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Sequential Chemotherapy and Circadian Rhythm in Human Solid Tumours

A Randomised Trial

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Summary. *Sixty-three patients were randomised into two groups each receiving the same 40-h sequential chemotherapy regimen (methotrexate or 5-FU, followed by vinblastine and cyclophosphamide). In the group receiving infusions of chemotherapeutic agents at times taking into account the circadian rhythm of tumoral proliferation, the antitumoral effectiveness appeared significantly better with respect to both tumoral regressions (85% contrasting with 58% in the other group) and the duration of response and survival.*

Introduction

The existence of a diurnal fluctuation of proliferation is well known in actively dividing normal tissues and in usually quiescent tissues triggered into active regeneration [2–4, 14, 15, 18, 19, 21].

In tumours, the same waves of proliferative activity have been demonstrated in some experimental systems [1, 5, 10, 16], e.g., in our experimental methylcholanthrene(MCA)-induced sarcomas of mice [10]. More recently, reinforcing earlier studies [12, 17, 20, 22], we have obtained evidence that such diurnal variations exist in some human tumours, such as epidermal tumours [8], subcutaneous metastases [7], and squamous-cell carcinomas of the oral cavity (C. Focan et al., in preparation), in which 29%–43% more cells were engaged in DNA synthesis during the morning than during the evening.

We have also obtained evidence of the importance of such a rhythm when the time and the sequence of administration of oncolytic drugs are chosen, first in MCA-induced sarcomas of mice [9, 11] and then in human tumours [6, 7]. We first carried out a nonrandomised clinical trial of sequential chemotherapy [6]. In this trial, an infusion of a phase-specific drug (methotrexate or 5-FU) given as an oncolytic, synchronising, and recruiting agent was followed after a 24-h interval (which appeared optimal according to in vitro-tritiated thymidine [^{3}H-TDR] incorporation [6, 7, 9, 11; C. Focan, in preparation]) by concomitant administration of a phase-specific (vinblastine) and a cell cycle-specific (cyclophosphamide) agent: more than 80% of treated solid tumours responded objectively to this combination [6, 7].

A randomised trial was obviously necessary to determine whether the quality of results resulted from the sequence used and/or from the choice of the times of drug administration.

Materials and Methods

Sixty-three patients (with 64 evaluable tumours) were randomly allocated to one of the two groups. The following characteristics were comparable in both groups: sex (group A: 19 males, 13 females; group B: 23 males, 8 females), median age [group A: 63 (47–82) years; group B: 67.5 (39–81) years, no statistical difference between A and B], number previously treated (group A: 5; group B: 7), type of cancer (classified according to both the site of the primary tumour and its histological characteristics: glandular or nonglandular, differentiated or undifferentiated) (Tables 1–3), and the number of chemotherapy courses applied (group A: 142 courses, 4.6 ± 0.7 per patient; group B: 115, 3.6 ± 0.6 per patient; no statistical difference between A and B). Moreover, as stated in Table 1, there was no difference in tumour burdens between groups A and B according to the TNM classification: the stage of the primary tumour was generally T3 or T4 and the vast majority of neoplasms were disseminated at presentation.

The regimen administered every third week to group A was that previously tested in a nonrandomised trial [6, 7] and consisted of methotrexate (60 mg/m^2) in nonglandular carcinomas, or 5-FU (15 mg/kg) in adenocarcinomas, on day 1, infused from 10 a.m. to 8 p.m., followed on day 2 by combined vinblastine (10 mg/m^2) and cyclophosphamide (300 mg/m^2) infused from 8 p.m. to 2 a.m. Group B received the same 40-h regimen, but beginning 12 h later (i.e., at 10 p.m.).

The tumoral responses were assessed as complete response (CR, apparent disappearance of all known lesions), partial response (PR, tumoral regression evaluated at between 50% and 99%), mixed response (MR, regression of one tumour site without progression of the

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Table 1. Clinical staging (TNM) and evolution during chemotherapy in patients in the trial

	No.	Sex	Age	Staging at diagnosis	Tumoral response[a] (duration in months)	Survival (months)	Remarks
Group A (33)							
Lung cancer,	1	M	58	T4-N1-M1c (CNS)	NC (2)	3	No response of CNS involvement
non-oat-cell	2	M	61	T2-M1c (liver)	PR (2+)	2.25	Died in PR from granulocytopenia
	3	M	65	T4	SR (2.5)	2.5	
	4	M	68	T3-N1-M1c (liver)	PR (2.5+)	3	Died in almost CR (cause unknown)
	5	M	51	T3	NC (11)	18	Tumour followed by bronchoscopy
	6	M	79	T4-M1c (bone)	SR (1.5)	1.5	
	7	M	57	T4-N1-M1b	CR (5.5)	9.5	
	8	M	73	T4-M1b-M1c (liver)	NC (2+)	2	Died in NC (cause unknown)
	9	M	65	T2-N1-Mo	CR (12)	19	CR of mediastinal syndroma
	10	F	56	T3-M1c (liver + CNS)	NC (2)	2	No response of CNS involvement
	11	M	66	T3-N1-M1b	NC (0.75+)	1 +	
	12	M	57	T3-M1b-M1c (CNS)	NC (1+)	1 +	
Lung cancer,	13	M	70	T4-N1-M1a	PR (4)	9 +	
oat-cell	14	M	55	T3-N1	CR (7)	9	
	15	M	65	T3	PR (10)	16 +	
	16	M	70	T4	NC (1.5)	2	
	17	M	55	T3	CR (7+)	7 +	
Ovary	18	F	71	T4-M1b (ascites, liver); M1c (pleura)	PR (1.5)	3	
	19	F	55	T4-N1-M1b (ascites)	CR (4)	8	
	20	F	51	T4-M1b (ascites, liver)	PR (3)	3 +	
	21	F	50	T4-M1b (ascites, liver)	CR (5)	8.5	
	22	F	47	T4-M1b (ascites, liver)	CR (10)	15	
GI tract	23	M	53	M. liver (primary unknown)	CR (6)	12.5	
	24	F	75	Pancreas, M1 (pleura)	F	1.5	
	25	F	54	Pancreas, M1 (supra clav., liver, bone)	NC (1+)	1 +	
	26	F	64	Stomach: T4-Nxc-M1 (P4)	PR (18)	19	
Breast	27	F	64	T4-N3-M1b-M1c (ascites, liver)	PR (11)	14	
	28	F	47	T4-N2-M1c (bone)	PR (7+)	8 +	
	29	F	64	Top-M1c (bone)	PR (18)	19	
Hypernephroma	30	F	40	T3-M1b-P3	PR (5)	6	
Prostatic	31	M	82	T4-M1a	NC (0.5)	0.5	Biological DT 12.5 days, fibrinolysis
Uterine cervix	32	F	72	Tx-M1 (bone)	SR (3+)	3 +	(stabilisation under
Hepatoma	33	M	65	M1 (liver + parietal tumor)	F	2 +	chemotherapy)
Group B (31)							
Lung cancer,	1	M	61	T4-N1-M1c (bone, CNS)	F	5	No response of CNS involvement
non-oat-cell	2	F	69	T3	PR (11)	17.25	
	3	F	49	T4-M1a	NC (2)	2	
	4	M	74	T2-M1a	F	3 +	
	5	M	69	T3-N1 + CIVD	NC (1)	1.5	
	6	M	75	T4-N1-M1c (bone)	PR (0.5 +)	0.5	Died in PR of granulocytopenia
	7	M	73	T3-M1a-M1c (liver)	NC (3)	4	
	8	M	54	T3-M1b	F	2	
	9	M	39	T4-N1-M1c (bone)	F	20	
	10	M	68	T3-M1c (liver)	PR (1 +)	1	Died from bronchopleural fistula
	11	M	56	T2-M1c (bone)	SR (2.5)	3	following massive
	12	M	71	T4-M1c (liver, bone)	NC (5.25)	8.75	tumoral necrosis
	13	M	56	T2-M1c (bone)	F	4	
	14	M	47	Tx-N1 (med. syndroma)	F	8.5	

Table 1 (continuation)

Group B (31)

	No.	Sex	Age	Staging at diagnosis	Tumoral response[a] (duration in months)	Survival (months)	Remarks
Lung cancer, oat-cell	15	M	78	T2-M1c (CNS, bone)	NC (1)	2	No response of CNS involvement
	16	F	65	T3-M1a-M1c (liver)	PR (2)	6	
	17	M	55	T3-M1c (liver)	CR (7)	9 +	
	18	M	74	T3-M1a	F	1.5	
GI tract	19	M	57	T4 sigmoid, M1 (liver, bone)	NC (1)	2	
	20	M	52	M1 liver	F	2.5	
	21	M	64	Tx (recto-sigm.), M1 adenop., liver, lung)	MR (1)	2	Regression of adenopathies
	22	F	79	Tx duod., M1 (liver, jaundice)	NC (0.75 +)	1 +	SR + normalisation of liver tests
	23	F	72	Tx sigm., M1 (liver)	NC (15 +)	16 +	
Breast	24	F	45	Top-M1c (pleura + bone)	SR (1 +)	1	Relief of pain – objective response
	25	F	59	T4-N3-M1 (pleura + bone)	NC (5)	7	inevaluable
Hypernephroma	26	M	69	To-M1b (lung, bone)	F	1	
Hepatoma	27	M	56	M1 (liver, ascites)	NC (2)	3	
Prostate	28	M	69	T3-M1a	F	5	
Cylindroma	29	M	67	T4 palate	PR (8)	12 +	
Angiosarcoma of scalp	30	M	81	T4-N3	PR (3)	5 +	
Ovary	31	F	68	T4-M1b (liver, ascites) M1c (pleura)	F	1.5	

NB: Case 26 = Case 29 in group A.

[a] NC, < 50%; PR, ⩾ 50% < 100%; CR, 100%; MR, mixed response; SR, subjective response; F, failure

Table 2. Primary solid tumours included in the trial

	Group A (33)	Group B (31)
Lung carcinoma	17	18
GI tract carcinoma	4	5
Ovary carcinoma	5	1
Breast carcinoma	3	2
Hepatoma	1	1
Hypernephroma	1	1
Prostate carcinoma	1	1
Terebrant cylindroma of palate	–	1
Angiosarcoma of scalp	–	1
Uterine cervix carcinoma	1	–

Table 3. Histological characteristics of solid tumours

Histology	Glandular	Non-glandular	Differentiated	Undifferentiated
Group A (33)	16[a]	17	26	7
Group B (31)	13[a]	18	26	5

[a] Including one lung cancer in each group

other sites), stabilised disease (NC, no change: tumoral regression by less than 50%), failure (F, progression of disease), and subjective response (SR, clinical improvement, relief of pain, without demonstrable objective tumoral response).

The χ^2-test permitted comparison of the frequency of tumoral responses in both groups.

The median duration of remission in responders and of survival was obtained by the actuarial method according to Gehan [13]. The plot of points (t, – ln S[t] or – ln R[t], where t represents the time in months and S[t] or R[t] a function of survival or remission) on semi-log paper suggested that it seemed reasonable to assume an exponential model: S[t] or R[t] = exp (–λ t). The estimation of the λ parameter after graphical adjustment of points allowed the calculation of median values of t corresponding to S[t] or R[t] = 0.5. To compare remission or survival rate distributions in various groups, we tested the null hypothesis Ho: λ A = λ B against the alternative hypothesis Ha: λ B > λ A or the 2-sided alternative Ha: λ A ≠ λ B by using the quotient of means considered as having an F distribution with (2 [n_1

$- S_1]$, 2 $[n_2 - S_2]$) degrees of freedom (n, total number of measurements; S, number of censored data – values +) [13]. As the graphical linear adjustment suggested elimination of some end points of distribution for low values of S[t] or R[t], we took these eliminatory data into account in the fixation of the total amount of data and in the amount of censored data.

Results

The objective tumoral regression (Tables 1 and 4) appeared significantly higher in group A (28/33–85%) than in group B (18/31–58%). Moreover, the quality of

Table 4. Tumoral responses to chemotherapy

Group A (33)			Group B (31)		
Responses (< 50%)	Responses (⩾ 50%)	Failures	Responses (< 50%)	Responses (⩾ 50%)	Failures
9	19 (8 CR)	5[a]	11	7 (1 CR)	13[a]
28/33 (85%)		(15%)	18/31 (58%)		(42%)

[a] Including three subjective responses (SR) in group A, two SR in group B
Statistical significance A/B: 1. Objective responses: $P < 0.05$
2. Responses ⩾ 50%: $P < 0.01$
3. Complete responses: $P < 0.05$

Table 5. Tumoral responses according to histological characteristics

Histology		Glandular	Nonglandular	Differentiated	Undifferentiated
Group A (33)	Tumoral responses	14/16 (88%)	14/17 (82%)	21/26 (81%)	7/7 (100%)
	Failures	2/16 (12%)	3/17 (18%)	5/26 (19%)	0/7 (0%)
Group B (31)	Tumoral responses	7/13 (54%)	11/18 (61%)	15/26 (58%)	3/5 (60%)
	Failures	6/13 (46%)	7/18 (39%)	11/26 (42%)	2/5 (40%)

Table 6. Statistical comparison between median values for remission and survival

Comparison	n_A[a]	n_B[a]	n_A[a]–S_A[b]	n_B[a]–S_B[b]	λ_A	λ_B	P_A (0.50) (months)	P_B (0.50) (months)	Significance (F test)
(1) Duration of remission	23	16	15	13	0.112	0.289	6.2	2.4	$P < 0.05$
(2) Total survival	28	24	19	21	0.093	0.180	7.5	3.9	$P < 0.01$
(3) Survival of responders	23	14	16	12	0.086	0.165	8.1	4.2	$P < 0.05$
(4) Survival of failures	4	9	3	8	0.424	0.252	1.6	2.8	NS
(5) Survival of responders/failures in group A	23	4	16	3	0.086	0.424	8.1	1.6	$P = 0.07$
(6) Survival of responders/failures in group B	14	9	12	8	0.165	0.252	4.2	2.8	$P < 0.01$

[a] n, total number of measurements (end points excluded according to linear adjustment)
[b] S, total number of censored measurements (values +)
λ = parameter of exponential distribution: S(t) or R(t) = exp ($-\lambda t$); S(t) or R(t) = function of survival or remission.
λ is expressed in months^{-1}

response was better in group A (19/33—58% responses by over 50%, including eight apparent CRs) than in group B (7/31—23% responses by more than 50%, with only one CR).

No difference was noted in the quality of response according to histology (Table 5): glandular or nonglandular, differentiated or undifferentiated tumours responded with the same frequency in both goups. The lung cancer patients responded significantly better in group A (15/17 including 5/5 oat-cell) than in group B (10/18 with 4/5 oat-cell): $P = 0.05$ (Table 1).

As seen in Table 6, the median duration of remission and survival was also significantly superior in group A: remissions in group A: 6.2 months, in group B, 2.4 months; survival of whole group A: 7.5 months, whole group B, 3.9 months; survival of responders in group A, 8.1 months, in groups B, 4.2 months. The survival of failures in the two groups is not statistically different (1.6 VS 2.8 months). Moreover, in group A, a clear-cut difference in survival between responders (8.1 months) and failures (1.6 months) nevertheless yielded a P value of 0.07 in the F test related to the few degrees of freedom of the denominator. In group B, the F test between the survival of responders and of failures is significant but the λ values are very similar: it could then be due to a statistical error of the first kind.

The clinical and haematological toxicity of the chemotherapy was acceptable, not statistically different in the two groups, and quantitatively comparable to the secondary effects already decribed [6, 7]. Five deaths during response, namely three in group A and two in group B, must be mentioned.

Discussion

The present trial demonstrates that the sequential chemotherapy regimen taking into account the circadian rhythm of tumoral proliferation (group A) gives significantly better results than the control regimen. We have discussed the mode of action of the chemotherapy used elsewhere [6, 7, 9, 11]. It should be recalled that we have demonstrated experimentally that 30—40% more cells are engaged in DNA synthesis during the morning and that around 30% more antitumoral efficacy is found in the group receiving methotrexate or 5-FU (S-specific agents) at this time of day.

We have confirmed by a mathematical approach that the diurnal variations of tumoral proliferation are not only of 'academic' of 'anecdotal' interest, but that the right choice of the time of administration significantly improves the effectiveness of a therapeutic sequence of oncolytic drugs. This fact is expressed not only in the tumoral responses, but also in the quality and duration of remissions induced and in the survival. Moreover, in the group of patients receiving the chemotherapy protocol at inadequate hours, despite a tumoral response of 58%, no real benefit in terms of survival was demonstrated.

The proposed protocol, which involves tolerable toxicity, seems advantageous for differentiated as well as undifferentiated carcinomas; the excellent responses of a terebrant cylindroma of the palate and of an haemangioendothelioma (angiosarcoma of the scalp) should also be emphasised.

As the duration of remission and survival remains rather short in spite of significant initial tumoral regression, some effort still needs to be put into the search for new combination protocols and new therapeutic combined modalities.

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