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Review

Interferon alpha delivery systems for the treatment of hepatitis C

Sumalee Thitinan, Jason T. McConville*

College of Pharmacy, The University of Texas at Austin, Austin, TX 78712-0231, USA

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ABSTRACT

Hepatitis C virus (HCV) infections are the most common chronic blood-borne viral infections in the world. The prevalence of HCV infections varies significantly by race or ethnicity, with a high prevalence of the disease displayed in the Hispanic population. Additionally, Hispanics with chronic HCV have also more advanced hepatic fibrosis and faster liver fibrosis progression rates than either African Americans or Caucasians. Furthermore, a higher prevalence of cirrhosis and extent of mortality from liver cirrhosis is also observed in the Hispanic population compared with other groups. Current recommendations for treatment of hepatitis C are interferon alpha (IFNα)-based monotherapy and combination of IFNα preparations with ribavirin. Future treatment regimens will still be based on IFNα therapy with or without other effective antiviral agents, currently under investigation. However, there are some inherent limitations, mainly their relative short systemic circulation lifespan, and their unwanted effects on some non-target tissues. New research focuses on the development of novel modified interferon molecules which demonstrate reduced side effects and extended systemic circulation time, which can ultimately provide greater efficacy. Alternative routes for IFNα delivery, such as oral delivery, demonstrate challenging but promising areas of research for improving future patient compliance.

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* Corresponding author. Tel.: +1 512 471 0942; fax: +1 512 471 7474.
E-mail address: jtmcconville@mail.utexas.edu (J.T. McConville).

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1. Introduction

The hepatitis C virus (HCV) is a common infectious agent, affecting about 170 million individuals worldwide (Fig. 1) (Kim, 2002). In The United States, the National Health and Nutrition Examination Survey (NHANES) conducted from 1999 to 2002 showed that the overall prevalence of anti-HCV was 1.6%, corresponding to 4.1 million persons who have been infected with HCV in the United States, and of those 3.2 million persons were chronically infected with HCV (Armstrong et al., 2006).

HCV is a blood-borne pathogen, which may be transmitted through parenteral exposure to contaminated blood or body fluids (Scannell et al., 2002). Factors most strongly associated with HCV infection are blood transfusion (56%), intravenous drug abuse (31%), and alcohol consumption (44%) (Barazani et al., 2007; Scannell et al., 2002). The Centers for Disease Control and Prevention (CDC) indicated other risk factors include use of inadequately sterilized medical equipment, high-risk sexual behaviors, and social or cultural practices such as body piercing, circumcision, and tattooing. Perinatal transmission occurs but is not very efficient, except in mothers with a HIV co-infection. Although HCV can be recovered from the saliva of infected persons, casual contact has not been associated with transmission (Alter et al., 1998).

CDC reported that there were approximately 19,000 new cases of HCV infections in the United States in 2006, and of those up to 85% developed a chronic hepatitis C infection (Wasley et al., 2008). A chronic case can progress to cirrhosis, end-stage liver disease, and hepatocellular carcinoma (Durand, 2002; El-Serag, 2002; Nguyen and Ho, 2001). The majority of chronic cases of are associated with liver inflammation and slow progression of hepatic fibrosis. This progressive course leads ultimately to cirrhosis in as many as 20% of patients, although it may take as long as 20–25 years from the time of infection (Strader and Seeff, 1996). It also has been estimated that, of patients with cirrhosis approximately 1–4% will develop hepatocellular carcinoma each year (El-Serag, 2002). In addition, cirrhosis resulting from chronic hepatitis C is the leading cause of liver transplantation in the United States (Rosen, 2002). CDC estimated that 8000–10,000 chronic liver disease deaths associated with HCV occur in the United States each year, and although the number of new cases of HCV infection per year in the United States is thought to be declining, HCV-associated morbidity and mortality have been predicted to increase substantially in the near future owing to the often prolonged period of time between infection and clinical disease (Wasley et al., 2008).

The prevalence of hepatitis C is different among ethnic groups, and is more common in African-Americans and Hispanics than in Caucasians (Bonacini et al., 2001). According to the 2002 census of The United States population, Hispanics were reported for 13.3% of population and they are the fastest growing minority group (Ramirez and Patricia de la Cruz, 2003). HCV was reported to be amongst the ten leading causes of death in Hispanics nationally in 2004, chronic liver disease and cirrhosis was ranked as the sixth. Moreover, chronic liver disease was the third most common cause of death in Hispanics in the 45–64 age range for both genders (Murphy, 2000). From the NHANES III study, it was shown that there are approximately 2.7 million Americans with chronic HCV infections and the prevalence of HCV at 2.1% in Hispanics, compared to 1.5% in Caucasians (Alter et al., 1999). Hispanics with chronic HCV have also more advanced hepatic fibrosis and faster liver fibrosis progression rates than either African Americans or Caucasians (Bonacini et al., 2001; Verma et al., 2006). Furthermore, there was a higher prevalence of cirrhosis and greater extent of mortality from liver cirrhosis in Hispanics with chronic HCV compared with Caucasians (Hoyumpa et al., 2002, 2005; Lepe et al., 2006; Yoon and Yi, 2006).

Hepatitis C is caused by the hepatitis C virus, a single-stranded RNA virus that belongs to the *Flaviviridae* family. HCV shows substantial nucleotide sequence diversity distributed throughout the viral genome, so it has provided six major genotypes (Ramadori and Meier, 2001) and now, more than 50 subtypes have been reported with different geographical distributions. Genotypes 1, 2 and 3 are mostly found with a predominance of genotype 1 in The United States and Europe (Blatt et al., 2000; Friedrich-Rust et al., 2007; Nainan et al., 2006). From the NHANES III study, 73.7% of 2.7 million American with chronic hepatitis C were infected with genotype 1 (56.7% with genotype 1a, and 17.0% with genotype 1b) (Alter et al., 1999). Focused on Hispanics, the prevalence of genotype 1 was 71% including genotype 1a at 46% and genotype 1b at 24%. Also, the prevalence of genotype 2/3 and 3a were 29% and 10%, respectively (Hoyumpa et al., 2002). Genotype 1 is associated with a lower rate of response to treatment (Scannell et al., 2002). Genotype 4 can be found primarily in North Africa and the Middle East, genotype 5 in South Africa, and genotype 6 in Vietnam and Thailand (Friedrich-Rust et al., 2007). HCV genotype is not only related to the likelihood of treatment response but may also determine the duration of treatment.

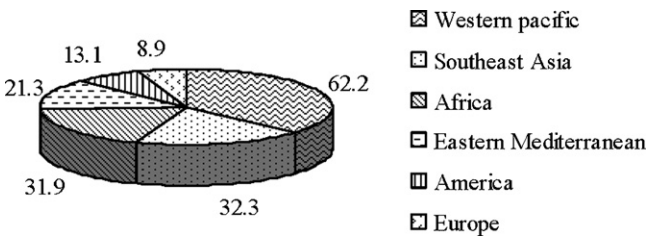

Fig. 1. Worldwide prevalence of hepatitis C in millions (total 169.7 million).

2. Current therapies for hepatitis C

The main goal of treatment of chronic hepatitis C is to eliminate detectable viral RNA from the blood. Lack of detectable HCV RNA from blood 6 months after completing therapy is known as a sustained response. Studies suggest that a sustained response is equated with a very favorable prognosis and that it may be equivalent to a cure. Therefore, sustained virological response (SVR) should be determined by measuring HCV RNA by a sensitive method (qualitative polymerase chain reaction or transcription-mediated amplification) 6 months after completing treatment (Davis, 2002).

Current recommendations for treatment of hepatitis C are interferon alpha (IFNα)-based monotherapy and combination of IFNα therapy with ribavirin, as shown in Table 1 (Ahmed and Keeffe, 1999; Bethesda, 2007; Sweetman, 2008; Tan et al., 2002) and their outcomes (SVR) as shown in Tables 2 and 3.

2.1. Monotherapy

Interferon alpha-2a (Roferon®A; Hoffmann-La Roche, Inc.), inteferon alpha-2b (Intron®A; Schering Corp.) and interferon alphacon-1 (Infergen®; Amgen, Inc.) are all approved in the United States for the treatment of adults with chronic hepatitis C as single agent. IFNα-2a and IFNα-2b are administered by intramuscular or subcutaneous injection. The starting dose of both IFNα is 3 million units given three times weekly for 24–48 weeks (Bethesda, 2007). Response rates as defined by a sustained non-detectable hepatitis C virus RNA in blood specimen were low as shown in Table 2. The average of SVR rates of IFNα-2b treatment are approximately 6% and 12–19% for 24 and 48 weeks, respectively. A SVR of a genotype 1 infection treated for 24 and 48 weeks is 2% and 7–11%, respectively. For genotypes 2 and 3, SVR has been achieved in 16% (24 weeks) and 28–33% (48 weeks) (Farrell et al., 1998; Lindsay et al., 2001; McHutchison et al., 1998; Poynard et al., 1998). Under the IFNα-2a treatment the mean SVR has been observed in 48 weeks,

Table 1
Current recommended therapy for the initial treatment of chronic hepatitis C in adults.

Drug names (brand names)	Manufacturer	FDA approval	Treatment regimen
Monotherapy			
IFNα-2b (Intron® A)	Schering Corporation	1991	3 MU, TIW, SC or IM for 48–72 weeks or for up to 96 weeks
IFNα-2a (Roferon® A)	Hoffman-La Roche, Inc.	1996–2007	Initial dose 3–6 MU, TIW, SC or IM for 24 weeks followed by 3 MU, TIW for an additional 24 weeks or 3 MU, TIW, SC or IM for 48 weeks
IFN alfacon-1/Concensus IFN (Intergen® A)	Amgen, Inc.	1997	9 μg TIW, SC for 24 weeks followed by 15 μg TIW for up to 48 weeks if necessary
IFNα-nl (Wellferon®)	GlaxoSmithKline	N/A[a]	3 or 5 MU, TIW, SC or IM for 48 weeks
PEG–IFNα-2b (PEG-Intron®)	Schering Corporation	2001	0.5 or 1 μg/kg QW, SC for 24–48 weeks
PEG–IFNα-2a (Pegasys®)	Hoffman-La Roche, Inc.	2002	180 μg QW, SC for up to 48 weeks
Combination therapy			
IFNα-2b + RBV (Rebetron™: Intron® A + Rebetol®)	Schering Corporation	1998	IFNα-2b 3 MU, TIW, SC or IM plus RBV 1000 mg daily for those weighing ≤75 kg or 1200 mg daily for those weighing >75 kg for 24–48 weeks
IFNα-2a (Roferon® A) + RBV	Hoffman-La Roche, Inc.	N/A	IFNα-2a 3–4.5 MU, TIW, SC or IM plus RBV 1000 mg daily for those weighing ≤75 kg or 1200 mg daily for those weighing >75 kg for 24–48 weeks
PEG–IFNα-2b + RBV (PEG-Intron® + Rebetol®)	Schering Corporation	2001	PEG–IFNα-2b 1.5 μg/kg QW, SC plus RBV 800 mg daily (400 mg BID) for 24–48 weeks
PEG–IFNα-2a + RBV (Pegasys® + Copegus®)	Hoffman-La Roche, Inc.	2002	PEG–IFNα-2a 180 μg QW, SC plus RBV 800–1200 mg daily (HCV genotype 1, 4: 500 mg BID in those weighing <75 kg or 600 mg BID in those weighing ≥75 kg for 48 weeks, HCV genotype 2, 3: 400 mg BID for 24 weeks)

N/A = not applicable, HCV = hepatitis C virus, RBV = ribivirin, MU = million units, SC = subcutaneous injection, IM = intramuscular injection, PO = oral administration, TIW = three times weekly, QW = once weekly and BID = twice a day.
[a] Not commercially available in the U.S. but commercially available in Canada.

and the SVR of genotype 1 and SVR of genotypes 2 and 3 have been achieved in 19–22%, 13% and 42% (Zeuzem et al., 2000a,b).

For interferon alfacon-1, the recommended dose is 9 μg given by subcutaneous injection three times a week for first time treatment and 15 μg three times a week for another 6 months for patients who do not respond or relapse (Bethesda, 2007). SVR of interferon alfacon-1 treatment (9 μg three times weekly by subcutaneous for 24 weeks) compared with a standard regimen of IFNα-2b (3 million units three times weekly by subcutaneous for 24 weeks) was 12.1 and 11.3%, respectively. In addition, there was also a better response rate for patients with genotype 1 who were treated with interferon alphacon-1 compared with those treated with IFNα-2b (24% versus 15% at end-of-treatment and 8% versus 4% sustained) (Tong et al., 1997).

IFNα-n1 (Wellferon®; GlaxoSmithKline) is not commercially available in The United States, but commercially available in Canada. The recommended dose is 3 or 5 million units three times weekly by subcutaneous or intramuscular injection for 48 weeks

Table 2
IFNα monotherapy treatment regimens for hepatitis C and therapeutic outcomes measured as a sustained virological response (SVR).

Monotherapy	Treatment regimen	SVR (%) followed treatment course						Reference
		24 Weeks			48 Weeks			
		Mean	GI	Non-GI	Mean	GI	Non-GI	
IFNα-2b	3 MU, SC, TIW	5.7						Farrell et al. (1998)
	3 MU, SC, TIW	6	2	16	13	7	29	McHutchison et al. (1998)
	3 MU, SC, TIW				19	11[a]	33	Poynard et al. (1998)
	3 MU, SC, TIW				12	6	28	Lindsay et al. (2001)
IFNα-2a	6 MU, SC, TIW				22	13	42	Zeuzem et al. (2000b)
	6MU, SC, TIW for 12 weeks, followed by 3 MU, SC, TIW for 36 weeks				19			Zeuzem et al. (2000a)
IFN alfacon-1	3 μg, SC, TIW	2.6	2	G2 = 3 G3 = 7				Tong et al. (1997)
	or 9 μg, SC, TIW	12.1	8	G2 = 21 G3 = 15				Tong et al. (1997)
IFNα-nl	3 MU, SC, TIW	9.9						Farrell et al. (1998)
	3 or 5 MU, SC, TIW				42	10.5	63	Garson et al. (1997)
PEG–IFNα-2b	0.5 μg/kg, SC, QW				18	10	35	Lindsay et al. (2001)
	or l.0 μg/kg, SC, QW				25	14	47	Lindsay et al. (2001)
	or l.5 μg/kg, SC, QW				23	14	49	Lindsay et al. (2001)
PEG–IFNα-2a	180 μg, SC, QW				39			Zeuzem et al. (2000a)
	90 μg, SC, QW				15	5	29	Heathcote et al. (2000)
	or l80 μg, SC, QW				30	12	51	Heathcote et al. (2000)
	180 μg, SC, QW				36	31	50	Reddy et al. (2001)
	180 μg, SC, QW				29	21	45	Fried et al. (2002)

G1 = genotype 1, non-G1 = non-genotype 1, RBV = ribavirin, MU = million units, SC = subcutaneous injection, PO = oral administration, TIW = three times weekly, QW = once weekly and BID = twice a day.
[a] Genotype 1, 4, 5, or 6.

(Sweetman, 2008). Mean SVR was significantly better for patients treated with IFNα-n1 compared with those treated with IFNα-2b (9.9% versus 5.7%) (Farrell et al., 1998). Moreover, patients infected with HCV non-type 1 genotypes were significantly more likely to achieve SVR than were those infected with HCV genotype 1 (63% versus 10.5%) (Garson et al., 1997).

However, these IFNs alone are rarely used for the treatment of patients with chronic hepatitis C because of their low response rate.

More recently polyethylene glycol interferon alpha (PEG–IFNα), sometimes called pegylated interferon, has been studied in patients with hepatitis C. PEG–IFNα is an antiviral agent that contains IFNα (recombinant DNA origin) covalently bound to polyethylene glycol (PEG) monomethoxy ether. PEG–IFNα increases the size of the IFNα molecule. The absorption rate is slower, and the overall time it remains in the body increases (prolonged biological half-life). As the rate of IFNα clearance decreases the duration of biological activity also increases compared to unmodified IFNα. As a result the PEG–IFNα has allowed a decrease in the weekly dosing frequency from 3 to 1. Two different PEGylated recombinant IFN-α species, i.e., PEG–IFNα-2a (Pegasys®; Hoffmann-La Roche, Inc.) and PEG–IFNα-2b (PEG-Intron®; Schering Corp.), are now approved for the treatment of chronic hepatitis C. While a recommend dose of PEG–IFNα-2b is 0.5–1.0 μg per kg body weight, PEG–IFNα-2a is dosed independent from body weight with 180 μg as shown in Table 1 (Bethesda, 2007; Sweetman, 2008).

PEG–IFNα-2b is a conjugation of recombinant IFNα-2b with a linear 12 kDa mono-methoxylated PEG (Gilbert and Park-Cho, 1997). The antiviral specific activity for PEG–IFNα-2b was 28%, relative to unconjugated IFNα-2b. After subcutaneous administration, both PEG–IFNα-2b (0.1–2.0 μg/kg once weekly) and the IFNα-2b (3 MIU three times weekly) have been shown to be rapidly absorbed (Grace et al., 2001). One compartment modeling of pharmacokinetic data has indicated that mean absorption half-life ($t_{1/2ka}$) for PEG–IFNα-2b was slightly longer than for nonpegylated IFNα-2b (2.3 h versus 4.6 h). However, because of sustained maximal concentrations, time to maximum plasma concentration (t_{max}) has been indicated to be longer for PEG–IFNα-2b (range of means was 15–44 h) compared with nonpegylated IFNα-2b (mean of 8 h). After absorption, PEG–IFNα-2b has displayed sustained maximal serum concentrations for 48–72 h after dose administration, followed by a slow elimination phase. In the same study, mean serum nonpegylated IFNα-2b concentrations declined rapidly after reaching peak

Table 3
Combination therapy treatment regimens for hepatitis C and therapeutic outcomes (SVR).

Combination therapy	Treatment regimen	SVR (%) followed treatment course						Reference
		24 Weeks			48 Weeks			
		Mean	GI	Non-GI	Mean	GI	Non-GI	
IFNα-2b + RBV	IFN 3 MU, SC, TIW and RBV PO, BID at a total daily dose of 1000 mg for patients who weighed ≤75 kg or 1200 mg for those who weighed >75 kg	31	16	69	38	28	66	McHutchison et al. (1998)
	IFN 3 MU, SC, TIW and RBV PO, BID at a total daily dose of 1000 mg (bodyweight <75 kg) or 1200 mg (bodyweight ≥75 kg) per day	35	18[a]	64	43	31[a]	64	Poynard et al. (1998)
	IFN 3 MU, SC, TIW and RBV PO, BID at a total daily dose of 1000 mg for patients who weighed ≤75 kg or 1200 mg for those who weighed >75 kg per day				44	36	61	Fried et al. (2002)
	IFN 3 MU, SC, TIW and RBV PO, BID at a total daily dose of 1000 mg (bodyweight <75 kg) or 1200 mg (bodyweight >75 kg) per day				47	33	79	Manns et al. (2001)
IFNα-2a + RBV	IFN 3 MU, SC, TIW and RBV PO, BID at a total daily dose of 1000 mg for patients who weighed ≤75 kg or 1200 mg for those who weighed >75 kg per day				44.6	28.2[b]	74.4	Mangia et al. (2005)
PEG–IFNα-2b + RBV	PEG–IFN 1.5 μg/kg SC, QW and RBV PO, BID at a total daily dose of 800 mg per day				54	42	82	Manns et al. (2001)
	or PEG–IFN 1.5 μg/kg SC, QW for the first 4 weeks followed by 0.5 μg/kg QW for the next 44 weeks and RBV PO, BID at a total daily dose of 1000 mg (bodyweight <75 kg) or 1200 mg (bodyweight ≥75 kg) per day				47	34	80	Manns et al. (2001)
	PEG–IFN 1.5 μg/kg SC, QW and RBV 800–1400 mg/day PO (<65 kg 800 mg; 65–85 kg 1000 mg; >85–105 kg, 1200 mg; >105 kg, 1400 mg).			G3 = 79				Zeuzem et al. (2004)
				G2 = 93				
PEG–IFNα-2a + RBV	PEG–IFN 180 μg SC, QW and RBV PO, BID at a total daily dose of 1000 mg for patients who weighed ≤75 kg or 1200 mg for those who weighed >75 kg per day				56	46	76	Fried et al. (2002)
	PEG–IFN 180 μg SC, QW and RBV PO, BID at a total daily dose of 800 mg per day		29	84		41	79	Hadziyannis et al. (2004)
	PEG–IFN 180 μg SC, QW and RBV PO, BID at a total daily dose of 1000 mg (bodyweight <75 kg) or 1200 mg (bodyweight ≥75 kg) per day		42	81	63	52	80	Hadziyannis et al. (2004)

G1 = Genotype 1, non-G1 = non-genotype 1, RBV = ribavirin, MU = million units, SC = subcutaneous injection, PO = oral administration, TIW = three times weekly, QW = once weekly and BID = twice a day.

[a] Genotype 1, 4, 5, or 6.

[b] Genotype 1, 4.

concentrations with final measurable concentrations at 15 h. Mean apparent volume of distribution was slightly higher for nonpegylated IFNα-2b (~1.4 L/kg) than for PEG–IFNα-2b (0.99 L/kg). Mean PEG–IFNα-2b half-life values ranged from 27.2 to 39.3 h, whereas mean nonpegylated IFNα-2b half-life was 4.3 h. Mean apparent clearance (CL/F) for PEG–IFNα-2b (22 mL/h kg) was approximately one tenth that of nonpegylated IFNα-2b (231 mL/(h kg)). Therefore, PEG–IFNα-2b has shown an improved pharmacokinetic profile, including 10-fold longer elimination half-life, 10-fold decreased mean apparent clearance and sustained maximal serum concentration, compared to nonpegylated IFNα-2b (Glue et al., 2000). In one study SVR rates of chronic hepatitis C patients treated with PEG–IFNα-2b 0.5, 1.0 and 1.5 μg/kg were 18, 25 and 23%, respectively. All 3 PEG–IFNα-2b dose groups had significantly higher SVR rates than the IFNα-2b group (12%). Moreover, SVR rates of genotype 1 infected patients with PEG–IFNα-2b treatment in this study were 10, 14 and 14%, for the different doses respectively and these rates were approximately 2-fold higher than seen in the IFNα-2b group (6%). Among genotype 2 or 3 infected patients, SVR rates were achieved in 35, 47 and 49%, for the different doses, respectively, considerably higher than that seen for the nonPEGylated IFNα-2b group which was 28% (Lindsay et al., 2001).

PEG–IFNα-2a is produced by the covalent attachment of recombinant IFNα-2a to a 40 kDa branched-chain PEG moiety (Zeuzem et al., 2000a). Reduced *in vitro* antiviral activity has also been observed with PEG–IFNα-2a compared to unmodified IFNα-2a. PEG–IFNα-2a had a relative antiviral specific activity of only 7% of the starting IFNα-2a (Bailon et al., 2001). However, this PEGylation reaction on IFNα-2a has led to an increased sustained absorption half-life, upon subcutaneous injection, from 2.3 to 50 h, an increased terminal half-life from 3–8 to 65 h, and a decreased clearance from 6.6–29.2 to 0.06–0.10 L/h (about 100-fold) (Harris et al., 2001). Results from randomized clinical trials indicate that PEG–IFNα-2a produces much higher end-of-treatment and SVR than standard IFNα in patients with a chronic hepatitis C virus infection, with or without cirrhosis (Fried et al., 2002; Heathcote et al., 2000; Reddy et al., 2001; Zeuzem et al., 2000a). For example, the SVR rate in patients without cirrhosis was twice as great in patients treated with PEG–IFNα-2a as in those treated with IFNα-2a (39% versus 19%) (Zeuzem et al., 2000a). In addition, the proportion of patients with HCV genotype-1 who had SVR was 31%, whereas SVR rate among the patients with non-1 genotypes was 50% (Reddy et al., 2001).

Patient tolerability of the PEG–IFNs is comparable to the nonpegylated formulations. Monotherapy with these agents produces a better response in some patients than monotherapy with the nonpegylated formulations (Backer, 2001; Glue et al., 2000; Heathcote et al., 2000; Lindsay et al., 2001; Reddy et al., 2001; Zeuzem et al., 2000a).

2.2. Combination therapy

It has been demonstrated that a combination therapy with IFNα-2a or IFNα-2b plus ribavirin for 48 weeks led to a marked improvement of hepatitis C therapy compared to monotherapy with IFNα. The result of IFNα-2b plus ribavirin treatment provided the mean SVR rate of 38–47% and SVR rate for patients with genotype 1 was 28–36% compared to 61–79% for patients with genotype-2 or 3 infections (Fried et al., 2002; Manns et al., 2001; McHutchison et al., 1998; Poynard et al., 1998). Following IFNα-2a plus ribavirin treatment, an SVR was achieved which was approximately 44% in all HCV genotype groups, 28% in the genotype-1 group and 74% in genotype-2 or 3 groups (Mangia et al., 2005). This combination was approved as a first-line therapy for treatment-naive patients.

Since 2001, the combination of PEG–IFNα and ribavirin has been established as standard therapy for chronic hepatitis C because of higher SVR rates compared with both conventional IFNα plus ribavirin therapy and PEG–IFNα monotherapy in patients with chronic hepatitis C (Shepherd et al., 2004). This combination therapy of PEG–IFNα-2b plus ribavirin and PEG–IFNα-2a plus ribavirin for 48 weeks provided an average SVR rate of 54 and 56%, respectively. The significant differences in SVR were apparent not only in the overall population, but also in those infected with hepatitis C virus genotype-1 and genotypes-2 or 3. SVR rates for patients with genotype-1 infection were 42 and 46%, respectively. Patients with genotype-2 or 3 infection showed high SVR rates of 82 and 76%, respectively (Fried et al., 2002; Manns et al., 2001). Furthermore, for patients with genotype-2 or 3 infections, SVR rates of 81–84% were achieved in patients treated with PEG–IFNα-2b plus ribavirin for 24 weeks. These results were not significantly lower than those of a 48-week therapy (79–80%) (Hadziyannis et al., 2004). Consequently, the 24-week therapy has been established as standard for genotypes-2 and 3 infections (Hadziyannis et al., 2004; Zeuzem et al., 2004). Therefore, the current treatment recommendations are therapy with combination PEG–IFNα (PEG–IFNα-2b, 1.5 μg/(kg week) or PEG–IFNα-2a, 180 μg/week) plus ribavirin (800–1200 mg/day) for 48 weeks in patients infected with genotype-1 and for 24 weeks in patients infected with genotype-2 and 3 (Bethesda, 2007; Scannell et al., 2002). The incidence of adverse event was similar in recipients of

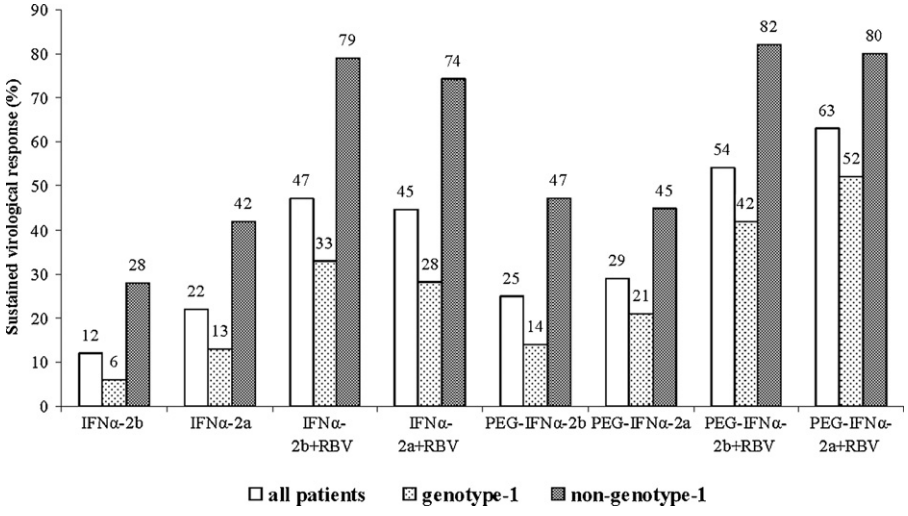

Fig. 2. Sustained virological response rates with treatment (for 48 weeks) and genotype of hepatitis C.

Table 4
Future IFNα based therapies for the treatment of hepatitis C.

	Clinical trial
New IFNα preparations	
Albuferon (albumin–IFNα-2b: IFN with prolonged half-life)	Phase 3
Locteron (controlled release)	Phase 2
Multiferon (highly purified natural IFNα)	Phase 2
Medusa Interferon (long-acting IFNα)	Phase 2
Belerofon injection (long-acting IFNα)	Phase 1
Belerofon oral IFNα (enteric coated tablet)	Phase 1
Other drugs combine with IFNα or with IFNα and ribavirin	
Hepatitis C virus inhibitors	
ISIS 14803 (anti-sense oligonucleotides)	Phase 1/2
Telaprevir (VX-950: NS3 protease inhibitor)	Phase 2
SCH-503034 (NS3 protease inhibitor)	Phase 2
Valopicitabine (NM-283: NS5B polymerase inhibitor)	Phase 2
HCV-796 (polymerase inhibitor)	Phase 2
Celgosivir (glucoside inhibitors)	Phase 2
Immunomodulations	
Merimepodib (inosine monophosphate dehydrogenase (IMPDH) inhibitor)	Phase 2b
Viramidine (prodrug of ribavirin)	Phase 3
CPG 10101 (synthetic agonist of TLR-9)	Phase 2
Thymalphasin (thymosin-alpha-1)	Phase 3
Histamine dihydrochloride (immunomodulatory/antioxidant)	Phase 2
Statins	
Fluvastatin	Phase 2
Atorvastatin	
Lovastatin	
Simvastatin	
TNF blockers	
Etanercept	Phase 2

PEG–IFNα plus ribavirin, versus conventional IFNα plus ribavirin (Fried, 2002).

We can see the higher SVR rates of combination therapy of PEG–IFNα plus ribavirin than combination therapy of IFNα plus ribavirin and monotherapy of IFNα (Fig. 2) (Fried et al., 2002; Hadziyannis et al., 2004; Lindsay et al., 2001; Mangia et al., 2005; Manns et al., 2001; Zeuzem et al., 2000b). Although the current treatment recommendations are likely to be used in combination with the combination of PEG–IFNα and ribavirin, we anticipate the potential for higher SVR rates than current treatments, and shorter treatment durations will make these new treatment options more appealing to patients and help improve treatment rates.

2.3. Interferon alpha (IFNα)-based therapy

All current treatments of hepatitis C are based on the use of various preparations of IFNα in the monotherapy or combination therapy with ribavirin. Furthermore, the data from the U.S. National Institutes of health (ClinicalTrials.gov) and many research showed that the tendency of future treatment regimens will still be IFNα-based regimens with or without other effective HCV antiviral agents which are currently under investigation (Table 4), such as HCV inhibitors, immune modulators, statins, and TNF blockers (Drittanti et al., 2007; Franciscus, 2008; Ikeda et al., 2006; Lurie et al., 2002; Parfieniuk et al., 2007; Zein, 2005).

2.3.1. Physicochemical properties

IFNα is a family of highly homologous, species-specific proteins and, occasionally, glycoproteins. It is a water-soluble protein. It is also a cytokine that not only acts through its antiviral effect, but also leads to an enhancement of immune response in hepatitis C virus treatment (Bethesda, 2007). The IFNα is classified as type 1 IFNs which is the same as IFNβ and IFNω. IFNα contains 165 or 166 amino acid residues. Comparisons among the known IFNα subtypes show primary sequence differences of 2–23%. These amino acid differences (sometimes only 3 amino acid residues) are believed to cause differences in binding affinities to the cell surface receptors, and ultimately differences in biological potency among the naturally occurring IFNα subtypes (Melian and Plosker, 2001).

There are currently four IFNα subtypes used in chronic hepatitis C: (1) IFNα-2a, (2) IFNα-2b, (3) interferon alfacon-1, and (4) IFNα-n1 (Sweetman, 2008). IFNα-2a and IFNα-2b are biosynthetic forms of IFNα (rHuIFNα) and are manufactured commercially in genetically modified *Escherichia coli* by use of recombinant DNA technology. They are composed of 165 amino acids but the peptide sequence of IFNα-2a differs from that of IFNα-2b by a single amino acid at position 23; IFNα-2a has a lysine (Lys) residue in that position, while and IFNα-2b has an arginine (Arg) residue at that position (Bethesda, 2007; Perry and Wilde, 1998; Sweetman, 2008). Both of IFNα-2a and IFNα-2b have molecular weights of approximately 19,000 daltons. Compared with other IFNα subtypes, IFNα-2a and IFNα-2b both have a deletion at position 44 in the amino acid sequence (Bethesda, 2007).

Interferon alfacon-1 (consensus interferon) is also a biosynthetic form of type 1 IFNs containing 166 amino acid residues and is manufactured in genetically modified *E. coli* by use of recombinant DNA technology. At the amino acid level, Interferon alfacon-1 shares similar structural features with IFNα (89% homology) and also has homology with IFNβ and IFNω (Alberti, 1999; Melian and Plosker, 2001).

IFNα-n1 (not commercially available in The United States, but commercially available in Canada) is a mixture of at least 8 IFNα proteins but is derived from cultured lymphoblastoid cells (from the Namalwa cell line that have been stimulated by a Sendai virus) rather than from leukocytes. The precise subtype composition of IFNα-n1currently is not known (Bethesda, 2007; Sweetman, 2008).

2.3.2. Pharmacological properties

IFNα limits amplification and spread of viruses during infections by direct mechanisms leading to the induction of an antiviral state that either protects cells from infection or attenuates the production of progeny in already infected cells, and indirect mechanisms leading to the activation of the adaptive immune response (Davis et al., 2007). Although the precise mechanisms of antiviral activity of IFNα have not been fully elucidated, IFNs with antiviral activity appear to bind to specific membrane receptors on cell surfaces and then activate tyrosine kinases which then upregulate the production of several gene products (e.g., 2′,5′-oligoadenylate synthetase, β2-microglobulin, neopterin and p68 kinases). These gene products are responsible for the antiviral, antiproliferative and immunomodulatory effects, cytokine induction and human leukocyte antigen (HLA) class I and II regulation characteristic of type I IFNs (Melian and Plosker, 2001). While both the amino and carboxy terminal regions of the molecules may be involved in eliciting antiviral activity, studies to determine which regions of the molecules confer various degrees of activity have yielded conflicting results. Some evidence indicates that different regions may be involved in eliciting various activities of the drug (Bethesda, 2007).

2.3.3. Pharmacokinetic properties

2.3.3.1. Absorption. IFNs are not absorbed from the gastrointestinal tract, and are degraded by gastric acid and proteolytic enzymes. Therefore, IFNs are administered by injection for systemic effects. Absorption of IFNα is high (greater than 80%) following either intramuscular or subcutaneous injection (Bethesda, 2007; Sweetman, 2008). Time to peak concentration (T_{max}) is 3.8 h for a single intramuscular dose of IFNα-2a, and 7.3 h for a subcutaneous dose (Perry and Wilde, 1998). Time to peak concentration of a single intramuscular or subcutaneous dose of IFNα-2b is usually 3–12 h (Bethesda, 2007). Interferon alfacon-1 T_{max} was reached at 0.3–4 h after intramuscular administration and at 3.1–6 h after subcutaneous

administration (Melian and Plosker, 2001). Although time to peak serum drug concentration after subcutaneous injection is slower than for intramuscular injection, the serum concentration–time profiles of IFNα of both administrations appear to be the same (Bethesda, 2007). IFNα is detectable in the plasma for 4–8 h after rapid intravenous injection or infusion, and for 16–30 h after intramuscular or subcutaneous administration of various doses (Bethesda, 2007).

2.3.3.2. Distribution. IFNα is widely and rapidly distributed into body tissues after parenteral administration with the highest concentrations occurring in spleen, kidney, liver, and lung (Bethesda, 2007; Sweetman, 2008). Animal studies of recombinant IFNα-2a or IFNα-2b showed that only the kidney which is the main site of IFN metabolism demonstrates substantial uptake of the drug. The volume of distribution of IFNα in humans reportedly approximates 20–60% of body weight (Bethesda, 2007). IFNα does not readily cross the blood–brain barrier following systemic administration of mixtures of naturally occurring human or recombinant IFNs in animals or humans, although low concentrations have been detected in cerebrospinal fluid following administration of large systemic doses (Bethesda, 2007; Sweetman, 2008). It is not known whether IFN crosses the placenta or is distributed into breast milk in humans, but studies in mice indicate that murine IFN is distributed into milk (Bethesda, 2007).

2.3.3.3. Elimination. Elimination of IFNα is rapid from plasma following intravenous injection or intravenous infusion in animals or humans, while more prolonged concentrations are observed following intramuscular or subcutaneous administration (Bethesda, 2007). The main route of elimination of IFNα is via renal catabolism and a negligible amount of IFNα is excreted in the urine, while hepatic metabolism and biliary excretion are minor pathways of elimination (Bethesda, 2007; Sweetman, 2008). Elimination half-lives of IFNα-2a, IFNα-2b and Interferon alfacon-1 are approximately 2–3 h after intravenous administration and 3–8 h following intramuscular or subcutaneous administration (Sweetman, 2008).

3. Novel approaches to deliver IFNα

The widespread use of IFNα suffers from some inherent limitations. Pharmacokinetic studies in humans (Radwanski et al., 1987; Wills et al., 1984) have shown that the *in vivo* half-life of IFNα is relatively short due to the small molecular size, hence rapid renal clearance, and susceptibility to serum proteases (Bailon et al., 2001; Shechter et al., 2001; Wang et al., 2002). Thus frequent injections over prolonged periods are required for efficacy. However, the thrice-weekly regimen results in a pharmacokinetic profile that exhibits a "peak and valley" pattern of blood levels (Schenker et al., 1997). In addition, IFNα treatment has many unwanted effects, such as fatigue, malaise, myalgias, headache, poor appetite, depression, irritability, anxiety, emotional lability, difficulty concentrating, forgetfulness, sleeplessness, bone marrow suppression, thrombocytopenia, and neutropenia (Bethesda, 2007; Liang et al., 2000). Thus increasing the dose to achieve longer lasting levels leads to undesirable side effects. Although pegylated interferons can improve pharmacokinetic features and provide the once-weekly regimen, the effectiveness of antiviral treatment remains unsatisfactory, especially as regards genotype-1 infections (Hugle and Cerny, 2003). Another limitation of PEG–IFN is the frequent occurrence of adverse events, which greatly influence adherence to the treatment regimen (Fried, 2002). Therefore, these constraints have become the focal points for research in the development of novel modified interferon molecules (more effective, less toxic, and more convenient than pegylated interferon), a variety of novel delivery systems and alternative routes of IFNα delivery.

3.1. Modified IFNα

3.1.1. Modified IFNα for long-circulation

3.1.1.1. Pegylated interferon. Pegylated interferon is produced by the covalent attachment of recombinant IFNα to a polyethylene glycol (PEG) moiety (PEGylation). The objective of PEGylation is to shield the IFNα molecule from enzymatic degradation, thereby reducing systemic clearance to provide long-circulating IFNα in the patient's serum. However, increasing the size of the associated PEG may result in a loss of bioactivity, although it increases the long-acting capacity of IFNα. Therefore, one of the most important considerations in PEGylation is associated with the proper selection of size and shape of the PEG moiety (Harris et al., 2001). Ramon et al. explored the conjugation of IFNα-2b to a branched-chain 40 kDa PEG ($PEG_{2,40k}$–IFNα-2b) (Ramon et al., 2005). The PEGylation reaction gave the mono$PEG_{2,40k}$–IFNα-2b product at approximately 30–55% and other byproducts, including poly$PEG_{2,40k}$–IFNα-2b and unmodified IFNα-2b. Compared to the poly$PEG_{2,40k}$–IFNα-2b species, the pure (96%) mono$PEG_{2,40k}$–IFNα-2b conjugate retained a significantly higher bioactivity. However, mono$PEG_{2,40k}$–IFNα-2b showed both an *in vitro* antiviral and antiproliferative activity of about 2% of the initial IFNα-2b. Immunorecognition against IFN was reduced by the $PEG_{2,40k}$ moiety in the conjugate due to PEG-related steric hindrance. PEGylation markedly enhanced both the thermal stability at 60 °C of IFNα-2b and the resistance to protease degradation. *In vivo* pharmacokinetic studies (after intravenous bolus injection in rats) of mono$PEG_{2,40k}$–IFNα-2b showed the distribution volume parameter about 5-fold lower than of the unconjugated IFNα-2b. However, mono$PEG_{2,40k}$–IFNα-2b showed sustained serum concentrations and a 330-fold and 708-fold increase in the serum half-life and plasma residence time compared to unconjugated IFNα-2b.

Another type of PEGylation of IFNα was developed by Jo et al. (2006). They conjugated IFNα-2a to a trimer-structured PEG (PEG_3, comprised of three PEG chains) of 43 kDa PEG to improve the pharmacokinetic properties and minimize the loss of IFNα-2a bioactivity. $PEGy_3$–IFNα-2a was prepared via an amide bond due to the reaction between an *N*-hydroxysuccinimide ester derivative of a 43 kDa PEG molecule and the free amino group of IFNα-2a. The PEGylation reaction gave approximately 25% monoPEG_3–IFNα-2a, 10% diPEG_3–IFNα-2a and 60% unmodified IFNα-2a. The *in vitro* specific antiviral activity of monoPEG_3–IFNα-2a was approximately 10% of that of the native IFNα-2a. Therefore, monoPEG_3–IFNα-2a conjugate exhibited a superior antiviral activity compared to branched 40 kDa PEG–IFNα-2a (PEG–IFNα-2a). Pharmacokinetic studies in rats of monoPEG_3–IFNα-2a had about a 31-fold and 54-fold increase in the serum half-life and plasma residence time compared to unconjugated IFNα-2b after intravenous injection. In addition, the serum half-life of monoPEG_3–IFNα-2a was increased approximately 36-fold longer and the mean plasma residence time was increased approximately 38-fold compared to unconjugated IFNα-2b after subcutaneous injection.

3.1.1.2. Albuferon (albumin–IFNα-2b). Albuferon is an 85.7 kDa protein consisting of the antiviral properties of IFNα-2b genetically fused to human serum albumin (HSA). It is designed to improve the pharmacokinetic and pharmacodynamic profile of IFNα and decrease dosing frequency (Table 5). The long half-life and relatively high stability of HSA makes it an attractive candidate for fusion to short-lived therapeutic proteins, using fusion technology. HSA is the most prevalent naturally occurring blood protein in the human circulatory system, persisting within the circulation for over 20 days. *In vitro* comparisons of albuferon and IFNα have shown similar antiviral and antiproliferative activities, although albuferon was less potent on a molar basis than IFNα, as expected. It can be concluded that the physiochemical properties, binding characteristics

Table 5
A summary of new IFNα delivery approaches.

New approaches	New IFNα preparations	Dosing schedule	Clinical trial
Genetic modification of IFNα for extended therapy	Albuferon: IFNα-2b genetically fused to human serum albumin	SC every 2 weeks	Phase 3
	Belerofon: single point amino acid mutation of human IFNα	SC once weekly	Phase 1
Microspheres for extended therapy	Locteron: formulation of IFNα-2b with copolymers of poly(ethylene glycol) and poly(butylenes terephthalate)	SC every 2 weeks	Phase 2
	Medusa: formulation of IFNα-2b using the polymer based of hydrophilic chain of poly-L-glutamate and hydrophobic molecules of α-tocopherol	SC once weekly	Phase 2
Alternative routes of administration	Belerofon: enteric coated tablet (single point amino acid mutation of human IFNα)	Oral daily	Phase 1

SC = subcutaneous injection.

and apparent biological activity of IFNα are preserved in the albumin–IFNα fusion protein. *In vivo* studies in monkeys have demonstrated that albuferon has a prolonged elimination half-life (68 and 93 h after 30 μg/kg intravenous and subcutaneous injection, respectively). In addition, in other monkey models albuferon showed 140-fold slower rate of clearance and 18-fold longer half-life than for IFNα given by subcutaneous route (Osborn et al., 2002).

A phase1 dose-ranging study, evaluated a limited exposure to albuferon to assess its safety, pharmacokinetics and pharmacodynamic parameters in patients who had failed prior IFNα therapy. The results showed that albuferon had a favorable safety profile at doses up to 1200 μg and there were no discontinuations associated with adverse events. Reduced clearance resulted in a mean elimination half-life of 159 h, which supports dosing at 2–4-week intervals (Balan et al., 2006). A phase 2 study in IFNα-naive chronic hepatitis C patients infected with hepatitis C virus genotype-1 showed that albuferon was well tolerated at dose up to 1200 μg, and that 69% of patients achieved a 2 log 10 IU/mL or greater reduction in HCV RNA at week 4 at higher doses of 900 and 1200 μg (Bain et al., 2006). Albuferon is now in phase 3 clinical studies to establish efficacy and safety for regulatory approval for treatment with 900 or 1200 μg doses every 2 weeks (Subramanian et al., 2007).

3.1.1.3. Highly purified natural leukocyte IFNα (nIFNα, Multiferon®). Highly purified natural leukocyte IFNα (nIFNα, Multiferon®) is a mixture of various physiologically glycosylated IFNα subtypes. Importantly, each subtype has an individual pharmacodynamic pattern of antiproliferative, antiviral and immunological properties. Therefore, the spectrum of activity and patient's tolerance of nIFNα is superior to a single IFNα subtype (Musch et al., 2004b). It could possibly be an alternative in the treatment of difficult-to-treat patients with chronic hepatitis C. For example, a patient with chronic hepatitis C (genotype-1b) and cirrhosis achieved complete biochemical and virological response after 14 and 16 weeks, respectively, of therapy with nIFNα (3 MU by subcutaneous injection every second day) (Musch et al., 2004b). No breakthrough occurred during a prolonged therapy (68 weeks) and no relapse occurred after treatment-free and follow-up period (44 months). The patient's tolerance of nIFNα treatment was reported to be excellent. Prior to this treatment, treatment approaches including recombinant IFNα-2b (rIFNα-2b) and natural IFNβ (nIFNβ) have been carried out but these have been unsuccessful due to breakthrough phenomena and thrombo-leukocytopenia. In contrast, after the patient was switched to nIFNα, the thrombocyte and leukocyte counts increased significantly. Furthermore, this treatment resulted in normal protein electrophoresis, spleen size and counts of thrombocytes and leukocytes. The other study reported that a patient with histologically proven chronic hepatitis C and chronic hepatitis B and additional compensated cirrhosis of the liver (Child A) were not successful with rIFNα-2b or a combination of nIFNβ and recombinant IFNγ (rIFNγ) but the patient achieved sustained complete biochemical and viral response following 5 and 14 months respectively of therapy with nIFNα 3 × 3 MU weekly (Musch et al., 2004a). Moreover, no relapse occurred after the end of treatment with nIFNα (76 months) and a follow-up period (30 months), and nIFNα was well tolerated by the patient and no substantial side effects were noted.

3.1.1.4. Belerofon. Belerofon, a modification of human IFNα with a single amino acid replacement, has been engineered by Nautilus Biotech to lower the susceptibility of IFNα to proteolytic degradation in blood and other tissues, and make it longer-lasting in serum (Table 5). It has an unchanged molecular weight and specific antiviral activity compared to native IFNα. Pharmacodynamic studies of Belerofon in cynomolgus monkeys using several surrogate markers to check anti-HCV, such as neopterin, MxA and 2′,5′-oligoadenylate synthetase, show excellent biological responses. Importantly, *in vivo* pharmacokinetics of Belerofon after subcutaneous and intravenous injection is superior to native IFNα and pegylated derivatives, and its safety profiles (toxicity, tolerability, or immunogenicity) are the same as those for native IFNα (Franciscus, 2004; Martin, 2006). Subcutaneous Belerofon is ongoing in a phase 1 clinical trial in the treatment of hepatitis C for a once weekly dosing schedule (Drittanti et al., 2007; Franciscus, 2008).

3.1.1.5. Polysialic interferon. Polysialylation is the conjugation of peptides and proteins to the biodegradable α-(2→8) linked polysialic acid (PSA) and can protect some peptides and proteins from proteolytic enzymes (Gregoriadis et al., 2005). Therefore, Hirst et al. (2002) prepared a polysialylated IFNα-2b (PAS–IFN(-2b)) using a chemical conjugation method. However, their results showed that the *in vitro* inhibitory activity of the conjugate was still 2.5–4.5 times lower than that of the native IFNα-2b due to the conditions of polysialylation which are still to be optimized for IFNα-2b.

3.1.1.6. FMS_7–IFNα-2. More recently, Shechter et al. (2001) have covalently linked seven molecules of 2-sulfo-9-fluorenylmethoxycarbonyl (FMS) to the amino groups of human IFNα-2 by a chemical conjugation method, to protect IFN from proteolysis and inactivation by serum proteases, and promote long circulation of IFN. The biological activity of the FMS_7–IFNα-2 conjugate was lower than that of the native IFNα-2. The FMS_7–IFNα-2 conjugate has approximately 4% of the biological potency and about 33% of the receptor binding capacity of the native IFNα-2. FMS_7–IFNα-2, being resistant to the proteolytic inactivation by human serum proteases *in vitro* and capable of slowly reverting to the native active IFNα-2 at physiological conditions *in vivo* and *in vitro*, maintains prolonged circulating antiviral activity in mice, exceeding 7–8 times the activity of intravenously administered native IFNα-2. A pharmacokinetic study of FMS_7–IFNα-2 in mice provided a lag time of 2 h, C_{max} at 20 h and half-life of 35 ± 4 h (about 9-fold compared to native IFNα-2). However, the optimization is still required to improve both of biological activity and pharmacokinetics of the conjugate for the further clinical studies.

3.1.1.7. IFNα-2b–AGPs. IFNα-2b–AGPs, a new method that not only provides high product yields of IFN, but also increases half-life, has been explored by Xu et al. (2007). The IFNα-2b is expressed as

arabinogalactan-protein (AGP) chimeras in cultured tobacco cells. Under this method IFNα-2b–AGPs secretion can reach to 350–1400 times higher yields than the non-glycosylated IFNα-2b control. Interestingly, IFNα-2b–AGPs exhibited antiviral specific activity units ranging from 87 to 95% that of the IFNα-2b standard. Furthermore, the IFNα-2b–AGPs showed up to a 13-fold increase as *in vivo* serum half-life, and had significant antiviral activity in the plasma during 40 h following intravenous injection. Therefore, the advantages of AGP glycomodules were to enhance production of IFNα-2b, increase the molecular size and extend serum half-life with almost complete retention of IFNα-2b biological activity, which in clinical applications potentially means decreased number of injections and a reduced cost.

3.1.2. Modified IFNα for liver-targeted delivery

One method to minimize the side effects during IFN therapy is to reduce the injection dose of IFN by targeting to its site of action, the liver. Passive trapping of microparticles by reticuloendothelium in the liver and active targeting based on hepatic receptor recognition are two main methods for liver-targeted delivery (Wu et al., 2002; Zhang et al., 2004). In chronic hepatitis there is some research focusing on active targeting of IFN to the liver by conjugation with water-soluble polysaccharides, with a high affinity for the liver, such as pullulan (Suginoshita et al., 2002; Tabata et al., 1999; Xi et al., 1996).

3.1.2.1. Pullulan conjugation. Pullulan, a linear, nonionic polysaccharide with a repeated unit of maltotriose condensed through α-1,6 linkage, has been used extensively in the food industry as well as the pharmaceutical industry. Pullulan was found to accumulate in the liver at significantly higher amounts than other water-soluble polymers, such as polyethylene glycol, polyvinyl alcohol, and dextran (Suginoshita et al., 2002; Xi et al., 1996). Therefore, Xi et al. (1996) developed an IFNα–pullulan conjugation using a cyanuric chloride method (chemical conjugation). They used a pullulan grade with the molecular weight of 200,000 to conjugate with IFNα. IFNα was shown to bind highly to pullullan, ranging from 70 to 90% of the initial IFNα amount. The IFNα–pullulan conjugate retained the antiviral activity of approximately 10% compared to unconjugated IFNα due to protein denaturation of IFNα and the steric hindrance of pullulan (impairing the receptor binding ability of IFNα). However, the IFNα–pullulan conjugate showed significantly increased liver accumulation of IFNα, greater than the unconjugated IFNα (about 36-fold at 1 h and 121-fold at 24 h following intravenous administration to mice). The biospecific interaction of some liver receptors with nonionic pullulan may be caused by the repeated sugar units of pullulan. Furthermore, IFNα–pullulan conjugate was more efficient than that of free IFNα, with respect to the IFN amount required for the induction of liver 2′,5′-oligoadenylate synthetase. In addition, the liver 2′,5′-oligoadenylate synthetase induced by conjugate injection was retained at a high level for 3 days, whereas it was lost within the first day for the free IFNα. However, the chemical coupling involves a multistep, complicated process that is poorly reproducible and loses a considerable amount of the drug activity, making it difficult to use IFN-polymer chemical conjugates clinically, despite their high pharmacologic efficacy.

Tabata et al. attempted to increase the antiviral activity of IFNα–pullulan conjugation. They developed a new IFNα–pullulan conjugation method by metal coordination instead of chemical binding (Tabata et al., 1999). Firstly, diethylenetriamine pentaacetic acid (DTPA) residues were introduced to hydroxyl group of pullulan (DTPA–pullulan), and then IFNα and the DTPA–pullulan were mixed in an aqueous solution containing Zn^{2+} ions, resulting in formation of an IFNα–DTPA–pullulan conjugate based on Zn^{2+} coordination. Approximately 60% of IFNα was conjugated to the DTPA–pullulan under Zn^{2+} coordination. The IFNα–DTPA–pullulan conjugate retained the antiviral activity of approximately 60% compared to unconjugated IFNα. This showed the higher efficiency of metal coordination than chemical conjugation. Moreover, IFNα–DTPA–pullulan conjugate showed significantly increased and prolonged liver accumulation of IFNα greater than unconjugated IFNα (about 16-fold at 1 h and 122-fold at 24 h after intravenous injection to mice). To induce activity in the liver of an antiviral enzyme 2′,5′-oligoadenylate synthetase, IFNα doses of the IFNα–DTPA–pullulan conjugate for intravenous injection were lower than those used for unconjugated IFNα injection. In addition, the induction level by the conjugate was also retained at a detectable level over 4 days, whereas induction by free IFNα was rapid lost by day 2. Prolonged retention of the large molecular weight DTPA–pullulan conjugate in the extravascular space of the liver, and suppression of intracellular metabolization is possible due to IFNα coordinately binding to pullulan. However, the lack of toxicity of the conjugate has not been finally confirmed.

3.2. Novel sustained release injectable drug delivery systems

3.2.1. Injection routes

3.2.1.1. Minipellet. The IFN minipellet is a matrix-type long-acting drug delivery system of IFNα incorporated in atelocollagen as a biodegradable carrier material. Fujioka et al. (1995b) prepared thin cylindrical pellets to be administered subcutaneously, in a similar fashion to conventional solutions for injection. Importantly, the minipellet is manufactured under mild processing conditions, without the use of organic solvents or heating. The release of IFNα from the collagen matrix varied with various processing conditions, based on the density of final collagen matrix. For the release mechanism, swelling of the matrix and diffusion of IFNα are considered to be the most important. *In vitro* release of IFNα from a cylindrical solid dosage form prepared by extrusion and air-drying of a 30% (w/w) collagen solution showed continuous release without an initial burst. Pharmacokinetic studies in mice showed that the peak concentration was reduced, minimizing side effects, and the serum IFNα concentration was maintained for a long time (5 days), but the blood IFNα concentration tended to be maintained at a lower level due to a slower release rate. The group then introduced the IFN-minipellets in which human serum albumin (HSA) was added to the collagen matrix, to promote and maintain IFNα-release (Fujioka et al., 1995a). *In vivo* results showed that serum IFNα concentrations were maintained at elevated levels for 7–10 days after administration of IFN minipellets containing 30% (w/w) HSA in the matrix.

3.2.1.2. Liposomes. Liposomes have the potential to provide controlled release and stabilize IFNα. Moreover, Liposomal IFNα preparations can alter the pharmacokinetics, tissue distribution and uptake of IFNα in comparison with free IFNα (Eppstein and Stewart, 1982). Therefore, IFNα-containing liposomes have been under investigation for many years. Karau et al. prepared IFNα-containing liposome as multilamella vesicles (MLV) by extrusion and homogenization techniques (Karau et al., 1996). The highest trapping efficiency, approximately 23% for IFNα, was found for negatively charged liposomes composed of egg phosphatidylcholine/cholesterol/dimyristoyl phosphatidylglycerol in a 6:4:1 molar ratio with a lipid concentration of 10% (w/v). The method was a one-step process and involved repeated extrusion through 0.2 μm polycarbonate filters, and mechanical high shear homogenization. Encouragingly, the activity of IFNα and the size distribution of the liposomes prepared by extrusion were stable over 4 months at 20 °C. However, the trapping efficiency for IFNα in liposomes prepared by homogenization using a microfluidizer according to the one-step method, was only approximately 2.5%.

Another idea for a sustained release injectable drug delivery system is the use of a multivesicular liposome (MVL). Since MVLs

contain a larger internal space than conventional liposomes, more drugs may be loaded. Their larger size may also deter rapid clearance by tissue macrophages, so that they may act as drug depots to enable sustained release of drugs (Howell, 2001). Therefore, encapsulation of IFNα in MVLs may also provide sustained therapeutic levels of drug and reduce systemic exposure and toxicity. Qiu et al. developed a sustained release MVL formulation of IFNα-2b using a typical double-emulsion (water-in-oil-in-water) method (Qiu et al., 2005). The IFNα-2b encapsulation efficiency in MVLs composed of lipids (molar ratio 1,2-dioleoyl-sn-glycero-3-phosphocholine:cholesterol:1,2-dipalmitoyl-sn-3-phosphoglycerol:triolein, 7:11:1:1) at a protein (triolein)-to-lipid ratio of 0.031 (w/w) was more than 60%. Moreover, with more triolein present, the lipid walls were more stable; this resulted in a longer release time. In addition, there were two different-sized populations obtained and the IFNα-2b release from MVL with smaller sizes (10–25 μm) was obviously slower than that from larger MVLs (40–60 μm). After subcutaneous injection in rat, the MVLs slowly released IFNα-2b into the systemic circulation for more than 5 days and the estimated serum half-life of IFNα-2b was approximately 30 h. Interestingly, Vyas et al. developed a sustained release formulation of IFNα-2a by using both pegylation and encapsulation in MVLs. IFNα-2a was pegylated with methoxy-polyethylene glycol of molecular weight 5000 (IFNα-2a–$mPEG_{5000}$) using a simple end-to-end mixing and rotation method (Vyas et al., 2006). The relative *in vitro* antiviral activity of pegylated IFNα-2a was found to 87.9% of the unmodified IFNα-2a. Pegylated IFNα-2a encapsulated in MVLs composed of both phospholipids and triglyceride components were also prepared by a water-in-oil-in-water double emulsification technique. Pegylated and nonpegylated IFNα-2a were entrapped in MVLs with good encapsulation efficiency approximately 65–75%. *In vitro* release profiles of IFNα-2a–$mPEG_{5000}$ containing MVLs showed a lower initial burst release less than 10% with sustained and incomplete release up to 6 days. On the contrary, unmodified IFNα-2a entrapped MVLs showed higher initial burst release nearly 35% followed by almost complete release. Moreover, MVLs were stable over 6 months at 4 °C.

In a recent study, Yang et al. could enhance the encapsulation efficiency of IFNα-2b in liposomes above 80% by using a novel modified film-hydration method employing polyvinylpyrrolidone K30 followed by homogenization (Yang et al., 2006). Polyvinylpyrrolidone K30 played an important role in improving the encapsulation of the drug and stability of liposomes. The liposomes produced by this technique were a mixture of mainly unilamellar vesicles and a small number of multilamellar vesicles. While the number of homogenization cycles increased, the size of liposomes was reduced and the activity of IFNα-2b was decreased. Under these limitations a mean size of 101 nm with 9.9% loss of IFNα-2b activity was an optimum size for the liposomes containing IFNα-2b and their physical stability for 22 months at 4 °C showed the mean size increasing slightly from 101 to 122 nm. Interestingly, the liposomes with size ≤100 nm can take IFNα-2b through the lymph system into the blood circulation by protecting IFNα-2b from the enzymes *in vivo*. This would result in a longer half-life, higher bioavailability, and higher levels in the liver. However, further *in vivo* pharmacokinetic studies are required to prove this hypothesis.

3.2.1.3. Microsphere drug delivery systems. Microsphere drug delivery systems based on polylactide (PLA), polylactide-co-glycolide (poly lactic-glycolic acid; PLGA) and poly-DL-lactide-poly(ethylene glycol) (PELA) have been extensively investigated to encapsulate protein or peptide drug, including IFNα. However, the potential of IFN formulated in these systems has been limited by the decreased activity of IFN during the encapsulation and/or release process. Consequently, new stabilization approaches are required. Zhou et al. developed a novel microsphere-based delivery system comprising calcium alginate microcores coated by a biodegradable PELA wall (Zhou et al., 2002). IFNα-2a was loaded within the alginate microcores using a high-speed stirrer and then microencapsulated into PELA copolymer using a water-in-oil-in-water solvent extraction method. This system showed the highest biological activity (48%) and encapsulation efficiency (68.7%) of IFNα-2a, compared to the conventional microspheres based on biodegradable PLGA and PELA. This was because the IFNα-2a was entrapped within alginate complex microcores, thus avoiding direct contact with organic solvents and preserving biological activity. From the *in vitro* release study the initial burst release of IFNα-2a from the core-coated microspheres was the least compared with PLGA and PELA microspheres. In addition, the core-coated microspheres showed sustained and gradual release of IFNα-2a over 13 days and preserved the biological activity of IFNα-2a for up to 11 days longer than the conventional microspheres. This is because the alginate microcores themselves could be protected by an acidic microenvironment which may be generated by the outer polymer degradation. The other stabilization approach was developed by Sanchez et al. (2003). IFNα was encapsulated within PLGA/poloxamer blend microspheres prepared by an oil-in-oil solvent extraction technique and also within PLGA micro- and nanospheres containing poloxamer, prepared by the water-in-oil-in-water solvent evaporation technique. These biodegradable micro- and nanoparticles used poloxamer 188 (polyethylene–polypropylene glycol) as a stabilizing agent to minimize IFNα denaturation during encapsulation and/or release. In addition, human serum albumin was also added in these systems to improve the encapsulation efficiency and stability of the encapsulated IFNα. *In vitro* release studies for these systems, showed a similar pattern of release. This was an initial burst release of IFNα approximately 2–24%, followed by small pulses of immunoenzymatically detected IFNα over 30 days providing therapeutic levels, whilst avoiding the adverse effects.

To achieve a long-acting formulation by using microsphere drug delivery systems Diwan and Park have used pegylated IFNα-2a encapsulated in PLGA microspheres (Diwan and Park, 2003). IFNα-2a was pegylated with mono-methoxylated polyethylene glycol (mPEG, MW 2000 or 5000 Da), and the optimized conjugates were microencapsulated in PLGA microspheres prepared by a double emulsion (water-in-oil-in-water) technique. *In vitro* release profiles from biodegradable PLGA microspheres, native IFNα-2a released only about 16% after 3 weeks, but IFNα-2a–$mPEG_{2000}$ and IFNα-2a–$mPEG_{5000}$ conjugates released approximately 73 and 57%, respectively after the same period. Therefore, the PEG chain length also influenced the release rate of IFNα-2a from microspheres. Moreover, the pegylated IFN showed significantly greater resistance towards aggregation induced under the harsh conditions for microencapsulation in contrast to native IFNα-2a.

Recently, Biolex Therapeutics Incorporation has developed a novel controlled release formulation of IFNα-2b (Locteron™) to be administered every 2 weeks for chronic hepatitis C treatment (Table 5). The biodegradable polymer used in Locteron™ is OctoPlus' PolyActive™, a series of poly(ether ester) multiblock copolymers, based on poly(ethylene glycol) and poly(butylenes terephthalate). This biodegradable polymeric microsphere can release unmodified IFNα-2b with near zero-order release kinetics to achieve steady serum levels (Bechet et al., 2006). In a phase 2a study, 480 μg Locteron™ administered subcutaneously every 2 weeks, plus weight-based ribavirin for 12 weeks, to treatment-naive patients with chronic hepatitis C (genotype-1) gave the highest efficacy compared to other doses, i.e.,160, 320 and 640 μg (Dzyublyk et al., 2007). After 12 weeks with 480 μg Locteron™ plus ribavirin, the average viral reduction was 4.2 logs, 63% of subjects were HCV negative, and early viral response (EVR: 12-week ≥2-log drop in HCV RNA) was achieved in 100% of subjects. Moreover, clinical adverse events were shown to be generally mild.

More recently, a Medusa II formulation of IFN alpha-2b (IFNα-2b XL) for slow release, has been developed by using the polymer-based Medusa® system (Flamel Technologies) (Chan et al., 2007) (Table 5). The Medusa II polymer consists of hydrophilic chains of poly-L-glutamate and hydrophobic molecules of α-tocopherol (vitamin E) randomly grafted to some of the glutamate units through a hydrolysable ester bond by a conventional esterification method in solution phase. Therefore, after purification, the polymer isolated in water forms a colloidal suspension of nanoparticles (10–50 nm), and then may be simply mixed with an aqueous solution of IFNα-2b, without the use of organic solvents. The physical binding of IFNα-2b to the hydrophobic nano-domains within the nanoparticles is fully reversible leading to a sustained drug release. Interestingly, the formulation process does not degrade IFNα-2b and also maintains the biological activity of IFNα-2b to approximately 97%. Pharmacokinetic profiles of IFNα-2b XL in three animal models (rats, monkeys and dogs) showed a 20-fold lower C_{max}, and clearly more extended IFNα-2b release (up to 6 days) with a loss of relative bioavailability of approximately 50%, in comparison with an IFN immediate release. In a phase 1/2 clinical trial a single subcutaneous injection over 1 week of IFNα-2b XL also showed 9-fold reduction in C_{max} and extended IFNα-2b release time over 7 days with a 50% relative bioavailability compared with standard IFNα-2b treatment. However, the number of adverse events was significantly lower for IFNα-2b XL compared with standard IFNα-2b treatment. Moreover, the reduction in the mean viral load was also higher after administration of IFNα-2b XL in the 27 MIU group than for standard IFNα-2b treatment (1.27 log versus 0.97 log), including patients with the genotype 1 (Trepo et al., 2006). Therefore, the Medusa formulation may support weekly dosing, and a phase 2 evaluation of weekly dosing of IFNα-2b XL in HCV patients is ongoing (Franciscus, 2008).

3.2.1.4. Injectable carrier. Hydroxyapatite ($Ca_{10}(PO_4)_6(OH)_2$, HAp) is a biodegradable material and the main mineral component of bones and teeth. Interestingly, it has already been reported that implanted HAp preparations of antibiotics and anticancer drugs showed extended release in animals. Therefore, Mizushima et al. developed injectable spherical porous hydroxyapatite microparticles (SP-HAp) as a biodegradable carrier for IFNα using a spray-drying method (Mizushima et al., 2006). The SP-HAp had an average diameter of 5 μm. Consequently, it could be injected subcutaneously as a suspension through a very narrow gauge needle, such as a 27-gauge needle (reducing pain on injection). The SP-HAp also had a porosity of approximately 58% providing a very large surface area for the absorption of IFNα. IFNα was adsorbed well to SP-HAp particles, but IFNα was observed to release faster from the particles than the particles could degrade (both *in vitro* and *in vivo*). After subcutaneous injection of the IFNα-containing SP-HAp preparation into mice no initial burst was observed, this was followed by a similar plasma profile to the control IFNα solution. However, the sustained release of IFNα (up to day 10) was achieved in the IFNα-containing SF-HAp preparation with human serum albumin and zinc.

3.2.2. Implants

Implantation is one of the interesting approaches to provide sustained release of IFNα and reduce the frequent injections. In 1999 Yamagata et al. prepared the cylindrical matrix implant by a heat extrusion technique using a lyophilized powder of IFNα-2a and different types of polyglycerol esters of fatty acid (PGEF) (Yamagata et al., 2000). PGEFs are synthetic substances that resemble glycerides in the structure, and are expected to be a biocompatible material. In both *in vitro* and *in vivo* release studies, IFNα-2a release from the matrices containing tetraglycerol diesters of palmitate and stearate showed a steady rate of release without a large initial burst. This maintained a high serum level of IFNα-2a for 7 days and the mean residence time of IFNα-2a was prolonged to between 76 and 93 h, following administration of matrices prepared from tetraglycerol dipalmitate and tetraglycerol distearate, respectively. The release from those matrices was concluded to be a diffusion-controlled process. In addition, the bioavailability of IFNα-2a after implantation of those matrices was high and almost equivalent to that after injection of IFNα-2a solution, indicating that in those matrices, it was stable during the release period. Around the same time Kajihara et al. also developed a sustained release formulation of IFNα, by mixing silicone as a carrier with lyophilized or spray-dried albumin powder that contained only traces of IFNα (Kajihara et al., 2000) a cylindrical matrix implant was formed. Human serum albumin (HSA) was used to control IFNα release from the silicone by promoting the penetration of water. No use of organic solvent or heating was required using this preparation method. Moreover, silicone formulations showed good chemical stability even during *in vivo* testing. In addition, they may be easily removed after finishing treatment. The IFNα release rate may be controlled by varying parameters such as the amount of additive and particle size of the IFN/HSA powder. However, the release of IFNα from a matrix type formulation is a first-order release process, i.e., the release rate decreases with time. In order to achieve a zero-order release, Kajihara et al. developed a new drug delivery system using silicone by improving the geometry of the formulation, which is named the covered-rod-type formulation (Kajihara et al., 2001). The preparation method of the covered-rod-type formulation was also conducted under mild conditions, without the need for organic solvents or heating. Covered-rod-type formulations were prepared by using the IFN/HSA powder containing different types of additives, e.g., glycine, sodium glutamate and sodium chloride, since these additives increased the osmotic pressure, and the subsequent IFNα release rate from the covered-rod-type formulations. To suppress the diffusion of IFNα in a covered-rod-type formulation, therefore, the inner part is the IFN/HSA/additive containing silicone, and the outer part which water does not penetrate is the IFN/HSA/additive-free silicone. *In vitro* data has indicated that the covered-rod-type formulation accomplishes release of IFNα at a constant rate for 30–100 days without a significant initial burst. In addition, the serum IFNα concentration was maintained at a constant level for 28 days after the IFNα covered-rod-type formulation was implanted in nude mice, indicating that IFNα was released from the covered-rod-type formulation at a constant rate, not only *in vitro*, but also *in vivo*. Therefore, the covered-rod-type formulation would be expected to reduce the frequency of administration and subsequent side effects associated with IFNα.

Another method to develop the implant device for IFNα delivery was explored by Mohl et al. The group developed a tristearin implant device containing lyophilized IFNα-2a and polyethylene glycol 6000 (PEG 6000) for continuous delivery of IFNα-2a by compression (Mohl and Winter, 2004). Importantly, IFNα-2a was stabilized by a lyophilization process using either trehalose or hydroxypropyl-β-cyclodextrin (HP-β-CD) as stabilizer. The *in vitro* release studies indicated that more than 90% of the incorporated IFNα-2a may be liberated continuously over 1-month from systems containing 10% PEG 6000, and the initial drug release reduced to values below 25%. The constant IFNα-2a release rates over prolonged periods of time result from the diffusion of PEG 6000 out of matrices, partially compensating for the increasing length of the diffusion pathways. Over time, this diffusion of PEG promotes the complete release of IFNα-2a, due formation of an interconnected porous network (Herrmann et al., 2007a,b). Moreover, integrating HP-β-CD into the matrices led to faster, and more complete release of IFNα-2a due to solubilizing effects (Mohl and Winter, 2004), and exhibited good stabilization of IFNα-2a within the matrices over 3 months and 6 months at 4 °C or room temperature (with 33%

relative humidity). In contrast to the matrices containing trehalose, the amount of total drug released was significantly reduced, and high levels of covalently linked IFNα-2a aggregates were observed, IFNα-2a dimer and trimer species, due to disulfide exchange (Mohl and Winter, 2006).

3.3. Alternative delivery routes

3.3.1. Transdermal

Transdermal delivery systems present an attractive, non-invasive delivery method to achieve steady blood levels and have the potential to limit the side effects of IFN therapy. Badkar et al. have demonstrated a novel transdermal delivery of IFNα-2b using microporation and iontophoresis in hairless rats (Badkar et al., 2007). They found that after applying a transdermal patch (Iomed TransQ1-GS-hydrogel) saturated with an IFNα-2b solution (600 mg/mL) for 4 h IFNα-2b was not delivered through intact skin by itself (passive delivery) or during iontophoresis. Interestingly, when microporation of the Altea Therapeutics PassPort™ System (2.0 cm^2; 72 micropores/cm^2) was applied on the rat abdomen followed by trandermal patch of IFNα-2b, passive delivery through micropores was shown to deliver a dose of 397 ± 67 ng over 6 h (0.067% of patch loading delivered into the systemic circulation), with steady state serum concentrations reaching a plateau at 1 h post-patch application. No "peak and valley" pharmacokinetic profile observed with subcutaneous injection occurred during patch application. Moreover, when iontophoresis was applied to microporated skin, the amount delivered was increased to 722 ± 169 ng in 6 h (0.12% of patch loading delivered into the systemic circulation). Iontophoresis did not have any effect on the bioactivity of the IFNα-2b as determined in an *in vitro* Franz diffusion cell set up. In addition, passive delivery of IFNα-2b through microporated skin was found to be dependent on the patch loading, concentration, gradient mechanism, and number of micropores per cm^2.

3.3.2. Buccal

The buccal administration route is an attractive way to deliver IFNα because this route is a convenient and safe transport method. It may reduce many related side effects by providing a constant, predictable level of drug in the blood. However, the efficiency of the buccal delivery is not currently able to compete with injection methods, therefore, an absorption promoter is required. Bile acid salts are the most efficient absorption promoters, but have a strong bitter taste, so may be unacceptable for good compliance. Starokadomskyy and Dubey have studied the possibility of IFNα delivery across the buccal mucosa by using lysalbinic acid as an absorption enhancer (Starokadomskyy and Dubey, 2006). Lysalbinic acid is a product of alkaline hydrolysis of albumin, and does not contain strong cationic or anionic functional groups. Consequently, lysalbinic acid can be classified as a non-ionic detergent with minimal irritation action and a neutral taste. In studies by the group, IFNα without absorption enhancers was detected in hamster cheek pouches after 15–30 min of incubation only at trace concentrations. Co-administration of IFNα with 1% lysalbinic acid increased the permeability of IFNα through the mucous by a factor of 5 times over 10 min. Furthermore, addition of 5% of lysalbinic acid increased IFNα transport by 6 times, in 2 min and 9 times in 10 min. Thus, lysalbinic acid has been shown to increase significantly the permeability of the hamster oral mucosa for IFNα. They have also demonstrated that lysalbinic acid has no irritating effect upon the rat buccal mucosa. The mechanism of lysalbinic acid to enhance absorption may be similar to that of other detergent enhancers (sodium dodecyl sulfate, bile salts, etc.) with paracellular routes being the major pathway through the buccal epithelium.

3.3.3. Nasal

The nasal route has been actively investigated in the last few years for IFN. However, the absorption of IFN by the nasal route was deemed poor due to many factors, such as the loss of administered drug into the throat, instability on the nasal mucosa due to pH and temperature, loss of activity on the nasal mucosa due to peptidase action, and binding of IFN to nasal mucosal cells. Therefore, the use of absorption enhancers seems necessary for significant absorption of IFN. Shim and Kim have compared the efficiency of several bile salt enhancers on the nasal absorption of IFNα in rats (Shim and Kim, 1993). Concentrations of 1% (w/w) of sodium cholate, sodium glycocholate, sodium taurocholate, sodium glycodeoxycholate, sodium taurodeoxycholate or polyoxyethylene-9-lauryl ether can improve the nasal bioavailability of IFNα. Of these bile salt enhancers, sodium taurocholate appeared to be less toxic and more effective in enhancing the nasal absorption of IFNα than other bile salt enhancers. Additionally, nasal bioavailability of IFNα was increased up to 32.3% by the addition of 1% sodium taurocholate. Therefore, sodium taurocholate seemed to be a potential candidate for a nasal absorption enhancer of IFNα. The major mechanism of bile salts in absorption enhancement is believed to be due to the disturbance of the nasal membrane integrity while hydrophobicity of the bile salts may not be an important factor to enhance the nasal absorption of IFNα.

3.3.4. Pulmonary

The pulmonary route would seem to be a promising alternative for delivering IFNα due to the large surface area of the alveolar epithelium and the short distance of the air to blood pathway from the lung. Yamada et al. examined the effects of chitosan and chitosan oligomers on the pulmonary absorption of IFNα by means of an *in vivo* pulmonary absorption experiment in rats (Yamada et al., 2005). They found that the bioavailability of IFNα in rats after pulmonary administration without chitosan was 6.9% of the amount when compared with that of an intramuscular injection. Therefore, some absorption enhancers are needed to enhance the pulmonary absorption of IFNα from the lung. In this study chitosan oligomers used as absorption enhancers, show a significant increase in bioavailability of IFNα, especially chitosan hexamer. The area under curve (AUC) of IFNα with chitosan hexamer increased 2.6-fold as compared with the control, and the bioavailability of IFNα was increased up to 17.8% compared with the intramuscular injection by the addition of 0.5% (w/v). However, chitosan polymers with high molecular weights (22–96 kDa) were not effective as absorption enhancers for the pulmonary delivery of IFNα due to their low solubility in water. Interestingly, chitosan oligomers did not cause any membrane damage to the rat pulmonary tissues, as determined by leakage of protein and lactate dehydrogenase (LDH) in bronchoalveolar lavage (BAL) fluid. However, the mechanisms for enhanced pulmonary absorption of IFNα by chitosan oligomers are not fully understood. It might be possible that chitosan oligomers may enhance the absorption of IFNα from the lung through a paracellular pathway, and inhibit the proteolytic degradation of IFNα in the lung.

More recently, Dumont et al. have developed IFNα-Fc fusions by conjugation of IFNα to the Fc fragment of IgG1 (an FcRn-binding ligand) to deliver via pulmonary route, because FcRn (the neonatal Fc receptor) is expressed in several absorptive epithelial tissues, including the lung (Dumont et al., 2006). They found that the *in vitro* antiviral activity of the IFNα-Fc monomer (a single molecule of IFNα linked to dimeric Fc) showed a significant increase efficacy compared with the dimer (two molecules of IFNα joined to dimeric Fc). In addition, the bioactivity of IFNα-Fc fusions was dependent on the length of the linker between the IFNα and Fc regions. Increasing the length of the linker (ranging from 0 to 15 amino acids) resulted in an increase in specific activity, and steric hinderance was reduced

for the dimer by the inclusion of a long linker. A pharmacokinetic study in the monkey of the IFNα-Fc dimer showed good transport properties with a pulmonary bioavailability of 50% compared to an intravenous administration, with a half-life that was approximately 20 times longer than the unconjugated IFNα. In the case of IFNα-Fc monomer, the pharmacokinetic parameters (i.e., C_{max}, AUC, and $t_{1/2}$) were similar to the dimer after inhalation of the same dose, irrespective of linker length.

3.3.5. Oral

Development of oral formulation of IFN would greatly expand its clinical application. However, bioavailability of oral IFN has been known to be low. Shim and Kim have examined the mechanism of the low oral bioavailability of IFNα and found that only about 40% of IFNα absorbed from the gastrointestinal lumen can reach the systemic circulation due to proteolysis by pancreatic enzymes such as trypsin and chymotrypsin, and the large molecular size of IFNα. Moreover, IFNα is also extracted by the GI mucosa and the liver (Shim and Kim, 1993).

More recently, Nautilus Biotech has formulated lyophilized Belerofon together with inactive ingredients to produce enteric-coated tablets for oral administration (Table 5). Molecular units of oral Belerofon are the same as units of subcutaneous Belerofon (a single point amino acid mutation of native IFNα, designed for higher resistance to proteolysis) (Drittanti et al., 2007). Oral bioavailability of Belerofon is based on its low sensitivity to protease degradation in the intestine which renders the molecule available for absorption into the bloodstream. In animal studies, appropriate oral doses of Belerofon can reache blood levels comparable to those obtained from subcutaneous injection of native IFNα. Furthermore, antiviral activity of Belerofon in rats after oral administration is significantly superior to that of native IFNα (Martin, 2006). Consequently, a clinical phase 1 study on Oral Belerofon will be initiated in the USA to evaluate oral Belerofon in healthy adult subjects for safety, tolerability and pharmacokinetics.

The relative benefits or limitation for various delivery systems for IFNα, are summarized in Table 6. Much work is still required for any of these listed systems to reach the patient populations as only Belerofon (Subcutaneous injection and the enterically coated oral formulation), Albuferon, and microsphere formulations are currently in Clinical Phase Studies, as indicated in Table 5.

Table 6
A comparison of IFNα delivery systems.

Delivery systems	Benefits	Limitations
Modified IFNα molecules	Long-acting of IFNα	Decrease antiviral activity of IFNα
• Chemical conjugation ◦ PEGylation ◦ Polysialylation ◦ Pullulan conjugation	Minimize side effects	
• Genetic modification	Specific site-targeting	Technically challenging to produce
◦ Albuferon	Remain high or has similar antiviral activity of IFNα	
◦ Belerofon ◦ IFNα-2b–AGPs		
Sustained release injectables	Sustained therapeutic level	Low encapsulation efficiency
• Liposomes • Microspheres	Minimizes potential side effects	Physical stability issues
• Injectable carrier • Minipellet and implants		Loss of IFNα activity Size limitation Biocompatibility issues
Alternative routes	Non-invasive	Lower bioavailability
• Transdermal • Buccal	More convenient	Technically challenging to produce (e.g., iontophoresis, microporation)
• Nasal • Pulmonary		Require absorption enhancers
• Oral		

4. Conclusion

The standard treatment of hepatitis C is with a course of IFNα, however, frequent injections and undesirable side effects can result. New research focuses on the development of novel modified interferon molecules which demonstrate reduced side effects and extended systemic circulation time, which can ultimately provide greater efficacy. Alternative routes for IFNα delivery, such as oral delivery, demonstrate challenging but promising areas of research for improving future patient compliance.

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