Timeframe of HBeAg and HBsAg Levels of Developing Transgenic Mice and Correlation with Liver Hepatitis B DNA Levels

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Image from Hepatitis B by Steven A Gonzalez MD, MS
What is Hepatitis B?

- DNA virus spread through transmission of bodily fluids
- Causes a wide spectrum of liver disease
  - 5-10% of infected adults develop chronic infection
  - 20-30% of Chronic infections lead to cirrhosis or hepatocellular carcinoma
- ~300 million HBV carriers worldwide
  - 686,000 deaths annually

-Liang et al 2009
HBsAg and HBeAg

• Surface antigen:
  • Major surface protein
  • Chronic HBV infection: presence of **hepatitis B surface antigen (HBsAg)** for >6 months
  • key diagnostic marker

• E antigen
  • Indicated viral replication and infectivity

-Amini et al, 2017
-Nguyen et al, 2008
-Milich D and Liang T, 2003
Transgenic Mouse Model Used by the IAR

- Genome of C57BL genetically engineered to produce hepatitis B
- Liver and kidney tissue produce complete viral particle with associated DNA, HBsAg, HBeAg, HBcAg
- No associated cytopathology

-Guidotti et al, 1995
What We Know

• Weak observed correlation between HBeAg levels and expressed viral DNA
• No record of correlation between HBsAg levels and expressed viral DNA
• Data suggest that HBsAg levels decrease over time in males
  • No data regarding decrease in females
Purpose of experiment

• Develop a timeline showing HBsAg levels over time in both males and females
• Determine correlation between HBsAg levels and expressed viral DNA
• Confirm correlation between HBeAg levels and expressed viral DNA
Methods: Samples

• Serum collected from pups weekly from 4-10 weeks of age
• Mice were euthanized and livers were harvested at 10 weeks
Methods: Antigen Analysis

• HBsAg-specific ELISA with calibrator of known WHO (IU/mL) units
  • Absorbance was matched to standard curve to determine HBsAg levels from each collection date

• HBeAg-specific ELISA with calibrator of known PEI units
  • Absorbance was matched to standard curve to determine HBsAg levels from each collection date
Methods: DNA Analysis

- Serum DNA was extracted using QIAamp cador Pathogen Mini Kit
- Liver DNA was extracted by phenol-chloroform extraction
- Extracted DNA was analyzed by real-time qPCR
Results

HBV Serum Over Time

HBV Serum Week 4 vs. Week 10

No significant difference
Results

HBV Serum DNA vs Liver DNA at Necropsy

\[ R^2 = 0.7612 \]
Results

HBeAg Wk 4 vs Wk 10

No significant difference

HBeAg 1:100

Upper limit of confidence (3OD)

Normal Experimental Range

HBeAg (PEI Units/mL)

Age in weeks

Week 4

Week 10
Results

HBeAg vs Serum HBV DNA Linear Regression

Week 4 HBeAg vs HBV Serum DNA

Week 10 HBeAg vs HBV Serum DNA

Week 5: $R^2=0.2$
Week 6: $R^2=0.59$
Week 7: $R^2=0.72$
Week 8: $R^2=0.46$
Week 9: $R^2=0.28$
Results

HBeAg vs Liver DNA at Necropsy

$R^2 = 0.7962$
Results

HBsAg wk 4 vs wk 10 ALL

p=0.0066 **

Individual HBsAg 1:200

Upper limit of confidence (3OD)
Results

HBsAg Wk 4 vs Wk 10 between genders

- Males Week 4
- Males Week 10
- Females Week 4
- Females Week 10

HBsAg (IU/mL WHO)

- p=0.016 **
- No Significant Difference Between Females Groups p=0.058

No Significant Difference Between Females Groups p=0.058
HBeAg vs Viral DNA

- HBeAg vs. Serum HBV DNA: Weak/moderate correlation
- HBeAg vs. Liver HBV DNA: Strong correlation
- HBeAg useful as surrogate marker for liver and serum HBV DNA
Serum HBV DNA vs. Liver HBV DNA

- Strong positive correlation
- Serum HBV DNA useful as surrogate marker for liver HBV DNA
HBsAg Levels

- Significantly decrease over time

- Implications:
  - HBsAg commonly used as diagnostic marker
  - Potential erroneous results in mouse studies
  - Not a reliable indicator of liver HBV DNA levels
Conclusion

• Serum HBV DNA levels remain constant overtime, suggesting constant liver HBV DNA over time

• HBsAg levels significantly decrease over time. Why?
Hypothesis

• HBsAg-specific immunoglobulins begin to be produced by the mice
  • HBsAg is neutralized and degraded by phagocytosis
• Suggests possible autoimmune response
Further research

• Repeat experiment with larger sample size
  • Support evidence that HBsAg levels significantly decrease in male mice
  • Clarify non-significant decrease in female mice

• Perform IgG-specific ELISA alongside HBsAg-specific ELISA
  • Analyze suspected inverse relationship between IgG and HBsAg levels
Works Cited


Thank You!