Modelling of early viral kinetics and pegylated interferon-α2b pharmacokinetics in patients with HBeAg-positive chronic hepatitis B

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Background: Pegylated interferon α2b (PEG-IFN-α2b) is effective for the treatment of hepatitis B e antigen (HBeAg)-positive chronic hepatitis B, although its mechanism of action remains unclear. HBeAg loss is achieved in 36% of patients after one year of PEG-IFN-α2b treatment and combination therapy with lamivudine is not superior to PEG-IFN-α2b monotherapy.

Methods: Early pharmacokinetics and viral kinetics were analysed in patients treated for 52 weeks with PEG-IFN-α2b with or without lamivudine.

Results: After 4 weeks of treatment, there was a median viral decline of 2.94 $\log_{10}$ copies/ml in those treated with PEG-IFN-α2b and lamivudine and only 0.45 $\log_{10}$ copies/ml in the PEG-IFN-α2b monotherapy group. Peak PEG-IFN-α2b levels were reached approximately one day after administration and subsequently declined exponentially, consistent with a viral load rebound near to baseline levels at the end of the dosing period in most patients receiving PEG-IFN-α2b monotherapy. Modelling of pharmacokinetics and viral kinetics data in this group revealed that viral load was minimal 3.6 days after PEG-IFN-α2b administration, the mean maximal and mean antiviral effectiveness was 70% and 48% with a mean infected cell loss rate of 0.07 per day, while no significant biphasic decline was observed.

Conclusions: PEG-IFN-α2b induces a sustained response in a considerable number of patients despite limited direct antiviral activity during the first weeks of antiviral therapy.

Introduction

Patients with hepatitis B e antigen (HBeAg)-positive chronic hepatitis B often have high levels of circulating virus and immune responses directed against the virus cause inflammation, which in turn can lead to cirrhosis and hepatocellular carcinoma [1]. Although treatment with nucleos(t)ide analogues, like lamivudine, adefovir and entecavir, is effective for viral load reduction, long-term treatment is often necessary and carries the risk of viral resistance [2–4]. Using interferon therapy, a durable treatment response can be achieved in 35–45% of HBeAg-positive and HBeAg-negative chronic hepatitis B patients.

Pegylated interferons induce HBeAg seroconversion in approximately one-third of HBeAg-positive patients [5–9]. In a recent trial, a durable loss of HBeAg was achieved in 36% of patients after a 52 week course of pegylated interferon α2b (PEG-IFN-α2b) treatment with a 26 week follow-up period [6]. The decline in viral load during PEG-IFN-α2b therapy was not uniform and different patterns of viral decline could be recognized both during treatment and follow up [10]. Remarkably, a marked viral decline between weeks 4 and 32 of treatment resulted in the highest rate of HBeAg loss [10]; in general, there was only minimal decline in viral load in the first month of treatment. Until now, no viral kinetic data were available during PEG-IFN treatment in HBeAg-positive chronic hepatitis B [11]. Therefore, we analysed the relationship

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between viral kinetics and pharmacokinetics of PEG-IFN-α2b in HBeAg-positive chronic hepatitis B. To our knowledge, this is the first analysis fitting data from both pharmacokinetics and viral kinetics during treatment in patients with chronic hepatitis B.

Material and methods

Patients

A total of 96 patients who participated in an international multicentre randomized double-blinded study reported previously [6], underwent frequent blood sampling in the first month of therapy. Eligible patients were men and women over 16 years of age with chronic hepatitis B, documented by liver biopsy and HBsAg positivity for over 6 months, and positive serum hepatitis B virus (HBV) DNA levels. All patients were HBeAg-positive and had alanine aminotransferase (ALT) levels ≥2 times the upper limit of normal on two occasions within 8 weeks before randomization. Patients received PEG-IFN-α2b 100 μg once weekly and were randomized to receive either lamivudine 100 mg once daily or placebo. The dose of PEG-IFN-α2b was reduced to 50 μg once weekly after 32 weeks of therapy. Patients were treated for 52 weeks and followed for 6 months post-treatment.

HBV DNA quantification

HBV DNA levels were measured frequently during the first month of therapy (at days 0, 1, 2, 3, 4, 7, 14, 21 and 28) in a randomly selected subgroup of 38 patients (19 patients in the monotherapy group and 19 patients in the combination therapy group) using an in-house developed TaqMan real-time PCR test with a dynamic range of 4×10^2–10^{10} copies/ml [12]. Monthly HBV DNA measurements were available in all 96 patients.

PEG-IFN-α2b concentration

PEG-IFN-α2b serum concentrations were also measured at days 0, 1, 2, 3, 4, 7, 14, 21 and 28 using a quantitative sandwich interferon enzyme-linked immunosorbent assay (ELISA, Bender MedSystems Diagnostics GmbH, Vienna, Austria) in all 96 patients. Binding of (pegylated) interferon to a murine monoclonal antibody directed against interferon adsorbed onto microwells was detected by a horse radish peroxidase (HRP)-conjugated monoclonal anti-interferon antibody. Following 2 h of incubation, unbound complexes were removed by washing (three times) after which tetramethyl-benzidine was used to determine the amount of interferon in the sample. Absorbency was read using a spectrophotometer using 450 nm as the primary wavelength. Standards were prepared from diluted series of pegylated interferon in normal human serum obtained from healthy volunteers. Patient sera and standards were tested in triplicate, on the same plate. Although optical densities obtained were related to a standard of pegylated interferon, the ELISA might also detect free recombinant INF-α2b molecules and natural interferon. The detection limit of the assay is 35 pg/ml and is linear up to a concentration of 2,000 pg/ml.

Modelling of pharmacokinetics

For modelling of the pharmacokinetics of PEG-IFN-α2b we used the absorption and elimination model recently applied by Powers *et al.* and Talal *et al.* [13,14]. This model describes the concentration of drug in the blood (C) following a single injection at time t=0 as follows:

$$C(t)=\frac{k_a D(F/V_d)}{(k_e-k_a)}\left(e^{-k_a t}-e^{-k_e t}\right) \quad (1)$$

where t is the time after injection, k_a is the rate of absorption, k_e is the rate of elimination, F is the bioavailability, D is the drug dose and V_d is the volume of distribution. We used a more general model for multiple weekly injections of PEG-IFN-α2b that accounts for random variability effects between subjects. The PEG-IFN-α2b concentration in the blood for individual i at the time point t is then described as the sum of the individual contributions of each injection d until time t, that is, $t_d<t$ is the injection day (that is, t_d=0, 7, 14, 21, …,) and D_d is the dose per injection d:

$$C_i(t)=\sum_{d:t_d<t}\frac{(FD_d)_i}{V_{di}}\frac{k_{a,i}}{(k_{e,i}-k_{a,i})}\left(e^{-k_{a,i}*(t-t_d)}-e^{-k_{e,i}*(t-t_d)}\right) \quad (2)$$

$k_{a,i}$, $k_{e,i}$ *and* $(F/V_d)_i$ consist of both a fixed-effect as well as an individual random effect parameter. Using this formula, the area under the curve (AUC) of the PEG-IFN-α2b concentration could be calculated. Furthermore, these changes in PEG-IFN-α2b concentration over time have an impact on the effectiveness of PEG-IFN-α2b in contrast to a constant effect. Assume that the effectiveness of PEG-IFN-α2b for individual i is given by:

$$\varepsilon_i(t)=\frac{C_i(t-t_0)^n}{IC_{50}{}^n+C_i(t-t_0)^n} \quad (3)$$

where IC_{50} is the concentration at which the drug's effectiveness is half its maximum, and n is the Hill coefficient, a parameter that determines the steepness of the rise of the effectiveness with increasing PEG-IFN-α2b concentration, and t_0 is a possible time delay [14].

Modelling of viral kinetics

Using the pharmacodynamic efficacy model (Equation 3), the viral kinetics for the first week of

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Table 1. Baseline characteristics

	PEG-IFN-α2b + lamivudine (*n*=48)	PEG-IFN-α2b + placebo (*n*=48)
Age, years*	33 ±12	32 ±12
Sex: male/female, % male	32/16 (67)	37/11 (77)
Weight, kg*	72 ±16	71 ±13
Race, *n* (%)		
Caucasian	42 (88)	43 (90)
Asian	2 (4)	3 (6)
Other	4 (8)	2 (4)
Genotype, *n* (%)		
A	13 (27)	15 (31)
B	1 (2)	2 (4)
C	2 (4)	2 (4)
D	31 (65)	29 (61)
E	1 (2)	0 (0)
ALT, U/l*	175 ±193	167 ±130
Mean HBV DNA, $\log_{10}$ copies/ml*	9.2 ±1.1	9.3 ±0.7

*Mean value ±SD. ALT, alanine aminotransferase; HBV, hepatitis B virus; PEG-IFN-α2b, pegylated interferon-α2b.

PEG-IFN-α2b monotherapy can be described by a model originally applied by Nowak *et al*. [15] and modified by Sypsa *et al*. [16] and Powers *et al*. [13]. In our approach the constant ε_i is substituted by $\varepsilon_i(t)$ in the differential equation system modelling viral kinetics:

$$\frac{d}{dt} V_i(t)=(1-\varepsilon_i(t))p_i I_i(t)-c_i V_i(t) \quad (4)$$

and

$$\frac{d}{dt} I_i(t)=\beta_i V_i(t) T_i(t)-\delta_i V_i(t) \quad (5)$$

The resulting model function $V_i(t)$ describes the viral load of individual i at time point t and depends on the virion clearance rate c_i and the infected cell loss rate d_i. The total number of cells (that is, infected target cells, I_i, and uninfected target cells, T_i) are assumed to remain constant in each individual during treatment motivated by a fast liver regeneration. As usual, the infection rate β_i and the viral production rate p_i were substituted by the other parameters assuming that they remained unchanged from the steady-state situation.

Modelling and data fitting

The PROC NLMIXED procedure of SAS 9.1 (SAS Institute Inc., Cary, NC, USA) was used to fit the first month pharmacokinetic data of all 96 patients with a non-linear mixed modelling approach. The NLME procedure of R (R Foundation for Statistical Computing) yielded highly comparable results (data not shown). The prediction of the PEG-IFN-α2b concentration (Equation 2) and the model for effectiveness (Equation 3) were thereafter incorporated in the model of the viral load as solution from Equations 4 and 5 of the patients treated with PEG-IFN-α2b monotherapy. The viral load was hereafter fitted with non-linear mixed modelling with the NLME procedure of R, including the ordinary differential equation solver LSODA from the ODESOLVE package in a nested way to estimate the infected cell loss rate d, the baseline levels of viral load as well as the IC_{50} levels and the time delay t_0. Because interindividual variation could already be modelled by baseline viral load and IC_{50} levels, the other parameters were set constant between patients (fixed effects). Furthermore, relatively few data points can lead to biased estimates of the viral clearance rate c [16]. Therefore, we fixed c to 1.3 per day. Different Hill coefficients (n=1, n=2, n=3 and n=4) were checked and we used a coefficient of 1 because this gave the best results.

SPSS (version 14.0.1, SPSS Inc., Chicago, IL, USA) was used for further data analyses. All tests for significance and resulting P-values were two-sided, with a level of significance of 0.05.

Results

Patient characteristics

Demographic and baseline characteristics of the 96 patients included in this study are shown in Table 1. Forty-eight patients received PEG-IFN-α2b monotherapy; the other 48 patients received combination therapy consisting of PEG-IFN-α2b and lamivudine. There were no significant differences between the two

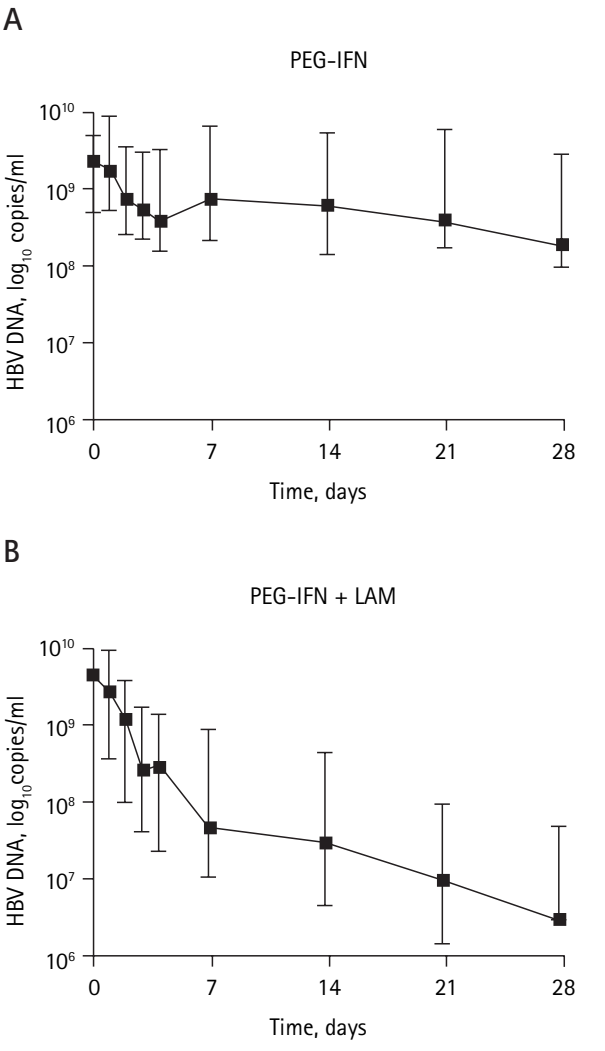
groups with respect to ALT, viral load, age, sex, weight and race. PEG-IFN-α2b concentration was measured in all 96 patients, whereas frequent HBV DNA measurements were obtained in a representative subset of 38 patients (19 in each treatment arm).

Viral kinetics

In the PEG-IFN-α2b monotherapy group (n=19), the median viral decline after 1 month of treatment was 0.45 $\log_{10}$ copies/ml (range -0.03–1.56) (Figure 1) and 0.40 $\log_{10}$ copies/ml (range -0.28–2.30) at week 8 of treatment. The median viral decline was 0.028 $\log_{10}$ copies/ml/day (range -0.069–0.165) for the first week and 0.017 $\log_{10}$ copies/ml/day (range -0.006–0.046) between week 1 and 4. In the first week of treatment, there was a median decline in viral load of 0.20 $\log_{10}$ copies/ml (range -0.48–1.15). There was an initial decline in viral load until 4 days after drug administration in all patients in the PEG-IFN-α2b monotherapy group. Thereafter there was a rebound towards the end of the week. The median slope of viral rebound at the end of the first week (day 4 to day 7) was 0.060 $\log_{10}$ copies/ml/day (range -0.117–0.393). There was no effect of the baseline viral load level on the amount of viral decline in the first month of treatment.

By contrast when viral decline was analysed in the PEG-IFN-α2b and lamivudine combination therapy group (n=19), a median decline in viral load of 2.94 $\log_{10}$ copies/ml (range, 0.55–5.02) after 1 month of treatment was observed (Figure 1). There was a viral decline of 3.43 $\log_{10}$ copies/ml (range 0.71–6.25) at week 8 of treatment. The median viral decline was 0.228 $\log_{10}$ copies/ml/day (range -0.037–0.337) for the first week of treatment and 0.055 $\log_{10}$ copies/ml/day (range 0.010–0.127) between week 1 and 4. All patients treated with combination therapy showed a biphasic HBV DNA decline pattern. The median decline in viral load was 1.59 $\log_{10}$ copies/ml (range -0.26–2.36) in the first week of treatment. The median slope of viral decline at the end of the first week (day 4 to day 7) was 0.083 $\log_{10}$ copies/ml/day (range -0.297–0.250) in the combination therapy group.

Figure 1. Median HBV DNA in patients with HBeAg-positive chronic hepatitis B in the first month of treatment with PEG-IFN-α2b alone or in combination with lamivudine



Median hepatitis B virus (HBV) DNA ($\log_{10}$ copies/ml) in patients with hepatitis B virus e antigen (HBeAg)-positive chronic hepatitis B in the first month of treatment **(A)** with pegylated interferon α2b (PEG-IFN-α2b) alone or **(B)** in combination with lamivudine (LAM).

Pharmacokinetics of pegylated interferon-α2b

In a first attempt to understand why HBV DNA levels showed a minimal decline during the first month, we analysed PEG-IFN-α2b levels in all 96 patients. Maximum levels of PEG-IFN-α2b concentration were reached 1 day after administration. Thereafter, a decline in the PEG-IFN-α2b levels was seen in all patients (Figure 2A). No significant differences in PEG-IFN-α2b levels between patients treated in the PEG-IFN-α2b monotherapy and the PEG-IFN-α2b plus lamivudine combination therapy group were observed. In 52/96 patients (54%), the PEG-IFN-α2b concentration had returned to undetectable levels 7 days after drug administration; this was still the case in 24/96 (25%) patients at day 28, 7 days after the fourth injection. In those with detectable PEG-IFN-α2b levels at day 7 and 28, these concentrations were in general low with a mean of 1,175 pg/ml and 1,645 pg/ml, respectively.

The pharmacokinetics were modelled using a non-linear mixed model. The fitted non-linear mixed model resulted in a population mean of the pharmacokinetic parameters k_a, k_e and F/V_d of all 96 patients, as well as an individual fit of these parameters (Figure 2B). The estimated population mean of k_a was 2.363 d^{-1}(SE

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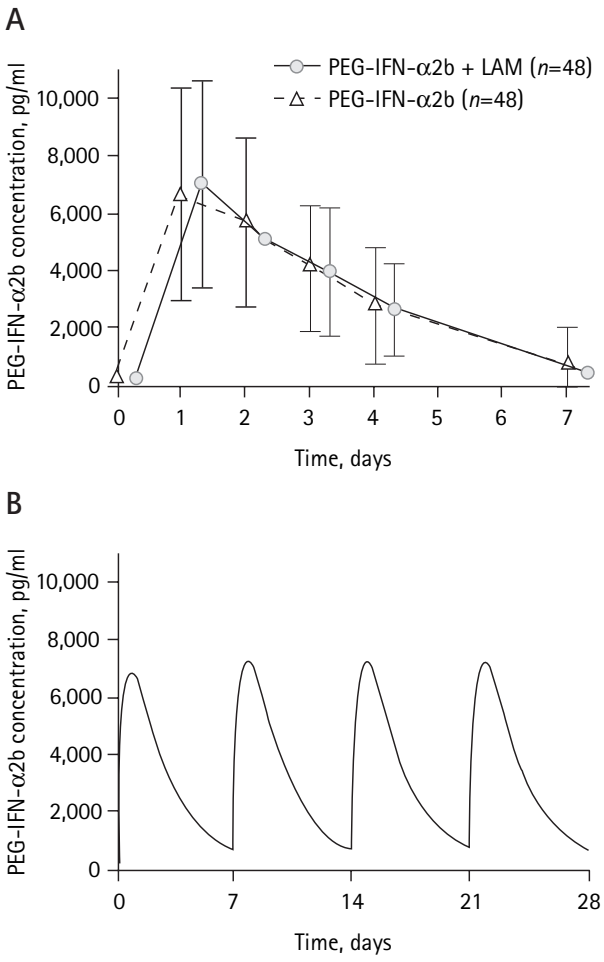
Figure 2. Pharmacokinetics of PEG-IFN-α2b in patients treated with PEG-IFN-α2b with or without lamivudine



(A) Pharmacokinetics of pegylated interferon α2b (PEG-IFN-α2b) in patients treated with PEG-IFN-α2b with or without lamivudine (LAM) in the first week of treatment. **(B)** Modelled pharmacokinetics in the first month of PEG-IFN-α2b treatment with and without LAM.

0.461), of k_e 0.420 d^{-1} (SE 0.029) and of F/V_d 1.023 pg/ml (SE 0.084) (Table 2 gives per patient data). The modelled interval between PEG-IFN-α2b administration and the maximum modelled drug concentration (t_{max}) was 0.89 day (0.71–1.24). There was a significant negative correlation between the per patient AUC of the PEG-IFN-α2b concentration for the first week of treatment and the body mass index (BMI) (P=0.024), as well as a significant relation between the AUC and sex: AUC was higher in females than in males (P=0.002).

Modelling of viral kinetics and its relation to pharmacokinetics and response

Using the non-linear mixed model it was possible to fit the first month viral kinetics data in the PEG-IFN-α2b monotherapy arm (n=19) using the results of the modelled pharmacokinetics for the first month of treatment with ε dependent on time (Figure 3; Table 2). Thus, a clear biphasic viral load decline is not observed using PEG-IFN-α2b monotherapy in HBeAg-positive chronic hepatitis B patients.

In the first week, the modelled viral load was minimal at 3.6 days (2.8–4.5) after administration of PEG-IFN-α2b. The mean and maximum estimated population antiviral effectiveness ε_{mean} and ε_{max} in patients receiving PEG-IFN-α2b monotherapy were 48% and 70% (range 24–80% and 39–98%), respectively. The infected cell loss rate d was estimated as 0.07 per day and the time delay of pharmacokinetics t_0 as 0.9 days. No clear association was found between the estimated maximum antiviral effectiveness and baseline HBV DNA levels, ALT levels, sex and BMI. Maximal effectiveness, but not mean effectiveness, was significantly smaller in older patients (P=0.046).

HBeAg loss at the end of follow up was observed in 9/19 patients. Despite the correlation between the AUC of the PEG-IFN-α2b concentration and BMI and sex, no significant difference was observed between the AUC in relation to treatment response (HBeAg loss at the end of follow up) or viral decline at the end of treatment and follow up. Furthermore, viral decline in the first month of treatment was 0.45 $\log_{10}$ copies/ml (range -0.12–1.56) in patients with a lower than median AUC and also 0.45 $\log_{10}$ copies/ml (range -0.03–1.87) in those with a higher than median AUC of the PEG-IFN-α2b concentration.

Discussion

In this study, we analysed early pharmacokinetics and HBV viral kinetics in HBeAg-positive chronic hepatitis B patients during the first 4 weeks of treatment with PEG-IFN-α2b and used the PEG-IFN-α2b pharmacokinetics to model viral decline. We observed only a minimal decline in viral load during the first month of PEG-IFN-α2b monotherapy, without a clear biphasic pattern. Given the fact that a significant number of patients is able to control the infection after 52 week of PEG-IFN-α2b treatment, immunomodulatory effects rather then direct antiviral activities of PEG-IFN-α2b might explain its beneficial effect.

In the first week of PEG-IFN-α2b treatment, we found highest drug concentrations 1 day after drug administration followed by a pronounced decline over time until the end of the week. At the end of the week, the PEG-IFN-α2b concentration returned to undetectable levels in the majority of patients. This is in accordance with previous PEG-IFN-α2b pharmacokinetic studies in patients with chronic hepatitis C [14,17–19]. On the basis of these pharmacokinetic

data, one could consider twice weekly administration of PEG-IFN-α2b. In chronic hepatitis C patients treated with twice weekly administration for 28 days, there were high PEG-IFN-α2b concentrations in the blood at all days during the week and there was no rebound in HCV RNA at the end of the week as was seen with once weekly injections [18]. Nevertheless, despite these suboptimal pharmacokinetic characteristics for PEG-IFN-α2b, the end of treatment and follow-up results of PEG-IFN-α2b and PEG-IFN-α2a – which has a prolonged higher concentration in blood – are comparable in chronic hepatitis B [6,7,9].

We analysed the pharmacokinetics during PEG-IFN-α2b therapy in all 96 patients using a model proposed by Powers *et al.* [13] and Talal *et al.* [14] for chronic hepatitis C infection. This model takes the decreasing efficacy of PEG-IFN-α2b at the end of the week into account during once weekly administration. We observed a significant correlation between the AUC of the PEG-IFN-α2b concentration and BMI and a correlation between sex and the AUC of PEG-IFN-α2b. On the basis of these findings, weight-based PEG-IFN-α2b dosing should also be considered in the treatment of chronic hepatitis B to optimize drug availability, as is the standard in hepatitis C treatment [20,21]. However, despite the influence of BMI on the pharmacokinetic constants of PEG-IFN-α2b, no clear effect of the PEG-IFN-α2b concentration was observed on treatment outcome or decline in viral load, as previously shown for PEG-IFN-α2a [22]. Furthermore, treatment of chronic hepatitis B patients with escalating doses of both PEG-IFN-α2a and -α2b did not lead to a better treatment outcome [5,16].

Next, we incorporated the pharmacokinetic model for multiple weekly PEG-IFN-α2b injections proposed recently [13,14] in a combined pharmacokinetic–pharmacodynamic model. Viral kinetics were modelled using Equations 3, 4 and 5. We were able to use per patient PEG-IFN-α2b pharmacokinetics as well as viral kinetics data in 19 patients of the PEG-IFN-α2b monotherapy group. With this approach, it was possible to fit the viral decline during the first month of PEG-IFN-α2b monotherapy in patients with HBeAg-positive chronic hepatitis B. The maximum antiviral effectiveness of PEG-IFN-α2b monotherapy (ε_{max}) was 70%, which is slightly lower than the antiviral

Table 2. Pharmacokinetic and viral kinetic parameters for the first week of treatment for the 19 patients treated with PEG-IFN-α2b monotherapy

	Pharmacokinetic parameters						Viral kinetic parameters*				
Patient	k_e, day^{-1}	k_a, day^{-1}	F/V_d, pg/ml	t_{max}, days	C_{max}, pg/ml	EC_{50}	AUC, pg/week/ml	v_0, $\log_{10}$ copies/ml	Decline week 1 $\log_{10}$ copies/ml	c, day^{-1}	ε
1	0.42	0.60	1.30	1.98	5,630	5,852	26,415	9.30	0.20	0.97	0.49
3	0.48	2.73	1.80	0.77	12,410	6,485	35,918	9.49	0.35	0.96	0.66
5	0.48	1.02	1.55	1.40	7,940	6,713	30,267	10.04	-0.48	0.90	0.54
8	0.47	4.47	1.62	0.56	12,430	1,400	33,228	9.06	0.30	0.80	0.89
9	0.55	2.93	1.86	0.70	12,610	1,480	32,823	8.68	0.34	0.91	0.89
11	0.46	0.91	1.43	1.52	7,160	3,782	28,890	8.69	0.53	0.77	0.65
12	0.43	1.34	1.31	1.25	7,630	3,969	28,079	8.36	0.24	0.87	0.66
22	0.56	4.29	1.63	0.54	12,020	276	28,295	9.57	1.15	0.97	0.98
24	0.50	2.07	1.93	0.91	12,300	2,017	37,259	8.65	0.71	0.55	0.86
27	0.37	2.08	0.88	1.01	6,020	5,881	21,372	9.03	-0.28	1.43	0.50
29	0.55	1.14	2.43	1.24	12,320	3,717	42,477	8.36	0.10	1.58	0.77
32	0.58	0.69	3.37	1.57	13,500	9,634	54,171	10.26	0.07	1.09	0.58
39	0.39	4.43	0.65	0.60	5,140	570	15,541	9.30	0.69	1.05	0.90
41	0.36	1.09	0.76	1.52	4,420	3,844	18,654	9.94	-0.07	1.09	0.54
43	0.45	1.23	1.43	1.29	8,030	12,684	29,735	9.53	0.11	1.08	0.39
45	0.37	1.56	0.87	1.21	5,540	1,078	21,063	9.13	0.42	1.26	0.84
50	0.58	3.34	1.83	0.64	12,660	5,325	31,017	9.91	0.03	0.89	0.70
51	0.58	0.71	3.29	1.56	13,360	5,282	53,428	8.85	-0.16	1.03	0.71
73	0.43	4.09	0.87	0.61	6,690	3,228	19,105	10.03	0.17	0.87	0.67
Median	0.47	1.56	1.55	1.21	8,030	3,844	29,735	9.30	0.20	0.97	0.67
Q25	0.42	1.02	0.88	0.64	6,020	1,480	21,372	8.69	0.06	0.87	0.54
Q75	0.55	3.34	1.86	1.52	12,430	5,881	35,918	9.91	0.39	1.09	0.86

*Identical estimates for all patients (fixed effects) were obtained for the pharmacokinetic time delay t_0 (0.9 day), the infected cell loss rate (0.07 per day) and the Hill coefficient (n=1). Decline week 1, the decline in viral load in the first week of treatment; Q, quartile. AUC, area under the curve.

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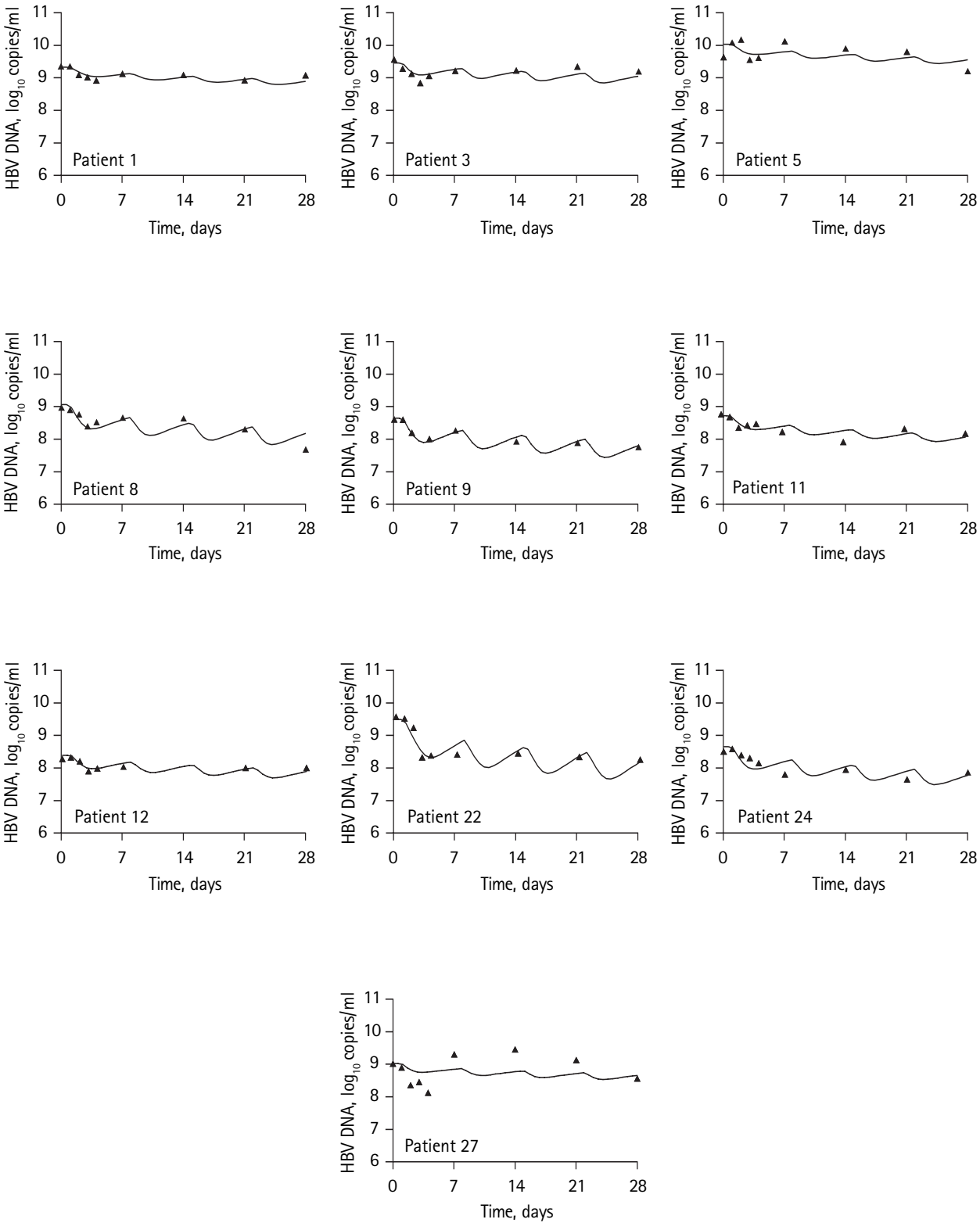
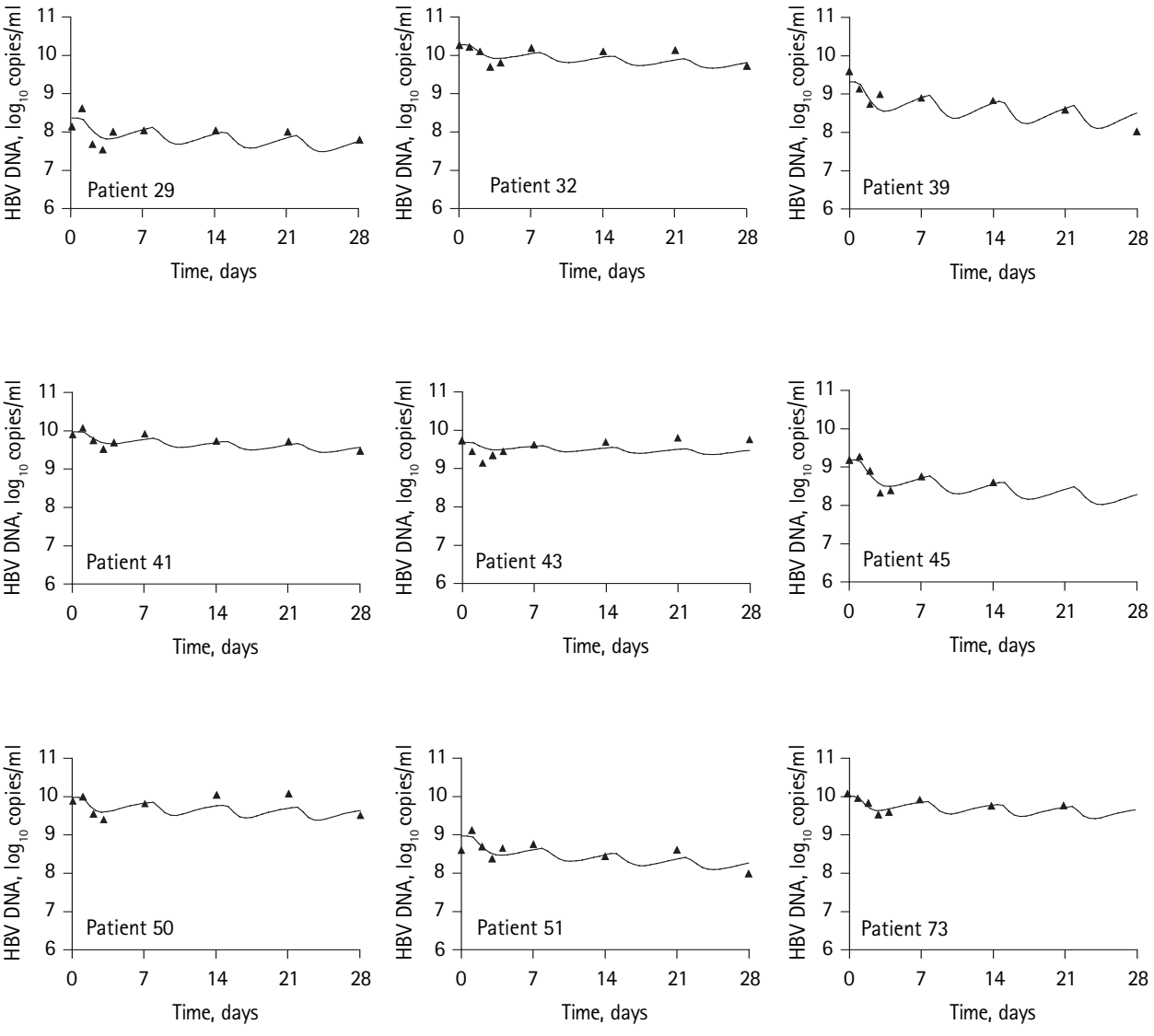
Figure 3. Modelled viral decline and observed viral load in all 19 patients treated with PEG-IFN-α2b monotherapy in the first month of treatment



effectiveness (83%) of PEG-IFN-α2b 100/200 μg in HBeAg-negative chronic hepatitis B patients reported in the study by Sypsa *et al.* [16] probably due to the lower PEG-IFN-α2b dose given. There was no clear association between the antiviral effectiveness and several baseline factors, only older patients showed a slightly reduced maximal antiviral effectiveness (P=0.046). This antiviral effectiveness is lower compared with the estimated antiviral effectiveness of approximately 92–99% for nucleos(t)ide analogues [23–26]. In the combination therapy group, viral load showed a biphasic decline pattern as a result of the addition of lamivudine. This pattern has already been extensively described in chronic hepatitis B patients treated with nucleos(t)ide analogues and, therefore, we did not model viral decline in the combination therapy group [15,24–26].

In the first week, there was a pronounced decline in viral load in the combination therapy group and after 1 month of treatment there was a 2.94 $\log_{10}$ copies/ml decline in viral load. In the monotherapy group, probably as a result of the decline in drug concentration associated with once weekly administration of PEG-IFN-α2b, we observed only a minimal decline in viral load with a rise towards the end of the week, as also recently reported by Sypsa *et al.* in HBeAg-negative

Figure 3. Continued



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chronic hepatitis B [16]. Therefore, there was only a limited decrease in viral load at the end of the first week of treatment in the monotherapy group and no clear biphasic decline pattern was observed as seen during PEG-IFN-α2a treatment in HBeAg-negative chronic hepatitis B [27]. After 1 month of PEG-IFN-α2b monotherapy there was still only a marginal decline of 0.45 $\log_{10}$ copies/ml in viral load. Regardless of this minimal decline in viral load early during treatment, treatment outcome was comparable in both treatment arms [6]. This emphasizes that a rapid early antiviral effect of PEG-IFN-α2b is not necessary for a sustained response 24 weeks post-treatment in HBeAg-positive chronic hepatitis B as it is in chronic hepatitis C infection. In line with these results, we previously showed that patients with a delayed rather than with an early viral load decline pattern exhibited the highest rates of HBeAg loss after PEG-IFN-α2b treatment [10].

In conclusion, the pharmacokinetics during the first week of therapy with PEG-IFN-α2b alone showed a peak 1 day after administration with a rapid decline thereafter. Concurrently, after an initial decline, an increase in HBV DNA was found during the second half of the week. Using the PEG-IFN-α2b pharmacokinetic data it was possible to model the HBV viral dynamics during the first month of treatment. Despite the minimal viral decline in the first weeks of PEG-IFN-α2b treatment, a sustained HBeAg response was achieved in a considerable proportion of patients.

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Additional files

An additional file containing details of all members of the HBV 99-01 Study Group can be accessed via the Volume 12 Issue 8 contents page for *Antiviral Therapy*, which can be found at www.intmedpress.com (by clicking on '*Antiviral Therapy*' then 'Journal PDFs').

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