Nucleic acid-based polymers effective against hepatitis B virus infection in patients do not harbour immune stimulatory properties in primary isolated blood or liver cells

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Background

Nucleic acid polymers (NAPs) block the release of HBsAg from infected hepatocytes in vitro. Although this mechanism is assumed to be responsible for the activity of NAPs against hepatitis B virus (HBV) in patients, the role of putative immunostimulatory effects has not been explored.

Objectives

Examine the immune stimulatory properties of NAPs in primary isolated human blood, parenchymal, and non-parenchymal liver cells.

Materials & Methods

- Peripheral blood mononuclear cells (PBMC, n=3) were isolated from healthy volunteers. Primary human hepatocytes (PHH, n=3) and Kupffer cells (KC, n=3) were isolated after two perfusion and digestion.
- Cells were treated with NAPs (see table) at 0.05 (x2OMe) and 5 µM (cytotoxic exposure) prior to analysis.
- NAPs treatment was performed by RT-qPCR on total RNA isolated from PBMC, PHH, and KC. RNA isolated from healthy volunteers. Primary human hepatocytes (PHH, n=3) were stimulated with DNA-based (REP2139 and REP2165), and RNA-based (REP2139 [2139] and REP2165 [2165]) NAPs or immune stimulatory controls (ODN 2216, TLR9 agonist; poly(I:C), TLR3 agonist; and ssRNA40, TLR7 ligand) for 6h. Gene expression was assessed by RT-qPCR. Primary human hepatocytes (PHH, n=3) were stimulated with DNA-based (REP2139 and REP2165), and RNA-based (REP2139 [2139] and REP2165 [2165]) NAPs or immune stimulatory controls (ODN 2216, TLR9 agonist; poly(I:C), TLR3 agonist; and ssRNA40, TLR7 ligand) for 6h.
- Analysis of IFN (IFNA4, IFNB1, IFNG, IFNL2) and inflammatory (TNF, IL6, and IL10) gene expression upon NAP treatment was performed by RT-qPCR on total RNA isolated from PHH. In PHH, no significant inflammatory or antiviral response was observed upon NAP treatment. In PBMC, NAP treatment did not significantly increase cytokine secretion. Values represent mean ± SEM.
- Peripheral blood mononuclear cells (PBMC, n=3), and primary human hepatocytes (PHH, n=3) were stimulated with ODN-2216 (200 µM), TLR9 agonist; Poly (I:C), TLR3 agonist; and Poly (ssRNA40), TLR7 ligand. Replicon treatment by specific ELISA on supernatants.
- Peripheral blood mononuclear cells (n=3), and primary human hepatocytes (PHH, n=3) were stimulated with ODN-2216 (200 µM), TLR9 agonist; Poly (I:C), TLR3 agonist; and Poly (ssRNA40), TLR7 ligand. Replicon treatment by specific ELISA on supernatants. Results suggest that clinically active NAPs do not induce significant antiviral responses in primary isolated blood or parenchymal and non-parenchymal liver cells.

Conclusions

- The intracellular uptake of CY3-labelled NAPs was confirmed using fluorescence microscopy.
- NAPs treatment by specific ELISA on supernatants. Peripheral blood mononuclear cells (PBMC, n=3) and primary human hepatocytes (PHH, n=3) were stimulated with ODN-2216 (200 µM), TLR9 agonist; Poly (I:C), TLR3 agonist; and Poly (ssRNA40), TLR7 ligand. Replicon treatment by specific ELISA on supernatants. Results suggest that clinically active NAPs do not induce significant antiviral responses in primary isolated blood or parenchymal and non-parenchymal liver cells.
- Peripheral blood mononuclear cells (n=3), and primary human hepatocytes (PHH, n=3) were stimulated with ODN-2216 (200 µM), TLR9 agonist; Poly (I:C), TLR3 agonist; and Poly (ssRNA40), TLR7 ligand. Replicon treatment by specific ELISA on supernatants. Results suggest that clinically active NAPs do not induce significant antiviral responses in primary isolated blood or parenchymal and non-parenchymal liver cells.
- Results suggest that clinically active NAPs do not induce significant antiviral responses in primary isolated blood or parenchymal and non-parenchymal liver cells.
- We therefore hypothesize that the antiviral activity of NAPs against HBV infection cannot be explained by direct induction of innate antiviral responses.

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