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Cell Kinetics and *in Vitro* Chemosensitivity as a Tool for Improved Management of Patients*

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INTRODUCTION

THE IMPROVEMENT of clinical results represents the main goal in the management of patients and necessarily requires the definition of biological aggressiveness of the tumor and the use of the most adequate treatment. The achievement of this aim is hampered by the variability of biological behavior and response to treatment, not only among but also within the different tumor types, as demonstrated by experimental and clinical findings.

The search for markers of risk has been carried out by considering general aspects such as morphology, immunology, cell kinetics and DNA features, or aspects specific for some tumors such as hormonal status. The outcome of these studies has led to establish a relationship among the different basic aspects, and between the latter and the clinical-pathologic patterns, and to define their significance as prognostic indicators.

The prediction of response to the chemical or physical agents has been approached by evaluating the cytocidal [1–5] or antimetabolic effects [6–14] in *in vitro* systems of human tumors or by early monitoring of the variations induced on cell kinetics [15–21].

For determination of the biologic aggressiveness and of sensitivity to drugs, the choice of experimental approaches, from those available, was mainly based on reliability and potential feasibility for routine use [22–24]. The potential proliferative activity was considered as an indicator of the aggressiveness of a tumor, and the clinical relevance of the proliferating cell fraction, as determined *in vitro* on fresh tumor material, was assessed for some tumor types [21, 25–32]. The small sample required and the quickness and relative simplicity of the procedure make the determination feasible on large series of consecutive patients and provide information in real time for clinical needs. Similarly, the use of a chemosensitivity test which evaluates the interference of drugs on incorporation of nucleic acid precursors in short-term cultures meets the requisites of shortness and feasibility for a tailored therapy [12, 33].

METHODOLOGIC APPROACHES

Cell kinetics

Tumor samples, fragments for solid tumors or cell suspensions for systemic diseases and effusions, were incubated with [^{3}H]thymidine for 1 hr at 37°C (Fig. 1) [29, 31]. The fraction of proliferating cells was evaluated by autoradiography and expressed as [^{3}H]thymidine labeling index (LI). Different areas from several fragments or triplicate smears were scored to reduce potential bias due to intratumor heterogeneity. The median LI defined for each tumor type on large series of patients was used as the cutoff to define slow and fast-proliferating tumors.

Chemosensitivity tests

Chemosensitivity was assessed with an antimetabolic assay by evaluating the interference on [^{3}H]thymidine and [^{3}H]uridine incorporation after 3 hr of *in vitro* treatment (Fig. 2) [12, 33]. Fragments were used for solid tumors and cell suspensions for systemic diseases and effusions. Drugs were tested individually, and the clinical equivalent dose was defined according to Tisman *et al.* [34]. All the drugs used in clinical protocols were tested *in vitro*. Radioactivity was expressed

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Abbreviations: A, adriamycin; AcD, actinomycin D; ABP, adriamycin, bleomycin and prednisone; AV, adriamycin and vincristine; BACOP, bleomycin, adriamycin, cyclophosphamide, vincristine (Oncovin) and prednisone; C, cyclophosphamide; PEB, *cis*-platinum, etoposide and bleomycin; PVB, *cis*-platinum, vinblastine and bleomycin; Vcr, vincristine.

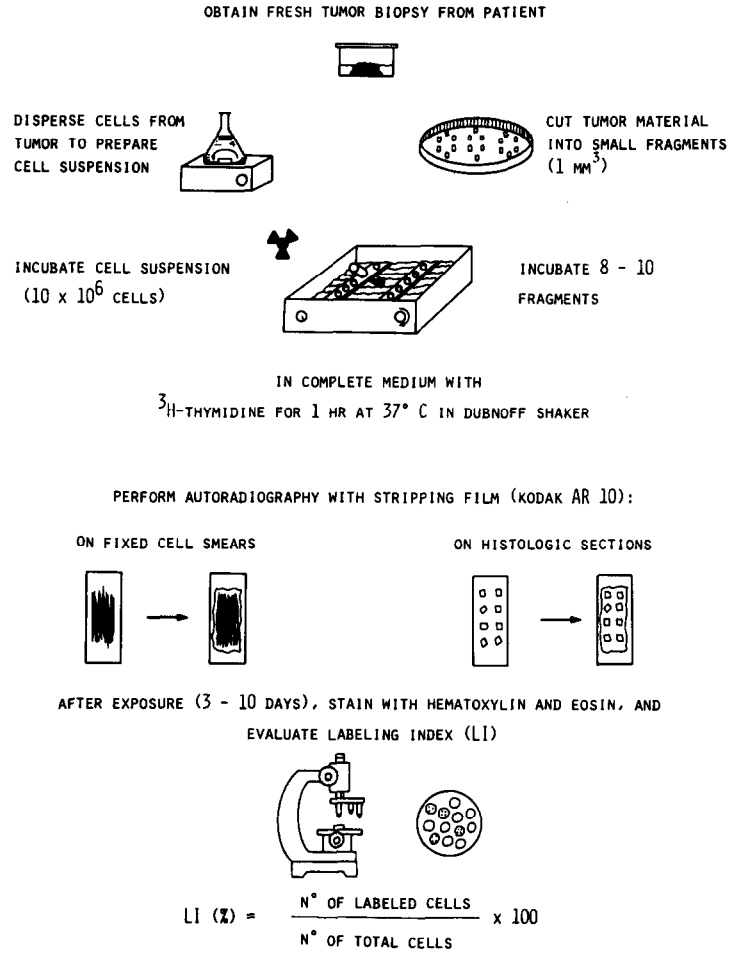

Fig. 1. Flow diagram of steps in labeling index determination.

as total dpm for cell suspensions and fractional incorporation for fragments [35], and the effect of drugs was evaluated as percentage variation in treated vs control samples. The variation coefficient (CV) of precursor incorporation in control samples (intratumor biologic variability) was used as cutoff to discriminate *in vitro* sensitive or resistant tumors. When the CV was lower than intertumor variability, as expressed by the mean value of *in vitro* effect evaluated for each drug on at least 25 tumors for each type, the mean value was used as the cutoff of sensitivity.

RESULTS

Cell kinetics

The kinetic characterization was performed on series of untreated patients with germ cell testicular tumors (TT), head and neck cancers (H&NC), breast cancers (BC) and non-Hodgkin's lymphomas (NHL) (Table 1). An exponential type of distribution of LI values was found for all tumor types except for H&NC, for which a log normal distribution was observed. The highest median value was observed for TT (26.2%), an intermediate value for H&NC, and the lowest ones for NHL and BC (4.0% and 2.8% respectively). However, notwithstanding the markedly different median value, a modulation of proliferative activity was observed within each tumor type, with wide and often overlapped ranges.

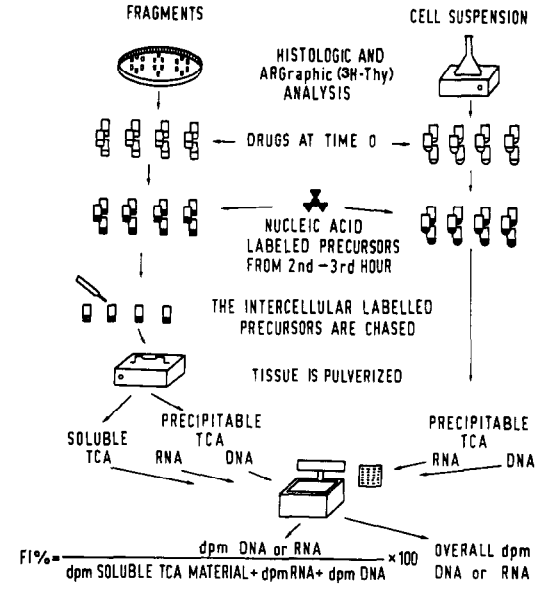

Fig. 2. Flow diagram of steps in drug sensitivity determination by an antimetabolic assay.

Table 1. *Kinetic patterns of different human tumor types*

Tumor type	No. of cases	Labeling index (%) Median	Range	Type of distribution
Testicular	97	26.2	0.01-77.3	exponential
Head and neck	147	10.9	0.01-50.0	log normal
Non-Hodgkin's lymphoma	134	4.0	0.01-30.0	exponential
Breast	541	2.8	0.01-40.7	exponential

Table 2. *Relationship between proliferative activity and various clinical and pathologic features*

Host or tumor characteristics	Existence of a relation for: TT	H&NC	NHL	BC
Age	no	yes	no	yes*
Histomorphology	yes	no	yes	—
TNM	no	yes	no	no
Estrogen receptor status	—	—	—	yes

*Menopausal status.

A subsequent analysis dealt with the relation between the kinetic variable and prognostic characteristics of the host and tumor, some of which are common to all tumors and others specific to some tumors, such as estrogen receptor (ER) status for BC (Table 2). An inverse relation between patient's age and LI was observed for H&NC [21, 36, 37] and BC; for the latter tumor a stronger association was observed when menopausal status instead of patient's age was considered [26, 27, 31, 38].

The proliferative activity proved to be strictly correlated with the histomorphology in TT and in NHL. More precisely, in TT the highest cell proliferative rate was observed for embryonal carcinoma histology in both pure and mixed forms, an intermediate one with a wide variability from tumor to tumor for seminomas and the lowest for mature teratomas [37, 39, 40]. In NHL a significantly lower proliferative rate was observed for nodular than for diffuse histology [29, 37], as well as for low- rather than high-grade malignancy tumors [37]. In H&NC only a trend of a correlation was observed between the proliferative activity and the histologic grading [21, 36, 37].

A significant association between high proliferative activity and the lack of ER, or low proliferative activity and the presence of ER was observed for BC [41-44].

An unexpected result was the lack of a correlation of cell proliferative rate with the extent of disease, which was observed for most tumors [21, 29, 31, 36, 37, 40].

The lack of a general association between cell proliferative activity and conventional risk indicators prompted us to evaluate the prognostic relevance of the kinetic variable. The analysis was performed at the present time on untreated patients with BC, H&NC or NHL (Table 3).

The series of patients with H&NC included 26 cases of epidermoid carcinomas at stages 3 and 4 submitted to surgery and postoperative radiation therapy on primary and locoregional fields. The pretreatment LI was indicative of clinical outcome; in fact, the probability of local recurrence at 2 yr was only 15% for patients with slow-proliferating tumors compared with 72% for those with fast-proliferating tumors ($P < 0.025$).

The study on NHL was performed on 116 adult patients with tumors at different pathologic stages and adequately treated, most of them with alternative cycles of CVP and ABP, the remainder with BACOP or miscellaneous treatments [25]. Again, the proliferative activity was an important prognostic discriminant, regardless of type of treatment or pathologic stage. In fact, patients with slow-proliferating tumors had a 60% probability of 5-yr survival, compared with only 20% observed for fast-proliferating tumors ($P < 10^{-7}$). A consistent finding was observed for a smaller series of patients (51 cases) homogeneously treated with CVP ~ ABP [25]. Moreover, the kinetic variable appeared to be a further discriminant of risk within homogeneous morphologic subsets of tumors according to Rappaport or Kiel classifications (unpublished data).

The clinical relevance of cell kinetics in BC was analyzed on a selected series of 221 patients with operable cancers without locoregional or distant metastases [45-47]. The patients were submitted to mastectomy without any other treatment until relapse. The overall disease-free survival (DFS) at 5 yr was 75%, which is quite similar to that

Table 3. Clinical relevance of pretreatment LI

Tumor type	Proliferative activity	Probability* (%) of: local recurrence (at 2 yr)	disease-free survival (at 5 yr)	survival (at 5 yr)
Head and neck	low	15		
		$P < 0.025$		
	high	72		
Non-Hodgkin's lymphoma	low			60
				$P < 10^{-7}$
	high			20
Breast	low		87	
			$P < 10^{-4}$	
	high		61	

*Evaluated with the actuarial life-table; the log-rank test was used to assess the statistical significance of the differences between the kinetic subgroups.

reported from clinical follow-up studies on large series of patients. A statistically higher DFS was observed for patients with slow-proliferating tumors (87%) than for those with fast-proliferating tumors (61%). The relevance of LI as a prognostic discriminant was consistently observed for the two distinctly analyzed pre- plus paramenopausal and postmenopausal subgroups [47]. Moreover, the analysis of DFS in relation to proliferative activity and ER status showed that the kinetic variable still discriminates within the two subgroups of estrogen-positive or -negative tumors [30, 47].

Chemosensitivity tests

By considering the potential utilization of an *in vitro* chemosensitivity test, the accuracy of the assay to predict tumor type sensitivity and individual tumor sensitivity was defined. Tumor type sensitivity was assessed on NHL, TT, BC and ovarian cancers (OC) by testing the drugs more frequently used in clinical trials in our institute (Table 4). A different *in vitro* sensitivity to the same drug by the different tumor types, as well as to the different drugs within the same tumor type, was observed, in agreement with clinical findings. Further analysis also evidenced a

Table 4. In vitro response rates (%) to conventional agents

Drug	Non-Hodgkin's lymphoma	Breast cancer	Germ cell testicular tumor	Ovarian carcinoma
Actinomycin-D			27 (30)	
Adriamycin	53 (102)	28 (119)	26 (42)	33 (39)
Alkeran		19 (31)		
Bleomycin	25 (59)		36 (78)	
Cisplatinum		16 (31)	43 (88)	35 (46)
Cyclophosphamide (4-OOH-cyclophosphamide)	63 (35)	30 (63)	50 (34)	33 (40)
Etoposide (Vp16-213)	54 (22)	48 (21)	54 (34)	
Mitomycin C		26 (36)		
Prednisolone	39 (83)			
Vinblastine			40 (84)	
Vincristine	26 (70)	19 (37)		

In parentheses, No. of cases.

generally good agreement between the *in vitro* response rate to individual drugs and the clinical response rate reported for the same drug used in monochemotherapy on the same tumor type [48–55]. The *in vitro* test appeared to slightly underestimate the clinical response to vincristine and bleomycin in NHL and overestimate that of BC to Vpl6.

As regards the accuracy of the test to predict individual tumor sensitivity, a retrospective analysis was performed on a series of 142 patients including NHL, locally advanced BC and advanced TT (Table 5). Generally, all the drugs planned for clinical treatment were tested *in vitro;* however, a limitation of this study is that the drugs were singularly tested *in vitro,* whereas they were used in association in clinical protocols. According to an empirical criterion, a tumor was thus defined clinically responsive on the basis of *in vitro* data when it was sensitive to at least one drug included in clinical protocols.

In vitro sensitivity appeared highly indicative of short-term clinical response in terms of complete response (CR) for NHL or CR plus partial response (PR ≥50%) for BC and TT (Table 6). An overall agreement between *in vitro–in vivo* sensitivity of 77% was observed for NHL, with true-positive and true-negative rates of 81 and 62% respectively. Similar results were observed for locally advanced BC with an even higher true-negative rate (81%).

The findings in TT clearly showed the importance of the tumor site on which the sensitivity is tested *in vitro.* In fact, the overall agreement regardless of the tumor site was 77%, but when the different lesions were distinctly analyzed, the sensitivity patterns of metastatic sites showed an accuracy to predict sensitivity or resistance of 93 or 80% respectively, whereas no significant association was observed between *in vitro* sensitivity of the primary tumor and clinical response.

CONCLUSIONS

The median cell proliferative rate is different for the various tumor types, but cell kinetics vary greatly within each tumor type. This biological finding is consistent with the clinical evidence of variability of the course of the disease for patients with the same tumor type. The estimation of proliferative activity cannot be adequately deduced from other morphologic, biologic, clinical or pathologic features of the tumor and host.

In fact, the kinetic variable is often unrelated or only roughly associated with the various factors that also have a relevant bearing on the biological

Table 5. *Tumor types and clinical characteristics of patients included in the retrospective correlative study* (142 *cases*)

Tumor type	Clinical treatment	Clinical response
Non-Hodgkin's lymphoma	CVP ~ ABP or BACOP	CR
Locally advanced breast cancer	AV	CR or PR ≥50%
Advanced testicular tumor	PVB, PEB or two drugs combinations including C, AcD, Vcr or A	CR or PR ≥50%

Table 6. *Evaluation of* in vitro *sensitivity to predict clinical response*

Tumor type	No. of cases	True-positive rate (%)	True-negative rate (%)	Overall agreement (%)	*P* value*
Non-Hodgkin's lymphoma	65	81 (40/49)	62 (10/16)	77	0.006
Locally advanced breast cancer	41	75 (15/20)	81 (17/21)	78	0.00032
Advanced testicular tumor:					
primary	16	100 (10/10)	0 (0/6)	62	N.E.
metastases	20	93 (14/15)	80 (4/5)	90	0.001
Total	142	84 (79/94)	64 (31/48)	77	<0.00003

*Evaluated by means of the kappa test.

and clinical aggressiveness of the tumor [21, 26, 29, 38]. However, cell kinetics has proved to be an important prognostic discriminant sometimes superior to those conventionally considered, so that it is worthy of the utmost consideration alone or in association with other more conventional factors in defining subgroups at different risk.

The clinical implication of such a finding could be to spare indolent tumors from heavy or in some cases from any treatment and to intensively treat the aggressive ones. The best results would be expected if the treatment was planned by taking into account the sensitivity pattern of individual tumors.

Since the analysis of different tumor features as potential predictors of sensitivity to drugs or to subgroups of drugs with similar action mechanisms [14] has failed to show any correlation and has stressed the unforeseeable and intrinsic chemosensitivity of individual tumors, the only way to assess the latter is by the use of a chemosensitivity test at the preclinical level. Among those available, the test here described, which considers the interferences on nucleic acid precursor incorporation as the endpoint of the drug effect, appears to meet the requisites to give information for a tailored therapy. Moreover, by considering the accuracy in predicting tumor type sensitivity, the antimetabolic assay represents an important system for cross resistance and *in vitro* phase II studies. For the latter, the *in vitro* system would have the advantage of overcoming the ethical limitations met in clinical experimentation and allowing the assessment of the effect of drugs from clinical phase I studies not only on tumors previously and often heavily treated, but also on previously untreated tumors.

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