Treatment of hepatocellular carcinoma with octreotide: a randomised controlled study

E Kouroumalis, P Skordilis, K Thermos, A Vasilaki, J Moschandrea, O N Manousos

Abstract

Background—Standard treatment of inoperable hepatocellular carcinoma has not been established. Somatostatin has been shown to possess antimitotic activity against a variety of non-endocrine tumours.

Aims—To assess the presence of somatostatin receptors in human liver and to treat advanced hepatocellular carcinoma with the somatostatin analogue, octreotide.

Methods—Somatostatin receptors were measured in liver tissue homogenates from patients with acute and chronic hepatitis, cirrhosis, and hepatocellular carcinoma. Fifty eight patients with advanced hepatocellular carcinoma were randomised to receive either subcutaneous octreotide 250 µg twice daily, or no treatment. Groups were comparable with respect to age, sex, Okuda classification, presence of cirrhosis, and liver biochemistry and virology.

Results—Various amounts of somatostatin receptors were identified in liver tissue of all patients including those with hepatocellular carcinoma. Treated patients had an increased median survival (13 months versus four months, $p=0.002$, log rank test) and an increased cumulative survival rate at six and 12 months (75% versus 37%, and 56% versus 13% respectively). Octreotide administration significantly reduced α fetoprotein levels at six months. When a multivariable Cox's proportional hazards model was fitted, variables associated with increased survival were: treatment administration, absence of cirrhosis, increased serum albumin, and small tumours. Treated patients clearly had a lower hazard (0.383) in the multivariate analysis.

Conclusions—Octreotide administration significantly improves survival and is a valuable alternative in the treatment of inoperable hepatocellular carcinoma.

(Gut 1998;42:442-447)

Keywords: hepatocellular carcinoma; octreotide; somatostatin receptors; liver disease

Hepatocellular carcinoma (HCC) is widely distributed in different geographical areas. There is a high prevalence in Asia and Africa¹ whereas the prevalence is very low in Western societies.² In Greece, the annual death rate has been estimated to be 10-23 per 100 000 population per year.³

Treatment of inoperable HCC remains unsatisfactory and different therapeutic regimens have been tested. Recently trials with tamoxifen,⁴ flutamide,⁵ and chemoembolisation with lipiodol⁶⁻⁹ have been reported.

Somatostatin, a hormone with well proven efficacy for the treatment of hormone producing tumours¹⁰ has been tested for efficacy in non-hormone producing tumours and somatostatin receptors have been identified in various adenocarcinomas including breast, kidney, large bowel, and ovary.^{11 12} Somatostatin receptors in human liver have not been adequately studied.

The present study had two aims: to study somatostatin receptors in human liver tissue in hepatic diseases; and to assess the clinical efficacy of octreotide administration in the treatment of HCC.

Patients and methods

SOMATOSTATIN RECEPTORS

Needle liver biopsy specimens from 23 patients were used for somatostatin receptor determination. Histological diagnoses were: acute hepatitis (four patients), chronic hepatitis (nine patients), cirrhosis (six patients), and HCC (four patients).

Liver biopsy specimens were homogenised in Tris-HCl buffer, pH 7.4, containing 200 µg/ml bacitracin, 10 µg/ml leupeptin, 5 mM MgCl₂, 5 mM EGTA and 0.1 M phenylmethylsulphonyl fluoride (buffer I) using a Polytron (Ultra-Turrax T-25) homogeniser. Due to the small quantity of tissue, the homogenate was used immediately for binding studies without any further membrane preparation. The amount of protein in each sample was determined according to the Bradford method.¹³

The concentration of somatostatin receptors in the biopsy specimens sampled was determined as previously described.^{14 15} Biopsy specimen homogenates were incubated for 90 minutes at 25°C with iodine-125 labelled tyr11-somatostatin (Amersham, specific activity 2000 Ci/mmol; 1.5 nM) and buffer I in a final volume of 500 µl. The binding reaction was terminated by vacuum filtration over GF/C filters (Whatman) presoaked with 0.1% polyethyleneamine for 30 minutes and 1% bovine serum albumin for 60 minutes. Filters were washed with 15 ml cold Tris-HCl buffer, pH 7.4, and the bound radioactivity was measured in a gamma counter (LKB 75% efficiency). Different concentrations (100 nM, 1, 10, and 20 µM) of somatostatin-14 or somatostatin-28 were used to define specific binding for somatostatin receptors. No difference in the specific

Department of
Gastroenterology,
University Hospital,
PO Box 1352,
Heraklion 71100,
Crete, Greece
E Kouroumalis
P Skordilis
O N Manousos

Department of
Pharmacology
K Thermos
A Vasilaki

Department of Social
Medicine, Biostatistics
Laboratory, Medical
School, University of
Crete
J Moschandrea

Correspondence to:
Professor Kouroumalis.

Accepted for publication
16 July 1997

binding was observed between the three concentrations of the peptide and the prohormone.

THERAPEUTIC STUDY

Fifty eight patients were included in the therapeutic trial between 1992 and 1996. Inclusion criteria were liver biopsy diagnosis of HCC and/or increased levels of α fetoprotein (AFP) over 500 ng/l with compatible liver ultrasound, computed tomography scan, or hepatic angiography. Patients with small tumours judged to be suitable for surgery were excluded. Patients with variceal bleeding or hepatic encephalopathy during the last 30 days were also excluded. All patients were classified according to Okuda staging,^{16 17} while cirrhotics were also classified according to Child-Pugh criteria. Patient consent for participation in the study was obtained.

TREATMENT SCHEDULE AND FOLLOW UP

All patients included in the study were randomised into two groups with the random number method. The first group received 500 μ g of octreotide (Sandostatin, Sandoz Hellas) subcutaneously in two divided doses. Treatment was given until patient withdrawal or death. The second group received no treatment and served as controls.

All patients had a monthly follow up with routine liver biochemical tests; every two months AFP concentrations were determined and a liver ultrasound was performed every three months. All biochemical tests were performed at the central hospital laboratory. Hepatitis B virus markers were detected with commercial enzyme linked immunosorbent assay (ELISA) kits (Abbott) while an ELISA II or III was used for anti-HCV detection. A radioimmunoassay method was used for AFP estimation. All percutaneous liver biopsies were ultrasound guided.

STATISTICAL ANALYSIS

Statistical analysis was performed by standard methods.¹⁸⁻²⁰ Ten possible prognostic variables were recorded: age; sex; place of residence; serum albumin; serum bilirubin; presence of cirrhosis; presence of hepatitis B surface antigen (HBsAg), anti-HCV (hepatitis C virus) or both; size of tumour (small, medium, large, multiple); AFP (initial and repeated AFP measurement); and treatment or no treatment. The two classifications (Child-Pugh A, B, C and Okuda I, II, III) were also used. Survival times were recorded in months. Initially, the χ^2 approach was used to test the homogeneity of the treatment and control groups with respect to the prognostic factors.

Somatostatin receptor levels in the four histological groups are displayed using dot plots and were tested using a non-parametric approach.

As the distribution of AFP concentration values was highly positively skewed, logarithms were taken of three values before any analyses were performed. The resulting variables had an approximately normal distribution.

Figure 1 Somatostatin receptors in liver tissue. Patients with either low or high levels of receptors can be identified in all disease groups. HCC, hepatocellular carcinoma.

Two approaches were used in the survival analyses. Firstly, Kaplan-Meier survival curves²¹ were plotted for each factor and the corresponding log rank tests were performed, on the null hypothesis that the risk of death is the same for each level of the factor. The continuous variables—age, AFP, bilirubin, and albumin—were each grouped into a three level factor, with approximately equal numbers in each level, for appropriate Kaplan-Meier curves to be plotted and log rank tests performed. Secondly, a multivariable Cox proportional hazards model²² was fitted using stepwise regression procedures (likelihood ratio tests) to determine significant variables.

Results

SOMATOSTATIN RECEPTORS

In the 23 patients classified according to the histological groupings of acute hepatitis, chronic hepatitis, cirrhosis, or HCC, receptor levels were either low (less than 50 fmol/mg) or high (greater than 210 fmol/mg) (fig 1). Median values in the four groups were 34.0 (range 5.5–226), 28.0 (3–320), 5.25 (4.5–453), and 137 (4.5–363) fmol/mg protein. There is no evidence of a difference in the mean levels between groups (Kruskal-Wallis test, $p=0.656$, $\chi^2=1.61$ on 3 df) but the power of the test is low as the sample sizes are small.

THERAPEUTIC TRIAL

The data consist of observations on 58 HCC patients. There were 56 deaths and two censored observations. Twenty eight patients were randomised to the treatment group and 30 patients served as the control group. Analysis was by intention to treat. There were four patient withdrawals from the treatment group (none from the control group). The mean

Table 1 Clinical and laboratory data of patients groups. No differences were found (χ^2 test)

	Group I: treated (n=28)	Group II: controls (n=30)
Median age (y) (range)	69 (53–84)	68 (52–87)
Sex		
Men	23 (82%)	25 (83%)
Women	5 (18%)	5 (17%)
Cirrhosis		
Present	24 (86%)	23 (77%)
Absent	4 (14%)	7 (23%)
Mean serum bilirubin (mg%) (range)	5.8 (0.6–17.0)	6.6 (1.0–21.0)
Mean serum albumin (g/l) (range)	33.3 (25–41)	31.4 (25–40)
Child-Pugh		
A	1 (4%)	2 (6%)
B	10 (42%)	12 (38%)
C	13 (54%)	16 (56%)
Hepatitis markers		
HBsAg	6 (21%)	8 (27%)
Anti-HCV	15 (54%)	16 (53%)
HBsAg+Anti-HCV	1 (4%)	1 (3%)
Negative	6 (12%)	5 (17%)
Tumour size		
Small (< 3 cm)	3 (11%)	1 (5%)
Medium (3–8 cm)	5 (18%)	6 (19%)
Large (> 8 cm)	11 (39%)	10 (33%)
Multiple	9 (32%)	13 (43%)
AFP (ng/ml)		
< 100	10 (36%)	10 (33%)
100–299	4 (14%)	5 (17%)
300–500	4 (14%)	5 (17%)
> 500	10 (36%)	10 (33%)
Okuda stage		
I	2 (7%)	3 (10%)
II	13 (46%)	10 (33%)
III	13 (46%)	17 (57%)

Table 2 Cumulative six and 12 month survival rates for the prognostic variables

Characteristics	Number of patients	Median survival (months)	Percentage surviving six months	Percentage surviving 12 months	p Value
Therapy					0.002
Octreotide	28	13.0	75	56	
Control	30	4.0	37	13	
Cirrhosis					0.029
Yes	47	6.0	47	31	
No	11	8.0	91	40	
Okuda index					0.020
Stage I	5	16.0	100	100	
Stage II	23	7.0	55	40	
Stage III	30	7.0	56	26	
Sex					NS (0.320)
Men	48	7.0	58	34	
Women	10	4.0	40	30	
Tumour size					NS (0.551)
Small	4	11.0	75	50	
Medium	11	9.0	89	44	
Large	21	4.0	44	31	
Multiple	22	7.0	56	39	
Age (years)					NS (0.065)
< 67	19	7.0	53	32	
67–72	20	5.0	50	22	
> 72	19	8.0	63	47	
Place of residence					NS (0.414)
Heraklion	21	7.0	62	38	
Reythymnon	23	7.0	56	37	
Lasithi	8	5.0	50	25	
Chania	4	1.0	25	25	
Other	2	6.0	50	0	
AFP					NS (0.054)
	19	8.0	58	31	
	20	6.0	45	25	
	19	7.0	63	46	
Serum bilirubin (mg%)					NS (0.190)
< 1	16	13.0	65	53	
1–6	22	7.0	52	33	
> 6	20	7.0	60	27	
Serum albumin (g/l)					0.016
< 3	18	3.0	36	14	
3–4	21	8.0	68	29	
> 4	19	14.0	69	69	
Hepatitis markers					NS (0.335)
Anti-HCV	31	8.0	61	39	
HBsAg	14	4.0	36	21	
HBsAg + anti-HCV	2	6.0	50	0	
	11	8.0	64	42	

(SEM) length of follow up was 14.1 (2.17) months (range 1–42 months) for the treated patients and 6.6 (1.36) months (range 1–33 months) for the controls. There were no major dissimilarities in the treatment group regarding the prognostic variables (χ^2 test) (table 1).

Kaplan–Meier survival curves

At the time of the final analysis (May 1996) only two patients (both from the treatment group) were alive. Table 2 shows the percentage of patients surviving six and 12 months. Log rank test results for the following factors were significant:

Treatment ($p=0.0024$, $df=1$). There is a significant survival difference between those who underwent treatment and those who did not. The median survival time for those patients who received treatment was 13.0 (1.90) months (95% confidence interval 9.3 to 16.7 months) while for those who did not receive treatment it was 4.0 (1.10) months (95% confidence interval 1.9 to 6.2 months). Thirty seven per cent of non-treated and 75% of those treated were alive at six months. At 12 months the figures were 13% and 56% and at 24 months 3% and 20% respectively (fig 2).

Cirrhosis ($p=0.0285$, $df=1$). This is significant evidence that those patients with cirrhosis have a higher instantaneous risk of death than those without cirrhosis.

Survival probability for treated versus non-treated cirrhotics with HCC is shown in fig 3 ($p=0.0114$, $df=1$).

Albumin ($p=0.0161$, $df=2$). Higher albumin concentrations are associated with a decreased hazard rate.

Okuda classification ($p=0.020$, $df=2$). Okuda I patients have a median survival time of 16.0 months (SE 9.00) whereas Okuda II and Okuda III patients have median survival time of 7.0 months (SE 2.24 and 0.78 respectively).

To test the effectiveness of the drug in patients while controlling for good and poor prognostic factors, log rank tests were performed controlling for Okuda I/II versus III and small/medium versus large/multiple tumours, for treated and untreated patients. Table 3 presents subgroup results. Treatment is effective after adjustment for Okuda staging or tumour size.

Multivariate analysis

The final model fitted contains the following variables: treatment, log (AFP), albumin, cirrhosis, and tumour size (table 4). Treated patients have a lower hazard (0.383) than those who did not undergo treatment, accounting for the prognostic variables. A unit increase in the albumin concentration decreases the hazard rates by 10.1%, all other covariates remaining unchanged (as the ratio of hazard rates for cases one unit apart is 0.899). The relative risk for those with cirrhosis is 5.47—that is, estimated risk of dying is 5.47 times greater for those with cirrhosis, adjusting for the other covariates. As the concentrations of albumin and AFP increase, the hazard decreases. Having medium, large, and multiple tumours is

Figure 2 Survival curves for treated and non-treated patients with HCC.

Figure 3 Survival curves for treated and non-treated cirrhotics with HCC.

Table 3 Comparison of survival distributions of treated patients versus controls while controlling for Okuda grouping (log rank $p = 0.0134$) and tumour size (long rank $p = 0.0085$)

	Treated	Untreated
Okuda I/II	13 (1.93)	6 (1.49)
Okuda III	9 (2.51)	3 (1.73)
Small/medium	19 (2.83)	11 (4.38)
Large/multiple	13 (2.12)	4 (0.65)

Values are median survival times in months (SE).

associated with a higher risk of death compared with those with small tumours.

Effect of treatment on tumour size

Small satellite tumours (below 3 cm in diameter) disappeared after six to 12 months of treatment in five patients (fig 4). In four additional patients, the size of the tumour remained unchanged during treatment. In all other treated patients and in all untreated patients the size gradually increased.

Influence of treatment on a fetoprotein concentration

Figure 5 shows the effect of treatment for those surviving for 6, 12, 18, or 24 months of treatment. Thirteen out of 20 had a reduction

Table 4 Multivariate analysis for significant variables

Variables in the equation	Significance	Exp(B)	95% CI for Exp(B)	
			Lower	Upper
Treatment	0.0098	0.3825	0.1845	0.7929
Log AFP	0.0703	0.8758	0.7586	1.0110
Cirrhosis	0.0017	5.4720	1.8974	15.7807
Albumin	0.0115	0.8992	0.8281	0.9764
Tumour size	0.1078			
Medium versus small	0.5583	1.4983	0.3869	5.8030
Large versus small	0.0418	3.7382	1.0503	13.3050
Multiple versus small	0.1957	2.2755	0.6549	7.9063

CI, confidence interval.

Figure 4 Liver ultrasound of a patient before (top panel) and after six months (bottom panel) of treatment with octreotide. Small satellite tumours have regressed while the large tumour remains unchanged.

in AFP after treatment, in some instances greater than 50% of the initial value. When pairwise comparisons were made of the initial AFP concentrations with those at six months (using the non-parametric Wilcoxon signed ranks test), there was evidence of a systematic difference between the measurements ($p=0.039$). There were too few 12 month readings for a repeated measures analysis.

A log rank test was performed to test for differences in the survival distributions of those patients who had undergone treatment and had survived for at least six months, according to whether their AFP concentration had increased or decreased. There was no evidence of a difference ($p=0.365$) for increased AFP (median 13 months, SE 1.85), or for decreased AFP (median 14 months, SE 0.65). In the control group AFP remained either constant or gradually increased.

Adverse effects

The drug was well tolerated. A mild diarrhoea was noted at the beginning of the treatment in 40% of the patients but this subsided on continuation of treatment. The only difficulty was the need for twice daily subcutaneous injections, which was the reason for discontinuation of treatment of four patients after four to six months.

Quality of life

Appetite, body weight, and the general feeling of well being were used as criteria: 15 of the 28 (53.6%) patients reported a remarkable improvement in the feeling of well being, while an increase in appetite was reported in 24 (85.7%)

Figure 5 AFP concentration in the treated group. M, months.

treated patients. Body weight improved in 12 (42.8%) of the patients treated. No differences were noted in the control group.

Discussion

Response of HCC to different therapeutic modalities is poor and no firm general recommendations can be made.²³ A new therapeutic approach for advanced HCC has been investigated in the present study. Somatostatin is a hormone with known antimitotic activity in various neoplasms. Synthetic analogues have been shown to delay tumour growth in animals.²⁴ The presence of somatostatin receptors in liver tissue, however, has not been adequately studied. A study of somatostatin receptor subtypes in rats reported the presence of type 3 receptors in the liver without data on other subtypes.²⁵ In a study using monoclonal antibodies in rat liver,²⁶ detection of subtype 2 receptor was not reported.

OctreoScan indium scintigraphy has also produced conflicting results. One study reports very low levels of liver uptake in rats.²⁷ Similar studies in humans reported either uptake by the liver without further details²⁸ or very low uptake.²⁹

In our study we have investigated the presence of somatostatin receptors in human diseased livers using a technique that detects all receptor subtypes. We found two distinct groupings of somatostatin receptors, unrelated to the underlying liver pathology. These findings could be attributed to fluctuation of the total receptor levels, to different subtype preponderance among individuals, or to a heterogeneous distribution of somatostatin receptors. The latter finding has been reported in certain adenocarcinomas,¹¹ pituitary adenomas,³⁰ and carcinoid tumours.³¹ Further studies are required to clarify the issue. The presence of somatostatin receptors led to the clinical trial of octreotide administration for the treatment of advanced HCC.

Results have clearly shown a significant improvement in both median survival time (13 versus four months) compared with the control group and estimated survival rates after 6, 12, 18, 24, and 30 months of follow up. Tolerance

to treatment was very good. In particular no problems with renal function were noted, a complication that has been reported in cirrhotic patients.³²

Improvement in quality of life was of particular interest in the present study. Although quality of life is very difficult or even impossible to assess objectively, the criteria used were improvement of appetite and increase in body weight (not related to ascites). A total of 53.6% of patients in the treated group and none in the controls had a positive response. Moreover, the general sense of well being improved dramatically in the treated group although the validity of this observation was limited by the fact that the study was not double blind.

In five treated patients small satellite tumours (diameter less than 3 cm) disappeared, while in four more patients, larger tumours (3–6 cm diameter) remained unchanged. No similar observations were found in the control group. AFP levels were reduced after six months of treatment.

Other therapeutic modalities for advanced HCC include lipiodol embolisation^{5,7,8} or hormonal manipulations.^{4,5} In a recent report⁶ intra-arterial iodine-131 labelled lipiodol administration gave comparable results to our study regarding tumour regression, but median survival and estimated survival rates at six and 12 months were higher in our study.

Our study also has more favourable results than those of a study on mainly Okuda II tumours treated with either lipiodol-epirubicin or ¹³¹I-lipiodol.³² Only when the majority of patients are Okuda I are the estimated survival rates better than ours although the median survival time is similar to ours.³³

In a recent study, Greek patients have been treated with tamoxifen.⁴ Median survival time and estimated survival rates are better in the present study group compared with tamoxifen treated patients (22% survival after 12 months compared with 58% in the present study). The Cox analysis showed no sex effect in our study as opposed to the tamoxifen study. However, this may be attributed to the fact that there were few women in our study. It should be noted that in both the lipiodol group and the tamoxifen group, the percentage of Okuda III tumours was lower than in the present study. In a separate study, the antiandrogen drug flutamide given to a group of advanced HCC patients with similar characteristics to our treated group showed no therapeutic effect, with a median survival time of 2.5 months.⁵

More favourable results than those in our study have only been reported in a recent paper of 122 cirrhotic HCC patients with resected tumours³⁴; an 80% 12 month survival rate is reported. However, the majority were Okuda I tumours, while survival of those with a tumour larger than 5 cm is only 51% at 12 months (which is lower than in our patients).

The mechanism of octreotide antimitotic activity has not been explored in the present study. It could be either direct through receptor subtypes 2 and 5^{8,11,12,35–38} or indirect through suppression of tumour trophic hormones such

as insulin or insulin-like growth factor 1 (IGF-1).³⁹

In rats, octreotide administration caused a reduction in regeneration after partial hepatectomy^{40 41} due to suppression of insulin secretion. This latter effect is improbable in HCC patients because in human HCC cell cultures a fourfold increase in expression of IGF-1 binding protein has been reported and therefore an increased effect of IGF-1 on HCC is to be expected.⁴² Evidence against the indirect effect is also the reported regression of hepatic adenocarcinoma metastases in rats without detectable effects on serum growth hormone, prolactin, and IGF-1. Of greatest importance is the fact that in this study only metastases bearing somatostatin receptors were arrested.⁴³

It has been reported recently that human HCCs express receptors for cholecystokinin B and gastrin, two hormones that could be trophic for the tumour.⁴⁴ Despite previous reports suggesting that there is no indirect effect it is still possible that somatostatin antimitotic activity in HCC might be partly related to suppression of these two hormones.

In conclusion, octreotide administration proved beneficial for the survival of patients with advanced HCC without serious adverse effects. Octreotide treatment is therefore a valuable alternative therapy for inoperable HCC. The expected availability of long acting somatostatin analogues might be very useful for this condition.

- 1 Scudamore CH, Ragaz J, Kluftringer AM, Owen DA. Hepatocellular carcinoma. A comparison of oriental and caucasian patients. *Am J Surg* 1988;155:659-62.
- 2 Wong F, Choi TK. Primary liver cell cancer. Asian experience. In: Blumgart LH, ed. *Surgery of liver and biliary tract*. London: Churchill Livingstone, 1988:1135-51.
- 3 Trichopoulos D, Tabor E, Gerety RJ, Xirouchaki E, Sparos L, Munoz N, Lincell CA. Hepatitis B and primary hepatocellular carcinoma in a European population. *Lancet* 1978; ii:1217-9.
- 4 Mannesis EK, Giannoulis G, Zoumpoulis P, Vafiadou I, Hadjiyannis SG. Treatment of hepatocellular carcinoma with combined suppression and inhibition of sex hormones: a randomized, controlled trial. *Hepatology* 1995; 21:1535-42.
- 5 Chao Y, Chan W-K, Huang Y-S, Teng H-C, Wang S-S, et al. Phase II of flutamide in the treatment of hepatocellular carcinoma. *Cancer* 1996;77:635-9.
- 6 Ram AM, Bhattacharya S, Novell JR, Dick R, Winslet MC, Hobbs KEF. Intra-arterial radiotherapy with 131 iodine-lipiodol for irresectable hepatocellular carcinoma [abstract]. *Gut* 1996;38(suppl 1):A17.
- 7 Madden MV, Krige JEJ, Bailey S, Beningsfield SJ, et al. Randomised trial of targeted chemotherapy with lipiodol and 5-epidoxorubicin compared with symptomatic treatment for hepatoma. *Gut* 1993;34:1598-600.
- 8 Rossi P, Rossi M, Clementi M, Pizzamiglio M, Bezzi M, Salvatori FM. Chemoembolization of hepatocellular carcinomas. *Proceedings, European seminars on diagnostic and interventional radiology*. Heraklion, September 1994:315-20.
- 9 Bhattacharya S, Novell JR, Dusheiko GM, Hilsen AJ, Dick R, Hobbs KE. Epirubicin-lipiodol chemotherapy versus 131 iodine-lipiodol radiotherapy in the treatment of unresectable hepatocellular carcinoma. *Cancer* 1995;76: 2202-10.
- 10 Lamberts SWJ, Van Der Lely AJ, de Herder WW, Hofland LJ. Octreotide. *N Engl J Med* 1996;334:246-54.
- 11 Reubi JC, Kvolis L, Krenning E, Lamberts SWJ. Distribution of somatostatin receptors in normal and tumour tissue. *Metabolism* 1990;39(suppl 2):78-81.
- 12 Lamberts SWJ, Krenning EP, Reubi JC. The role of somatostatin and its analogues in the diagnosis and treatment of tumours. *Endocr Rev* 1991;12:450-82.
- 13 Bradford M. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem* 1976;72:248-54.
- 14 Thermos K, Reisine T. Somatostatin receptor subtypes in the clonal anterior pituitary cell lines AtT20 and GH3. *Mol Pharmacol* 1988;33:370-7.
- 15 Liapakis G, Thermos K. Characterization of [125I] tyr11-somatostatin binding sites in the rabbit retina. *Neuropeptides* 1992;21:13-19.
- 16 Okuda K, Obata H, Nakajima Y. Prognosis of primary hepatocellular carcinoma. *Hepatology* 1984;4:36-65.
- 17 Okuda K, Kojiro M, Okuda H. Hepatocellular carcinoma. In: Schiff L, Schiff FR, eds. *Diseases of the liver*. 7th edn. Philadelphia: Lippincott, 1993:1243-96.
- 18 Pocock S J. *Clinical trials. A practical approach*. New York: Wiley, 1983.
- 19 Armitage P, Berry G. *Statistical methods in medical research*. 2nd edn. Oxford: Blackwell Scientific Publications, 1987.
- 20 Everitt BS. The analysis of repeated measures: a practical review with examples. *The Statistician* 1995;44:113-35.
- 21 Kaplan E, Meier P. Nonparametric estimation from incomplete observations. *J Am Stat Assoc* 1959;53:457-81.
- 22 Cox DR. Regression models and life tables. *J R Stat Soc* 1972;34:248-75.
- 23 Colombo M, Piva A. Treatment of hepatocellular carcinoma. In: Miguet P, Dhumeaux D, eds. *Progress in hepatology* 93. Paris: John Libbey Eurotext, 1993:13-22.
- 24 Schally AV. Oncological applications of somatostatin analogues. *Cancer Res* 1988;48:6977-85.
- 25 Bruno JF, Xu Y, Song J, Berelowitz M. Tissue distribution of somatostatin receptor subtype messenger ribonucleic acid in the rat. *Endocrinology* 1993;33:2561-7.
- 26 Theveniau MA, Yasuda K, Bell GI, Reisine T. Immunological detection of isomorphs of the somatostatin receptor subtype, SSTR2. *J Neurochem* 1994;63:447-55.
- 27 Bruns C, Stolz B, Albert R, Marbach P, Pless J. OctreoScan 111 for imaging of a somatostatin receptor positive islet cell tumour in rat. *Horm Metab Res* 1993;27(suppl S1):5-11.
- 28 Krenning EP, Bakker WH, Kooij PP, Breeman WA, Oei HY, de Jong M, et al. Somatostatin receptor scintigraphy with indium-111-DTPA-D-Phe-1-octreotide in man: metabolism, dosimetry, and comparison with iodine-123-Tyr-3-octreotide. *J Nucl Med* 1992;33:652-81.
- 29 Dorr U, Wurm K, Horing E, Guzman G, Rath U, Bihl H. Diagnostic reliability of somatostatin receptor scintigraphy during continuous treatment with different somatostatin analogs. *Horm Metab Res* 1993;27(suppl 1):36-43.
- 30 Greenman Y, Melmed S. Expression of three somatostatin receptor subtypes in primary adenomas: evidence for preferential SSTR5 expression in the mammosomatotroph lineage. *J Clin Endocrinol Metab* 1994;79:724-9.
- 31 Reubi JC, Schaer JC, Waser B, Mengod G. Expression of localization of somatostatin receptor SSTR1, SSTR2, and SSTR3 messenger RNAs in primary human tumours using in situ hybridization. *Cancer Res* 1994;54:3455-9.
- 32 Gines A, Salmeron JM, Gines P, Jimenez W, Salo J, et al. Effects of somatostatin on renal function in cirrhosis. *Gastroenterology* 1992;103:1868-74.
- 33 Bronowicki JP, Boudjema K, Chone L, Nisand G, Basin C, Plumbo F, et al. Comparison of resection, liver transplantation and transcatheter oily chemoembolization in the treatment of hepatocellular carcinoma. *J Hepatol* 1996;24:293-300.
- 34 Di Carlo V, Ferrari G, Castoldi R, Nadalin S, Marengi C, et al. Surgical treatment and prognostic variables of hepatocellular carcinoma in 122 cirrhotics. *Hepatogastroenterology* 1995;42:222-9.
- 35 Patel YC, Sricant CB. Subtype selectivity of peptide analogs for all five cloned human somatostatin receptors (hsstr 1-5). *Endocrinology* 1994;135:2814-7.
- 36 Kubota A, Yamada Y, Kagimoto S, et al. Identification of somatostatin receptor subtypes and an implication for the efficacy of somatostatin analogue SMS 201-995 in treatment of human endocrine tumours. *J Clin Invest* 1994; 93:1321-5.
- 37 Patel YC, Greenwood MT, Warszynska A, Panetta R, Sricant CB. All five cloned human somatostatin receptors (hsSTR1-5) are functionally coupled to adenylyl cyclase. *Biochem Biophys Res Commun* 1994;198:605-12.
- 38 Liebow C, Reilly C, Serrano M, Schally AV. Somatostatin analogues inhibit growth of pancreatic cancer by stimulating tyrosine phosphatase. *Proc Natl Acad Sci USA* 1989;86: 2003-7.
- 39 Schally AV, Redding TW. Somatostatin analogues as adjuncts to agonists of luteinizing hormone-releasing hormone in the treatment of experimental prostate cancer. *Proc Natl Acad Sci USA* 1987;84:7275-9.
- 40 Buthi RS, Faruk M, Tsai WH, Michalopoulos G, et al. The effect of octreotide on hepatic regeneration in rats. *Surgery* 1993;113:84-9.
- 41 Takeda Y, Escibano MJ. Effects of insulin and somatostatin on the growth and the colony formation of two human pancreatic cancer cell lines. *J Cancer Res Clin Oncol* 1991;117:416-20.
- 42 Ren SG, Ezzat S, Melmed S, Braunstein GD. Somatostatin analog induces insulin-like growth factor binding protein-1 (IGFBP-1) expression in human hepatoma cells. *Endocrinology* 1992;131:2479-81.
- 43 van Eijck CH, Slooter GD, Hofland LJ, Kort W, Jeekel J, et al. Somatostatin receptor-dependent growth inhibition of liver metastases by octreotide. *Br J Surg* 1994;81:1333-7.
- 44 Caplin ME, Michaeli D, More L, Pounder RE, Dhillon AP. The CCK-B/gastrin receptor in hepatocellular carcinoma [abstract]. *Gut* 1996;38(suppl 1):A16.