Received: 10 December 2020 | Revised: 19 February 2021 | Accepted: 24 February 2021

DOI: 10.1111/cts.13016

ARTICLE

Safety, tolerability, pharmacokinetics, and pharmacodynamics of a TLR7 agonist prodrug RO6870868 in healthy volunteers

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Funding information
Hoffman La Roche provided all funds for the design and conduct of this study.

Abstract

RO6870868 is an oral prodrug of the toll-like receptor 7 (TLR7) specific agonist, RO6871765. TLR7 agonists augment host immune activity and are in development to treat hepatitis B infection. We evaluated the safety, tolerability, pharmacokinetics (PKs), and pharmacodynamics (PDs) of RO6870868 in a first-in-human, phase I, randomized, single ascending oral dose study in 60 healthy volunteers at 6 dose levels (200–2000 mg). Single oral doses were generally well-tolerated with a predictable safety profile associated with dose-dependent increases in systemic interferon. No serious adverse events (AEs) were reported and no subject withdrew from the study due to an AE. No clinically significant changes were observed in vital signs, electrocardiograms, or laboratory parameters. Following oral RO6870868 doses, plasma RO6871765 concentrations increased rapidly, exhibiting mean terminal half-life ranging 2–6 h across all cohorts, with area under the plasma concentration versus time curve extrapolated to infinity ($AUC_{0-\infty}$) increasing proportionally with dose. A pattern of dose and time-dependent PD activity was demonstrated consistent with engagement of the TLR7 system. Single RO6870868 doses activated components of the TLR innate immune system in a dose-dependent manner with adequate safety and tolerability. Single-dose data in healthy volunteers are useful to evaluate safety, PK, and PD activity of TLR7 agonists and help to guide dose and regimen selection for further trials in patients with chronic hepatitis B.

Study Highlights

WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC?

Toll-like receptor 7 (TLR7) agonists induce broad immune-enhancing effects and may play a role in overcoming the adaptive and innate immune defects in chronic hepatitis B infection.

WHAT QUESTION DID THIS STUDY ADDRESS?

The safety, tolerability, pharmacokinetics, and pharmacodynamics of single oral doses of RO6870868 (a prodrug of the specific TLR7 agonist RO6871765) in healthy volunteers.

ClinicalTrials.gov Identifier: NCT01911611.

WHAT DOES THIS STUDY ADD TO OUR KNOWLEDGE?

RO6870868 was safe and acceptably tolerated across the dose range in healthy volunteers. Oral administration results in the rapid appearance of the active TLR7 agonist RO6871765 and leads to a profile of gene expression typical for TLR7 agonism, including activation of interferon and interferon-response genes. Gene activation occurs at RO6871765 exposure associated with single RO6870868 doses greater than or equal to 800 mg, with a plateau for several markers at doses between 1200 mg and 1600 mg.

HOW MIGHT THIS CHANGE CLINICAL PHARMACOLOGY OR TRANSLATIONAL SCIENCE?

The study results help to guide dose and regimen selection for clinical trials with RO6870868 and other potent TLR7 activators.

INTRODUCTION

Chronic hepatitis B (CHB) due to hepatitis B virus (HBV) infection is a major global health problem with significant morbidity and mortality.[1] Without appropriate management, CHB leads to serious liver diseases, such as cirrhosis and hepatocellular carcinoma, in up to 40% of patients.[1–3] Among the proteins transcribed from HBV, hepatitis B surface antigen (HBsAg) plays a prominent role in maintaining HBV infection.[2–6] HBsAg is thought to suppress cellular immune responses to HBV, including virus-specific CD8+ T-cell responses.[7,8] Clearance of HBsAg from the serum, currently considered as "functional cure," may help restore HBV-specific immune function and improve long-term prognosis.[3,9–11] Functional cure is rarely achieved with currently approved direct-acting antiviral agents, such as with nucleos(t)ide analogs (NUCs), or with immune modulation by pegylated interferon-α (PEG-IFN α).[3,9,10,12] Combining strong antiviral agents with immune modulators is emerging as an attractive treatment modality for CHB. One class of small molecule activators of innate and adaptive immunity that could be used in combination regimens for CHB therapy are the toll-like receptor (TLR) agonists.

TLRs are a family of highly conserved transmembrane receptors capable of activating both innate and adaptive immune response. Stimulation of TLRs results in the release of multiple cytokines, including several isotypes of IFN and induction of antigen-presenting cells, with the subsequent activation of the adaptive immune response and antigen-specific immunity.[13–16] TLR3, TLR7, and TLR9 are considered the best targets for antiviral therapy.[17] Effort has been made to discover agonists of TLR7 that may be used to stimulate the immune system in various therapeutic indications, including oncology and infectious diseases. To this end, a number of small molecule agonists of TLR7 have been identified.[17] Due to their broad immunoenhancing properties, TLR7 agonists have potential to strengthen antiviral responses and have greater therapeutic impact than IFNs alone.[17–20] In addition to having potentially improved antiviral efficacy,[21–23] endogenous IFN induction with a TLR7 agonist has the potential to be better tolerated than parenteral administration of PEG-IFNα.[24–26]

Promising antiviral activity has been observed with TLR7 agonists in preclinical models of HBV,[18,27,28] but significant HBsAg decline has yet to be demonstrated with TLR7 agonists administered alone or in combination with NUCs in the clinical CHB setting.[19,29–31] Although TLR7 agonists would not directly lower HBsAg, it has been suggested that TLR7 agonists could be used effectively in combination with direct-acting antivirals or drugs that have complementary immunomodulatory activity.[19,32–34] This concept remains to be determined in the phase II setting and clinical trials are currently ongoing in patients with CHB to test this hypothesis.

RO6870868 is an oral single prodrug that is rapidly converted by aldehyde oxidase to the TLR7-specific agonist, RO6871765. A prodrug approach was chosen to improve oral bioavailability and limit potential TLR7 activation in the gastrointestinal (GI) tract, which may be associated with GI intolerability. Neither RO6870868 nor its minor metabolite RO6872373 have TLR7 agonist activity (data not shown). Previously, another prodrug RO6864018 (ANA773) has been evaluated in healthy White volunteers and patients with chronic hepatitis C virus (HCV) infection.[35,36] RO6864018 is a double prodrug that is rapidly converted first by nonspecific esterases to RO6870868, and then to the active TLR7-specific agonist RO6871765 by aldehyde oxidase. Additionally, RO6864018 has been evaluated in Asian healthy volunteers and patients with CHB (manuscripts in preparation). In these study populations, oral dosing of RO6864018 had acceptable safety and tolerability, and upregulated biomarkers of TLR7 activation, including IFNs and IFN-stimulated gene products. Relative ease of large-scale production of the single prodrug, RO6870868 prompted its preclinical and clinical evaluation as a TLR7 agonist prodrug.

The current study (NP28628) was a first-in-human, phase I, randomized, double-blind, placebo-controlled, single

ascending dose study to evaluate the safety, tolerability, and pharmacokinetic (PK), and pharmacodynamic (PD) profiles of RO6870868 and its metabolites following oral administration to healthy volunteers.

METHODS

Study drug

The Investigational Medicinal Product (IMP) and matching placebo were supplied to the investigational site and administered to study subjects as 100 and 400 mg RO6870868 or placebo tablets. The IMP and matching placebo were manufactured according to current Good Manufacturing Practice.

Subjects

Healthy male and female subjects aged 18–65 years with a body mass index (BMI) of 18–30 kg/m^2 at screening were eligible to participate in the study. Inclusion and exclusion criteria are summarized in the Supplementary Information S1.

Study design

This study was a randomized, double-blind, placebo-controlled, single ascending dose study following oral administration of RO6870868 to healthy volunteers. For dose escalation, 10 subjects were sequentially enrolled into cohorts, receiving 200, 400, 800, 1200, 1600, or 2000 mg RO6870868 or placebo (8 active/2 placebo). Rationale for starting dose is described in Supplementary Information S2. Subjects were housed in a clinical research unit for 4 nights from day −2 to day 3. On day 1, subjects were administered a single oral RO6870868 dose or placebo in the fasted state. Escalation to the next dose group was based upon review of safety, tolerability, and PK data with mutual agreement between the sponsor and the investigator. In all cohorts, follow-up was conducted with a visit 7 days after the single dose, and a follow-up call on postdose day 28.

The study protocol and its amendments were reviewed and approved by the responsible ethics committee (Stichting Beoordeling Ethiek Biomedisch Onderzoek [Stichting BEBO]) and by the Netherland's Competent Authority (The Centrale Commissie Mensgebonden Onderzoek [CCMO]): Sponsor-code NP28628, CRO-code RHE390EC-123901, CCMO-code NL45513.056.13. Written informed consent was obtained from each subject participating in the study. The study was conducted at the phase I unit of PRA Health Sciences (Groningen, The Netherlands) in accordance with the principles of the Declaration of Helsinki and Good Clinical Practice guidelines.

Safety assessments

Safety and tolerability of RO6870868 were assessed by monitoring AEs, vital signs, laboratory tests, and electrocardiograms (ECGs) from baseline through the follow-up visit. For AE classification purposes, the most up-to-date version of the Medical Dictionary for Regulatory Activities terminology for AEs and diseases and the Roche INN (International Nonproprietary Name) Drug Terms and Procedures Dictionary for medications and treatments were used.

Pharmacokinetic and pharmacodynamic assessments

Blood samples for PK determination of plasma concentrations of RO6870868 and its metabolites (including RO6871765 and RO6872373) as applicable, were collected at predose and at 0.25, 0.5, 1, 1.5, 2, 3, 4, 8, 12, 18, 24, 36, and 48 h postdose. Urine samples were collected for measurement of concentrations of the drug (RO6870868) and its metabolites (RO6871765 and RO6872373) in pooled urine samples at 0–4, 4–8, 8–12, 12–24, 24–36, and 36–48 h postdose. Plasma and urine concentrations were measured by a specific and validated liquid chromatography–mass spectrometry method. Details are provided in Supplementary Section S3.

Blood samples were collected to evaluate the changes of PD markers as well as transcriptional changes of TLR7/IFN-response genes at predose and 3, 6, 12, 18, 24, 36, and 48 h postdose in all subjects. IL-6, TNF-α, IL12-p40, IL-10, and IP-10 levels were determined using Luminex X-MAP; IFN-α, using Simoa; neopterin by ELISA; ISG15, OAS1, MX1, and TLR7 mRNAs via TaqMan, as previously described.[37]

Statistical analyses

The primary study variables were safety and tolerability, including incidence and severity of AEs, safety laboratory abnormalities, ECGs, and vital sign abnormalities. The main PK study variables were area under the plasma concentration-time curve from time 0 to infinity ($AUC_{0-\infty}$) and maximum plasma concentration (C_{max}) of the active metabolite RO6871765. Other PK variables included the $AUC_{0-\infty}$ and C_{max} of RO6870868 and a minor metabolite RO6872373. Plasma PK parameters were estimated by a standard noncompartmental method using WinNonlin version 6.4 (Pharsight Corporation) and presented with summary statistics. PD variables were regarded as secondary outcomes. In all analyses,

placebo subjects from each cohort were pooled into one group. Descriptive statistics were used to summarize PK and PD variables. Detailed methods for determination of PK dose proportionality, PD response, and PK/PD relationships are described in the Supplementary Information S4.

RESULTS

Study population

A total of 60 subjects were randomized in 6 dosing cohorts (48 subjects received active drug and 12 subjects received placebo). All but one subject completed the study, including the follow-up visit on day 7 and follow-up telephone call on day 28; one subject in the 2000 mg dose cohort was lost to follow-up after day 3. The study population comprised 12 female subjects (women of nonreproductive potential) and 48 male subjects aged 19–65 years with a BMI of 18.7–29.4 kg/m^2 (Table S1). All subjects were White, except for one subject, who was of mixed race (White and Asian). Two female subjects were included in each dosing cohort and only one female subject was randomized to receive placebo (this subject was in the 1200 mg dose cohort). No remarkable differences were noted between the treatment cohorts.

Safety

All single doses of RO6870868 from 200 to 2000 mg were considered safe with acceptable tolerability. No serious AEs were reported, and no subjects withdrew from the study due to an AE. No clinically significant changes were observed in vital signs, ECGs, or laboratory parameters.

A total of 55 AEs were reported in 24 subjects (Table 1). The most common AEs were pyrexia, headache, dizziness, myalgia, and nausea. Most AEs (41 events in 23 subjects) were considered mild in intensity. Events considered moderate (7 events in 4 subjects) or severe (7 events in 2 subjects: one subject with myalgia and headache, and another subject with dizziness, malaise, nausea, headache, and myalgia) occurred only in the 1600 and 2000 mg dose groups and could be described as "flu-like." These events were reported within 24 h of dosing and were treated with analgesics (paracetamol). All flu-like symptoms resolved within 1–2 days.

A total of 29 AEs reported by 8 subjects were considered related to the study drug. Although the incidence of AEs increased with dose, the percentage of subjects who reported related AEs did not increase across the dose range, with 2 of 8 subjects at dose levels of 400, 1200, 1600, and 2000 mg reporting related AEs. No subjects at 200 and 800 mg dose levels reported related AEs.

Mean values for all hematology parameters per cohort were within normal ranges for all dose levels. There were some individual slight decreases in neutrophil, lymphocyte, and platelet counts that were considered nonclinically significant and returned to baseline levels at the follow-up visit. No clinically significant dose-related trends in chemistry or urinalysis parameters were observed in any cohort.

Pharmacokinetics

Following oral doses of RO6870868, mean plasma RO6871765 concentrations increased rapidly, with a median time to C_{max} (T_{max}) of 1 h (range 0.5–3.0 h) across all dose cohorts (Figure 1). RO6871765 plasma exposure ($AUC_{0-\infty}$ and C_{max}) increased with increasing RO6870868 dose (Table 2). The intersubject variability (coefficient of variation percentage) for RO6871765 was low across all cohorts for $AUC_{0-\infty}$ (14%–24%) and for C_{max} (13%–32%). Elimination of RO6871765 from plasma appeared to be biphasic, with mean terminal half-life in the range of 2.2–6.1 h across all cohorts (Table 2).

Following oral doses of RO6870868, RO6871765 was the most common metabolite detected in plasma with little or none of the minor inactive metabolite RO6872373 (Figure S1). Details of relative exposures of these compounds are presented

TABLE 1 Overview of adverse events

AEs, *n* subjects (*n* events)	Placebo (*N* = 12)	RO6870868 200 mg (*N* = 8)	RO6870868 400 mg (*N* = 8)	RO6870868 800 mg (*N* = 8)	RO6870868 1200 mg (*N* = 8)	RO6870868 1600 mg (*N* = 8)	RO6870868 2000 mg (*N* = 8)
Total AEs	4 (4)	4 (6)	3 (5)	3 (4)	3 (9)	3 (14)	4 (13)
Related AEs	0	0	2 (2)	0	2 (7)	2 (10)	2 (10)
AE intensity							
Mild	4 (4)	4 (6)	3 (5)	3 (4)	3 (9)	2 (7)	4 (6)
Moderate	0	0	0	0	0	2 (5)	2 (2)
Severe	0	0	0	0	0	1 (2)	1 (5)

Abbreviation: AE, adverse event.

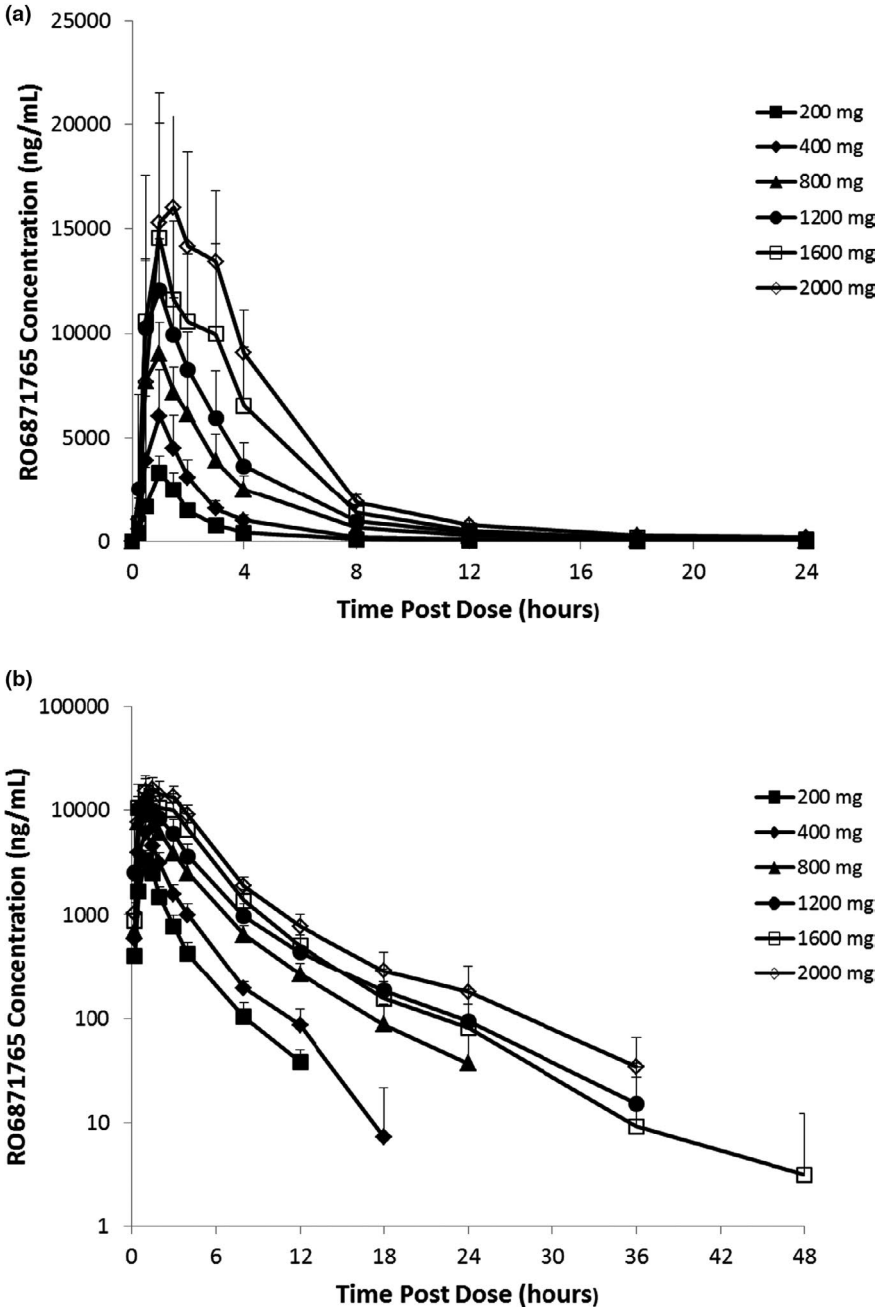

FIGURE 1 Mean (± SD) RO6871765 concentration vs time profiles (a) linear and (b) semi log scale following single doses of RO6870868

in the Supplementary Information S5 and RO6870868 PK parameters are presented in Table S2. $AUC_{0-\infty}$ of RO6871765 but not C_{max} increased proportionally with RO6870868 dose (Figure S2). Using a statistical power model, the expected fold in $AUC_{0-\infty}$ of RO6871765 with a 2-fold increase in dose (95% confidence interval [CI]) was 2.06 (range 1.97–2.16) and for C_{max} was 1.60 (range 1.50 to 1.70). In this analysis, slope (95% CI) was 1.05 (0.98–1.11) for $AUC_{0-\infty}$ and 0.68 (0.58–0.77) for C_{max}.

Following oral administration, 93%–95% of the dose of the prodrug RO6870868 was found in urine as the active TLR7 agonist, RO6871765. Approximately 3%–4% of each dose was eliminated as the unchanged parent compound RO6870868.

Pharmacodynamics

All subjects enrolled in the study provided samples for PD analysis, including the one subject who did not return for follow-up. In general, there was little or no change in concentrations of cytokines TNF-α and IL-12P40 at all RO6870868 doses, whereas IL-6 and IL-10 exhibited increased levels only at higher doses (Figure S3). IFN-α, IP-10, neopterin,

TABLE 2 Summary statistics for RO6871765 pharmacokinetic parameters

Parameter	Dose (mg)[a]	Mean ±SD (%CV)	Median (Min–Max)
$AUC_{0-\infty}$, ng·hr/ml	200	7160 ± 1650 (23.1)	6860 (5490–9620)
	400	14,600 ± 2890 (19.8)	13,900 (11,500–20,700)
	800	30,700 ± 5400 (17.6)	29,300 (25,200–39,500)
	1200	44,900 ± 7770 (17.3)	45,100 (34,300–58,300)
	1600	61,300 ± 14,700 (24.0)	60,400 (44,900–87,600)
	2000	80,700 ± 11,600 (14.4)	77,000 (68,000–99,400)
C_{max}, ng/ml	200	3630 ± 941 (25.9)	3450 (2410–5050)
	400	7100 ± 2240 (31.6)	7130 (3080–10,500)
	800	9500 ± 1260 (13.3)	9230 (7520–11,300)
	1200	12,600 ± 1890 (15.0)	12,700 (9820–15,000)
	1600	16,100 ± 4900 (30.5)	15,900 (9900–24,300)
	2000	18,000 ± 4310 (24.0)	16,660 (11,500–24,400)
$t_{1/2}$, h	200	2.23 ± 0.29 (13.0)	2.30 (1.62–2.52)
	400	2.41 ± 0.77 (31.9)	2.16 (1.91–4.27)
	800	4.14 ± 1.45 (35.2)	3.75 (2.52–7.19)
	1200	4.99 ± 2.66 (53.4)	4.33 (2.19–10.95)
	1600	4.59 ± 2.22 (48.4)	3.73 (2.25–8.75)
	2000	6.09 ± 2.55 (42.0)	6.31 (2.99–10.52)

Abbreviations: $AUC_{0-\infty}$, area under the concentration time curve extrapolated to infinity; C_{max}, maximum concentration; CV, inter-subject variability measured as coefficient of variation; $t_{1/2}$, terminal half-life.
[a]$n = 8$ for all dose levels.

and the mRNA species ISG-15, OAS-1, MX-1, and TLR7 exhibited increased levels at doses of 800 mg and higher.

Serum IFN-α

Serum IFN-α increased in a dose-dependent manner after single RO6870868 doses of 800 mg or higher (Table S3), peaking ~6 h after dosing. The time course of the IFN effect was independent of dose and was observed up to 48 h postdose in some subjects at higher doses. Table S3 provides a summary of subjects in each dose cohort who exhibited serum IFN-α levels greater than the lower limit of quantification (0.043 pg/ml) within the first 24 h following a single dose of RO6870868. The incidence and the magnitude of the measurable IFN-α responses appeared to increase with increasing RO6870868 dose. Intersubject variability in the IFN-α response was high. IFN-α levels were highest in the 1600 and 2000 mg doses, reaching maximum levels of 6.89 and 5.30 pg/ml, respectively.

IP-10 and Neopterin

Blood concentrations of the chemokine IP-10 and immune marker neopterin demonstrated clear dose- and time-dependent increases after single RO6870868 doses 800 mg or higher (Figure 2). IP-10 exhibited a peak response at 12 h postdose that remained above background for up to 48 h. As expected, the neopterin response peak appeared at 36–48 h after the single RO6870868 dose, but some subjects exhibited neopterin peaks at 24 h postdose. The decay of the neopterin signal could not be visualized because timepoints past 48 h were not collected.

Neopterin or IP-10 responses were evident at the 400 mg dose, but both markers began to increase in incidence and magnitude with doses 800 mg and higher (Table S4). There was considerable intersubject variability in the extent of the increase, particularly for IP-10, which exhibited a range of response from 6 to 33-fold at the 2000 mg dose.

ISG-15, OAS-1, MX-1, and TLR7 mRNA Species

The expression of select mRNA species expected to be modified by TLR7 agonism (e.g., ISG-15, OAS-1, MX-1, and TLR7) exhibited dose- and time-dependent increases following single RO6870868 doses. Figure 3 illustrates the geometric mean fold change from baseline versus time for each of these genes following a single RO6870868 dose. Time-dependent responses for ISG-15, OAS-1, and MX-1 mRNA occurred with RO6870868 doses 800 mg or higher. For TLR7, trends in increases in gene expression were more evident at higher doses, beginning at the 1200 mg dose, and

maximum fold changes were relatively small compared with the other genes evaluated.

Mean responses for ISG-15, OAS-1, and MX-1 appeared to peak ~12–18 h postdose. TLR7 appears to respond somewhat earlier, peaking between 6 and 12 h postdose. The duration of response was dependent on RO6870868 dose. ISG-15, OAS-1, and MX-1 levels remained elevated for 48 h postdose for 1200 mg or higher and for 36 h following the 800 mg dose, whereas TLR7 appeared to remain elevated at 36 h postdose for RO6870868 doses of 1200 and 2000 mg.

Nearly all subjects exhibited response patterns for ISG-15, OAS-1, and MX-1 at doses of 1600 mg or higher and nearly 50% or more subjects responded at doses of 800 and 1200 mg (Table S5). For TLR7, the response was generally

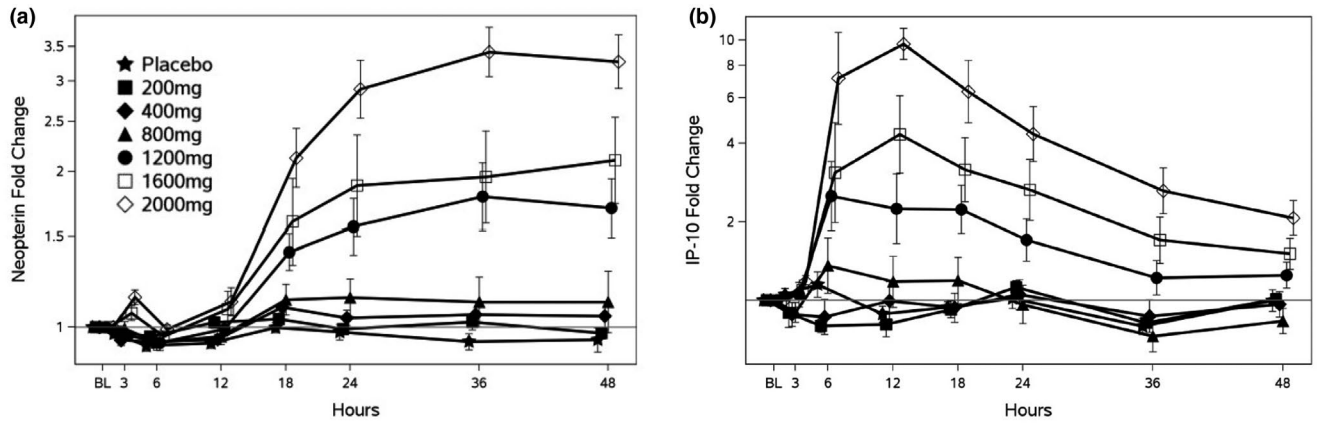

FIGURE 2 Geometric mean (±SEM) fold change from baseline versus time following a single dose of RO6870868 for (a) neopterin and (b) IP-10

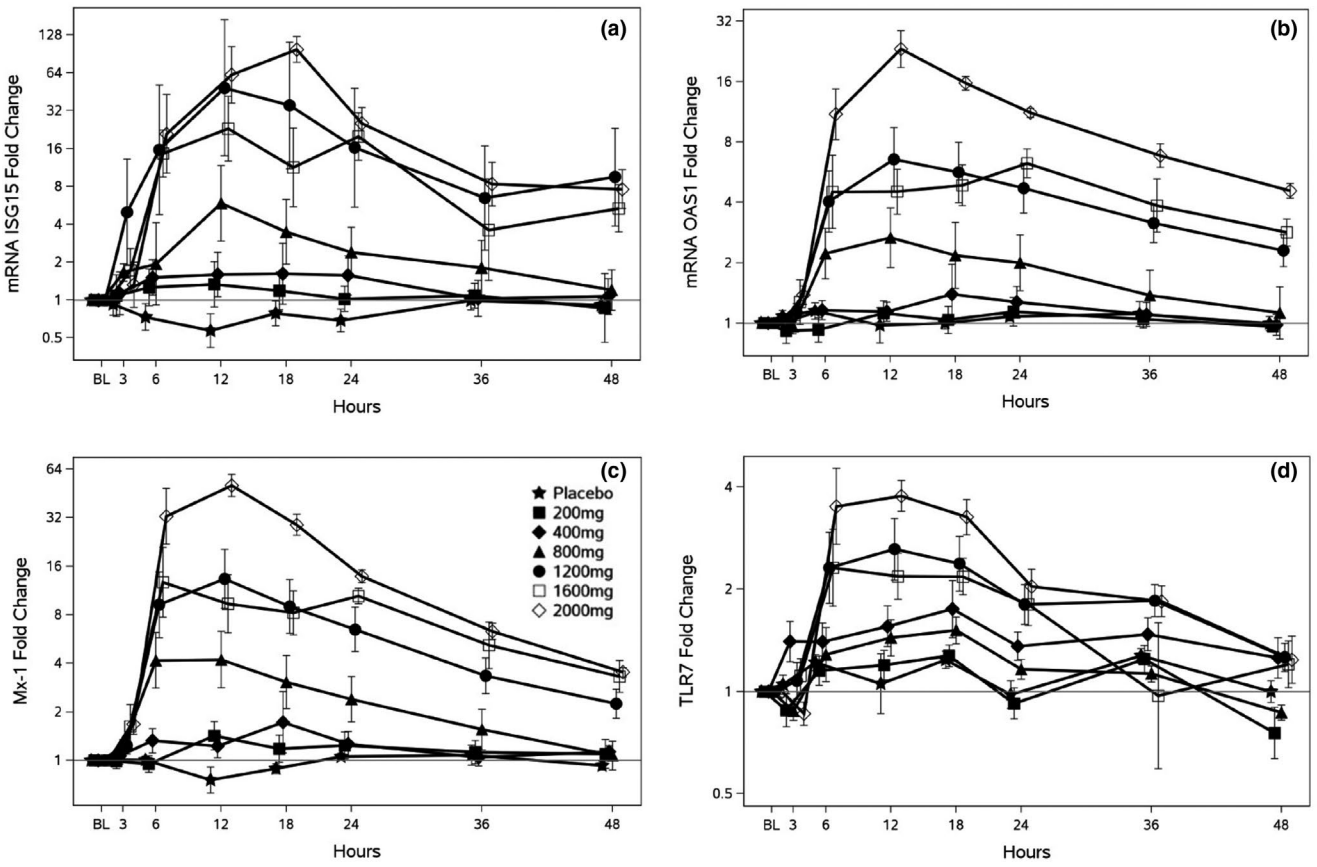

FIGURE 3 Geometric mean (±SEM) fold change from baseline versus time following a single dose of RO6870868 for mRNA species (a) ISG-15, (b) OAS-1, (c) MX −1, and (d) TLR7

weaker than for the other mRNA species, and with a cutoff of 4.4-fold for background, only 3 of 8 subjects responded at the 2000 mg dose. In general, responses to mRNA species were quite variable; for example, at the 1200 mg dose, ISG-15 ranged from ~7.5- to 1800-fold change from baseline.

Pharmacokinetics and pharmacodynamics relationship

A consistent temporal relationship can be demonstrated between the appearance of active TLR7 agonist in plasma and the response markers of TLR7 activation at all doses where PD can be detected (≥800 mg). To best represent the range of response signal strengths for IFN-α, OAS-1, and neopterin, Figure 4 illustrates this temporal relationship at the highest RO6870868 dose (2000 mg). RO7861765 plasma concentration peaks within 1 h and is undetectable within 24 h postdose. IFN responds early and peaks at ~6 h postdose. This event is followed by the secondary appearance of IFN-response genes, such as OAS-1, that appear to peak at ~12 h postdose. MX-1 and ISG-1 respond in a similar fashion to OAS-1 (data not shown). Neopterin responds later and peaks within 36–48 h postdose.

There appears to be a threshold level of plasma RO6871765 that initiates the cascade of TLR7 response. In Figure 5, the individual geometric mean fold change from baseline is plotted against the individual $AUC_{0-\infty}$ for each subject for IP-10 and neopterin. The figure shows that little or no response occurs below a RO6871765 plasma AUC of ~30 μg·h/ml. This level correlates with the mean plasma level achieved with the 800 mg dose of RO6780868, which exhibits values of RO6871765 $AUC_{0-\infty}$ from 25.2 μg·h/ml to 39.5 μg·h/ml. Responses appear to increase with higher RO6871765 exposure. For ISG-15, MX-1, and TLR7, there appears to be a plateau between the 1200 and 1600 mg doses where little additional effect is seen, whereas OAS-1 appears to respond further with increasing RO6871765 exposure (Figure S4).

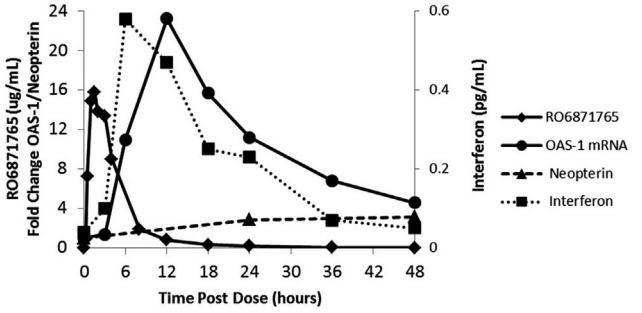

FIGURE 4 Temporal relationship between RO6871765 concentration and responses for interferon-α, OAS-1 mRNA, and neopterin following a single dose of RO6870868 2000 mg. Curves represent arithmetic mean concentrations of RO6871765 (μg/ml), geometric mean fold-change from baseline for OAS-1 mRNA and neopterin, and geometric mean interferon-α concentration (pg/ml). Errors bars are omitted for ease of visualization

DISCUSSION

The current strategy for using TLR7 agonists as therapeutic agents for CHB involves the use of these agents to stimulate immune activation in combination with a direct acting antiviral agent. It may be challenging to pick the optimal dose and regimen to limit safety issues associated with elevated systemic IFN with very potent TLR7 activators. Development of PF-4878691, a potent TLR7 agonist evaluated for treatment of chronic HCV infection (oral dose range of 3 to 9 mg), was abandoned as the compound failed to demonstrate sufficient antiviral activity in the absence of severe safety signals associated with systemic IFN release.[38] Another potent TLR7 agonist, GS-9620 (vesatolimod), was evaluated in patients with HBV with

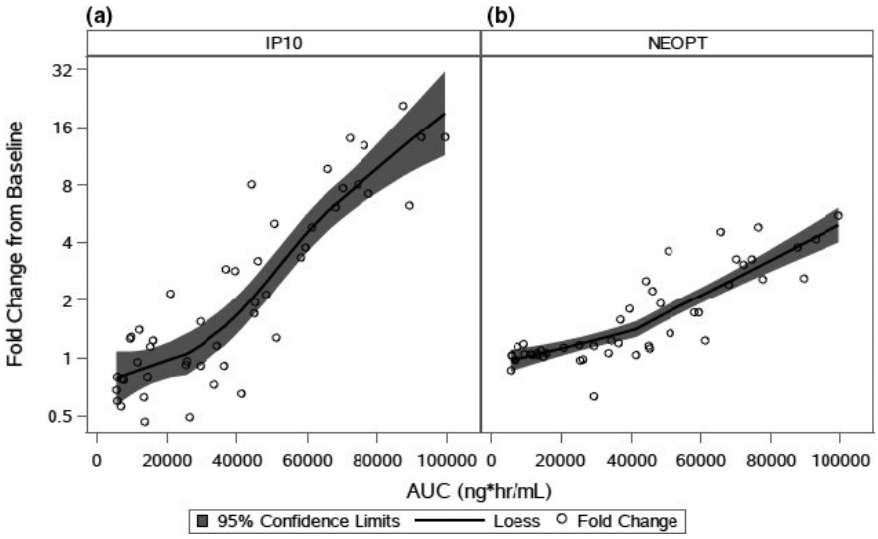

FIGURE 5 Geometric mean fold change from baseline versus RO6871765 $AUC_{0-\infty}$ for (a) IP-10 at 12-hours post-dose and (b) neopterin at 36-hours post-dose for each subject after a single dose RO6870868. $AUC_{0-\infty}$, area under the plasma concentration versus time curve extrapolated to infinity

a once weekly regimen (oral dose range of 1 to 4 mg) designed to stimulate TLR7 responses while minimizing systemic IFN levels.[25,29,31] GS-9620 did not achieve clinically significant declines in HBsAg and development for HBV has been discontinued.[29,31,39] Compared with GS-9620, RO6871765 is ~100 fold less potent in vitro (data not shown) and the RO6870868 dose range necessary to stimulate TLR7-dependent PD activity is between 800 and 2000 mg. One hypothesis is that the use of less potent TLR7 agonists in combination with antiviral agents may allow for better dose titration of the immune activation required for achieving HBsAg loss. To this end, another TLR7 agonist in development, RO7020531 exhibits a good balance between safety and PDs at a 150 mg dose.[37] By comparison, the choice of doses suitable for phase II testing of RO6870868 would be in the range of 800 to 1200 mg. Careful safety monitoring and formal safety evaluations are warranted in clinical studies with immune agonists. In this single ascending dose study, single doses of RO6870868 were generally well-tolerated by study subjects. A proportion of subjects in high-dose cohorts developed reversible flu-like symptoms that are part of the known safety profile of therapeutic IFNs. These AEs are expected for TLR7 agonists at doses that induce serum IFN. Similar types of AEs have been reported in phase I studies both in healthy subjects and patients infected with chronic HCV receiving RO6864018, the double prodrug of RO6871765.[35]

Measurement of the PKs of RO6871765 following oral administration of the prodrug RO6870868 has shown that the active metabolite RO6871765 entered the plasma compartment rapidly. Analysis of renal elimination indicated that the majority of the prodrug was metabolized to the active TLR7 agonist. Taken together, these data suggest that oral delivery of an active TLR7 agonist by this prodrug route represents a plausible therapeutic approach. Of equal importance, intersubject variability for exposure of the active TLR7 agonist RO6871765 was low. Elimination half-life for RO6871765 is short and plasma is cleared of RO6870868 and its metabolites within 24 h postdose. Nevertheless, analysis of response markers including IFN, select IFN-responsive genes (OAS-1, MX-1, and ISG-15) and markers of immune activation, such as neopterin show that this transient increase in RO6871765 exposure is enough to set into motion a cascade of events typically associated with TLR7 activation of the innate immune system.

One of the primary roles of plasmacytoid dendritic cells (DCs) is synthesis and release of IFNs, an action that is triggered by activation of TLR7 receptors on the DCs.[21] As anticipated, one early response seen following single oral dose administration of RO6870868 is the appearance of IFN in the systemic circulation and the subsequent upregulation of expression of IFN-responsive genes, such as OAS-1 and ISG-15. Among the cytokines and chemokines tested, IP-10 appeared to have the most robust response to single doses of RO6870868. IP-10 is an 8.7 kDa protein belonging to the CXC chemokine family with several roles related to TLR activation, such as chemoattraction for monocytes/macrophages, T cells, NK cells, and DCs, and promotion of T cell adhesion to endothelial cells.[40,41] Activation by RO6871765 may enable recruitment of elements of the adaptive immune system adding to the therapeutic potential of this agent as an immune modulator.

Associated with IFN and ISG-15, the neopterin response was dose- and time-dependent following oral doses of RO6870868 and, as expected, peaked at later times postdose. Neopterin is synthesized by human macrophages upon stimulation with the cytokine IFN-gamma and is indicative of a pro-inflammatory immune status. Thus, neopterin serves as a marker of overall cellular immune activation to be expected downstream of TLR7 agonism.

Among the selection of mRNA species examined, ISG-15, OAS-1, and MX-1 exhibited dose- and time-dependent responses following RO6870868 doses, peaking after the appearance of IFN in the systemic circulation. Interestingly, TLR7 mRNA exhibited approximately the same response time as IFN and may represent a mechanism to enhance TLR7 signaling in the presence of an appropriate stimulus.

The safety, PK, and PD data collected in the current study in healthy volunteers provides a framework for evaluation of PK/PD properties of a TLR7-specific immunomodulatory agent. Mean systemic IFN levels in this study were relatively modest in subjects administered single 800 and 1200 mg doses of RO6870868. Increasing the dose to 1600 and 2000 mg results in 60% to 100% of subjects showing measurable systemic IFN levels and, more importantly, to higher and more variable spikes of IFN in some individuals. This event is associated with higher incidence and severity of AEs. The 800 mg dose is the minimum dose in which TLR7 responses were recorded in this study and 1200 mg exhibited in some cases the first PD plateau for increase in gene expression. These doses represented plausible choices for initiating phase II clinical studies with either RO6870868 or the double prodrug RO6864018 balancing safety and activation of TLR7 responses.[42] In addition to dose selection, the choice of dose regimen has been explored with RO7020531[37] and with the double prodrug RO6864018 in a study in patients with CHB where the TLR7 agonist was given either once weekly or every other day to gain insight into the relationship among PD, dose, and dose regimen (Study NP28938, manuscript in preparation).

Although it is not clear which biomarker may be indicative of antiviral activity, the PD activation seen with oral dosing of single and double prodrugs of TLR7 agonists provides evidence that the TLR7 target can be engaged and that a prodrug approach is plausible for development of TLR7 agonists as potential therapeutic agents for treatment of patients with CHB.

It should be noted that TLR7 agonists alone are not expected to have a direct impact on lowering HbsAg levels. A goal in using these agents will be to modestly stimulate an impaired host immune system in patients with CHB offering indirect support to a direct acting antiviral agent in a combination drug paradigm. The work ahead for development of TLR7 agonists is to identify a safe balance using dose and regimen that provides tolerable immune activation.

ACKNOWLEDGMENTS

The authors thank all healthy volunteers and their families for participation in this trial. We also thank PRA Health Sciences and Jeroen van de Wetering de Rooij, as well as Roche employees who contributed to the subject enrollment, study conduct, and analysis. Editorial and medical writing support was provided by Weber Shandwick Hong Kong, funded by F. Hoffmann-La Roche.

CONFLICT OF INTEREST

All authors participated in this study while employed by Hoffmann La Roche and declare no additional competing interests for this work.

AUTHOR CONTRIBUTIONS

J.F.G. and I.F. wrote the manuscript. J.F.G. designed the research. All authors performed the research and all authors analyzed the data.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

How to cite this article: Grippo JF, Folitar I, Passe S, et al. Safety, tolerability, pharmacokinetics, and pharmacodynamics of a TLR7 agonist prodrug RO6870868 in healthy volunteers. *Clin Transl Sci*. 2021;14:1524–1534. https://doi.org/10.1111/cts.13016