Circadian Rhythms, microbiota and inflammatory disorders

circadian disruption: A Trigger for Gut-Derived Inflammation

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Outline

• What are circadian rhythms?
• Why should we consider circadian in inflammation?
• “Two-hit” hypothesis of circadian rhythm disruption that triggers/promotes inflammatory disorders
• Circadian disruption and gut derived inflammation [alcohol model of gut derived inflammation]
  – intestinal Barrier
  – Intestinal microbiota
What are Circadian Rhythms?

What are Circadian Rhythms?

Zeitgeber Time (ZT)
ZT0 = lights on
ZT12

What are Circadian Rhythms?
Central & Peripheral Circadian Rhythms: Misalignment

Normal Central / Peripheral Rhythms
Central Circadian Clock
Suprachiasmatic nucleus (SCN)

Peripheral Circadian Clocks

Disorganized Central / Peripheral Rhythms
(e.g., wrong-time eating)
Central Circadian Clock
Suprachiasmatic nucleus (SCN)

Peripheral Circadian Clocks
Clock shifts earlier in time

Clock shifts later in time
Interaction of Environmental and genetic factors

- Inflammatory disorders
- Environmental Factors
- Genetic Predisposition
How May Central Circadian Timing Impact Health?

- **Multi synaptic projections from SCN**
  - heart, kidney, intestine, adrenal cortex, liver, pancreas, spleen, white and brown adipose tissue
    - Konturek et al. J Physiol Pharmacol 2011

- **Inflammation**
  - shift work: ↑leukocytes, CRP
  - 2 h sleep lost/night for 1 week: ↑IL-6, TNFα (men)
    - Vgontzas et al. JCEM 2004

  - SCN → circadian oscillation in leukocyte recruitment
    - Scheiermann et al. Immunity 2012

- **Melatonin binding to melatonin receptors**
  - kidney, intestine, heart, pancreas (beta-cells)
    - Ekmekcioglu, Biomed Pharmacother 2006
    - Lyssenko et al. Nat Genet 2009

- Melatonin prevents beta cell “overcharge”
Two-Hit Hypothesis: Circadian Rhythm Disruption

- Inflammatory trigger(s) e.g. Alcohol consumption
- Clinical relevance organ damage
- Disrupted Circadian Rhythms
- Normal Circadian Rhythms

Inflammation & Organ Pathology

Inflammatory trigger(s) e.g. Alcohol consumption
P65 RelA mRNA vs. b-actin expression in proximal colon tissue from BL/6 mice was analyzed with Affymetrix array after 22 weeks circadian treatment. Some mice were subjected to weekly 12h L/D phase shift (circadian disruption). Mice killed at ZT0 (ZT0=lights on; ZT12=lights off). Data are means ± SE of n=5-6 mice.

Circadian disruption upregulates colonic NF-kB expression

* p<.05
Change in Intestinal Permeability After Moderate Alcohol Consumption

Day Workers

Night Workers

% Change in 24 hour Urinary Sucralose

*p=0.63

**p<0.05
The Gut Landscape: Maintaining Intestinal Homeostasis
Interplay between the Microbiota and the Gut Circadian Clock

Cell, Volume 161, Issue 1, 2015, 84 - 92
Intestinal Microbiota Exerts Diurnal Oscillations

A. Wild-type mice, food ad libitum

B. 16S rDNA sequencing

C. Shotgun metagenomic sequencing

D. Bacterial family

E. Relative abundance (%)

F. Dehalobacterium spp.

G. Relative abundance (normalized to no. of reads)

H. KEGG pathways

Cell Press
Human Microbiota Undergoes Diurnal Oscillations in Composition and Function

Data from 16S rRNA sequencing and shotgun metagenomic sequencing show diurnal changes in the human microbiota. Panels A and B illustrate the temporal variation in relative abundance across time, with panels C and D focusing on specific pathways and their abundance over time. Panels E and F display oscillating operational taxonomic units, while panels G and H visualize relative abundance profiles for different metabolic pathways. The diagram concludes with a summary of key pathways and their temporal variation, highlighting the dynamic nature of the human microbiome throughout the day.

KEGG pathways:
- Sulfur metabolism
- Nitrogen metabolism
- Carbohydrate degradation
- D-Arginine and D-ornithine metabolism
- Oxidation degradation
- Nitroaromatic degradation
- Flagellar assembly
- Trypto, tryptophan and pyridine alkaloid biosynthesis
- Glycosylated glycosidase
- Pentose phosphate pathway
- Riboflavin metabolism
- Folate biosynthesis
- Bile-Escherichia resistance
- Carbon fixation in photosynthetic organisms
- Amino sugar and nucleotide sugar metabolism
- Galactose metabolism
- Fructose and mannose metabolism
- Starch and sucrose metabolism
- Seraphin gene family
- Inositol phosphate metabolism
Dysbiosis in Per1/2-Deficient Mice
Jet Lag Leads to Loss of Diurnal Microbiota Oscillations and Dysbiosis
Two-Hit Hypothesis: Circadian Rhythm Disruption

- Sleep deprivation worsens inflammation and delays recovery in a mouse model of colitis (Tang et al, Sleep Med 10(6): 597-603, 2009)
Models of Circadian Rhythm Disruption

Genetic Circadian Rhythm Disruption

Environmental Circadian Rhythm Disruption

Experimental Outcomes

Intestinal Barrier Function: Intestinal Permeability

Administer: Sucralose, Sucrose, Lactulose, Mannitol
Measure: Urinary sugar content

Gene Expression

Microbiome: Stool Microbiota

End Organ Damage: Liver Pathology
Steatosis & Lobular Inflammation
Genetic Circadian Disruption Promotes Intestinal Hyperpermeability

Models of Circadian Rhythm Disruption

Environmental Circadian Rhythm Disruption

Environmental Circadian Rhythm Disruption Promotes Intestinal Permeability

Change in Melatonin AUC compared to Colonic Permeability

$R^2 = 0.28, p < 0.05$
Environmental Circadian Rhythm Disruption Alters Gene Expression in the Proximal Colon

Voigt et al., in preparation
Genetic Circadian Rhythm Disruption Promotes Intestinal Dysbiosis

Genotype Effect

<table>
<thead>
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<th>Genotype Effect</th>
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<tbody>
<tr>
<td>W-2 WT v ClockΔ19</td>
<td>0.30</td>
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<tr>
<td>W8 WT Control v ClockΔ19</td>
<td>0.024*</td>
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Alcohol Effect

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<td>W8 WT Control v WT Alcohol</td>
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<tr>
<td>W8 ClockΔ19 Control v ClockΔ19 Alcohol</td>
<td>0.002*</td>
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</tr>
</tbody>
</table>

Voigt et al, Manuscript in preparation
Circadian Rhythm Disruption Promotes Intestinal Dysbiosis

Environmental Circadian Rhythm Disruption

Circadian-Induced Effects

- NSC v SC p=NS
- NSD v SD p=0.04
- NSA v SA p=NS

B

Week: -14 -12 -10 -8 -6 -4 -2 0 2 4 6 8

Non-shifted:

LD

Shifted:

LD

Control diet

Alcohol diet

Non-shifted, chow fed

Shifted, chow fed

Non-shifted, control diet

Shifted, control diet

Non-shifted, alcohol diet

Shifted, alcohol diet

Similarity

20

40

60

Circadian Rhythm Disruption Promotes Intestinal Dysbiosis

Environmental Circadian Rhythm Disruption

Intestinal Microbiota Correlate with Proximal Colon Gene Expression

- CLDN4 mRNA: $R=0.56$, $R^2=0.31$, $P=0.04^*$
- HNF1A mRNA: $R=-0.65$, $R^2=0.42$, $P<0.00^*$
- PER1 mRNA: $R=-0.57$, $R^2=0.33$, $P=0.03^*$
- OCLN mRNA: $R=0.57$, $R^2=0.33$, $P=0.04^*$
- HNF4A mRNA: $R=0.67$, $R^2=0.45$, $P<0.00^*$
- ARNTL (BMAL) mRNA: $R=-0.58$, $R^2=0.33$, $P=0.04^*$
Conclusion

Circadian Rhythm Disruption

Disrupted Expression of Circadian genes & TJ Proteins
Lumen contents
Intestinal epithelial cells

Systemic Inflammation

Intestinal Hyperpermeability
Dysbiosis
Endotoxemia
Circadian and Microbiota Summary

Intact host circadian clock and feeding habits

Detoxification
Motility
Environmental sensing

Microbiota diurnal rhythmicity
Energy metabolism
Cell growth, DNA repair

Metabolic homeostasis

Loss of diurnal rhythmicity

Impaired host circadian clock and feeding habits (jet lag / shift work)

Dysbiosis

Obesity glucose intolerance
They don’t call it “the second brain” for nothing. Your digestive tract is a smart system that is acutely sensitive to your feelings. Here’s how to keep it healthy (and happy).
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Figure 5 – Lipopolysaccharide (LPS) and LPS bind protein (LPB) in Alcoholic and Control subjects

- A
- B
- C
- D

P<0.01

Cosinor Model Plot of LBP levels in Alcoholics and Controls

Cosinor Model Plot of LPS levels in Alcoholics and Controls

P=0.57

P=0.46