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REVIEWS

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Poly(Ethylene Glycol)-Poly(Lactic Acid) TLR7 agonist as a novel HBsAg immune response stimulant

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A – Study Design, **B** – Data Collection, **C** – Statistical Analysis, **D** – Data Interpretation, **E** – Manuscript Preparation, **F** – Literature Search, **G** – Funds Collection

Summary Hepatitis B is caused by a hepatitis B virus infection, causing inflammation of the liver. The therapeutic approach used to help treat HBV infection has been based on re-establishing a functioning T cell response and limiting additional negative consequences. Due to inadequate outcomes, new strategies for enhancing the immune response and inducing a particular HBV immune response have been developed. Activation of toll-like receptor 7 plays a role in mediating the immune response. TLR7 has shown to increase T cell activity, increase production of cytokines, increase the number of NK cells and lower serum concentrations of HBsAg, thus showing high potential to prevent and treat chronic hepatitis B. Evaluation of NK cell cytokine generation and degranulation reveals a considerable improvement in every cell function when compared to baseline values. However, TLR agonists are microscopic molecules that are rapidly eliminated from the system following ingestion and may cause significant systemic adverse effects. Poly(Ethylene Glycol)-Poly(Lactic Acid) can be utilised to increase the effectiveness of the toll-like receptor 7 agonist and lower the negative effects of the TLR7 agonist. TLR7 agonist distribution may be enhanced by an effective drug carrier, and conjugating a macromolecule may increase pharmacokinetics without toxicity or other negative side effects. The Poly(Ethylene Glycol)-Poly(Lactic Acid) TLR 7 agonist is potentially beneficial in treating hepatitis B as an immune response stimulant.
Key words: Hepatitis B, immunity, TLR7 agonist.

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Background

The World Health Organization (WHO) Global Health Sector Strategy formulated a viral hepatitis elimination strategy as a public health problem in 2016 due to the high incidence and prevalence of hepatitis cases [1]. Hepatitis B, a crucial health concern around the world, is a viral infection caused by the hepatitis B virus and primarily causes inflammation in the liver [2]. In 2015, around 2 billion people worldwide had been infected by this virus, and it was estimated that in 2019, around 300 million people had developed chronic hepatitis B, along with more than 800,000 deaths annually. Among the 300 million people experiencing chronic hepatitis B, 75% was predominantly found in Asia [3, 4]. Moreover, the prevalence of hepatitis B infection in Indonesia was found to be 7.1% in 2013, while total deaths caused by hepatitis B in 2020 was roughly 3,000 [5, 6]. Additionally, a majority of hepatitis B related deaths were due to severe complications including cirrhosis and hepatocellular carcinoma (HCC), making it the top 10th cause of death globally [4].

Hepatitis B Virus (HBV) activates the cell mediated immune response when entering the body. As a result, cytotoxic T cells and Natural Killer cells (NK) release inflammatory cytokines and eventually eliminating the virus [7]. However, because of hepatocyte's ability to proliferate, HBV will also be continually shed into the circulation and causes chronic infection. Up to now, the therapeutic method implemented to aid in treatment of HBV infection focuses on restoring a functional T cell response and preventing further adverse effects and complications. As a result of inadequate outcomes, novel methods are being explored to enhance the immune response and promote a targeted immune response against HBV. [8, 9].

Toll-like receptors (TLRs) are a type of receptor commonly found in many white blood cells. The function of TLRs is to mediate the immune response and also link the adaptive and innate immunity [10]. TLR7 is one of the types of TLRs, and its agonist has shown to be able to induce a suppressive HBV replication effect and sustainable control of HBV replication in efforts to prevent chronic HBV infection and complications. TLR7 activation increases the capabilities of dendritic cells and macrophages to function as antigen presenting cells (APCs) and subsequently begin T cell responses [11].

Despite the potential brought on by TLRs agonist, the microscopic size of TLR7 has caused it to have inefficient tissue distribution, fast metabolism and ineffective pharmacodynamics when presented with a TLR7 agonist within the body [8]. Therefore, an efficient drug carrier poses an opportunity to improve TLR7 agonist distribution, and conjugation of a macromolecule can provide better pharmacokinetics and specificity without causing toxicity and other side effects [9].

Material and methods

This article was written using a literature review writing methodology. Relevant journals from Pubmed, PLoS ONE and Google Scholar were used as sources. Search engines were used to look for the terms "Toll-Like Receptor 7", "Peg-PLA" and "Hepatitis B". The authors included all relevant publications that discussed the use of

TLR7 in treating hepatitis B. The research materials could not be more than twenty years old, and no subsequent study could dispute earlier conclusions. The assessment revealed that some of the assessed journals could be utilized as references for this study. The information was gathered, examined and checked for accuracy and consistency before being drafted into a single scientific literature review.

Hepatitis B infection pathophysiology

Hepatitis B is caused by an HBV infection of hepatocytes in the liver [12]. HBV is a partially double-stranded DNA virus which belongs to the *Hepadnaviridae* family [13, 14]. The virus may spread through contact with infected blood and bodily fluids such as semen, saliva or vaginal fluids [13, 15, 16]. Although uncommon, faecal-oral transmission can also occur [12]. On average, HBV usually goes through an incubation period of 90 days, but it can range from around 30 to 180 days [12, 13].

HBV replication is a multistep process, in which the virus binds with a receptor and then undergoes translational and transcriptional processes within the hepatocyte before it exits the cell through vesicles and infects other hepatocytes [17]. HBV has to go through low-affinity binding, which will then promote high-affinity binding in order to enter the cell. In low-affinity binding, HBV binds with heparin sulphate proteoglycans (HSPGs), and in high-affinity binding, it binds with sodium taurocholate co-transporting polypeptide (NTCP), triggering endocytosis, allowing entry of HBV through the caveolin-1-mediated entry (CME) pathway [18].

Acute hepatitis B (AHB) infection with persistent HBsAg for longer than 6 months is classified into CHB [12]. This is thought to occur due to the ability of HBV to modulate the immune response through various methods. CHB is mainly an immune-mediated disease which is caused by the failure of the immune system to completely eradicate the virus from the hepatocytes. This leads to inflammation and fibrosis of the liver. It has also been mentioned that HBV can lead to CHB infection through T cell anergy and immune suppression, reducing the immune response of the host [17]. In other cases, HBV can also trigger T cells to induce lysis of the infected hepatocytes [12].

Role of TLR7 agonist in hepatitis B

The Toll-Like Receptor (TLR) 7 agonist works within two mechanisms. It can work by decreasing the HBsAg level as a surface antigen that binds to the receptor during infection or increasing NK cell and T cell responses without any reduction of HBsAg level [19–21]. In increasing the immune response to HBV, some medication therapies are added with a type of TLR7 agonist called GS-9620, one of which is NUC therapy [21]. This induces dose-dependent activation of the type I interferon pathway, in which the entire treatment cohort is linked to diverse types of T cell responses, showing various individual effects [21].

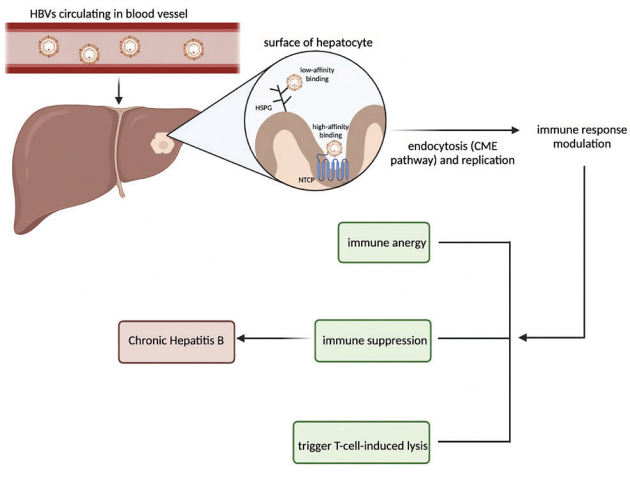

Figure 1. Pathophysiology of chronic hepatitis B

This leads to the increase of IL-2 production in CD4 and CD8 cell subsets. The increase in this response is attributed to HBV antigens, while the improvement of TNF-α production between CD8 cells actively continues by responses opposed to the HBV core. The improvement of IL-2 production is not temporary, as IL-2 positive T cells are higher than the baseline. Medication therapy with the TLR7 agonist GS-9620 has a big impact on the frequency, function and phenotype of a cell. Its modulatory effect on them is assessed during treatment, with further monitoring and follow up. During the therapy, the positive cells and expression frequency increase, which specify the treatment activating effect [21].

This effect makes the phenotype of NK cells become more similar to chronic naive patients. Analysis of the production of the NK cell cytokine and degranulation indicates a specific increase of all cell function in comparison to baseline levels. It shows that the activation of NK cells in GS-9620 therapy is a productive situation related to increased functionality compared to chronic untreated infection, in which NK cell activation is related to a lower function of NK cells. GS-9620 increases NK cell activation progressively, which causes an increase in NK cell antiviral potential. This is indicated by increased antiviral cytokines production and the reduction of the NK cell inhibitory effect on HBV-specific T cells [21].

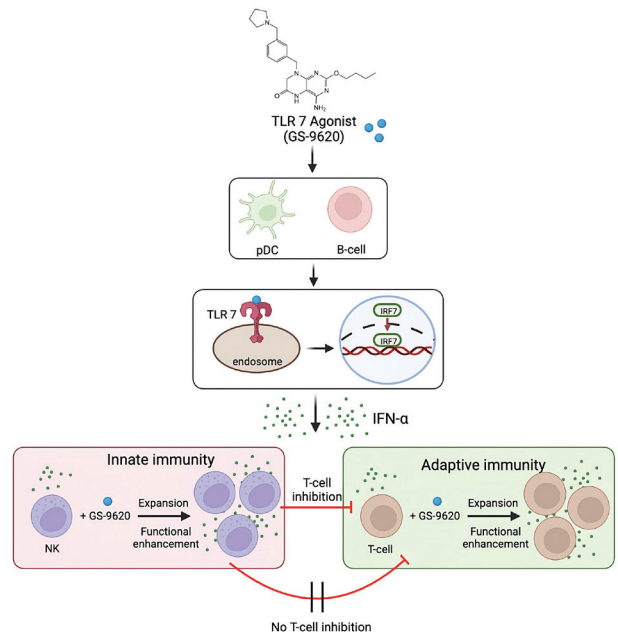

Figure 2. Role of TLR7 agonist in immunity

Construction and administration method

0.25 g or 4.1 mmol of PEG and 10.6 mg of 1,8-diazabicycloundec-7-ene is dissolved in 1 ml methylene chloride (DCM) at low heat. Lactide is then added to the solution, followed by rapid stirring as long as 10 minutes. The mixed reaction will then be given 7 ml of acetone and will then be precipitated to be separated from diethyl ether, filtrated and dried in a vacuum. This will result in a white polymer that can be used when added with ethyl acetate and 35 ml of diethyl ether [8, 22]. 1 mL of PEG-PLA block copolymers are added to 10 mL of distilled water in a dropwise manner while being vigorously stirred (600 rpm). In the ultracentrifugation of NPs, a filter with specifications of molecular weight cut-off at 10 kDa is used [23]. Afterwards, resuspension in phosphate buffered saline is done to reach a final concentration of 200 mg/mL. Dynamic light scattering (DLS) is used to characterise NPs to find out their diameters and potential [24]. The TLR7 agonist will then be encapsulated in PEG-PLA nanoparticles by employing a modified method of double evaporation of water and oil, followed by lyophilisation with the addition of 5% trehalose (a stabiliser) to produce encapsu-

lated-TLR7-PEG-PLA NPs [25, 26]. The encapsulation efficiency (EE) can be determined as follows: EE = A - B/A 100 per cent, where A is the total TLR7 agonist quantity, and B is the free TLR7 agonist quantity, extrapolated from measurements of the total TLR7 agonist contained inside PLA-PEG [27, 28].

Encapsulated-TLR7-PEG-PLA NPs are then injected intraperitoneally with a buffer of 200 µL containing the encapsulated-TLR7-PEG-PLA NPs. By drawing blood from the tail vein at appropriate intervals, the serum can be obtained and kept at -80°C. The enzyme-linked immunosorbent assay (ELISA) will then be used to assess the serum IFN level (ELISA) [8, 22].

Pharmacokinetics of the TLR7 agonist

GS-9620, as one type of TLR7, is used to make a presystemic response that is known as local stimulation of innate immune cells. This stimulation occurs in plasmacytoid dendritic cells in GALT (Gut-Associated Lymphoid Tissue). It also occurs in the liver, but the introduction of a systemic IFN-a response does not happen. TLR7 GS-9620 absorption is very high in the gastrointestinal tract, but at first-pass hepatic metabolism, it has a moderate clearance [29].

These effects and characteristics reduce TLR7 GS-9620 systemic exposure after oral administration. TLR7 GS-9620 is specifically tailored to target the stimulation and activation of innate immune cells located in the liver. TLR7 GS-9620 has high absorption in the intestines, but the level of its metabolic stability and bioavailability is low to medium. Its metabolism occurs by CYP3A4 at hepatic first-pass extraction to raise the exposure to interferon-producing cells in the liver and GALT during systemic exposure minimisation [29].

TLR7 GS-9620 has various hepatic metabolic clearance within the species, from medium to high. This can be seen in an *in vitro* experiment by using hepatic microsomal fractions from cynomolgus monkeys, humans and CD-1 mice, which show the diverse numbers of GS-9620, such as 5.9, 30.5 and 4.9 minutes. It shows that TLR7 GS-9620 has lower oral bioavailability in mice at 0.2% and cynomolgus monkey at 1.1% [29].

Pharmacodynamics of TLR7 agonist

Based on the previously described *in vitro* experiments, the oral administration of progressively increased oral doses of GS-9620 to CD-1 mice and cynomolgus monkeys resulted in dose-dependent induction of serum cytokines and chemokines. The increasing oral doses of GS-9620 in CD-1 mice from 0.1 to 50 mg/kg created dose-dependent induction of IFN-α, cytokines and chemokines. Near to the results obtained from mouse spleen cell cultures *in vitro*, the proinflammatory cytokines interleukin-1β and TNF-α were induced in mice concomitant with IFN-a induction, consistent with the expression variation of TLR7 in mice [29].

The *in vivo* pharmacodynamic profile of rats aligns with the outcomes of the *in vitro* peripheral blood mononuclear cell (PBMC) study conducted on cynomolgus monkeys. When administered with a single oral dose of GS-9620 (ranging from 0.05 to 10 mg/kg), the cynomolgus monkeys exhibited a dose-dependent increase in interferon-alpha (IFN-α) at lower oral doses. However, the induction of TNF-α was observed only at a dose of 1.5 mg/kg. This data indicates that GS9620 can induce selective TLR7-dependent pharmacodynamic responses *in vivo* to TLR8-dependent cytokines and chemokines, which can be observed only after high-dose oral administration [29].

Both the *in vitro* and *in vivo* profiles of pharmacodynamics induced by GS-9620 in mice are not the same as those that are observed in cynomolgus monkeys and which were previously reported in humans. Oral administration of GS-9620 to CD-1 mice resulted in dose-dependent induction of IFN-α and other cytokines and chemokines. However, induction of the pro-inflammatory cytokines interleukin-1β and TNF-α occurred at relatively low doses and was observed together with IFN-α induction in mice, which is different from the cynomolgus monkeys [29].

Clinical effects of PEG-PLA TLR7 agonist in the treatment of hepatitis B

Based on the TLR and target cell, stimulation of TLRs may potentially cause cancer and resistance. TLR agonists are tiny substances that may pose harmful systemic adverse effects and are quickly removed from the body after administration. Targeting individual cells to achieve the best outcome is a significant issue because of the conflicting consequences of TLR activation [30]. It is clear that effective nanoparticle delivery strategies for TLR agonists that can precisely target the innate immune system are required in order for optimal delivery. Core-shell NPs made of PEG-PLA block copolymers are a versatile platform that are often used as drug carriers. They have undergone extensive testing in human clinical trials and are regarded as being relatively biocompatible [31, 32]. Since TLR7 molecules were conjugated to the PEG-PLA NP construct, the circulatory concentrations of numerous cytokines that are largely attributed to the harmful effects of TLR7/8a were dramatically lowered in TLR7/8a NPs. This resulted from the changed biodistribution and pharmacokinetics [8]. Following nanoprecipitation, the density of conjugated TLR compounds on the NP surface may be easily controlled by physically combining synthesised PEG-PLA polymers with unaltered PEG-PLA polymers in differing amounts [33]. With self-assembly utilising nanoprecipitation, the PEG-PLA nanoparticle platform enables precise regulation of TLR7/8a valency and the consequent surface presentation [8]. A preliminary report on TLR7-PLA addressed the following impact on adaptive immune responses, as it increased and maintained stimulation of innate immune cells. TLR7-NPs significantly lengthen both germinal centre and extrafollicular B cell responses, generate a substantial antigen-specific $CD8^+$ T cell response, induce B cell proliferation, elicit production of antibodies and reduce systemic immune toxicity [34].

Recovery of the immunological response, accomplished via immunomodulators such TLR7 agonists, is vital to improve the prognosis of chronic infections, since the immune system response is repressed in chronic HBV infection [40]. TLR agonists are important in regulating the outcomes of immunotherapy. Due to the release stimulation of IFN, inflammatory cytokines and chemokines, which may have anti-HBV effects, TLR agonists have received attention for their use as immunological modulators [41, 42]. Several studies on animals showed the promising potential of immunoregulatory therapies for HBV treatment. The TLR7 agonist GS-9620 has been shown to reduce HBV in animal models by raising the secretion of IFN-α, cytokines and chemokines in infected chimpanzees. The study found lowered serum HBsAg and HBeAg levels by 50% and viral loads by over 2 logs [37]. Other studies proceeded to point out that increased levels of TLR7 agonists mediate the activation of natural killer cells, $CD8^+$ cells, IFN-α, IL-1RA, I-TAC, Eotaxin, MIG, MCP-1, IL-1β, IL-6 and IP-10 in HIV viral infected subject [43, 44]. The TLR7 agonist RG-7854 also showed similar effects in regard to the SVR. In a subgroup of woodchucks with CHB, therapy with RG7854 – alone or in combination with ETV – led to an SVR and anti-WHs antibody seroconversion. Oral administration of the TLR7-specific agonist dual prodrug to individuals has the potential to result in persistent immunological suppression of chronic HBV infection and may provide a novel therapeutic alternative in the pursuit of HBV treatment [38]. Similarly, research done on HBV transgenic mice showed beneficial antiviral properties in managing the infection [39].

A human clinical trial demonstrated the therapeutic effects of GS-9620 for the treatment of CHB. In this study, GS-9620 stimulated the production of cytokines that could stimulate the antiviral signal transduction function of various immune mediators, such as natural killer cells, HBV-specific $CD8^+$ T cells and $CD4^+$ follicular helper T cells [21]. Natural killer cells are becoming more well recognised for their role in the treatment of hepatitis B. Natural killer cells have contradictory activities; they have antiviral and immunoregulatory effects, while also participating in the pathophysiology of liver damage [45]. The assessment of NK cell dysfunction reversal aiming to reverse T cell fatigue in chronic illnesses should be taken into consideration [46]. pDCs express the TLR7 and 9, positioning them as potential antiviral defences. As a result, decreased IFN-α production by pDCs from CHB patients suggests that lower expression of both TLRs may result in functional abnormalities and be the cause of pro-

Table 1. Clinical trials of TLR7 in hepatitis B subjects

TLR7 Agonist	Subjects	Route	Doses	Period	Results	
GS-9620	28 chronic HBV patients	Oral	1 mg, 2 mg and 4 mg	Weekly for 12 weeks	T cells generated larger quantities of cytokines, Natural killer cell activation and activity improved, and serum concentrations of HBsAg did not drop substantially	[21]
Loxoribine	32 chronic HBV patients and 13 healthy volunteers	IV	500 µM and 30 lg/mL single dose	24 hours	Upon TLR7 or TLR9 activation, HBV infection reduced the plasmacytoid dendritic cells' (pDCs') ability to produce IFN-α, affecting T cell responses and inflammation	[35]
R-837	12 chronic HBV patients and 7 liver stenosis patients	Oral	4, 6 and 8 µg/ml	72 hours	By activating TLR7, HBV multiplication and viral protein synthesis were suppressed. R837 likely activates the Jun N-terminal Kinase (JNK) pathway to initiate antiviral activity	[36]
GS-9620	3 HBV infected chimpanzees	Oral	1 mg/kg for 4 weeks and 2 mg/kg for 4 weeks	3 times each week for 8 weeks	HBV DNA was permanently suppressed after short-term oral dosing of GS-9620. As hepatocyte apoptosis progressed, a reduction of serum levels of HB surface antigen, HBeAg and hepatocytes positive for the HBV antigen were found. IFN-α and other cytokines and chemokines were produced as a result of the delivery of GS-9620, and ISGs, natural killer cells and lymphocyte were all stimulated	[37]
RG-7854	16 woodchucks	Oral	30/120 or 60 mg/kg	24 weeks	The Sustained Viral Response (SVR) was aided by cytokine-mediated non-cytolytic and T cell-mediated cytolytic processes. Oral RG7854 caused an SVR based on the reconstitution of antiviral innate and adaptive immune responses	[38]
R-848	18 HBV transgenic mice	IV	10 µg single dose	24 hours	The delivery of 10 µg of TLR3, 4, 5, 7 and 9 ligands virtually eliminated HBV replication. TLR ligands activate diverse signalling pathways suppressing HBV replication, suggesting that co-administration of several TLR ligands may have synergistic antiviral benefits in individuals with chronic infection	[39]

longed HBV infection [35]. Therefore, in response to various stimuli by a TLR7 agonist, cytokines like IL-12 stimulate the induction of TH1 responses on T cells, which are marked by the output of IFN by T cells and activation of cell-mediated immunity, triggering the release of phagocytes, antigen-specific cytotoxic T lymphocytes and cytokines [47]. In another study, an inverse correlation among HBV DNA load and TLR7 in histopathology samples was found, showcasing the antiviral function of TLR7 in HBV infection. HBV replication in HepG2.2.15 cells was suppressed by the TLR7 agonist R837, further confirming the lowered presence of TLR7 in HBV-replicating HepG2.2.15 cells and in liver histopathology samples from patients with CHB [36]. It is hypothesised that a persistent HBV infection causes the host's innate immune system to become dysregulated, resulting in the inhibition of cytokine cascades and a TLR response. The use of TLR agonists to induce a host's innate immune function may help one to better comprehend these processes, specifically the relationship among TLRs with viral components [48].

Conclusions

The TLR7 agonist triggers various immune responses resulting in the suppression of HBV replication and improved adaptive and innate immunity of the host. PEG-PLA lowers the negative impacts of TLR7. After treatment with TLR7, results show an increased T cell production of cytokines, increased number of NK cells, increased number of lymphocytes and lowered HBsAg levels. In summary, targeted therapy with encapsulation of a TLR7 agonist with PEG-PLA shows promising results as novel preventive and curative therapy for CHB.

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Conflicts of interest: The authors declare no conflicts of interest.

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