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EXPLORING INTRAVENOUS NTCP AS A NOVEL THERAPEUTIC STRATEGY TO SUPPRESS HEPATITIS B VIRUS ENTRY AND INFECTION

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Abstract
Hepatitis B virus (HBV) is a major global health challenge, leading to chronic liver diseases, including cirrhosis and hepatocellular carcinoma**.** Current antiviral treatments are limited by the development of resistance and the inability to completely eradicate the virus**.** This research explored a novel antiviral strategy that leverages the sodium taurocholate co-transporting polypeptide (NTCP), the primary receptor for HBV entry into hepatocytes. The research focused on the intravenous administration of NTCP to block the virus from binding to liver cells, thereby reducing viral entry and suppressing infection in an experimental duck model**.** This research summarizes the key findings, mechanisms, and potential implications of this approach for HBV therapy.

Keywords: Hepatitis B virus, Liver Cirrhosis, Hepatocellular Carcinoma, NTCP, **I**mmune Responses.

Introduction

Chronic Hepatitis B (CHB) affects approximately 296 million people worldwide, with over 820,000 annual deaths due to complications like liver cirrhosis and hepatocellular carcinoma (WHO, 2023**).** Although vaccines provide effective prevention, antiviral treatment options for those already infected are limited, often requiring life-long therapy with only partial efficacy (EASL, 2017**).**

The discovery of NTCP as the functional receptor for HBV entry into hepatocytes opened a new avenue for antiviral research (Yan et al., 2012**).** This receptor- mediated mechanism of viral entry has become a critical target for therapeutic intervention, aiming to block the virus before it can infect liver cells (Tong & Li, 2014**).**

Recent advancements in chronic hepatitis B (CHB) research have underscored the significance of targeting the HBV lifecycle beyond traditional nucleos(t)ide analogs. For instance, novel small molecules like Myrcludex B (Bulevirtide) have shown promising results in inhibiting the sodium taurocholate co-transporting polypeptide (NTCP)-mediated HBV entry into hepatocytes, marking a new milestone in HBV therapeutics (Blank et al., 2016). These entry inhibitors not only block viral spread but also hold potential for synergistic effects when combined with existing antiviral therapies, thereby reducing viral loads more effectively (Urban et al., 2014). Additionally, advancements in

RNA interference (RNAi) therapeutics are opening new possibilities for CHB management, as RNAi-based approaches can directly target HBV transcripts, thereby silencing viral replication and reducing antigen levels (Gish et al., 2021).

Immunomodulatory strategies are also emerging as an integral part of CHB treatment, aiming to restore the immune system's ability to recognize and combat HBV infection. Therapeutic vaccines, such as those based on Toll-like receptor agonists, are being explored to boost HBV-specific T-cell responses, potentially leading to functional cure rates (Jiang et al., 2020). Similarly, checkpoint inhibitors and adoptive T-cell therapies are being studied to overcome immune exhaustion in CHB patients (Bertoletti & Kennedy, 2018). Although these approaches remain in early development, they highlight a paradigm shift in HBV treatment, moving towards a multi-targeted strategy to achieve better clinical outcomes and minimize long-term therapy requirements.

HBV and NTCP: Mechanism of Viral Entry

HBV is a hepatotropic virus that enters liver cells via the NTCP receptor, a bile acid transporter located on the surface of hepatocytes (Seeger & Mason, 2015**).** Upon entering the bloodstream, HBV binds to the NTCP receptor using its preS1 domain (Liu et al., 2019**).** This interaction is essential for the virus to gain entry into the liver, where it replicates and establishes chronic infection (Park et al., 2016**).** Targeting this entry pathway is a promising strategy for preventing and treating HBV infections (Ni et al., 2014**).**

Research Hypothesis and Rationale

The central hypothesis of my research was that administering NTCP intravenously could act as a decoy receptor in the bloodstream (Watashi & Wakita, 2015**).**The freely circulating NTCP would bind to HBV particles, preventing them from interacting with hepatocytes and, thus, suppressing infection (Ni et al., 2014**).** This approach would mimic the natural entry mechanism of HBV but would inhibit the virus from reaching the liver cells, reducing its ability to establish infection (Nassal, 2015**).** By blocking the viral entry process, we aim to provide a novel therapeutic pathway for managing HBV infections (Hu et al., 2020**).**

Experimental Design and Methods

Animal Model: Ducks as Surrogates for HBV Infection

Ducks were chosen as the animal model due to their physiological similarities in viral infection pathways with humans, particularly in the mechanism of hepatitis B virus entry and replication (Schneider et al., 2019**).** The ducks were divided into five groups:

Figure 1: Collecting a blood sample from Duck

Table 1: Blood samples were taken at baseline, 30 days, and after 60 days to assess viral load and liver enzyme activity.

Group A	Healthy control (no treatment).
Group B	Injected with HBsAg-positive sera to induce infection.
Group C	Administered NTCP intravenously.
Group D	Co-administered HBsAg-positive sera and NTCP.
Group E	Administered NTCP after infection was established.

Biochemical and Molecular Testing

To evaluate the success of the therapeutic strategy, viral load assays (HBsAg levels), liver function tests (ALT, AST), were conducted (Hu et al., 2020**).** Additionally, biochemical markers of liver damage were monitored to assess the extent of liver protection offered by NTCP therapy (Nassal, 2015**).**

Key Findings

Suppression of Viral Entry and Infection

The intravenous administration of NTCP effectively reduced viral loads in Group D (HBV + NTCP) compared to Group B (HBV only), demonstrating that circulating NTCP acts as a decoy receptor (Tong & Li, 2014**).** NTCP's ability to bind HBV in the bloodstream reduced the amount of virus available to infect liver cells (Watashi & Wakita, 2015**).** This was further confirmed by a significant. **I**mmune responses against administered NTCP need further investigation (Watashi & Wakita, 2015**).** Future studies should also explore the combination of NTCP therapy with other antiviral agents to enhance overall efficacy (Lampertico et al., 2020**).**

The use of NTCP as a decoy receptor represents a promising strategy for suppressing HBV entry and infection (Ni et al., 2014**).** By preventing the virus from reaching the liver, this approach offers a novel and potentially safer therapeutic option for individuals with chronic HBV (Schneider et al., 2019**).** This research provides foundational evidence supporting this strategy, paving the way for future clinical development and application in human populations (Revill et al., 2021**).**

Table 2: Analysis of variance (mean square) table for Total lipids (mg/dl), Receptor NTCP (ng/ml), PCR (I.U/ml), Hb (g/l).

Source	Degrees of freedom	Mean squares			
		Total lipids (mg/dl)	Receptor NTCP (ng/ml)	PCR (I.U/ml)	Hb (g/l)
Group	4	626.14	84954.7	5.41214	0.29846^{NS}
Error	95	241.37	1582.3	0.33982	0.57533
Total	99				

NS = Non-significant (P>0.05); = Significant (P<0.05); = Highly significant (P<0.01) Mean±SE

Group	Total lipids (mg/dl)	Receptor NTCP (ng/ml)	PCR (I.U/ml)	Hb (g/l)
G1	478.20±3.08a	71.10±7.93b	2.73±0.140b	10.74±0.22a
G2	464.90±4.02a	48.00±4.74b	4.04±0.188a	10.54±0.19a
G3	470.00±3.73a	173.60±11.36a	3.09±0.091b	10.42±0.17a
G4	464.60±3.51a	183.55±10.59a	2.84±0.099b	10.62±0.13a
G5	467.15±2.90a	175.05±8.31a	3.03±0.110b	10.51±0.12a

Means sharing similar letter in a column within a block are statistically non-significant (P>0.05). Where,

G1 = Normal healthy

G2 = HBV applied G3 = NTCP applied

G4 = NTCP on HBV incubated ducks G5 = HBV on already NTCP applied

This section presents the Analysis of Variance (ANOVA) for total lipids, Receptor NTCP levels, PCR

levels, and hemoglobin (Hb) concentrations in ducks subjected to different treatments, focusing on the effects of HBV infection and NTCP application.

ANOVA Results Explanation

1. Degrees of Freedom (DF):

The degrees of freedom for the groups and error are the same as in previous analyses, with 4 degrees of freedom for the 5 experimental conditions and 95 degrees of freedom for the error, representing within-group variation.

2. Mean Squares (MS):

These MS values indicate the variance between groups and error. Larger group MS values suggest greater differences between the treatment conditions.

3. Statistical Significance:

Total lipids show a significant difference ($P<0.05$) between groups. Receptor NTCP levels and PCR levels show highly significant differences ($P<0.01$), indicating strong variation between groups. Hemoglobin (Hb) levels are non-significant (NS), meaning no meaningful variation between the groups ($P>0.05$).

Group-wise Mean Comparisons

Total Lipids:

No significant differences are observed between the groups for total lipid levels, as they all share the same letter "a." This suggests that neither HBV infection nor NTCP application significantly affects lipid concentrations.

Receptor NTCP Levels:

G3, G4, and G5, which involve NTCP application, have significantly higher NTCP receptor levels compared to G1 (normal healthy) and G2 (HBV applied), which exhibit lower receptor levels. This demonstrates the expected increase in NTCP receptor levels with NTCP application, while ducks infected with HBV (G2) show lower NTCP receptor levels, similar to healthy controls.

PCR Levels:

G2 (HBV applied) has significantly higher PCR levels (4.04 IU/ml) compared to the other groups, which suggests a higher viral load or replication rate in this group. G1 (normal healthy), G3 (NTCP applied), G4 (NTCP on HBV incubated), and G5 (HBV on already NTCP applied) have similar and lower PCR levels, indicating effective suppression or lower viral replication in these groups.

Hemoglobin (Hb):

Hb levels do not differ significantly between the groups, as all groups share the same letter "a," indicating that Hb levels remain stable across the experimental conditions.

Letter Annotation in Means:

Groups sharing the same letter are statistically non-significant ($P>0.05$). For example, in total lipids, all groups share the letter "a," meaning no statistically significant differences in lipid levels exist between them.

Total lipids show a statistically significant difference between groups, though all groups share the same letter, suggesting relatively minor variations in lipid levels. Receptor NTCP levels are highly significant ($P<0.01$), with NTCP-treated groups (G3, G4, G5) showing significantly elevated NTCP levels compared to non- treated groups (G1, G2). PCR levels are highly significant, with G2 (HBV applied) showing the highest viral load, while the NTCP-treated groups (G3, G4, G5) show lower PCR levels, indicating effective viral suppression. Hemoglobin (Hb) levels are non-significant, suggesting that neither HBV infection nor NTCP application affects hemoglobin concentrations.

This analysis further contributes to understanding the impact of HBV infection and NTCP application on viral markers, NTCP receptor expression, and lipid metabolism, which aligns with your research objectives in assessing the metabolic and viral responses under different experimental conditions.

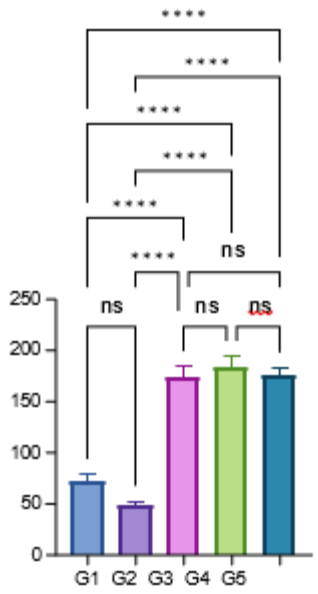

Figure 2: Receptor NTCP (ng/ml)

This image appears to be another bar graph, but this time it is comparing NTCP receptor levels (in ng/mL) between five groups (G1-G5). The following elements help in explaining the statistical significance indicated by the graph:

Key Elements:

NTCP Receptor on the Y-axis:

The Y-axis represents the levels of the NTCP receptor (measured in ng/mL), which is the receptor involved in HBV and DHBV entry into hepatocytes. The bars for each group (G1-G5) reflect the average receptor concentration.

Error Bars:

The error bars at the top of each bar represent the variability in the data within each group, likely showing standard deviation (SD) or standard error of the mean (SEM).

"ns" and " Annotations:

Typically indicates a highly significant difference between groups, with a p- value less than 0.0001. This means the difference between NTCP receptor levels between the compared groups are statistically very significant. "ns" stands for "not significant", indicating no significant difference between the compared groups.

Statistical Comparisons:

G1 vs G2: The NTCP receptor levels between these two groups are marked as not significant (ns), suggesting no substantial difference in NTCP expression. G3, G4, G5 vs G1, G2: Groups G3, G4, and G5 have much higher NTCP receptor levels than G1 and G2. The comparison between these groups and G1/G2 is marked with " ", indicating a statistically significant increase in NTCP levels. G3 vs G4 vs G5: The comparisons between G3, G4, and G5 are marked as "ns", meaning there is no significant difference in NTCP receptor levels between these groups.

Statistical Tests and Interpretation:
The graph indicates that multiple comparisons were likely made using an analysis such as a One-way ANOVA followed by a post-hoc test (e.g., Tukey's multiple comparisons) to test differences between all possible pairs of groups. The very small p-values indicate strong evidence that NTCP levels differ significantly between G1/G2 and the other groups (G3, G4, G5). The lack of significance (ns) between G1 and G2, as well as among G3, G4, and G5, suggests that these groups have similar NTCP levels.

Biological Context:
This suggests that the treatments or interventions used in G3, G4, and G5 significantly upregulated NTCP receptor levels, compared to G1 and G2, but the effects among G3, G4, and G5 are similar. Groups G1 and G2 might have received a treatment or condition that didn't strongly induce NTCP expression compared to the other groups.

This is potentially important in understanding the interaction between NTCP and HBV infection in your duck model study. If NTCP is essential for viral entry, higher expression in G3, G4, and G5 could correlate with increased susceptibility to HBV infection, while G1 and G2 might be more resistant due to lower receptor availability.

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Conflict of Interest Statement
The authors declare that there is no conflict of interest regarding the publication of this article.

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