TABLE I – CHARACTERISTICS OF THE STUDY SUBJECTS

Variables	Controls (n = 291)	Cases			
		Colon cancer (n = 59)	p value[1]	Rectal cancer (n = 243)	p value[1]
Gender (male %)	182 (62.5)	40 (67.8)	0.45	157 (64.6)	0.62
Age groups					
≤40 yr	99 (34.0)	21 (35.6)		72 (29.6)	
>40 yr	192 (66.0)	38 (64.4)	0.30	171 (70.4)	0.03
Median (range)	50 (20–75)	50 (22–72)		50 (17–75)	
Mean (sd)	47.3 (12.6)	48.5 (12.0)	0.51	49.1 (14.1)	0.12
Current BMI (kg/m^2)[2]					
<25	221 (76.0)	51 (89.5)		205 (86.5)	
≥25	32 (11.0)	3 (5.3)		14 (5.9)	
≥27	38 (13.0)	3 (5.3)	0.08	18 (7.6)	0.01
Median (range)	21.7 (14.0–36.1)	19.5 (12.3–28.5)		20.0 (13.1–33.3)	
Mean (sd)	21.9 (4.3)	20.0 (3.8)	<0.01	20.5 (4.0)	<0.01
Education					
<Middle	88 (30.2)	18 (30.5)		85 (35.0)	
Middle and high	164 (56.4)	31 (52.5)		126 (51.9)	
>High	39 (13.4)	10 (17.0)	0.75	32 (13.2)	0.49
Religion					
Hindu	256 (88.0)	47 (79.7)		219 (90.1)	
Muslim	27 (9.3)	7 (11.9)		16 (6.6)	
Christian	8 (2.8)	5 (8.5)	0.08	8 (3.3)	0.50
Household income (rupees)					
<500	82 (28.2)	17 (28.8)		104 (42.8)	
500–1500	148 (50.9)	25 (42.4)		79 (32.5)	
>1500	61 (21.0)	17 (28.8)	0.35	60 (24.7)	<0.01
Mother tongue					
Tamil	185 (63.6)	27 (45.8)		127 (52.3)	
Telugu	77 (26.5)	20 (33.9)		90 (37.0)	
Urdu	9 (3.1)	3 (5.1)		7 (2.9)	
Other	20 (6.9)	9 (15.3)	0.04	19 (7.8)	0.05
Family history					
None	174 (59.8)	47 (79.7)		226 (93.0)	
Colorectal cancers	0 (0.0)	3 (5.1)		1 (0.4)	
Other cancers	117 (40.2)	9 (15.3)	<0.01	16 (6.6)	<0.01

[1]By chi-square test or t test.–[2]Data missing for 2 subjects with colon cancer and 6 subjects with rectal cancer.

cases had lower current BMI than controls. In respect to household income, a lower annual income (<500 rupees) was more often found among rectal cancer cases. However, controls had a higher frequency of family history of other cancers than cases, because controls were selected from relatives/visitors to the patients having nongastrointestinal cancers. There was a slight difference in the distribution of mother tongue between cases and controls. Marital status and types of residence were also compared between cases and controls, but there were no differences (data not shown).

Data for smoking status, drinking status, chewing habit and vegetarians and risks to colon and rectal cancer are shown in Table II. Nonsmokers and nondrinkers were more common in both cases and controls. For categories of tobacco products, cigarette smoking was more frequent among colon cancer cases (18.6%) and less among rectal cancer cases (11.1%) compared to controls (14.4%). Both bidi and chutta smokers exhibited an increased rectal cancer risk (bidi: OR = 1.44, 95% CI 0.71–2.94; chutta: OR = 4.47, 95% CI 1.12–23.9) but without statistical significance for bidi and with a wide 95% confidence interval for chutta because of the small numbers. Although total pack-years were stratified into 2 groups, no significant risk derived from pack-years was found.

With respect to alcohol, no significant differences were found between colon or rectal cancer cases and controls in distribution of non-Indian-alcohol drinkers (p = 0.72; 0.71, respectively), but Indian-alcohol drinkers may be at a somewhat higher rectal cancer risk (OR = 2.26, 95% CI 0.86–6.36). According to stratification by drinking duration, alcohol consumption for more than 20 years was associated with the tendency for an increased risk of rectal cancer (OR = 1.55, 95% CI 0.73–3.33). Regarding the amount of consumed alcohol, for both colon and rectal cancers, the group with less than eight-hundred gram-years showed weakly decreased risk (colon: OR = 0.77, 95% CI 0.21–2.21; rectal: OR = 0.66, 95% CI 0.32–1.35), and that with more than 800 gram-years showed a slightly elevated risk (colon: OR = 1.53, 95% CI 0.55–3.86; rectal: OR = 1.56, 95% CI 0.82–3.02).

The distribution of betel chewing showed no statistical differences between cases and controls. A decreased colon or rectal cancer risk was found for chewing habits but had not reached statistical significance. However, a significantly increased rectal cancer risk was found for vegetarianism.

The frequencies of *MTHFR* genotypes and the association between genotypes and cancers are summarized in Table III. The allele frequency for *MTHFR* 677T was 0.05 among colon cancer cases and 0.08 among rectal cancer cases, compared with 0.06 among controls. The *MTHFR* 677TT genotype in the Indian population is extremely rare, absent among colon cancer cases and controls, and was present in only 2 rectal cancer cases. The observed frequencies of MTHFR 677 genotypes among controls (CC, 87.6%; CT, 12.4%) were in accordance with the Hardy-Weinberg equilibrium (p = 0.26). The *MTHFR* 677T allele was found no association with colon cancer (OR = 0.82, 95% CI 0.28–2.05) and a nonstatistically significantly elevated risk with rectal cancer (OR = 1.51, 95% CI 0.86–2.68). The allele frequencies for *MTHFR* 1298C were 0.27, 0.33 and 0.41 in the colon and rectal cancer groups and controls, respectively. The distribution of *MTHFR* 1298 genotypes among controls (AA, 36.1%; AC, 46.4%; and CC, 17.5%) also agreed with that expected from the Hardy-Weinberg equilibrium (p = 0.54), which was significantly different from colon cancer cases (AA, 54.2%; AC, 37.3%; and CC, 8.5%; p = 0.02) and rectal cancer cases (AA, 44.9%; AC, 44.4% and CC, 10.7%; p = 0.03). As compared with their counterparts with the *MTHFR* 1298 AA genotype, subjects carrying the *MTHFR* 1298

TABLE II – DISTRIBUTION OF SMOKING, DRINKING, CHEWING AND VEGETARIANS AND ORS FOR COLON AND RECTAL CANCER

Habit	Controls (n = 291)	Colon cancer (n = 59)	OR (95% CI)	Rectal cancer (n = 243)	OR (95% CI)
Smoking status[1]					
Cigarette					
Never	249 (85.6)	48 (81.4)	1.00 (Ref)	216 (88.9)	1.00 (Ref)
Smokers	42 (14.4)	11 (18.6)	1.30 (0.54–2.96)	27 (11.1)	0.63 (0.34–1.15)
Bidi					
Never	266 (91.4)	54 (91.5)	1.00 (Ref)	218 (89.7)	1.00 (Ref)
Smokers	25 (8.6)	5 (8.5)	1.12 (0.34–3.20)	25 (10.3)	1.44 (0.71–2.94)
Chutta					
Never	288 (99.0)	58 (98.3)	1.00 (Ref)	232 (95.5)	1.00 (Ref)
Smokers	3 (1.0)	1 (1.7)	1.63 (0.07–16.84)	11 (4.5)	4.47 (1.12–23.95)
Pack-years[2]					
0	225 (77.3)	44 (74.6)	1.00 (Ref)	188 (77.4)	1.00 (Ref)
≤3	33 (11.3)	8 (13.6)	1.38 (0.51–3.49)	19 (7.8)	0.72 (0.35–1.44)
>3	33 (11.3)	7 (11.9)	1.07 (0.37–2.85)	36 (14.8)	1.26 (0.67–2.39)
Drinking status[3]					
Non-Indian alcohol					
Never	247 (84.9)	49 (83.0)	1.00 (Ref)	209 (86.0)	1.00 (Ref)
Drinkers	44 (15.1)	10 (17.0)	1.25 (0.53–2.72)	34 (14.0)	1.02 (0.59–1.77)
Indian alcohol					
Never	282 (96.9)	57 (96.6)	1.00 (Ref)	227 (93.4)	1.00 (Ref)
Drinkers	9 (3.1)	2 (3.4)	1.22 (0.18–5.35)	16 (6.6)	2.26 (0.86–6.36)
All alcohol					
Never	238 (81.8)	48 (81.4)	1.00 (Ref)	198 (81.5)	1.00 (Ref)
Drinkers	53 (18.2)	11 (18.6)	1.13 (0.50–2.38)	45 (18.5)	1.08 (0.66–1.79)
Duration (years)[4]					
<20	35 (12.0)	8 (13.6)	1.19 (0.46–2.79)	25 (10.3)	0.83 (0.44–1.53)
≥20	18 (6.2)	3 (5.1)	0.99 (0.22–3.31)	20 (8.2)	1.55 (0.73–3.33)
Amount (gram-years)[4]					
≤800	29 (10.0)	4 (6.8)	0.77 (0.21–2.21)	16 (6.6)	0.66 (0.32–1.35)
>800	24 (8.2)	7 (11.9)	1.53 (0.55–3.86)	29 (11.9)	1.56 (0.82–3.02)
Chewing habit[5]					
No	236 (81.1)	50 (84.7)	1.00 (Ref)	202 (83.1)	1.00 (Ref)
Yes	55 (18.9)	9 (15.3)	0.61 (0.25–1.34)	41 (16.9)	0.78 (0.47–1.30)
Vegetarianism[6]					
No	258 (88.7)	49 (83.0)	1.00 (Ref)	195 (80.2)	1.00 (Ref)
Yes	33 (11.3)	10 (17.0)	1.87 (0.77–4.29)	48 (19.8)	1.83 (1.04–3.26)

[1]Adjusted for gender, age, household income, education, religion, mother tongue, drinking, chewing and vegetarianism.–[2]Pack-years calculated by different tobacco products (weight of 1 for cigarettes, 0.25 for bidis and 0.5 for chuttas).–[3]Adjusted for gender, age, household income, education, religion, mother tongue, smoking, chewing and vegetarianism.–[4]Never drinkers of all alcohol as the referent group.–[5]Adjusted for gender, age, household income, education, religion, mother tongue, smoking, drinking and vegetarianism.–[6]Adjusted for gender, age, household income, education, religion, mother tongue, smoking, drinking and chewing.

TABLE III – GENOTYPE FREQUENCIES, AND ADJUSTED ORS(95% CIS)[1] FOR COLON, RECTAL AND COLORECTAL CANCERS WITH POLYMORPHISMS OF MTHFR 677 AND 1298

Genotype	Control subjects (n = 291) n (%)	Colon cancer (n = 59) n (%)	OR (95% CI)	Rectal cancer (n = 243) n (%)	OR (95% CI)	Colorectal cancers (n = 302) n (%)	OR (95% CI)
MTHFR 677							
CC	255 (87.6)	53 (89.8)	1.00 (Ref)	204 (84.0)	1.00 (Ref)	257 (85.1)	1.00 (Ref)
CT	36 (12.4)	6 (10.2)	0.82 (0.28–2.05)	37 (15.2)	1.40 (0.79–2.49)	43 (14.2)	1.22 (0.72–2.09)
TT	0 (0.00)	0 (0.00)	NA	2 (0.8)	NA	2 (0.7)	NA
CT or TT	36 (12.4)	6 (10.2)	0.82 (0.28–2.05)	39 (16.0)	1.51 (0.86–2.68)	45 (14.9)	1.31 (0.78–2.23)
MTHFR 1298							
AA	105 (36.1)	32 (54.2)	1.00 (Ref)	109 (44.9)	1.00 (Ref)	141 (46.7)	1.00 (Ref)
AC	135 (46.4)	22 (37.3)	0.43 (0.22–0.82)	108 (44.4)	0.70 (0.45–1.06)	130 (43.0)	0.62 (0.42–0.92)
CC	51 (17.5)	5 (8.5)	0.30 (0.09–0.81)	26 (10.7)	0.43 (0.23–0.80)	31 (10.3)	0.40 (0.22–0.70)
AC or CC	186 (63.9)	27 (45.8)	0.40 (0.22–0.74)	134 (54.1)	0.62 (0.42–0.93)	161 (53.3)	0.56 (0.38–0.81)
Combined genotypes							
CC and AA	83 (28.5)	28 (47.4)	1.00 (Ref)	83 (34.2)	1.00 (Ref)	111 (36.8)	1.00 (Ref)
CC and AC	121 (41.6)	21 (35.6)	0.42 (0.21–0.83)	95 (39.1)	0.69 (0.43–1.11)	116 (38.4)	0.61 (0.39–0.93)
CC and CC	51 (17.5)	4 (6.8)	0.22 (0.06–0.64)	26 (10.7)	0.45 (0.23–0.86)	30 (9.9)	0.39 (0.21–0.70)
CT or TT and AA	22 (7.6)	4 (6.8)	0.54 (0.13–1.74)	26 (10.7)	1.17 (0.56–2.51)	30 (9.9)	0.99 (0.49–2.00)
CT or TT and AC	14 (4.8)	1 (1.7)	0.16 (0.01–0.98)	13 (5.3)	0.97 (0.38–2.48)	14 (4.6)	0.71 (0.29–1.73)
CT or TT and CC	0 (0.0)	1 (1.7)	NA	0 (0.0)	NA	1 (0.3)	NA

[1]Adjusted for gender, age, household income, education, religion, mother tongue, smoking, drinking, chewing and vegetarianism.

AC genotype were at a low risk for either colon cancer (OR = 0.43, 95% CI 0.22–0.82) or possibly rectal cancer (OR = 0.70, 95% CI 0.45–1.06). Moreover, individuals carrying the *MTHFR* 1298CC genotype showed a significantly decreased risk for both colon cancer (OR = 0.30, 95% CI 0.09–0.81) and rectal cancer (OR = 0.43, 95% CI 0.23–0.80). The p values for trend tests of the MTHFR 1298 genotypes were 0.005 (χ^2 = 7.93) for colon cancer and 0.007 (χ^2 = 7.41) for rectal cancer.

The distribution of cases and controls for *MTHFR* polymorphisms is in line with C677T and A1298C being in complete linkage disequilibrium (p = 0.000, χ^2 test). Estimation of *MTHFR* haplotype frequencies for combinations of C677T and A1298C alleles also demonstrated the following statistically significant case-control differences (p = 0.004): 0.61 677C/1298A; 0.07 677T/1298A; 0.31 677C/1298C; and 0.004 677T/1298C among cases and 0.53 677C/1298A; 0.06 677T/1298A; 0.40 677C/1298C; and 0.000 677T/1298C among controls.

Combined effects of the *MTHFR* 677 and 1298 genotypes on risk of colon, rectal and colorectal cancer were also analyzed (see Table III). No subject in our study carried homozygous mutant alleles at both sites (677TT/1298CC). Only 1 case carried the 677CT/1298CC genotype, and individuals who carried 677CT(TT)/1298AC were rare. When *MTHFR* 1298AA genotype was only considered, *MTHFR* 677T showed an inverse association with colon cancer risk (OR = 0.54, 95% CI 0.13–1.74), and combined 677CT/1298AC genotypes appeared a decreased risk for colon cancer compared with the homozygous wild-type 677CC/1298AA (OR = 0.16, 95% CI 0.01–0.98), but these results need to be confirmed because of small numbers.

Interactions for alcohol, vegetable intake and *MTHFR* polymorphisms are presented in Table IV. For alcohol consumption, no significant link was found between the *MTHFR* 677 polymorphism and rectal cancer. A nonstatistically significant association was observed among drinkers with the *MTHFR* 1298AA genotype for rectal cancer (OR = 1.97, 95% CI 0.88–4.57). With regard to vegetable intake, nonfried and fried categories were individually analyzed for their effects. With high intake of nonfried vegetables, a clearly decreased risk was found for both colon cancer (adjusted OR = 0.40; 95% CI, 0.20–0.84) and rectal cancer (adjusted OR = 0.47; 95% CI, 0.28–0.75), comparing to the low intake group. However, with fried vegetables, the lower risk was observed with low consumption for both colon cancer (adjusted OR = 0.78; 95% CI, 0.40–1.46) and rectal cancer (adjusted OR = 0.62; 95% CI, 0.40–0.96). For rectal cancer with the *MTHFR* 677T allele, there appeared to be risk reduction among those with high intake of nonfried vegetables (OR = 0.66, 95% CI 0.30–1.42). The lowest risk for rectal cancer (OR = 0.22, 95% CI 0.09–0.52) was found among the high intake group of nonfried vegetables with the *MTHFR* 1298CC genotype. Similarly, interactions of fried vegetable intake with *MTHFR* genotypes were apparent, but not as strong as in the nonfried case.

In addition, high intake of fruit also was associated with a somewhat reduced risk for both colon cancer (adjusted OR = 0.65; 95% CI, 0.35–1.23) and rectal cancer (adjusted OR = 0.75; 95% CI, 0.50–1.13). There was no significant interaction with *MTHFR* genetic polymorphisms regarding susceptibility to colon or rectal cancer.

TABLE IV – RELATIONSHIP OF ALCOHOL AND VEGETABLE INTAKE TO RECTAL AND COLORECTAL CANCER RISK STRATIFIED BY MTHFR GENOTYPE

	MTHFR677 genotype				MTHFR1298 genotype					
	CC[1]	OR (95% CI)[2]	CT or TT[1]	OR (95% CI)[2]	AA[1]	OR (95% CI)[2]	AC[1]	OR (95% CI)[2]	CC[1]	OR (95% CI)[2]
Alcohol										
Never drinker										
Rectal cancer	166/208	1.00 (Ref)	32/30	1.53 (0.82–2.86)	85/91	1.00 (Ref)	93/105	0.88 (0.55–1.41)	20/42	0.44 (0.22–0.86)
Colorectal cancer	208/208	1.00 (Ref)	38/30	1.38 (0.77–2.47)	110/91	1.00 (Ref)	111/105	0.75 (0.48–1.56)	25/42	0.42 (0.22–0.78)
Drinker										
Rectal cancer	38/47	1.10 (0.64–1.88)	7/6	1.46 (0.41–5.42)	24/14	1.97 (0.88–4.57)	15/30	0.47 (0.21–0.99)	6/9	0.72 (0.20–2.49)
Colorectal cancer	49/47	1.05 (0.64–1.73)	7/6	1.09 (0.31–3.89)	31/14	1.69 (0.79–3.66)	19/30	0.45 (0.22–0.90)	6/9	0.51 (0.15–1.70)
Non-fried vegetables[3]										
Low intake[4]										
Rectal cancer	58/40	1.00 (Ref)	14/4	1.97 (0.60–7.78)	33/19	1.00 (Ref)	33/17	0.68 (0.27–1.72)	6/8	0.39 (0.09–1.59)
Colorectal cancer	73/40	1.00 (Ref)	15/4	1.84 (0.58–7.09)	45/19	1.00 (Ref)	36/17	0.57 (0.24–1.39)	7/8	0.37 (0.10–1.38)
High intake[4]										
Rectal cancer	146/215	0.50 (0.29–0.84)	25/32	0.66 (0.30–1.42)	76/86	0.46 (0.21–0.95)	75/118	0.33 (0.15–0.67)	20/43	0.22 (0.09–0.52)
Colorectal cancer	184/215	0.49 (0.30–0.80)	30/32	0.58 (0.28–1.17)	96/86	0.45 (0.22–0.88)	94/118	0.30 (0.15–0.58)	24/43	0.19 (0.08–0.43)
Fried vegetables										
Intake										
Rectal cancer	138/158	1.00 (Ref)	29/24	1.63 (0.83–3.24)	78/63	1.00 (Ref)	71/84	0.58 (0.34–0.99)	18/35	0.39 (0.18–0.82)
Colorectal cancer	171/158	1.00 (Ref)	31/24	1.27 (0.67–2.44)	101/63	1.00 (Ref)	83/84	0.54 (0.33–0.88)	23/35	0.40 (0.20–0.79)
Non-intake										
Rectal cancer	66/97	0.66 (0.41–1.04)	10/12	0.73 (0.26–2.05)	31/42	0.45 (0.23–0.90)	37/51	0.46 (0.24–0.86)	8/16	0.24 (0.08–0.67)
Colorectal cancer	81/97	0.70 (0.46–1.06)	14/12	0.96 (0.39–2.40)	40/42	0.59 (0.31–1.09)	47/51	0.48 (0.27–0.85)	8/16	0.21 (0.07–0.56)

[1]Numbers of cases/controls.–[2]Adjusted for gender, age, household income, education, religion, mother tongue, smoking, drinking and chewing.–[3]These vegetables as the predominant source of folate in Indian population.–[4]Based on a sum of assigned weights of various vegetables (low intake group ≤21, high intake group >21).

Discussion

Several epidemiological studies have focused on associations between *MTHFR* polymorphisms and colon cancer in Caucasians.[23,30–33] Two demonstrated an inverse association between *MTHFR* 677TT genotype and colorectal cancer when either folate intake was high or alcohol consumption was low, and a positive association with low folate intake or high alcohol intake.[30,31] Furthermore, 2 studies revealed weak inverse associations between *MTHFR* 677TT genotype and colon cancer independent of intake of folate or alcohol.[32,33] One study found no association between the low activity *MTHFR* 677TT genotype and colon cancer,

and no relations with the use of alcohol, but a weak positive association was shown with low folate intake (<400 μg/day). Moreover, a significant inverse association was demonstrated between *MTHFR* 1298CC genotype and colon cancer.[23] In addition, Curtin *et al.* recently reported a strong inverse association between colon cancer and *MTHFR* 1298CC genotype among women in a largely Caucasian population.[24]

The participants in our study were mainly of Tamil and Telugu language groups. Although slight differences in the distribution were present between cases and controls, both belonging to the same Dravidian race, and the distribution of *MTHFR* genotypes demonstrated no significant variation between Tamil and Telugu participants. We also adjusted for mother tongue in our analysis.

Our results showed that *MTHFR* 677T allele was extremely rare (0.06) in healthy Dravidian Indians, in accordance with the low prevalence of 677 mutation reported in Asian Indians and Tamilians,[34,35] differing from the case with Whites (0.30–0.35)[23,30,31] and other Asian peoples (0.41–0.44).[36,37] In contrast, the frequency of the *MTHFR* 1298C allele (0.41) is higher than in either[23,36,37] which is also similar to that reported among Tamilians.[35] To date, associations of *MTHFR* genetic polymorphisms with coronary artery diseases have been evaluated in Indians.[34,38]

The *MTHFR* 677T allele was found no association with colon cancer [OR = 0.82 (0.28–2.05)] in our study, similar to the earlier studies,[23,24] albeit not as strong as the reports in the meta-analysis undertaken by Houlston *et al.* [OR = 0.77 (0.62–0.92)].[39] Furthermore, an indication of an increased rectal cancer risk with the *MTHFR* 677T allele was also found [OR = 1.51 (0.86–2.68)]. The inconsistent results in our study may be due to the rare 677T allele in Indians and result in our sample size was insufficient to evaluate the association of *MTHTR* 677 genotypes with colon or rectal cancer.

In agreement with the findings of Keku *et al.*[23] and Curtin *et al.*,[24] our study demonstrated strong inverse associations between *MTHFR* 1298CC genotype and colon cancer [OR = 0.30 (0.09–0.81)] or rectal cancer [OR = 0.43 (0.23–0.80)], and confirmed that *MTHFR* 1298 may be more important than 677 genotypes for colorectal cancer risk. Because the location of 677 (NH_2-terminal) and 1298 (COOH-terminal) is distinct, and the amino acid affected by 1298 single nucleotide substitution (A→C, glu to ala) is located near the binding site for the allosteric MTHFR inhibitor S-adenosyl-methionine, may possibly affect feedback inhibition. In addition, the balance of DNA synthesis and DNA methylation determined by *MTHFR* polymorphisms may play an important role in the regulation of gene expression influencing cancer risk. It has been suggested that relationships between *MTHFR* polymorphisms and colorectal cancer may be different by gender and age distribution, we also detected the associations between *MTHFR* 1298 genotypes and rectal cancer risk by gender and age groups, but no significant differences were found.

The haplotype frequencies of *MTHFR* 677 and 1298 were also estimated, and significant case-control differences were found. However, we have not examined the association with cancer risk because the sample size was small and haplotypes might be unreliable.

We evaluated the associations of smoking status and colon or rectal cancer risk. Although specific categories of tobacco, bidi and chutta exhibited an increased rectal cancer risk, for bidi no statistical significance was found and for chutta with a wide confidence interval. Furthermore, total pack-years of 3 tobacco categories were not found to be associated with rectal cancer risk. For alcohol consumption, indigenous Indian alcohol drinkers may be at a somewhat higher rectal cancer risk [OR = 2.26 (0.86–6.36)], and drinking duration for more than 20 years was found to be an increased risk tendency for rectal cancer [OR = 1.55 (0.73–3.33)]. Furthermore, in order to detect the association between cumulative doses of alcohol consumption and colon or rectal cancer risk for light drinkers, we made an attempt to calculate the amount (gram-years) and found that the amount for less than 800 gram-years was associated with a somewhat lower colon or rectal cancer risk [colon: OR = 0.77 (0.21–2.21); rectal: OR = 0.66 (0.32–1.35)] and that with over eight-hundred gram-years was associated with a somewhat higher colon or rectal cancer risk [colon: OR = 1.53 (0.55–3.86); rectal: OR = 1.56 (0.82–3.02)]. In our study, although the definition of alcohol drinkers (who drink at least once a month for more than 1 year) may be too inclusive, if drinkers were defined as usual (who drink at least once a week or a day), then there were not drinkers in our study. In addition, there may be underreporting of alcohol intake relating to religion, which should be taken into account. We also assessed the interaction of alcohol consumption and *MTHFR* polymorphisms with susceptibility to rectal cancer, and found that drinkers with *MTHFR* 1298AA genotype were related to an increased risk tendency for rectal cancer [OR = 1.97 (0.88–4.57)]. As introduced above, *MTHFR* 1298 wild-type (AA) with high enzyme activity may promote the conversion of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate, producing a low 5,10-methylenetetrahydrofolate pool level that leads to misincorporation of uracil for thymidine during DNA synthesis. Moreover, some investigators have hypothesized that colorectal cancer risk associated with alcohol was related to its anti-folate effect[40] or more specifically to its effects on DNA methylation.[41] These may explain the elevated risk among drinkers carrying the high activity *MTHFR* genotype.

High intake of nonfried vegetables or fruit showed inverse association with both colon and rectal cancer in our study, and these vegetables and fruit are thought to be the predominant source of dietary folate intake in Indian population. Especially, the combination of high intake of non-fried vegetables and *MTHFR* 1298CC genotype demonstrated the lowest risk for rectal cancer [OR = 0.22 (0.09–0.52)].

In conclusion, this case-control study exhibited that the frequency of *MTHFR* 677T allele is rare, while *MTHFR* 1298C allele is common among Indians, and *MTHFR* 1298CC genotype was significantly associated with decreased colon and rectal cancer risk. Furthermore, our study confirmed the suggestion that *MTHFR* 1298 polymorphism may be more important than *MTHFR* 677 polymorphism for colorectal cancer. The intake of vegetables is frequent on the whole in Indian population, and the high intake of nonfried vegetables clearly showed a reduced risk for both colon and rectal cancers. Furthermore, the combination of high intake of nonfried vegetables and *MTHFR* 1298CC genotype was found to be associated with the lowest rectal cancer risk. These may explain why the incidence rate of colorectal cancer is very low in Indian populations, taken together with high level of physical activity and walking, high intake of dietary folate from vegetables and fruit but very limited alcohol consumption. For the light drinkers, long-term alcohol consumption and going beyond a certain cumulative amount also showed an increased risk trend for rectal cancer. However, the low prevalence of colorectal cancer in India may be associated with other dietary factors such as high curry intake and low red meat intake as well as the other genetic variations in metabolic enzymes and DNA repair enzymes, which remain to be confirmed.

Acknowledgements

The authors are grateful to Dr. V. Shanta and Dr. T. Rajkumar (Cancer Institute, Chennai, India) for their support and cooperation, and Dr. M. A. Moore for linguistic advice. Jingwen Wang was also financially supported by the Japan Society for the Promotion of Science and its postdoctoral fellowship for researchers from abroad.

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Investigational New Drugs **22:** 169–176, 2004.

Phase II trial of intra-arterial chemotherapy using a novel lipophilic platinum derivative (SM-11355) in patients with hepatocellular carcinoma

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Key words: intra-arterial chemotherapy, SM-11355, hepatocellular carcinoma (HCC)

Summary

SM-11355, a lipophilic platinum derivative, is a novel intra-arterial chemotherapeutic agent for hepatocellular carcinoma (HCC). A phase II study of SM-11355 was conducted to evaluate the antitumor activities and the toxicity in chemotherapy-naive patients with HCC. Sixteen patients were treated with transcatheter arterial injection of SM-11355–lipiodol emulsion (20–120 mg/body). The responses were evaluated by computed tomography 3 months after treatment. Complete response (CR) was defined as disappearance or 100% necrosis of all tumors, and lipiodol accumulation in tumors was regarded as indicating necrosis. Nine patients achieved CR (56%; 95% confidence interval, 29.9–80.2%). The grade 3 toxicities were neutropenia (19%), total bilirubin elevation (19%), AST elevation (44%), and ALT elevation (19%). None of the patients showed grade 4 toxicities or episodes of renal dysfunction. Other common adverse effects were eosinophilia (100%) and pyrexia (94%). Intra-arterial chemotherapy with SM-11355, which was well tolerated, showed promising antitumor activity in patients with HCC.

Introduction

Hepatocellular carcinoma (HCC) is one of the most common malignant tumors throughout the world. Although screening of high-risk populations for HCC using ultrasonography and tests of serum α-fetoprotein (AFP) levels has recently caused an increase in the number of candidates for effective local treatments such as hepatic resection and local ablation therapy, many patients exhibit multiple HCC at the time of initial diagnosis or at the time of recurrence after the local treatment. In these patients, various anticancer agents have been employed as intra-arterial chemotherapy alone [1–9] or in combination with transcatheter arterial embolization (TAE) [10–14]. However, it has not yet been proven which anticancer agent is most effective, and the impact of intra-arterial chemotherapy on survival is still uncertain.

SM-11355 (*cis*-[((1R,2R)-1,2-cyclohexanediamine-*N*,*N*′) bis(myristato)]-platinum (II) monohydrate, Sumitomo Pharmaceuticals Co., Osaka, Japan; Figure 1) is a novel lipophilic cisplatin derivative that can be suspended in lipiodol, a lipid lymphographic agent [15]. When lipiodol is injected into an artery feeding HCC nodules, it selectively accumulates in the tumor [16]. Accordingly, a SM-11355–lipiodol emulsion is deposited within the HCC nodules and releases active platinum compounds into tumor tissues gradually [17]. In a phase I study, a concentration escalation study for this agent, the recommended concentration was determined to be 20 mg/ml when the maximum

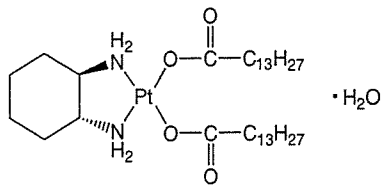

Figure 1. Chemical structure of SM-11355 (*cis*-[((1R,2R)-1, 2-cyclohexanediamine-*N*,*N*')bis(myristato)]-platinum (II) monohydrate).

volume was fixed at 6 ml [18]. To evaluate the antitumor effect and the toxicity of SM-11355, a phase II study of this agent was conducted in patients with HCC. The pharmacokinetics of SM-11355 was also investigated in this study.

Patients and methods

TAE-naive and chemotherapy-naive patients with unresectable HCC were eligible if they had no indication for local ablation therapy. Diagnosis was confirmed histologically and/or clinically by angiography and computed tomography (CT). Each patient was required to meet the following criteria: at least one measurable intrahepatic lesion that showed tumor staining by angiography, adequate hematological function (white blood cells $\geq$3000/mm^3, platelets $\geq$50,000/mm^3, hemoglobin $\geq$9.5 g/dl), adequate hepatic function (serum total bilirubin $\leq$3.0 mg/dl, serum albumin $\geq$3.0 g/dl, serum aspartate aminotransferase (AST) $\leq$200 U/L, serum alanine aminotransferase (ALT) $\leq$200 U/L), adequate renal function (serum creatinine $\leq$ the upper limit of normal value), Eastern Cooperative Oncology Group [19] performance status of 0–2, 20–74 years of age, a minimum life expectancy of more than 2 months, and written informed consent. Eligible patients were also with clinical stage I or II in accordance with the classification of underlying liver cirrhosis by the Liver Cancer Study Group of Japan [20], defined as ICG retention at 15 min (ICG R-15) $\leq$40%, prothrombin time $\geq$50%, and no refractory ascites. Patients who had previous hepatic resection and/or local ablation therapy were eligible if they had no evidence of local tumor recurrence in the treated lesions. The previous anticancer treatment had to have been discontinued for at least 4 weeks before enrollment in this study. Patients were excluded if they met the following criteria: a history of allergy to iodine-containing agent and/or contrast material, concomitant malignancy, extrahepatic metastasis or tumor thrombus in the portal vein and/or the hepatic vein, intrahepatic arteriovenous shunting, pregnant or lactating women and patients of reproductive potential, or other serious medical conditions. The study was approved by the ethics committee of each participating center.

SM-11355/lipiodol emulsion (20 mg/ml) was injected slowly under fluoroscopic monitoring into the artery feeding the HCC by use of a catheter. The emulsion was prepared by suspending SM-11355 (120 mg) in lipiodol (6 ml), which was shaken just before injection. The volume of the emulsion, up to a maximum of 6 ml (containing 120 mg of SM-11355), was adjusted according to the tumor size and tumor distribution, that is, the injection was discontinued when full accumulation of the emulsion in the tumor vessels was obtained, as defined in the protocol. When tumor staining was noted in enhanced CT or angiography after the initial treatment, the second treatment was performed within 3 months after the initial treatment. Patients who showed no tumor staining after the initial treatment did not undergo a second treatment.

The antitumor effect was evaluated by CT, which was performed 3 months after the second treatment. In patients who did not receive a second treatment, CT was performed 3 months after the initial treatment to evaluate the response. Tumor size was measured by the sum of the products of the perpendicular longest diameters of all measurable lesions. Lipiodol accumulation in tumors was regarded as an indication of necrosis [21,22]. We defined complete response (CR) as disappearance or 100% necrosis of all tumors, and partial response (PR) as more than 50% reduction and/or more than 50% necrosis. Progressive disease (PD) was defined as more than 25% enlargement of the tumor. No change (NC) was considered as disease not qualifying for classification as CR, PR, or PD. Survival curves were calculated from the day of initiation of this treatment using the Kaplan–Meier method. Toxicity was assessed according to the criteria of the Japanese Society for Cancer Therapy [23], which are fundamentally similar to the World Health Organization (WHO) criteria [24] and National Cancer Institute (NCI) Common Toxicity Criteria.

Pharmacokinetic evaluation was performed in patients who gave written informed consent. Peripheral blood samples (5 ml) were collected in a heparinized tube before initiation of the treatment, and 7 days, 3 weeks, 4–6 weeks, 12–15 weeks, 6–8 months, and 10–14 months after the initial treatment. In patients who underwent a second treatment, the

scheduled blood samples collections after the initial treatment were discontinued. Instead, blood samples were drawn 7 days, 3 weeks, 4–6 weeks, 12–15 weeks, 6–8 months, and 10–14 months after the second treatment. All samples were centrifuged immediately after sampling and stored below −25 °C. Platinum concentrations in the treated tumor and nontumorous tissues were examined in patients who underwent hepatic resection after administration of SM-11355. The plasma and the tissue total platinum concentrations were determined by inductively coupled plasma mass spectrometry (ICP-MS). The maximum plasma concentration (*C*max) and the maximum plasma concentration time (*T*max) were analyzed.

The sample-size goal of this study was set at 15 eligible patients. This number of patients was planned using a design based on the assumptions that the CR rate in conventional TAE was 9–11% [25], and the expected CR rate in this trial were 40%. If the CR rate in this trial were 40% in 15 patients, the lower limit of the value of 95% confidence interval in the CR rate would be more than 15%.

Results

A total of 17 patients were enrolled in this study between July 1998 and April 1999. Of these 17 patients, 16 were eligible and one was ineligible and was not treated because of the presence of intrahepatic arteriovenous shunting. The patient characteristics in the 16 eligible patients before treatment are summarized in Table 1. Of the 16 patients, 12 underwent a second treatment, while the remaining four who showed no viable lesions after the initial treatment did not receive a second treatment.

All of the 16 patients were assessed for toxicity. Response could be assessed in 15 of the 16 patients. One patient was not evaluated for response because he underwent TAE with gelatin sponge before the response evaluation for SM-11355, at his request.

Antitumor effect

Nine patients (56%) achieved CR, three patients (19%) showed NC, three patients (19%) had PD, and one patient (6%) was not evaluated. The CR rate was 56% (9/16) with a range of 30–80% within the 95% confidence limits. In nine CR patients, three had local recurrence of the treated tumor, four showed recurrence in the lesion isolated from the treated tumors, and one showed no recurrence at the time of analysis

Table 1. Patient characteristics

Total patients		16	
Gender (men : women)		15 : 1	(94% : 6%)
Age (years)	Median (range)	65.5	(49–74)
PS*[1] (0 : 1 : 2)		16 : 0 : 0	
HBs Ag*[2] positive		2	(13%)
HCV Ab*[3] positive		12	(75%)
Albumin (g/dl)	Median (range)	3.6	(3.2–4.1)
Total bilirubin (mg/dl)	Median (range)	0.95	(0.4–2.4)
Clinical stage*[4] (I : II : III)		8 : 8 : 0	
History of PEI*[5] and/or resection		4	(25%)
Intrahepatic nodules	1	8	(50%)
	2–4	7	(44%)
	5–	1	(6%)
Maximum tumor diameter (mm)	Median (range)	20	(14–50)
Tumor stage*[6] (I : II : III : IV–a)		5 : 5 : 5 : 1	
α-fetoprotein > 100 ng/ml		4	(25%)

*[1]PS: performance status.
*[2]HBs Ag: hepatitis B surface antigen.
*[3]HCV Ab: hepatitis C virus antibody.
*[4]Grading of underlying liver cirrhosis by the Liver Cancer Study Group of Japan [18].
*[5]PEI: percutaneus ethanol injection.
*[6]Tumor stage according to the criteria of the Liver Cancer Study Group of Japan [20].

(23.6 months after the achievement of CR). In one CR patient, hepatic resection was carried out 3 months after the second treatment at his request. The pathological examination showed more than 95% necrosis in this tumor. In CR patients except for the patient who underwent the resection, the median duration of response (from the date CR was first recorded to the date on which progressive disease was first noted) was 7.1 months (range, 2.5–23.6 + months). Regarding the additional assessment of tumor response in terms of the WHO criteria, seven patients (44%) showed 50% or greater decrease in total tumor size of the treated lesions, as determined by two observations not less than 4 weeks apart.

Change in serum α-fetoprotein levels

Serum AFP levels were measured serially after treatment. Of the four patients whose serum AFP levels

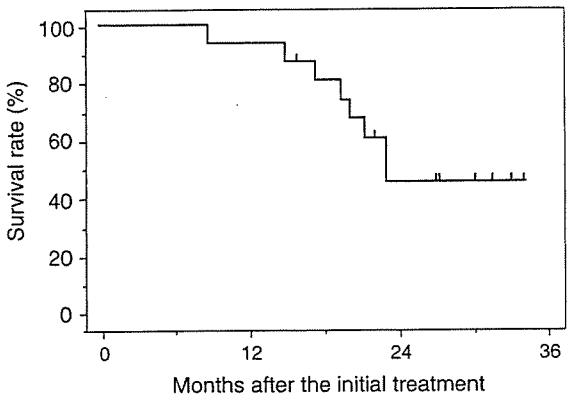

Figure 2. Survival curve for HCC patients who received intra-arterial chemotherapy using SM-11355.

Table 2. Hematologic toxicities (per patient)

	Grade			
	1	2	3	4
Hemoglobin	2 (13%)	1 (6%)	0 (0%)	0 (0%)
Leucocytes	6 (38%)	2 (13%)	0 (0%)	0 (0%)
Neutrophils	6 (38%)	1 (6%)	3 (19%)	0 (0%)
Platelets	2 (13%)	5 (31%)	0 (0%)	0 (0%)
Eosinophils >500/mm^3	16 (100%)			

were over 100 ng/ml before treatment, three patients (75%) showed at least 50% reduction in the level within 1 month after treatment.

Survival

The overall survival curve is shown in Figure 2, with a median follow-up period of 23.1 months (range, 8.7–34.0 months). Of the sixteen patients studied, eight had died of cancer at the time of analysis. Eight patients were still alive 15.7–34.0 months after the initial treatment. The 1-year and 2-year survival rates and median survival time were 94%, 45%, and 23 months, respectively.

Toxicity

Hematological toxicity per patient is summarized in Table 2. It was relatively mild and transient, although three patients (19%) showed grade 3 neutropenia. All 16 patients showed an increase of the eosinophil count to more than 500/mm^3; the maximum eosinophil count was 502–4743 (median 817) /mm^3, which was observed 2–3 weeks after the initial treatment. However, the eosinophil counts in all patients recovered to initial levels. While experiencing eosinophilia, fourteen patients (88%) developed grade 1–2 fever and three patients (19%) showed deterioration of performance status (ECOG performance status: 1–2). After the second treatment, five of twelve patients (42%) had an increase of the eosinophil count to more than 500/mm^3. The maximum count was 214–2030 (median 405)/mm^3, and occurred 1–2 weeks after the second treatment. Of these five patients, one showed grade 2 fever and declining performance status temporarily when eosinophilia was observed.

A summary of nonhematological toxicity in each patient is shown in Table 3. Grade 3 toxicity was observed as elevated total bilirubin in three patients (19%), as elevated AST in seven patients (44%), and as elevated ALT in three patients (19%). Among 13 patients who showed grade 1 or worse hepatic toxicity, the peaks of the toxicity were achieved within 2 weeks after the treatment in six patients (46%), 3–5 weeks in three patients (23%), and 9–11 weeks in four patients (31%). The liver function returned to the initial level within 8 weeks after the peak in all patients except one whose total bilirubin improved but did not attain to the initial level during his follow-up period (4 weeks after the peak). None of the patients showed grade 4 toxicity. Fifteen patients (94%) developed grade 1–3 fever after the initial treatment, although it was alleviated within a week. Grade 3 fever, which was observed in one patient, was caused by bacteremia induced from the indwelling intravenous catheter. This was unlikely to be related to the treatment and was not classified as a toxicity. Fourteen of the fifteen patients redeveloped grade 1–2 fever 7 days or later after the initial treatment while experiencing eosinophilia. Among the 12 patients who underwent the second treatment, fever occurred immediately after the treatment in 10 patients (83%), and recurred 7 days or later after the treatment in 1 patient (8%). All serum IgE levels, which were measured serially in 10 patients, were normal.

Pharmacokinetics

Plasma samples for pharmacokinetic studies were obtained from all 16 patients. Figure 3 shows changes in the plasma total platinum on a log scale. The median *C*max and *T*max were 9.95 ng/ml (range, 6.3–22.0) and 28 (range, 18–37) days, respectively, after the initial treatment, and 16 ng/ml (range, 8.2–54.0) and 21 (range, 7–34) days, respectively,

Table 3. Nonhematologic toxicities (per patient)

	Grade				
	1	2	3	4	
Nausea, vomiting	0 (0%)	4 (25%)	0 (0%)	–	(25%)
Diarrhea	3 (19%)	2 (13%)	0 (0%)	0 (0%)	(31%)
Stomatitis	1 (6%)	0 (0%)	0 (0%)	0 (0%)	(6%)
Total bilirubin	–	2 (13%)	3 (19%)	0 (0%)	(31%)
AST*1	0 (0%)	2 (13%)	7 (44%)	0 (0%)	(56%)
ALT*2	2 (13%)	2 (13%)	3 (19%)	0 (0%)	(44%)
Alkaline phosphatase	3 (19%)	4 (25%)	0 (0%)	0 (0%)	(44%)
Abdominal pain	6 (38%)	2 (13%)	0 (0%)	0 (0%)	(50%)
Serum creatinine	4 (25%)	0 (0%)	0 (0%)	0 (0%)	(25%)
Proteinuria	1 (6%)	2 (13%)	0 (0%)	0 (0%)	(19%)
Rash	0 (0%)	1 (6%)	0 (0%)	0 (0%)	(6%)
Fever	2 (13%)	13 (81%)	0 (0%)	0 (0%)	(94%)

*1aspartate aminotransferase.
*2alanine aminotransferase.

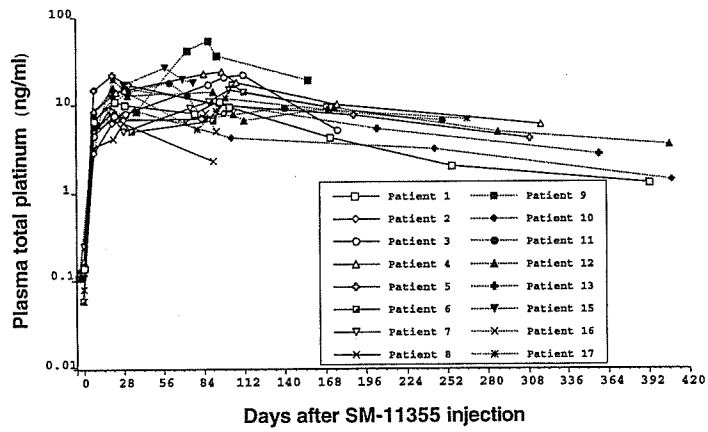

Figure 3. Plasma concentration of total platinum after administration of SM-11355.

after the second treatment. The plasma total platinum concentrations gradually decreased after *C*max was reached: it represented 47.3 ± 12.5%, 31.0 ± 6.4%, and 17.1 ± 3.7% of the *C*max at 12–15 weeks, 6–8 months, and 10–14 months after the final administration of SM-11355, respectively.

One patient underwent hepatic resection 3 months after the second treatment (Figure 4). In this patient, the dose of SM-11355 was 40 mg/body in the first treatment and 20 mg/body in the second treatment. The total platinum concentration was 250 μg/g tissue in the central part of tumor, 190 μg/g tissue in the peripheral part of the tumor, and 29 μg/g tissue in the nontumorous tissue.

Discussion

Several intra-arterial chemotherapy regimens using adriamicin [1], fluorouracil [2], fluorodeoxyuridine (FUDR) [3], mitomycin C [4], cisplatin [5], epirubicin [6], and mitoxantrone [7] administered singly or in combination [8] have been reported as treatments for HCC. Although some regimens have shown a high response rate, most of them resulted in only short survival or had significant adverse effects. Accordingly, the optimum regimen for intra-arterial chemotherapy for HCC is still unknown.

Among the anticancer drugs noted above, cisplatin is one of the most promising agents for

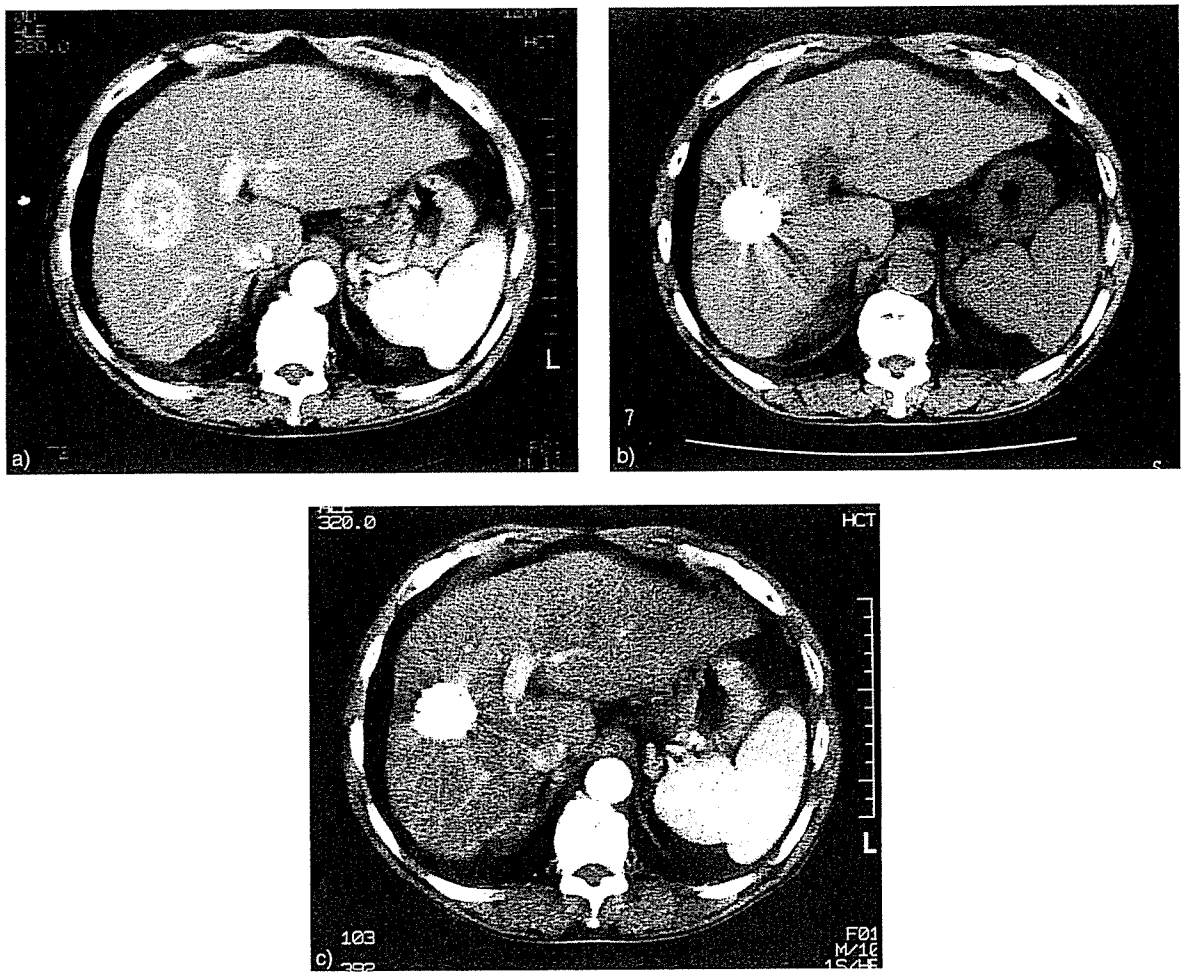

Figure 4. (a) CT scan before SM-11355 injection showed a hyperenhancing tumor in the right lobe of the liver; (b) CT scan which was performed 3 months after the second treatment showed dense accumulation of lipiodol throughout the whole tumor; (c) CT scan which was carried out 5 months after the second treatment showed maintenance of the lipiodol accumulation.

intra-arterial chemotherapy against HCC. A remarkable antitumor response was reported in patients who received cisplatin–lipiodol suspension intra-arterially [5]. However, cisplatin is rather hydrophilic and barely soluble in lipiodol (which is a carrier of anticancer agents for targeting chemotherapy for HCC) even when cisplatin is prepared as a powder to increase its solubility in lipiodol. Therefore, only a small volume of cisplatin remains in the tumor for a long period, and most of the agent is released briefly into bloodstream in the systemic circulation and cause systemic side effects such as nausea/vomiting and renal dysfunction. SM-11355 has been developed as a lipophilic platinum complex in an effort to produce a superior antitumor effect for HCC and lower toxicity than cisplatin [15,26]. SM-11355–lipiodol suspension is a stable and colloidal emulsion that is deposited within HCCs and releases active derivatives of SM-11355 there gradually [17]. SM-11355 itself, unlike cisplatin, is not an active agent against HCC, but SM-11355–lipiodol has greater stability and longer sustained release of active platinum compounds that bind to nuclear DNA in comparison with cisplatin–lipiodol [17,26,27]. In a rat intra-arterial chemotherapy model, SM-11355–lipiodol showed higher antitumor activity and lower hepatic toxicity than cisplatin–lipiodol [27].

In the present study, intra-arterial chemotherapy with SM-11355 had a remarkable antitumor effect in terms of both tumor necrosis and tumor reduction. Tumor necrosis was evaluated by lipiodol accumulation as revealed by CT in this study, because the

lipiodol accumulation area in the tumor corresponds to the tumor necrotic area [21,22]. In a previously conducted phase II study for styrene maleic acid neocarzinostatin (SMANCS) (zinostatin stimalamer), which is now the only commercially available lipophilic agent for HCC in Japan, only 27% of patients achieved CR [9]. A phase II study of TAE with SMANCS and gelatin sponge showed a CR rate of 56% [13]. In intra-arterial chemotherapy with cisplatin, approximately 40–50% of the patients showed reduction in tumor size more than 50% [5,28]. These results indicate that SM-11355, which showed a CR rate of 56% and produced tumor size reduction of more than 50% in 44% of the patients in the present study, may have equivalent efficacy to that of cisplatin or SMANCS plus gelatin sponge.

The toxicity profile in the present study was mild and acceptable. The major toxicities with this treatment were neutropenia and liver dysfunction, but neither grade 4 toxicities nor episodes of febrile neutropenia were observed. The hepatic toxicity of SM-11355 was relatively milder than that of TAE; elevation of the serum total bilirubin level was more frequent with SMANCS plus gelatin sponge compared with SM-11355 (68% vs. 31%) [13]. This study also showed favorable results in terms of renal toxicity (0%) and nausea/vomiting (25%), whereas intra-arterial chemotherapy using cisplatin causes vomiting in 62% patients (the incidence of renal toxicity was ambiguous) [5]. Eosinophilia was observed 7 days or later after the treatment, concomitantly with fever, which was likely due to drug allergy to SM-11355, but the precise mechanism remains unknown.

Our pharmacokinetic study revealed that the plasma concentration of total platinum in patients receiving SM-11355 was very low: the *C*max was approximately 300-fold lower than that reported in a study of intra-arterial administration of cisplatin at 40–100 mg/body [5]. The *T*max ranged from 18–37 days, which was much longer than the 10–60 min in the cisplatin study. In a preclinical study in laboratory animals, more than 80% of the administered total platinum dose was delivered to the liver both 24 h and 168 h after intra-arterial administration of SM-11355 (unpublished data). In the patients who underwent hepatic resection, the platinum concentration in the tumor tissue was more than six times higher than that in the nontumorous tissue. These results indicate that SM-11355 suspended in lipiodol selectively targeted liver tumors, and released platinum into the bloodstream gradually.

Cyclohexane-1,2-diamineplatinum(II) dichloride (DPC) is one of the platinum compounds released from SM-11355, which is presumed to have active antitumor activity [17], but analytical techniques have not been developed to measure DPC concentration under physiological conditions.

In conclusion, intra-arterial chemotherapy with SM-11355 was effective and well tolerated in patients with advanced HCC. This agent appeared to show a marked antitumor effect and reduced toxicity. A large-scale Phase II trial is now being conducted to confirm the results found in this study.

Appendix

Efficacy and safety evaluation committee

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Shoji Fukushima (*chairperson)	Kobe Gakuin University

Judgment committee

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Acknowledgments

This study was supported by Sumitomo Pharmaceutical Co. Ltd. We wish to thank Ms K. Kondo and Ms Y. Kawaguchi for help with the manuscript preparation.

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Cancer Chemother Pharmacol (2004) 54 (Suppl 1): S78–S82
DOI 10.1007/s00280-004-0891-1

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New approaches for pancreatic cancer in Japan

Published online: 14 August 2004

Abstract Pancreatic cancer is the fifth leading cause of cancer-related mortality in Japan, with an estimated annual incidence rate of approximately 20,000 cases. Even in patients with resectable disease, the long-term outcome remains unsatisfactory due to early recurrence after resection. However, surgical resection has offered the only curative strategy for pancreatic cancer. Currently available chemotherapeutic agents have little impact on survival, although the development of gemcitabine has renewed interest in clinical research for pancreatic cancer. To further improve the prognosis of patients with pancreatic cancer, the development of more effective nonsurgical treatment is essential. Studies to identify more effective treatments, such as chemotherapy, interventional therapy and gene therapy, are ongoing in Japan. The expanding understanding of molecular and genetic biology should facilitate research to develop novel molecular-targeted agents and to establish individualized therapy regimens for this disease.

Keywords Pancreatic cancer · Chemotherapy · Gemcitabine · Gene therapy

This work was presented at the 19th Bristol-Myers Squibb Nagoya International Cancer Treatment Symposium, "State of the Arts for Digestive Organs", 14–15 November 2003, Nagoya, Japan.

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Introduction

Pancreatic cancer is the fifth leading cause of cancer-related mortality in Japan. The estimated annual incidence is approximately 20,000 cases, which is similar to its mortality [26]. Of all the treatment modalities for pancreatic cancer, only resection offers the opportunity for cure. However, because of local extension and/or metastatic disease, only a small minority of pancreatic cancer patients are candidates for resection with curative intent. Moreover, even for these selected patients, the prognosis remains unsatisfactory because of postoperative recurrence, indicating that surgery alone has limited value in the treatment of pancreatic cancer. Accordingly, to improve the overall survival of patients with pancreatic cancer, there is an urgent need to develop effective nonsurgical treatment for this disease. Various studies have been conducted to identify more effective nonsurgical treatments for pancreatic cancer in Japan. This review focuses on new approaches for chemotherapy in patients with advanced pancreatic cancer, and introduces other approaches including nonmyeloablative allogeneic stem cell transplantation and gene therapy.

Fluoropyrimidine-based chemotherapy in Japan

Of all chemotherapeutic drugs, the thymidylate synthase inhibitor fluorouracil (5-FU) has been the most extensively evaluated and most widely used agent for pancreatic cancer in Japan. Since the results with this agent remain poor, with reported response rates reaching 20% [17], there have been various attempts at biochemical modulation to enhance the antitumor activity of 5-FU through different agents. In Japan, sequential administration with methotrexate and 5-FU has been examined, but the antitumor activity of this regimen appears to be only marginal [9]. UFT is an orally administered drug developed in Japan that is a combination of tegafur, a prodrug of 5-FU, and uracil,

a competitive inhibitor of dihydropyrimidine dehydrogenase. Unfortunately, clinical trials of this agent have demonstrated little superiority in therapeutic effect to 5-FU alone against advanced pancreatic cancer [22, 31].

S-1 is an oral anticancer drug, which consists of tegafur (FT), 5-chloro-2,4-dihydroxypyridine (CDHP), and potassium oxonate (Oxo). The drug was developed in Japan to improve the tumor-selective toxicity of 5-FU by two biochemical modulators, CDHP and Oxo. CDHP is a competitive inhibitor of dihydropyrimidine dehydrogenase involved in degradation of 5-FU, and maintains efficacious 5-FU concentrations in plasma and tumor tissues. Oxo, a competitive inhibitor of orotate phosphoribosyltransferase, inhibits phosphorylation of 5-FU in the gastrointestinal tract and reduces the serious gastrointestinal toxicity of 5-FU. S-1 has already demonstrated a potent antitumor effect in various solid tumors in clinical studies [7, 11, 12, 16, 25, 27]. We conducted an early phase II study of S-1 in patients with metastatic pancreatic cancer [19]. This study showed promising results with a 21% response rate in 19 evaluable patients and a manageable toxicity profile of this agent. We are conducting a multi-institutional late phase II study of S-1 for metastatic pancreatic cancer to confirm these results.

There has been hope that improved therapeutic results might be obtained with 5-FU-based multiagent chemotherapy, since several agents having at least some activity have been identified. Cisplatin has been the most extensively used agent as a potential modulator of 5-FU, and has itself demonstrated some antitumor activity against pancreatic cancer. The combination of continuous infusion of 5-FU and bolus administration of cisplatin has been found to have limited antitumor activity, with only an 8% response rate in 37 Japanese patients [15]. With this treatment, 4 (21%) of 21 patients obtained remarkable symptom relief [20]. Based on laboratory data suggesting a profound schedule dependency for the cytotoxicity of this combination, Tsuji and colleagues conducted a phase II trial of continuous-infusion 5-FU and low-dose consecutive cisplatin in 39 patients with advanced pancreatic cancer [30]. 5-FU (160 mg/m^2 per day) was continuously infused over 24 h for seven consecutive days and cisplatin (3 mg/m^2 per day) was administered over 30 min for 5 days followed by a 2-day rest period, every 4 weeks. The objective response rate was 28.2%, with a clinical benefit response rate of 48.7% and a median survival time of 6.5 months.

Most studies of 5-FU-based multiagent chemotherapy have documented little reproducible impact on patient survival, while all of these regimens exhibit great toxicity. Takada and coworkers failed to demonstrate a survival benefit for combination chemotherapy consisting of 5-FU, doxorubicin and mitomycin for Japanese patients with unresectable pancreatic and biliary tract cancer compared to palliative surgery alone [29]. Based on the results to date, 5-FU-based multiagent chemotherapy cannot be recommended outside clinical trials.

Chemotherapy using gemcitabine

Gemcitabine is a deoxycytidine analog that is capable of inhibiting DNA replication and repair. Gemcitabine has the potential for great activity against various solid tumors including pancreatic cancer. This is because of gemcitabine's prolonged inhibition of both cell synthetic function and progression through the cell cycle. In a randomized trial comparing gemcitabine with 5-FU, gemcitabine showed significantly better results in terms of clinical benefit and survival [3]. Accordingly, gemcitabine has been accepted as first-line chemotherapy for advanced pancreatic cancer. In the phase I trial conducted in Japan before this randomized trial, the recommended dose schedule of gemcitabine was 800 mg/m^2 weekly ×3 followed by 1 week of rest, with leukocytopenia as the dose-limiting toxicity [28]. However, in most trials of gemcitabine for pancreatic cancer including the previous randomized study, a dose of 1000 mg/m^2 has been employed and approved in Western countries. Therefore, we conducted a phase I trial to confirm the tolerability of a weekly schedule of gemcitabine at a dose of 1000 mg/m^2 in Japanese patients with advanced pancreatic cancer [18]. This study showed a low incidence of dose-limiting toxicity, suggesting that gemcitabine at 1000 mg/m^2 weekly ×7 followed by 1 week rest and weekly ×3 every 4 weeks may be tolerated in Japanese patients with advanced pancreatic cancer. In this trial, a partial response was obtained in 2 (18%) of the 11 enrolled patients with metastatic pancreatic cancer and a clinical benefit response was achieved in 2 (29%) of the 7 evaluable patients. Based on the consistency in response and toxicity of this study with those of previous Western trials, gemcitabine was approved in Japan for the treatment of pancreatic cancer in 2001.

Despite worldwide agreement on the role of gemcitabine as a first-line treatment in advanced pancreatic cancer, only a minority of patients obtain clear benefits such as symptom relief and prolongation of survival from the administration of gemcitabine. Accordingly, it is important to establish effective methods for estimating individual drug response and toxicity. We are currently conducting a pharmacogenomics study for gemcitabine to identify polymorphisms of genes encoding drug-metabolizing enzymes and membrane-transporter proteins for gemcitabine and its metabolites, and their correlation with pharmacokinetics, toxicity and tumor response in pancreatic cancer patients. In this study, evidence for functional single-nucleotide polymorphisms responsible for gemcitabine metabolism is accumulating. This gene-based information has the potential to aid in the establishment of individualized therapy regimens using gemcitabine for pancreatic cancer.

Based on preclinical and clinical data showing the favorable antitumor effects of gemcitabine in combination with other cytotoxic agents, additional trials of gemcitabine-based regimens including gemcitabine plus S-1 are in progress in Japan.

Other new agents

Several novel chemotherapeutic agents developed in Japan, such as irinotecan, exatecan, UCN-01, NK911, capecitabine and S-1, have been evaluated in clinical trials for pancreatic cancer in Japan and/or other countries. It is hoped that improved therapeutic results might be obtained using these agents either singly or in combination with gemcitabine. This section focuses on irinotecan and NK911, clinical trials of which are ongoing for pancreatic cancer patients in Japan.

Irinotecan, a semisynthetic, water-soluble derivative of the plant alkaloid camptothecin, induces antitumor activity by inhibition of topoisomerase I. The single-agent antitumor activity of irinotecan in pancreatic cancer has been demonstrated in two phase II studies [24, 33]. In the first study conducted in Japan, administration of irinotecan at 100 mg/m^2 weekly or 150 mg/m^2 every other week to previously untreated patients resulted in a response rate of 11% in the 35 assessable patients treated [24]. In the second study, conducted by the European Organization for Research and Treatment of Cancer (EORTC), an irinotecan regimen of 350 mg/m^2 every 3 weeks induced partial responses in 9% of the 32 assessable patients [33]. A confirmatory phase II study is now underway in Japan. While no significant survival improvement with the combination of irinotecan and gemcitabine over gemcitabine alone has been reported recently [23], this agent may have the potential to be used in gemcitabine-refractory patients.

A new agent, developed based on the pathobiology of pancreatic cancer, is also being studied in a clinical trial for treatment of this disease. NK911 is a doxorubicin-encapsulated polymeric micellar nanoparticle [10]. The polymeric micelle carrier of NK911 consists of a block copolymer of polyethyleneglycol and polyaspartic acid. Polyethyleneglycol is expected to be in the outer shell of the micelle. NK911 has a highly hydrophobic inner core, and therefore can entrap a sufficient amount of doxorubicin. After the NK911 is extravasated from the tumor vessels, doxorubicin is released from NK911. It is suggested that pegylated liposomal doxorubicin (known as Doxil) can deliver doxorubicin to a solid tumor, via the enhanced permeability and retention (EPR) effect, more efficiently than NK911. This is because pegylated liposomal doxorubicin is more stable in the bloodstream. However, it is expected that NK911 can distribute more doxorubicin into cancer cells distant from the tumor vessel than can pegylated liposomal doxorubicin, once NK911 is extravasated from the tumor vessel. It is, therefore, suggested that NK911 may be more effective against cancers where the tumor vessel network is rough due to an abundant collagen-rich matrix, e.g. pancreatic cancer. In a phase I trial, NK911 was well tolerated and produced only moderate nausea and vomiting at myelosuppressive dosages. A partial response was obtained in one patient with gemcitabine refractory pancreatic cancer [13]. A phase II study of NK911 is ongoing in Japan.

A novel arterial infusion chemotherapy

Homma and coworkers have reported a novel arterial infusion chemotherapy for advanced pancreatic cancer [8]. To restrict the blood flow into the pancreas, the peripancreatic blood vessels were embolized superselectively with microcoils. The catheter tip for continuous arterial infusion of 5-FU and cisplatin is placed in the splenic artery just proximal to the branching of the great pancreatic artery for treatment of the primary tumor, and in the common hepatic artery for treatment of metastatic liver lesions. In 31 patients with advanced pancreatic cancer, 2 achieved a complete response and 16 showed a partial response. The median survival period of all patients was 18.3 months. They concluded that this treatment is effective against both primary tumor and metastatic lesions in unresectable pancreatic cancer patients.

Other approaches in Japan

Allogeneic stem-cell transplantation has been proven to have potent antitumor effects not only in patients with hematologic malignancies but also in those with solid tumors [6, 32]. Successful nonmyeloablative allogeneic peripheral blood stem-cell transplantation has been reported in patients with metastatic renal cell carcinoma, and the results with this treatment are consistent with a graft-versus-tumor effect [4, 5]. Omuro and colleagues described a patient who showed continuous regression of unresectable pancreatic tumor following nonmyeloablative allogeneic peripheral blood stem-cell transplantation, which was considered to be attributed to a graft-versus-tumor effect [21]. Based on the results of the report and those for other malignancies, clinical trials of nonmyeloablative allogeneic peripheral blood stem-cell transplantation are being conducted with pancreatic cancer patients in several institutes in Japan.

Increased understanding of the biology of pancreatic cancer could provide the potential to develop entirely novel treatment options. One innovative approach for therapy is a combination of interferon α and antisense K-ras [14]. We have shown that interferon α gene transduction into pancreatic cancer cells induces growth suppression and cell death in the cells; an effect that appears to be more prominent when compared with other types of cancers and normal cells. Another strategy developing for pancreatic cancer targets its characteristic genetic aberration, K-ras point mutation. It has been reported that the expression of antisense K-ras RNA significantly suppresses the growth of pancreatic cancer cells [1, 2]. When these two gene therapy strategies are combined, the expression of antisense K-ras

RNA significantly enhances interferon α-induced cell death (1.3- to 3.5-fold), and suppresses subcutaneous growth of pancreatic cancer cells in mice. Because the 2′,5′-oligoadenylate synthetase/RNaseL pathway, which is regulated by interferon and induces apoptosis of cells, is activated by double-strand RNA, it is plausible that the double-strand RNA formed by antisense and endogenous K-ras RNA enhances the antitumor activity of interferon α. This study suggested that the combination of interferon α and antisense K-ras RNA is a promising gene therapy strategy against pancreatic cancer.

Conclusion

Pancreatic cancer is a major cause of cancer-related mortality in Japan. At present, nonsurgical therapy is of limited value in the treatment of pancreatic cancer, but various approaches are being attempted that we hope will result in improved patient survival. The evolving understanding of molecular and genetic biology should facilitate research to develop novel target-based agents and to establish individualized therapy regimens for this disease.

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British Journal of Cancer (2004) 91, 673–677

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Phase II study of radiotherapy combined with gemcitabine for locally advanced pancreatic cancer

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Gemcitabine has been reported to be a potent radiosensitiser in human pancreatic cell lines. This study was conducted to evaluate the efficacy and toxicity of radiotherapy combined with gemcitabine for locally advanced pancreatic cancer. In all, 42 patients with pancreatic cancer that was unresectable but confined to the pancreatic region were treated with external-beam radiation (50.4 Gy in 28 fractions over 5.5 weeks) and weekly gemcitabine (250 mg m^{-2}, 30-min infusion). Maintenance gemcitabine (1000 mg m^{-2} weekly $\times$ 3 every 4 weeks) was initiated 1 month after the completion of the chemoradiotherapy and continued until disease progression or unacceptable toxicity. Of the 42 patients, 38 (90%) completed the scheduled course of chemoradiotherapy. The major toxicity was leucopenia and anorexia. There was one death attributed to duodenal bleeding and sepsis. The median survival time was 9.5 months and the 1-year survival rate was 28%. The median progression-free survival time was 4.4 months. In 35 patients with documented disease progression at the time of analysis, 34 (97%) showed distant metastasis as the cause of the initial disease progression. The chemoradiotherapy used in this study has a moderate activity against locally advanced pancreatic cancer and an acceptable toxicity profile. Future investigations for treatment with more systemic effects are warranted.
British Journal of Cancer (2004) **91**, 673–677. doi:10.1038/sj.bjc.6602001 www.bjcancer.com
Published online 29 June 2004

Keywords: pancreatic cancer; chemoradiotherapy; gemcitabine; radiosentitiser

Clinical

Pancreatic cancer is the fourth leading cause of cancer death in the United States and the fifth leading cause in Japan. The statistics indicate a rapid increase in the number of deaths and the death rate due to pancreatic cancer in Japan, but the precise reasons are not clear, except for smoking. Pancreatic cancer in most patients is surgically unresectable at the time of diagnosis because of the difficulty of early detection of this disease. For patients with locally advanced pancreatic cancer, chemoradiotherapy has been accepted as standard treatment because the results of previous randomised trials have indicated that concurrent external-beam radiation therapy and 5-fluorouracil (5-FU) therapy results in a significantly longer survival time than radiotherapy (Moertel *et al*, 1969; Gastrointestinal Tumor Study Group, 1981) or chemotherapy alone (Gastrointestinal Tumor Study Group, 1988). In attempts to improve the efficacy of the treatment, numerous trials using modified approaches of chemoradiotherapy have been conducted (Chakravarthy and Abrams, 1997; Okada, 1999). However, there has not yet been a regimen that has demonstrated superiority over conventional chemoradiotherapy performed in randomised controlled trials.

Gemcitabine is a novel deoxycytidine analog, which has demonstrated significant clinical benefit and survival improvement compared with 5-FU in patients with advanced pancreatic cancer (Burris *et al*, 1997). Gemcitabine has also been shown to be a potent radiosensitiser in human pancreatic and other solid tumour cell lines (Lawrence *et al*, 1996; Shewach and Lawrence, 1996; van Putten *et al*, 2001), suggesting that the combination of radiotherapy and gemcitabine may improve survival in patients with locally advanced disease. A phase I trial that was conducted in our hospital determined the recommended dose of weekly gemcitabine for the phase II chemoradiotherapy trial to be 250 mg m^{-2} (Ikeda *et al*, 2002). We report our results of the phase II study that was conducted to clarify the efficacy and toxicity of concomitant chemoradiotherapy with gemcitabine in patients with locally advanced pancreatic cancer.

PATIENTS AND METHODS

Patients eligible for this study had locally advanced pancreatic cancer for which they had not received any anticancer treatment. Each patient was required to meet the following eligibility criteria: pathological proof of adenocarcinoma of the pancreas; an Eastern Cooperative Oncology Group (ECOG) performance status of 0–2; adequate bone marrow reserve (white blood cell count $\geqslant$4000 mm^3, platelet count $\geqslant$100 000 mm^3, haemoglobin level $\geqslant$10 g dl^{-1}); adequate renal function (normal serum creatinine and blood urea nitrogen levels, and a creatinine clearance level $\geqslant$60 mg min^{-1}); a serum aspartate aminotransferase (AST) level $<$2.5 times upper normal limit (UNL); a serum alanine aminotransferase (ALT) level $<$2.5 times UNL; and written informed consent. Patients with obstructive jaundice were

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Received 29 March 2004; accepted 24 May 2004; published online 29 June 2004

required to have a serum total bilirubin level of less than 2.0 mg dl^{-1} after biliary drainage. Pretreatment staging included ultrasonography and dynamic computed tomography (CT) scans of both the abdomen and the chest. The possibility for resection of the local tumour was assessed by dynamic CT and/or angiography. Obstruction or bilateral invasion of the portal vein and/or tumour encasement of the celiac or superior mesenteric arteries was considered to be unresectable. Patients were excluded if they met the following criteria: concomitant malignancy, pleural and/or peritoneal effusion, active ulcer of the gastrointestinal tract, active infection, severe heart disease, pregnant or lactating females, or other serious medical conditions. The goal was set at 40 eligible patients. This number of patients was planned using a design based on the assumptions that the median survival time in conventional chemoradiotherapy was 10 months, expected median survival time was 14 months, type I error was 5% (one-tailed) and statistical power was 70%.

Radiotherapy was delivered via a racetrack microtron (MM50, Scanditronix, Upsala, Sweden) with a 25 MV X-rays. A total dose of 50.4 Gy was delivered in 28 fractions over 5.5 weeks. All patients had treatment planning, CT scans (X-vision, Toshiba, Tokyo) and FOCUS (version 3.2.1, CMS, St Louis, MO, USA) was used as a radiotherapy treatment planning system. Clinical target volume (CTV) included the primary tumour, nodal involvement detected by CT scan and regional draining and paraaortic lymph nodes, which included the peripancreatic nodes, celiac and superior mesenteric axes. Planning target volume was defined as CTV plus a 10-mm margin. Four field techniques (anterior, posterior and opposed lateral fields) were used. Spinal cord dose was maintained below 45 Gy and $\geqslant$50% of liver was limited to $\leqslant$30 Gy, $\geqslant$50% of both kidneys were limited to $\leqslant$20 Gy.

Gemcitabine at a dose of 250 mg m^{-2} was given intravenously over 30 min starting 2 h before radiotherapy weekly for 6 weeks. This schedule was based on an *in vitro* study which revealed that gemcitabine induced its radiosensitising effect in cells within 2 h (Lawrence *et al*, 1997). Toxicity was assessed according to the National Cancer Institute – Common Toxicity Criteria version 2.0. When grade 3 haematological toxicity, serum creatinine of 1.5–2.0 times UNL, total bilirubin level of 3.0–5.0 times UNL, serum AST/APT of 5.0–10 times UNL and/or grade 2 nonhaematological toxicity (excluding nausea, vomiting, anorexia, fatigue, constipation, alopecia and dehydration) were observed, gemcitabine administration was omitted and postponed to the next scheduled treatment day. The radiotherapy was also suspended, and then resumed when the toxicities recovered. In patients who experienced the above adverse effects, dose reduction of gemcitabine to 200 mg m^{-2} was allowed in subsequent administrations. The combined treatment was discontinued when grade 3 leucopenia and/or neutropenia with high fever, grade 4 haematological toxicities after dose reduction of gemcitabine, serum creatinine of >2.0 times UNL, total bilirubin level of >5.0 times UNL, serum AST/APT of >10 times UNL, grade 3 or 4 nonhaematological toxicities (excluding nausea, vomiting, anorexia, fatigue, constipation, alopecia and dehydration), grade 4 vomiting, a total of 2 weeks of delay due to toxicity for any reason or tumour progression were observed. At 1 month after the completion of chemoradiotherapy, maintenance chemotherapy of gemcitabine at a dose of 1000 mg m^{-2} was administered as a 30-min intravenous infusion weekly for 3 weeks with 1-week rest until disease progression or unacceptable toxicity. Follow-up CT was performed within 1 week after the completion of chemoradiotherapy, and thereafter every 2 months to evaluate tumour response according to the WHO criteria (World Health Organization, 1979).

Progression-free and overall survival times were calculated from the first day of treatment using the Kaplan–Meier method (Kaplan and Meier, 1958). Serum CA 19-9 levels were measured monthly by a radioimmunometric assay using the Centocor radioimmunoassay kit (Centocor, Inc., Malvern, PA, USA).

RESULTS

Patients and treatments

In all, 42 patients were enrolled in the study between July 2001 and July 2002. Patient characteristics are listed in Table 1. A total of 38 patients (90%) received the full regimen of chemoradiotherapy, and the remaining four patients (10%) discontinued the treatment after 18.0–45.0 Gy. The reasons for the treatment discontinuation were elevated serum ALT of >10 times UNL (two patients), duodenal bleeding (one), and patient's refusal of treatment due to general fatigue (one). After discontinuation of the chemoradiotherapy, the two patients who showed the ALT elevation suspected as gemcitabine-related toxicity received chemoradiotherapy using 5-FU, and the other two patients underwent only supportive care. Of 241, 30 (12%) planned gemcitabine injections (0.7 injections per patient) were omitted owing to adverse events including grade 3 or more leucopenia and/or neutropenia, grade 2 fever, grade 2 skin rash and patient's refusal due to nausea, vomiting or fatigue. In three patients who showed grade 4 leucopenia and/or neutropenia, the dose of gemcitabine was modified in subsequent injections. Maintenance chemotherapy was initiated in 23 of the 38 patients who completed the full regimen of chemoradiotherapy. Of the remaining 15 patients, seven showed deterioration of general condition due to disease progression before initiating the chemotherapy, seven refused the treatment due to appetite loss (4) or general fatigue (3) and one transferred to another hospital (1).

Response and survival

Tumour response was determined in 40 patients. Two patients were excluded from the protocol efficacy analysis because their treatment was switched over to chemoradiotherapy using 5-FU before the response evaluation due to the ALT elevation. Nine patients (21%) achieved a partial response, 26 (62%) remained stable and five (12%) showed progressive disease demonstrated by the development of distant metastases. No patients could undergo tumour resection even after the completion of chemoradiotherapy because of infiltration of the adjacent large vessels. In 22 (76%) of the 29 patients with a pretreatment serum CA19-9 (carbohydrate antigen 19-9) level of 100 U ml^{-1} or greater, the level was reduced more than 50% within 14 weeks after initiation of treatment.

Table 1 Patient characteristics

Number of patients	42
Gender	
Male	19 (45%)
Female	23 (55%)
Age (years)	
Median (range)	59 (43–73)
ECOG performance status	
0	12 (29%)
1	30 (71%)
Tumour location	
Head	21 (50%)
Body–tail	21 (50%)
CEA (ng ml^{-1})	
Median (range)	11 (1.0–62.7)
CA19-9 (U ml^{-1})	
Median (range)	2775 (1–15 620)

ECOG = Eastern Cooperative Oncology Group; CEA = carcinoembryonic antigen; CA19-9 = carbohydrate antigen 19-9.