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Circadian rhythms, alcohol and gut interactions

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ABSTRACT

The circadian clock establishes rhythms throughout the body with an approximately 24 hour period that affect expression of hundreds of genes. Epidemiological data reveal chronic circadian misalignment, common in our society, significantly increases the risk for a myriad of diseases, including cardiovascular disease, diabetes, cancer, infertility and gastrointestinal disease. Disruption of intestinal barrier function, also known as gut leakiness, is especially important in alcoholic liver disease (ALD). Several studies have shown that alcohol causes ALD in only a 20–30% subset of alcoholics. Thus, a better understanding is needed of why only a subset of alcoholics develops ALD. Compelling evidence shows that increased gut leakiness to microbial products and especially LPS play a critical role in the pathogenesis of ALD. *Clock* and other circadian clock genes have been shown to regulate lipid transport, motility and other gut functions. We hypothesized that one possible mechanism for alcohol-induced intestinal hyperpermeability is through disruption of central or peripheral (intestinal) circadian regulation. In support of this hypothesis, our recent data shows that disruption of circadian rhythms makes the gut more susceptible to injury. Our in vitro data show that alcohol stimulates increased Clock and Per2 circadian clock proteins and that siRNA knockdown of these proteins prevents alcohol-induced permeability. We also show that intestinal Cyp2e1-mediated oxidative stress is required for alcohol-induced upregulation of Clock and Per2 and intestinal hyperpermeability. Our mouse model of chronic alcohol feeding shows that circadian disruption through genetics (in Clock$^{\Delta 19}$ mice) or environmental disruption by weekly 12h phase shifting results in gut leakiness alone and exacerbates alcohol-induced gut leakiness and liver pathology. Our data in human alcoholics show they exhibit abnormal melatonin profiles characteristic of circadian disruption. Taken together our data support circadian mechanisms for alcohol-induced gut leakiness that could provide new therapeutic targets for ALD.

Circadian rhythms in our lives

The circadian clock establishes rhythms throughout the body with an approximately 24-h period; these rhythms are present at the cell, tissue, organ, and behavioral levels of organisms (Lowrey & Takahashi, 2011; Panda, Hogenesch, & Kay, 2002). The 24-h molecular clocks regulate the diurnal timing of the expression of hundreds of clock-controlled genes (Bozek et al., 2009; Lowrey & Takahashi, 2011; Panda, Antoch, et al., 2002). The circadian system enables the organism to synchronize to the external 24-h light/dark cycle, and also coordinates the timing of internal physiological systems necessary for optimal function. There is a central circadian clock located in the SCN in the hypothalamus that is entrained via light from the environment. In addition, there are also peripheral clocks in nearly all tissues and cells of the body including the liver, kidneys, heart, lungs, skeletal and smooth muscle, adipose tissue, and the intestines (Bell-Pedersen et al., 2005; Hastings, Reddy, & Maywood, 2003; Hoogerwerf et al., 2007; Sládek et al., 2007; Zvonic et al., 2006). Central and peripheral rhythms are generated by core molecular transcriptional-translational circadian regulatory

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feedback loops that cycle approximately once every 24 h (Mohawk, Green, & Takahashi, 2012). While the SCN is often considered the "conductor" of the "orchestra" of peripheral clocks, precisely how the central clock regulates the peripheral clocks is not yet entirely clear. Central regulatory circadian mechanisms that have been detailed include hormonal (e.g., melatonin), neural, and body temperature regulation. In addition to the central pacemaker, peripheral rhythms can also be entrained by environmental factors. For example, time of eating can alter the circadian clock in the intestine and liver, creating a situation where peripheral and central clocks are "uncoupled" or "misaligned" (Froy & Miskin, 2010; Malloy, Paulose, Li, & Cassone, 2012; Mohawk et al., 2012).

Chronic circadian rhythm disruption is a common feature of modern day society (Reddy & O'Neill, 2010). In humans, chronic circadian misalignment occurs most commonly in occupations that require working during the night or in the very early morning, such as in shift workers who make up 15–20% of the workforce (United States Department of Labor and Bureau of Labor Statistics, 2005), and also occurs in people who regularly cross multiple time zones, such as international flight crew (pilots, flight attendants) or even in the more common case of 'social jetlag' (Golombek et al., 2013; Roenneberg, Allebrandt, Merrow, & Vetter, 2012; Wittmann, Dinich, Merrow, & Roenneberg, 2006). Epidemiological data suggest this chronic circadian misalignment significantly increases the risk for a myriad of diseases, including cardiovascular disease, obesity, metabolic/diabetes, cancer, infertility, and gastrointestinal disease (Golombek et al., 2013; Knutsson, 2003; Megdal, Kroenke, Laden, Pukkala, & Schernhammer, 2005; Monk & Buysse, 2013). Other factors that could also account for this increased risk for disease include, but are likely not limited to: poor diet, sleep disruption, melatonin suppression, and exposure to ionizing radiation at high altitude (Blask et al., 2005; Gangwisch et al., 2006; Knutson, Ryden, Mander, & Van Cauter, 2006; Sigurdardottir et al., 2013; Zeeb, Hammer, & Blettner, 2012). Nonetheless, laboratory studies in healthy humans have demonstrated that even short-term circadian misalignment increases metabolic, autonomic, and endocrine predictors of obesity, diabetes, and cardiovascular risk (Golombek et al., 2013; Scheer, Hilton, Mantzoros, & Shea, 2009).

Much less is known about the impact of circadian rhythm disruption on gastrointestinal health in humans, despite the fact that there is significant circadian regulation of digestive system activity (Hoogerwerf, 2009; Hoogerwerf et al., 2007, 2008; Polidarová, Sládek, Soták, Pácha, & Sumová, 2011; Scheving, 2000; Scheving & Russell, 2007). Disruption of gastrointestinal barrier function (by disease or environmental factors such as alcohol) can increase intestinal permeability ("gut leakiness"), thus enabling the translocation of bacterial products such as endotoxin, from the intestine into the circulation, triggering inflammatory cascades that can promote or exacerbate inflammatory-based diseases (Farhadi, Banan, Fields, & Keshavarzian, 2003; Turner, 2009; Wang, Gao, Zakhari, & Nagy, 2012). Epidemiological studies reveal higher rates of irritable bowel syndrome, gastric and duodenal ulcers, inflammatory bowel disease, and colorectal cancer in shift workers compared to daytime workers (Drake, Roehrs, Richardson, Walsh, & Roth, 2004; Knutsson, 2003; Nojkov, Rubenstein, Chey, & Hoogerwerf, 2010; Schernhammer et al., 2003; Segawa et al., 1987; Sonnenberg, 1990), and our recent publication demonstrates that circadian rhythm disruption in mice promotes intestinal hyperpermeability and exacerbates alcohol-induced intestinal hyperpermeability (Summa et al., 2013). Thus, circadian rhythm disruption may directly impact gastrointestinal health, but may also weaken or even play a role in the organism's response to injurious agents, such as promoting excessive alcohol consumption (Spanagel, 2009; Spanagel, Pendyala et al., 2005) or immune dysregulation and inflammation (Curtis, Bellet, Sassone-Corsi, & O'Neill, 2014; Voigt, Forsyth, & Keshavarzian, 2013). Some potential mechanisms by which circadian disruption may adversely impact gastrointestinal health, especially through regulation of intestinal permeability, have been investigated in our *in vitro* studies using intestinal models as well as studies in animal models and alcoholics, as discussed below.

Alcohol, intestinal permeability, and disease

The intestinal tract has many important functions including the regulation of water balance and nutrient absorption, a significant role in immunity, and also forming a selective barrier to the pro-inflammatory microbial gut contents (Farhadi et al., 2003; Turner, 2009). Disruption of this barrier function, also known as gut leakiness, has been shown by many studies to be especially important in the pathogenesis of alcohol-induced pathologies and, in particular, alcoholic liver disease (ALD) (Bjarnason, Peters, & Wise, 1984; Keshavarzian et al., 1999; Purohit et al., 2008). Several epidemiological studies have shown that alcohol causes ALD in only a 20–30% subset of alcoholics (Grant, Dufour, & Harford, 1988; O'Shea, Dasarathy, & McCullough, 2010). These data support the theory that alcohol is required but is not sufficient for development of ALD. Thus, additional factor(s) that are involved in the pathogenesis of alcohol-associated pathologies such as ALD should be investigated because these factors could be ideal therapeutic targets for the prevention and/or treatment of pathologies in alcoholics. The underlying mechanisms for this differential susceptibility to alcohol-induced pathologies have not been well-established; however, multiple studies have demonstrated that inflammation and oxidative stress are required mechanisms for alcohol-induced pathologies, providing a strong rationale to examine the source of "sterile" inflammation in alcoholics (Wang, Zakhari, & Jung, 2010). This led to the discovery that LPS is required to induce alcohol-related tissue injury and pathologies like alcoholic liver disease (Adachi, Moore, Bradford, Gao, & Thurman, 1995; Keshavarzian et al., 2009; Wang et al., 2010). Since the intestinal microbiota is the primary source of LPS, our research group as well as others began to study the impact of alcohol consumption on intestinal barrier (permeability) function in rodent models of alcohol-induced pathologies and in human alcoholics. Studies found compelling evidence that increased gut leakiness to microbial products and especially LPS play a critical role in the pathogenesis of ALD (Bode & Bode, 2005; Enomoto et al., 2000; Parlesak, Schäfer, Schütz, Bode, & Bode, 2000). Our laboratory and others have shown that *in vitro*, animal, and human studies support the hypothesis that both alcohol and alcohol metabolites including acetaldehyde cause increased intestinal permeability ("leaky gut") and endotoxemia that drive ALD pathogenesis (Elamin, Masclee, Dekker, & Jonkers, 2013; Keshavarzian et al., 2009; Rao, 2009). Although alcohol uniformly causes leakiness in Caco-2 cell *in vitro* models of the intestine, only a subset of alcoholics that have intestinal hyperpermeability (20–30%) actually develop liver disease (Bode & Bode, 2005; Keshavarzian et al., 1999). Thus, gut leakiness to endotoxins (LPS) could be a susceptibility factor promoting alcohol-induced pathologies such as ALD in a subset of alcoholics. But what makes the intestine leaky after excessive consumption of alcohol in only a subset of alcoholics? The answer to this question and identifying the mechanisms through which alcohol promotes this gut leakiness could provide a tool for risk stratification/assessment as well as new avenues for prevention and/or treatment of ALD.

Circadian rhythms and intestinal injury

Several key points support a role for circadian regulation of intestinal permeability and possible pathology including: 1) The core circadian clock molecular machinery is within essentially all the