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Long-term survival following treatment of hepatocellular carcinoma in Singapore: evaluation of Wellferon in the prophylaxis of high-risk pre-cancerous conditions*, **

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Summary. The present paper reviews several studies performed between 1977 and 1986 in Singapore on the 10-year survival outcome of treatment for stage I and II hepatocellular carcinoma (HCC). Of 801 HCC patients evaluated, only 2 survivors (0.3%) remained in complete remission for 13 and 14 years, respectively. One had received four weekly cycles of prednisolone, Adriamycin, vincristine and 5-fluorouracil for an inoperable HCC with a 10-cm diameter, and the other had received localised synchronised hepatic irradiation and Adriamycin. As follow-up, the use of localised hepatic irradiation consisting of ^{131}I-labeled (30 mCi) iodised oil in lipiodol infused via the hepatic artery appeared to benefit patients with small residual tumours but did not affect larger tumours measuring 2 cm in diameter. Prophylactic, intermittent long-term administration of lymphoblastoid interferon-alpha (Wellferon) was carried out in pre-cancerours, high-risk hepatitis B surface antigen (HBsAg)-positive patients with cirrhosis, in immediate male relatives of liver cancer patients, and in persons who had undergone hepatic resection. In the untreated group, 10/162 (6%) cirrhotics, 3/18 (17%) male family members, and 6/10 (60%) post-resection cases developed single or multiple HCCs within 1 year of screening done at 3-month intervals on the basis of alpha-fetoprotein (AFP) levels and real-time hepatic ultrasonography. In contrast, none of the Wellferon-treated group consisting of 518 cirrhotic patients, 82 male relatives of HCC patients and 20 post-resection cases developed HCC. Two HBsAg-positive individuals who had not been treated with interferon (IFN) developed hepatic nodules which that showed dysplasia, AFP elevation and chromosomal changes. These studies demonstrate the poor results of late diagnosis and show that early intervention and prophylaxis with Wellferon can reduce the incidence of HCC in high-risk persons. In addition, transhepatic chemoembolisation and liver resection are suitable methods for treating small HCCs (single or multiple) that are detected by screening. However, some of these early-detected HCCs remain highly malignant. Prophylactic treatment of pre-cancerous conditions appears to be a better option as a long-term programme for HCC.

Introduction

Despite the many medical advances in the treatment of hepatocellular carcinoma (HCC), the disease in Singapore continues to take the lives of many adults [10, 12, 14, 16, 18], mainly because of its late presentation, with treatment being started only after symptoms have arisen. However, a better understanding of the aetiological factors has enabled short- and long-term preventive measures to be implemented. These include the introduction of prophylactic hepatitis B immunisation, discouragement of alcohol and tobacco consumption, screening for aflatoxin in imported human food materials and testing for non-A non-B viruses, e.g., hepatitis C.

However, a large number of individuals remain at risk of developing HCC. These are particularly hepatitis B surface antigen (HBsAg)-positive persons, especially immediate male relatives of HCC patients, in whom the risk appears to be relatively higher than in others [15]. Moreover, genetic factors [2] may play as important a role as do environmental factors such as HBsAg expression, aflatoxin and nitrosamines. Another large at-risk group comprises asymptomatic, HBsAg-positive chronic carriers, many of whom develop insidious cirrhosis without showing any classic clinical features, and diagnosis can often be established only on the basis of liver biopsies or characteristic ultrasonographic changes. At least 40% of

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Table 1. Treatment by different chemotherapeutic regimens for stages I and II HCC initiated between 1977 and 1982: 10-year survival

Treatment regimens and schedules	Number treated	Median survival (weeks)	10-Year survival (weeks)
Regimen I:			
Prednisolone, 10 mg daily, days 1–7 Adriamycin, 50 mg/m^2, day 1 Vincristine, 1 mg/m^2, day 1 5-Fluorouracil (5-FU), 250 mg, days 1–5 (cycled every 4 weeks)	65	12	1 (CR to 14 years)
Controls	70	13	0
Regimen II:			
Prednisolone, 10 mg daily for 7 days Adriamycin, 50 mg/m^2, day 1 (cycled every 4 weeks)	205	12.5	0
Controls	180	10	0
Regimen III (use of Adriamycin as a radiosensitiser):			
Adriamycin, 10 mg i. v. daily for 30 days (to total dose not exceeding 300 mg) given synchronously with split-dose hepatic tumour irradiation (not exceeding a total dose of 3150 rad)	50	8	1 (CR)
Controls	60	12	0
Regimen IV (hormonal regimens):			
(a) Tamoxifen, 25 mg b. i. d. for 6 months	22	13	0
(b) Megace, 150 mg daily for 6 months	20	12.5	0
(c) Controls	45	12	0
Other agents:			
Levamisole	12	13	0
Carbimazole, 10 mg t. d. s. for 3 months	12	12	0
Oral 5-FU, 500 mg daily	30	10	0
Controls	30	11	0
Total number of patients treated	801		

CR, Complete remission

these chronic HBV cases are found to be 'e' antigen ('e'Ag)-positive and show evidence of concurrent hepatitis B viral DNA replication [6].

In an effort to improve both the treatment and the long-term survival of patients with HCC, a review of all previous clinical trials and studies reported between 1977 and 1986 was carried out. These included initial trials using conventional chemotherapeutic agents, radiosensitisers, localised therapy with ^{131}I-iodised lipiodol, and, more recently, prophylactic interferon-alpha (Wellferon) in patients with HBsAg-related cirrhosis and in those who had undergone surgery. The purpose of this investigation was to identify better ways of improving the treatment of HCC through the analysis of some of these clinical studies.

Patients and methods

1977–1985 studies

Patients with stage I and II disease [12] and histologically confirmed HCC were entered into various clinical trials between 1977 and 1985. Informed consent was obtained from all patients, and on the completion of each trial, the next one was initiated. Patients were entered and then randomised by lottery into treated and control groups. The treatment regimens used in each trial are shown in Tables 1 and 2. All patients were followed up monthly for the first 6 months and every 3 months thereafter. Telephone communications were established with all relatives, and confirmation of death was established through the patient's national identity card registration and/or with Cancer and Death Registries. The treatment schedules and responses are compiled in Tables 1 and 2.

1985–1986 studies

Between 1985 and 1986, two independent open-ended prophylactic studies were carried out using a natural interferon-alpha (Wellferon) as prophylaxis.

Randomised study. In the first study, 30 patients who had undergone an HBsAg-related HCC resection were randomised to receive either 50 mg/m^2 Adriamycin (one dose) and 10 mg mitomycin intravenously (one dose) followed by 3 MIU intramuscular Wellferon daily for 10 days (IFN-treated group) or the chemotherapy alone (control group). This course was given monthly for 6 months. Thereafter, IFN alone was given every 3 months. Both groups were monitored every 8 weeks for evidence of disease recurrence on the basis of AFP levels and hepatic ultrasonography.

Long-term administration of IFN for the prevention of HCC. In the second prophylactic study, randomisation was not possible because some patients preferred only to be monitored every 3 months with AFP and ultrasound, whereas others preferred to receive IFN. However, these

Table 2. Treatment by TAE using different embolisation agents in combination with anticancer agents: 1- and 3-year survival

Treatment regimens and schedules		Number treated	1-year survival	3-year survival
I	Gelfoam impregnated with 20 mg Adriamycin and 10 mg mitomycin given every 4 weeks	40	15	8 CR (20%)
	Controls	38	0	0
II	Lipiodol mixed with 20 mg and Adriamycin 10 mg mitomycin, given every 4 weeks	50	25 (50%)	8 CR (16%)
	Controls	65	0	0
III	Lipiodol mixed with 20 mg Adriamycin and 10 mg mitomycin, followed by i.v. infusion of 300 mg carboplatin in D/saline over 3 h on day 2	62	30 (20 CR, 10 PR)	20 CR (32%)
	Controls	60	0	0
IV	Iodised ^{131}I-labeled lipiodol (30 mCi) given as one dose	10	3 CR	3 CR (30%)
	Controls	22	2	1

CR, Complete remission; PR, partial remission (a 50% fall in the AFP base level and/or a 50% reduction in the tumour mass measurable by ultrasonography or CT scan)

patients were matched for sex and age. Of the 180 patients entered in the untreated group, 90% had histologically and/or ultrasonographically demonstrated cirrhotic changes, whereas the rest showed normal histology. Their ages ranged from 22 to 70 years (median, 45 years), and the ratio of men to women was 14 : 1.

The 600 persons in the IFN group received Wellferon intramuscularly at 3 MIU daily for 10 days every 3 months. Of these patients, 85% were cirrhotic, whereas the remainder had normal livers. The age range was 25–68 years (median, 46 years), and the ratio of men to women was 15 : 1. IFN was given every 3 months because our previous data had shown that it was possible to suppress HBVDNA with antiviral agents and that recrudescence occurred at between 4 and 12 weeks after termination of antiviral therapy [17]. In approximately 60% of CHBV-positive patients, the polymerase chain reaction (unpublished observations) detected HBVDNA that was not detectable by standard molecular hybridization techniques. The 3-month schedule was also chosen because of easier patient compliance with IFN therapy, and there was also evidence that other cytokines with antiviral and anti-oncogene properties were produced in response to Wellferon administration [1, 4]. These were alpha tumour necrosis factor and interleukin 1-β, which persisted in the patient for 12 weeks [4].

Results

Early intravenous chemotherapy regimens

The results of early intravenous or oral chemotherapy combinations and the use of hormones to inhibit progression of HCC were disappointing, although testosterone receptors were detectable in subjects with HCC [13, 23]. Of the 801 patients seen during the period between 1977 and 1982, only 2 survived for more than 10 years (Table 1). These two patients had contrasting diseases. The first, a Chinese man aged 55 years, was HBsAg-positive, anti-HBe-positive and HBVDNA-negative and had undergone unsuccessful laparotomy in 1977 to remove a 10-cm tumour located between the right and the left lobes of the liver. He completed six courses of combination therapy with 5-FU, Adriamycin, mitomycin C and prednisolone and remained disease-free for 14 years, showing no sign of recurrence. The second patient, a Chinese man aged 50 years, had alcoholic cirrhosis. He was HBsAg-negative and had a large right lobe tumour located posteriorly. He was treated with synchronised Adriamycin and localised hepatic irradiation of the tumour. This led to complete destruction of the lesion, but the patient continued to have problems with oesophageal variceal bleeding and hypersplenism. He survived for 13 years and remained tumour-free.

There were many early deaths in the hepatic irradiation group due to hepatic failure, gastrointestinal bleeding and haematuria. Nevertheless, in some patients in whom the irradiation could be effectively localised because of its location or focal appearances, tumour ablation was achievable. Although some of these tumours disappeared during this therapy, relapses occurred in the remaining liver. Gastrointestinal bleeding was also a problem because of hepatic venous occlusion or progression of the hepatic disease.

Transhepatic arterial chemoembolisation chemotherapy

Transhepatic arterial chemoembolisation (TAE) was introduced in 1985, and with the use of ultrasonic liver scanning, many minute HCCs measuring less than 3 cm in diameter became detectable. Better results were obtained in single tumours (less than 3 cm) or multiple smaller tumours located in a region that had previously been treated by TAE. The 3-year survival value determined for each of these procedures (Table 2) was 20% for Gelfoam with Adriamycin and mitomycin C, 16% for lipiodol mixed with Adriamycin and mitomycin C, and 32% for lipiodol, Adriamycin, and mitomycin C followed by intravenous carboplatin. Since these studies were non-concurrent, they were not statistically analysable. The best long-term survival occurred in patients who were HBsAg- and HCV-negative and had small HCCs measuring less than 2.5 cm in size.

Another method that was used to reduce the damage caused by total hepatic irradiation was the administration

Table 3. Effect of intermittent Wellferon given after Adriamycin and mitomycin C therapy as a long-term prophylactic agent in post-resection HCC cases

	Total number of cases	Number of relapses		
		1 year	2 years	3 years
IFN-treated group	20	0 (0)	0 (0)	0 (0)
Control group (no IFN)	10	6 (60%)	1 (10%)	3 (30%)

Both groups received 50 mg/m^2 Adriamycin on day 1 and 10 mg mitomycin on day 2, cycled every 4 weeks for 6 courses. The IFN-treated group also received 3 MIU i.m. Wellferon daily for 10 days after mitomycin C, after 6 courses, Wellferon was continued at the same dose every 3 months

of lipiodol tagged with ^{131}I (30 mCi). This was given as a single dose to ten patients, three of whom had mildly elevated AFP levels of 16–20 ng/ml (normal, 0–15 ng/ml) following repeated TAE. Following single-dose administration, the isotope was detectable in the hepatic nodule(s) for 48 h, but thereafter, all ^{131}I had been excreted in the urine [18]. The three patients with minimal residual disease showed complete regression with no further tumor relapse. The remaining seven patients showed no evidence of early or late regression.

Interferon as prophylaxis

Post-hepatic resection. In the group treated with Adriamycin and mitomycin C alone, 6/10 (60%), 1/10 (10%) and 3/10 (30%) patients experienced post-resection recurrences in the remnant lobe after 1, 2 and 3 years, respectively (Table 3). Most of the early relapses occurred between 6 months and the end of the 1st year. Later relapses occurred within the next 2 years in 40% of the patients. In contrast, HCC did not develop in any of the 20 patients who received IFN in conjunction with Adriamycin and mitomycin C.

Chronic HBsAg-positive cirrhotic and non-cirrhotic patients. In the second study, in which patients were only age- and sex-matched, none of the 600 IFN-treated HBsAg-positive cirrhotic and non-cirrhotic male relatives of HCC patients actually developed HCC (Table 4).

Effect of IFN on male family members

The non-cirrhotic IFN-treated group contained 82 (14%) adult male relatives of recent HCC patients, many of the latter being either the father or brother. All of these male family members were followed up regularly for 2 years while on regular IFN treatment, and none developed HCC. In contrast, 3/18 (17%) of the untreated male relatives developed the disease. Two developed multicentric tumours that were unresectable and were refractive to many courses of repeated TAE using Adriamycin, mitomycin C and carboplatin combinations. The one survivor, who has remained disease-free for 2 years, responded to wedge resection of a right lobe tumour and is currently receiving IFN.

Of the 162 untreated HBsAg-positive cirrhotics, 10 (6%) had HCC; 6 of these patients had a single nodule located in the right lobe of the liver (diameter, 1–3 cm). The most common site was the sub-diaphragmatic portion of the right lobe of the liver. Four of these HCCs were eliminated by repeated TAE, whereas in the remaining two, wedge resections were carried out for tumours in the lower pole of the right lobe. Two patients (1%) had solitary nodules (size, 0.8–1.5 cm) associated with elevated AFP levels. Resection of these two tumours under intra-operative ultrasonic guidance showed that they were dysplastic nodules displaying chromosomal changes. None of the 518 HbsAg-positive cirrhotics treated with IFN developed HCC over a continuous follow-up period of at least 2 years.

Side effects of Wellferon

Overall, IFN was well tolerated and did not affect the patients' life-styles. In all, 70% of the patients receiving

Table 4. Effect of intermittent (four times a year) administration of Wellferon on inhibition of HCC development in chronic HBV-positive patients at risk

HBV patients	Age/sex ratio	Total number detected (1-year follow-up)	Single or multiple HCC detected	1 year post-treatment: follow-up outcome
Untreated (180): Group I–162, cirrhosis	20–70 years M:F = 12:1 (median, 45 years)	10 (+2 dysplastic nodules with chromosomal breaks)	6 single 4 multiple	6 (CR) alive 3 (PR) 1 (CR) (2 cases of dysplasia: CR)
Group II–18, male family members of HCC patients	30–65 years (median: 48 years)	3 (17%)	3 multiple	1 (CR) alive 2 dead
IFN-treated (600): Group III–518, cirrhosis	25–68 years M:F = 10:1 (median, 46 years)	0	0	N/A
Group IV–82, male family members of HCC patients	35–70 years (median, 53 years)	0	0	N/A

CR, Complete remission; PR, partial remission

IFN developed mild 'flu'-like symptoms of fever, chills, myalgia and headache, which were alleviated by paracetamol. Fatigue occurred in 50% of the patients. Reversible mild leucopenia and thrombocytopenia were seen in all subjects. An erythematous pruritic rash occurring over the trunk and limbs was observed in 2 patients (0.3%) and required discontinuation; this effect was not reproduced on subsequent administration of IFN. Syncope with postural hypotension was seen in 5 patients (0.8%); it resolved when IFN was discontinued, and subsequent doses were spaced out at 3 MIU given thrice weekly.

Discussion

HCC is fatal once the tumours have become symptomatic. In contrast, lesions detected by screening every 3 months are small and more amenable to treatment. The favored treatments, which appear to be comparable in effectiveness in terms of survival, are resection and TAE. Early-detected HCCs do not always occur singly and sometimes appear synchronously at multiple sites such as the superior aspect of the right lobe of the liver, the caudate lobe and the left lobe in nodular livers. In general, the median survival of more than 1 year for cases detected by screening is far better than the survival of those detected by symptomatic presentation (3 months). In high-risk, HBsAg-positive cirrhotics, growth from an undetected tumour to one measuring about 4 cm in diameter takes about 3 months. Some of the familial-related HBsAg-positive HCCs detected by screening appeared to be highly malignant. For instance, two of the three tumours detected were rapid-growing and fatal despite their early detection. Recurrence or new lesions occurred in advanced cases of HCC, although treatment with resection or TAE had produced a transient improvement.

This rapid malignant progression of hepatitis B liver disease may be attributable to the transactivation and transformation of infected hepatocytes by previously integrated HBV virus. Such integrated genomes may alter the normal regulation of the growth of these hepatocytes. Transcription of integrated human HBV genomes such as the X gene in transgenic mice has been demonstrated to induce liver cancer [8].

Human lymphoblastoid natural IFN-α (Wellferon), expressed by cultured lymphocytes, has been shown to exert potent growth-inhibitory activity in a dose-dependent manner as judged from the uptake of [^{3}H]-thymidine by human PLC/PRF cell lines [5]. A slowing of the growth of tumour transplants was also observed in athymic mice treated with this interferon at 2×10^5 IU per day. Furthermore, very high doses of alpha 2aIFN (18 MIU/m^2 daily) induced more significant tumour regression than did doxorubicin in Chinese patients when given continuously [9]. The mode of action appeared to be a direct antiproliferative effect on tumour cells. Wellferon also stimulates the production of other antiviral inhibitory cytokines, e.g., tumour necrosis factor α and interleukin-1β [4]. It also causes the expression of a major histocompatibility class I antigen on the surface of these tumours, making them susceptible to natural killer (NK) cells, monocytes and cytotoxic cell destruction. IFN also inhibits the activity of tumour cellular protein production by depressing the tumour's 2,5-oligoadenylic acid synthetase activity [5].

Among all of the prophylactic groups studied in which there was a high risk for the early occurrence of HCC, no HCC developed in the Wellferon-treated HBsAg-positive groups. These included patients with known HBsAg cirrhosis, those with resected HCC associated with cirrhosis and immediate male relatives of patients with HCC. Although one group (HBsAg-positive cirrhotics) could not be randomised because of the patients' preference of treatment, the groups were comparable in terms of age and sex and were sufficiently large during the follow-up period.

The present studies suggest that a natural IFN-α such as Wellferon given regularly in small doses may exert an antitumour prophylactic effect. Whether this effect is durable will depend on further long-term follow-up. Wellferon may have acted through many different mechanisms beyond just an action on HBV replication, since all patients remained HBsAg-positive regardless of their HBVDNA 'e'Ag status. In the sera of six HCC patients who were HBVDNA-positive, we found non-random multiple sites of DNA mutation in the 'S' gene (unpublished observation) that were not detected in healthy carriers.

IFN can also exert an effect on transforming cells [22], and such transformation may occur as a result of altered functions of hepatitis B viral proteins (following viral DNA integration) that are known to lead to hepatic transformation [20]. Moreover, truncations and deletions of either the X gene or the pre-S/S gene can lead to alterations in the function of the integrated viral genome in infected hepatocytes. Two findings of HBVDNA integration were recently reported. First, HBVDNA was found to be integrated into a retinoic acid receptor gene and the cyclin A gene in human HCC [20]. Both of these genes are known to regulate cell division. Second, in transgenic mice containing large envelope polypeptide proteins of HBV (containing pre-S_1, pre-S_2 and S domains), liver changes were observed that ranged from necrosis to multifocal nodular hyperplasia and, several months later, to the almost simultaneous appearance of multiple malignant foci in the regenerating nodules [3].

Involvement of hepadna viruses has not been directly linked to mutations of *ras* proto-oncogenes, but activated H-*ras* and K-*ras* genes have been detected in a high percentage of human HCCs, HBV carriers [11] and mice [19]. In human HCCs, clonal mutational hot spots in the p53 gene (known to mutate in diverse human cancers) have also been found in HCC tissues [7]. In HBV-infected hepatocytes, IFNs are known to stimulate an increase (by HLA class I glycoprotein on the infected hepatocytes and tumor cells) in NK cell maturation and actively lead to inhibition of HBV proliferation and improvement in the liver histology [21].

However, other complex mechanisms involving the role of hepatocyte growth factors, tumour growth factors and insulin-like growth factors also promote the growth of pre-cancerous nodules [24]. When given for 1 year, Wellferon has been shown to inhibit and cause a decrease in hepatic fibrogenesis in patients with chronic hepatitis C

and cirrhosis as well as to normalise elevated levels of transforming factor β-1.

In contrast to many previous short-term studies involving 1–3 months' administration of IFN, all of our patients continue to undergo long-term intermittent Wellferon therapy, in some cases for as long as 5 years. None of these high-risk, HBsAg-positive patients have thus for developed HCC, whereas many subjects in previous trials of other agents have developed HCC following the cessation of treatment.

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