol decreased AA uptake, at least partially, by attenuating SVCT-2 transcription. Chronic ethanol use is known to alter gene transcription through several mechanisms. Ethanol exposure can affect expression and activity of a variety of transcription factors (10). Notably, ethanol and its metabolites are also regulators of several epigenetic mechanisms including DNA methylation and histone methylation and acetylation (10). It was, therefore, interesting to determine whether epigenetic mechanisms are involved in mediating the observed effects of ethanol on AA uptake in PACs.

Subramanian et al. investigated several potential epigenetic mechanisms by which ethanol could reduce SVCT-2 transcription in 266-6 cells. They determined by bisulfite sequencing that the methylation status of the *Slc23a2* promoter is unchanged by chronic ethanol exposure, suggesting that decreased SVCT-2 transcription was not the result of altered promoter methylation. Notably, they identified alterations in histone methylation by chromatin immunoprecipitation in 266-6 cells that are expected to be functionally important. Following ethanol exposure, the *Slc23a2* promoter was enriched when immunoprecipitated with anti-H3K27me3 and was depleted when immunoprecipitated with anti-H3K4me3. Both of these epigenetic marks indicate repression of SVCT-2 transcription. Furthermore, the authors noted that the transcription factors KLF-4 and Sp-1 have been previously shown to drive *Slc23a2* promoter activity in hepatocytes, and that chronic ethanol exposure leads to decreased expression of both KLF-4 and Sp-1 (7). Although the role of these transcription factors was not directly tested in this study, it is reasonable to assume that decreased KLF-4 and Sp-1 expression contributes to reduced SVCT-2 expression following chronic alcohol exposure. A proposed mechanism of the effects of ethanol on vitamin C homeostasis in PACs is shown in Fig. 1.

Overall, the study by Subramanian et al. supports the concept that chronic alcohol exposure reduces availability of ascorbic acid in pancreatic acinar cells, possibly contributing to the pathogenesis of pancreatitis. This is an important conclu-

Address for reprint requests and other correspondence: W. A. Alrefai, Jesse Brown VA Medical Center, Medical Research Service (600/151), 820 South Damen Ave., Chicago, IL 60612 (e-mail: walrefai@uic.edu).

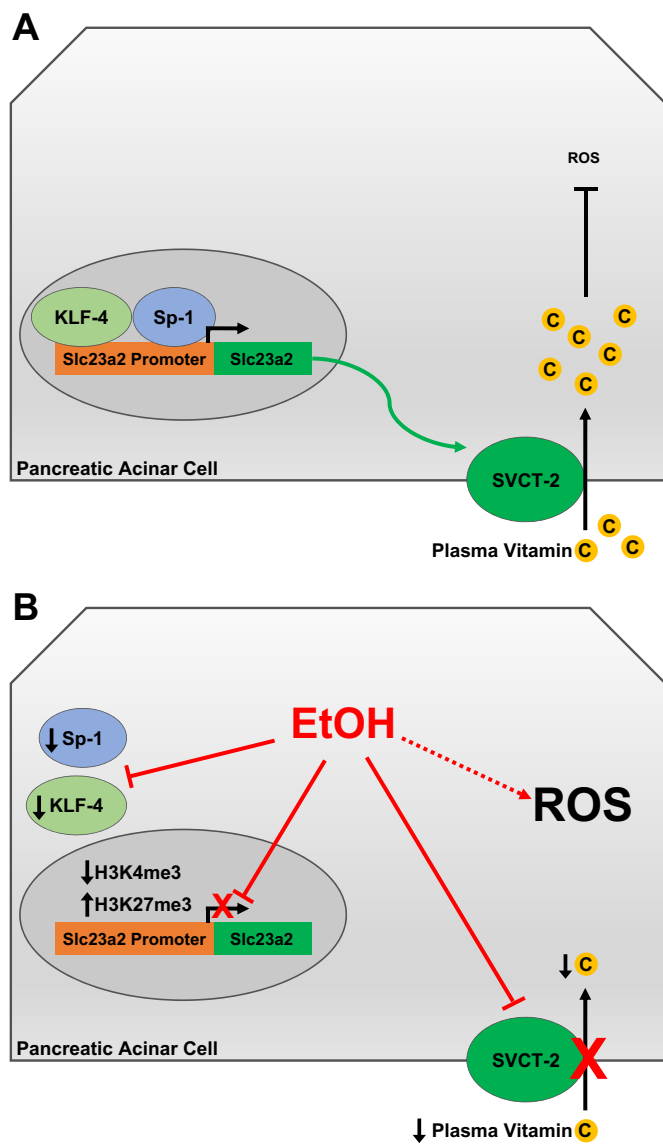


Fig. 1. Proposed mechanism of alcohol-induced disruption in ascorbic acid homeostasis. *A*: under normal conditions, SVCT-2 transcription is driven by KLF-4 and Sp-1. SVCT-2 imports vitamin C, which acts as an antioxidant to decrease intracellular reactive oxygen species. *B*: chronic ethanol exposure represses SVCT-2 expression through modulation of histone methylation and repression of KLF-4 and Sp-1.

sion that is based on novel data presented in this article and could be further supported by additional studies in the future. For example, it will be interesting to measure the effect of ethanol exposure on intracellular levels of AA and reactive oxygen species (ROS) in PACs. Additionally, the role of the SVCT-2 in the observed effect could be further supported by transfection of 266-6 cells to overexpress SVCT-2 and reverse the effects of ethanol.

It is important to note that disruption in AA homeostasis with chronic ethanol exposure has been implicated in tissues other than the pancreas. For example, a recent article published by Tian et al. (9) describes the role of SVCT-2 in protecting neurons against ethanol-induced oxidative damage. Taken together, these two studies suggest that reduced cellular uptake of AA may be a general mechanism by which chronic exposure to ethanol causes cell damage. By repressing SVCT-2 transcription, chronic ethanol use may increase susceptibility to oxidative damage in multiple cell types including pancreatic acinar cells. Therefore, interventions that induce SVCT-2 transcription may prove to be useful treatments for a variety of complications of alcohol use, including pancreatitis.

GRANTS

The research in the authors' laboratory is supported by Department of Veterans Affairs Grant BX000152 (to W. A. Alrefai) and National Institutes of Health National Institute of Diabetes and Digestive and Kidney Diseases Grant DK-71596 (to W. A. Alrefai).

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

A.L.T. and W.A.A. drafted manuscript; A.L.T. and W.A.A. edited and revised manuscript; W.A.A. approved final version of manuscript.

REFERENCES

1. Ahmed Ali U, Jens S, Busch ORC, Keus F, van Goor H, Gooszen HG, Boermeester MA. Antioxidants for pain in chronic pancreatitis. *Cochrane Database Syst Rev* 8: CD008945, 2014.
2. Clemens DL, Schneider KJ, Arkfeld CK, Grode JR, Wells MA, Singh S. Alcoholic pancreatitis: new insights into the pathogenesis and treatment. *World J Gastrointest Pathophysiol* 7: 48–58, 2016.
3. Corpe CP, Tu H, Eck P, Wang J, Faulhaber-Walter R, Schnermann J, Margolis S, Padayatty S, Sun H, Wang Y, Nussbaum RL, Espey MG, Levine M. Vitamin C transporter Slc23a1 links renal reabsorption, vitamin C tissue accumulation, and perinatal survival in mice. *J Clin Invest* 120: 1069–1083, 2010.
4. Devgun MS, Fiabane A, Paterson CR, Zaremski P, Guthrie A. Vitamin and mineral nutrition in chronic alcoholics including patients with Korsakoff's psychosis. *Br J Nutr* 45: 469–473, 1981.
5. Hornig D. Distribution of ascorbic acid, metabolites and analogues in man and animals. *Ann NY Acad Sci* 258: 103–118, 1975.
6. Scott P, Bruce C, Schofield D, Shiel N, Braganza JM, McCloy RF. Vitamin C status in patients with acute pancreatitis. *Br J Surg* 80: 750–754, 1993.
7. Subramanian VS, Srinivasan P, Said HM. Uptake of ascorbic acid by pancreatic acinar cells is negatively impacted by chronic alcohol exposure. *Am J Physiol Cell Physiol* (April 27, 2016). doi:10.1152/ajpcell.00042.2016.
8. Tandon RK, Garg PK. Oxidative stress in chronic pancreatitis: pathophysiological relevance and management. *Antioxid Redox Signal* 15: 2757–2766, 2011.
9. Tian H, Ye X, Hou X, Yang X, Yang J, Wu C. SVCT2, a potential therapeutic target, protects against oxidative stress during ethanol-induced neurotoxicity via JNK/p38 MAPKs, NF-κB and miRNA125a-5p. *Free Radic Biol Med* 96: 362–373, 2016.
10. Zakhari S. Alcohol metabolism and epigenetics changes. *Alcohol Res* 35: 6–16, 2013.