Commentary

A Murine Model of Alcoholic Cardiomyopathy

A Role for Zinc and Metallothionein in Fibrosis

W. Keith Jones
From the Department of Pharmacology and Cell Biophysics, University of Cincinnati, Cincinnati, Ohio

Chronic alcohol use in humans results in functional changes and pathology in multiple organ systems. Particularly affected are the liver, the heart, and the pancreas. Alcohol-induced disease of these organs often is a significant clinical problem, resulting in serious or fatal illness, particularly in cases of chronic alcohol abuse (defined as greater than 90 g of alcohol per day for 5 years or more). In humans, such exposure to alcohol typically results in a form of dilated cardiomyopathy that is characterized by reduced contractility, ventricular dilatation, cardiomyocyte apoptosis, and fibrosis, often progressing to heart failure. Cardiac fibrosis involves fibroblast proliferation and transdifferentiation to myofibroblasts, extracellular matrix remodeling, and stiffening of the ventricular wall. Because fibrosis exacerbates the decline of ventricular function and contributes to dilatation and progression to failure, it represents an attractive therapeutic target. To date, features of alcoholic cardiomyopathy (ACM), notably fibrosis, are not reproducibly recapitulated in animal models. In this issue of The American Journal of Pathology, Wang and colleagues detail a novel model of ACM and investigate the effects of metallothionein (MT) and zinc on ACM and myocardial fibrosis.

Alcohol affects the heart both directly and indirectly. Direct effects involve perturbation of the structure/function and/or metabolism of cardiac constituent cells. Indirect effects are mediated by other organs and by neurohormonal factors. Although the liver is the primary site of alcohol metabolism, alcohol also acts on the myocardium via direct effects on cellular metabolism. Myocardial cells metabolize ethanol via alcohol dehydrogenase to produce acetaldehyde, which enhances release of catecholamines and directly impairs cardiac function. Alcohol also interferes with excitation-contraction coupling by affecting the calcium transit. Ethanol can combine with fatty acids to produce fatty acid ethyl esters that damage mitochondria and membranes, and alcohol enhances lipid peroxidation, which damages cellular membranes. Cardiac muscle from alcohol-fed animals has a reduced capacity for oxidative metabolism and increased glycolysis, resulting in a more acidic intracellular environment. Alcohol is known to alter mitochondrial metabolism and to enhance production of oxygen-derived free radicals, including superoxide (O$_2^-$), and thus has direct effects on generation of reactive oxygen species. Acute alcohol exposure reduces the levels of the myofibrillar proteins and reversibly reduces cardiac contractile function while chronic exposure to alcohol leads to further irreversible reduction of myofibrillar protein.

Chronic alcohol administration increases the probability of fibrillation and affects excretion and tissue levels of metal ions. Thus, alcohol exposure to the heart generally leads to decreased myocardial contractile function, cellular damage, and increased oxidative stress.

Effects of Alcohol on Minerals

In both the human liver and heart, alcohol use is associated with perturbation of metal ion homeostasis. It is not known whether this is a primary effect of alcohol or an indirect result of tissue damage, but serum zinc deficiency is common among patients with idiopathic cardiomyopathy. In both liver and heart, chronic alcohol use results in reduced levels of zinc. In the liver, where this is better studied, zinc depletion occurs in association with high tissue levels of copper, iron, and manganese and increased fibrosis. Numerous studies have reported that low zinc levels associate with alcohol abuse and correlate with the severity of liver cirrhosis and mortality.

Accepted for publication April 26, 2005.

Address reprint requests to W. Keith Jones, Department of Pharmacology and Cell Biophysics, 231 Albert Sabin Way ML0575, University of Cincinnati, Cincinnati, OH 45267-0575. E-mail: joneswk@uc.edu.
weeks was reversible by withdrawal of the carbon tetra-
chloride. However, damage resulting from an 8-week
course of carbon tetrachloride treatment induced irre-
versible damage and fibrosis associated with reduced hepatic MT levels. These investigators went on to show
that, in mice deficient for MT-I and -II (MT-KO), the
4-week treatment regimen induced irreversible liver dam-
age and fibrosis. Importantly, adenoviral delivery of the
human MT-II gene to the liver reversed fibrosis and acti-
vated hepatocyte regeneration in both MT-knockout (KO)
(4 weeks) and wild-type mice (8 weeks) with irreversible
fibrosis. The increased MT activity was associated with
increased collagenase activity, suggesting that the MT-II
gene therapy activated anti-fibrotic pathways in the dam-
egaged livers. This was the first study to implicate MT in the
modulation of fibrosis, but whether this acts directly or via
the reactive oxygen species scavenging activity of MT
was not addressed. Until now, there has been no data
bearing on a role for MT in cardiac fibrosis.

Zinc, copper, iron, and manganese play important
roles as co-factors for several of the enzymes involved in
collagen synthesis/degradation and extracellular matrix
remodeling. Low zinc levels enhance prolylhydroxylase
activity and inhibit collagenases, favoring collagen depo-
sition.19,20 Also, low zinc levels impair superoxide dis-
mutase activity21 that would normally counter increased
generation of free radicals subsequent to alcohol expo-
sure. Zinc and copper are required co-factors for the
activity of matrix metalloproteinases (MMPs), which are
critically involved in extracellular matrix remodeling.
MMPs are grouped into four major categories; collagen-
ases (eg, MMP1, MMP13), gelatinases (eg, MMP2,
MMP9), stromelysin-specific (eg, MMP3), and membran-
ous types (eg, MT1-MMP). MMPs have multiple roles in the
events that underlie ventricular remodeling after in-
farction and development of ventricular failure in the
heart. For instance, mice deficient for MMP9, a gelati-
nase, have reduced remodeling after infarct and im-
proved function, suggesting that MMP9 activity contrib-
utes to fibrosis.22 On the other hand, MMPs with collagenase activity are anti-fibrotic.23 Dysregulation of
MMP activity is generally associated with adverse myo-
cardial remodeling.23,24 Thus, zinc potentially plays mul-
tiple regulatory roles relating to collagen deposition and
degradation. The article by Wang and colleagues3 in this
issue of The American Journal of Pathology confirms this
by demonstrating that zinc deficiency alters the cardio-
myopathic response to alcohol in the mouse.

Animal Models of ACM

Curiously, although alcohol induces hypertrophy and car-
diac dysfunction in mice, fibrosis is not typically part of
the pathophysiological picture. Thus, murine models of
ACM have been problematic and of dubious clinical ap-
lication. Investigators have turned to other animals, in-
cluding the dog, rabbit, rat, and chicken, to develop more relevant models. Still, the features of ACM in these animal models do not exactly mirror those of human
ACM and are completely reversible by withdrawal of
alcohol, unlike the human condition.7 Nevertheless, use
of these models has enabled a great deal of research and
has revealed several important aspects of alcohol
toxicity in the cardiovascular system. Although animal
models have their limitations, many of the experimental
approaches that have been used with these models
could not be performed with humans due to obvious
ethical dilemmas. However, the paucity of murine models in
particular poses a severe limitation because one can-
not take advantage of the powerful approaches available,
including genetically engineered mice (transgenics and
gene targeting), the mouse genome, gene expression
and proteomic databases, the numerous inbred, outbred,
mutilt strains, and the inbred recombinant and congenic
lines that have been engineered. Currently, many such
resources for the mouse exist and their number and
availability increase almost daily. Mouse models of car-
diovascular disease have proven to be extremely power-
ful tools for the elucidation of pathophysiological mech-
isms. Thus, developing mouse models of ACM that more
closely mirror the human condition is paramount.

The Murine MT-KO Model of ACM

In their study, Wang and colleagues3 demonstrate that
mice homozygous for a deficiency of both the MT-I and II
genes (MT-KO) present with an ACM more typical of the
human disease because fibrosis occurs. In this model,
alcohol feeding was accomplished using a Lieber and
DeCarli liquid diet modified by replacing the alcoholic
diet with the control diet on the last day of each week; this
prevented the weight loss that otherwise is associated
with long-term alcohol feeding regimens of this type. The
content of alcohol was increased during the 2-month
regimen, from 26 to 36% of total calories. Importantly,
the blood alcohol levels in this model are close to those
measured in alcoholic patients.3 At the 2-month time
point, wild-type and MT-KO mice were euthanized, and
the effects of alcohol on the morphological and histolog-
ical features of cardiomyopathy were ascertained. Anal-
ysis of areas of collagen accumulation showed a signifi-
cant twofold increase in fibrosis in MT-KO relative to
wild-type mice treated with alcohol. This reflects in-
creases in both perivascular and interstitial fibrosis. In
MT-KO mice, this was associated with neutrophil infla-
tion and cardiomyocyte hypertrophy in regions of focal
fibrotic lesions. This likely represents replacement fibro-
sis, which is quite characteristic of cardiomyopathy, in-
cluding human ACM. This is the first important aspect of
the article by Wang and colleagues,3 the development of
the first mouse ACM model that recapitulates fibrosis, a
critical aspect of pathophysiology associated with human
ACM.

The second important aspect of this work is that fibro-
sis in the MT-KO/ACM model is reversible by zinc.3 The
investigators used zinc supplementation with 100 mg of
zinc per L liquid diet and found this sufficient to prevent
the alcohol-induced decrease of zinc in the livers of
experimental animals. In the MT-KO/ACM model, zinc
supplementation significantly reduced fibrosis overall
and nearly prevented interstitial fibrosis. The zinc supplementation did not affect the alcohol-induced cardiac hypertrophy measured by heart-to-body weight ratio. Importantly, hypertrophy was similar in wild-type and MT-KO mice subjected to the alcohol diet for 2 months. Zinc supplementation also suppressed some of the alcohol-induced mitochondrial dysmorphogenesis and was more effective in this respect in wild-type relative to MT-KO mice. In addition to delineating a role in fibrosis, these results suggest that zinc and MT may play important anti-oxidant roles in this model.

The seminal discovery by Wang and colleagues, that alcohol-induced fibrosis occurs in mice deficient for MT and is suppressed by zinc supplementation, demonstrates that zinc homeostasis is critical for development of fibrosis in murine ACM. Although reduced tissue zinc levels were previously associated with alcoholic pathophysiology in the heart and liver, the causal relationship was unknown. Fibrosis has been well studied in multiple organs and appears to be a critical component of wound healing after injury. As such, fibrosis has multiple critical components and is intimately linked to inflammatory signaling and cell-cell interactions between tissue-resident components and is intimately linked to inflammatory signaling, leading to increased cytokine levels. In the human, this is sufficient to result in cardiomyopathy associated with irreversible tissue remodeling including fibrosis. In the mouse, however, further perturbation of zinc concentration, via abrogation of endogenous MT, is required to tip the balance far enough to result in fibrosis. Dietary supplementation with zinc can oppose these changes and significantly reduce fibrosis in the MT-KO/ACM model. Light arrows are anti-fibrotic effects while dark arrows are pro-fibrotic effects.

Reduction of zinc and MT act in multiple ways to exacerbate fibrosis. As discussed above, zinc may play a critical role in regulating the activities of proteases involved in extracellular matrix remodeling and may also be necessary for the activity of anti-oxidant proteins. MT has been shown to be critically involved in zinc homeostasis and to release zinc subsequent to oxidative stress. MT may therefore act as a sink for zinc, in which case its absence contributes to decreased tissue zinc levels and exacerbation of oxidative stress after ischemia or alcohol exposure. This is supported by the fact that MT and zinc levels are decreased coordinately in tissues of humans and animals chronically exposed to alcohol and the observation that zinc levels are reduced in the livers of MT-KO mice. MT is known to have reactive oxygen species scavenging capability and may also act directly as a reactive oxygen species scavenger to reduce the oxidative effects of alcohol. Thus, after ethanol exposure, low zinc levels and MT deficiency would tend to tip the homeostatic balance that exists in the normal myocardium further toward fibrosis (Figure 1). One would therefore predict that zinc supplementation would at least partially restore the decreased pool of zinc and improve the function of MT and collagenase MMPs, thereby reducing fibrosis (Figure 1). If zinc works partially through MT, this effect would be predicted to be stronger in wild-type than in MT-KO mice, exactly as observed by Wang and colleagues.

Summary

The study by Wang and colleagues in this issue of The American Journal of Pathology describes a novel murine model of ACM. Results from experiments that use this model support the hypothesis that zinc availability critically affects fibrosis in ACM in vivo. In humans, chronic alcohol abuse is associated with irreversible fibrosis and dilated cardiomyopathy. In animal models, however, fibrosis can be reversed by removing alcohol from the diet or, as in this study, by zinc supplementation. As discussed above, zinc supplementation has been previously reported to reverse alcoholic liver fibrosis in the carbon tetrachloride liver injury model. It is currently unknown whether the reversibility of fibrosis and other features of ACM in animals, whether by alcohol withdrawal or zinc supplementation, is related to differences in basic mechanisms or to the fact that human disease is most often associated with much longer duration of alcohol usage. Despite this conundrum, studying the mechanisms that underlie the effects of alcohol on cardiac function and remodeling will likely result in significant new knowledge that is clinically relevant. Whether it proves efficacious to completely block the fibrotic component of remodeling remains to be seen; certain aspects of fibrosis and wound healing are likely required for the heart to sustain functional integrity in certain circumstances. Nevertheless, understanding the mechanisms that underlie reversal of fibrosis is of great interest. The existence of the MT-KO/ACM model will allow investigators to bring the power of mouse genetics to bear on the problems of...
ACM and cardiac fibrosis, increasing the probability that detailed aspects of the molecular mechanism will be forthcoming. These results will eventually have great impact in identifying novel therapeutic targets for limiting pathological fibrosis in the clinic.

Acknowledgments
I thank Maria Brown and Suwen He for critical reading of the commentary.

References