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Predictive Testing of Vulvar and Cervical Cancers to Chemotherapy by the Subrenal Capsule Assay*

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Abstract—*The 6-day subrenal capsule assay (SRCA) in normal immunocompetent mice was used to assess response of vulvar and cervical cancers to chemotherapy. Twenty-five out of 31 assays (81%) were evaluable. The previously treated tumors tended to be less sensitive than the untreated tumors (20 vs 33%). Three to five cytotoxic drugs or drug combinations were tested against each individual tumor. In the whole material the combination of cisplatin and etoposide (VP-16) was significantly more effective than bleomycin ($P < 0.01$, χ^2 test). The combinations adriamycin + cyclophosphamide + cisplatin and cyclophosphamide + methotrexate were also rather effective. Preliminary clinical correlations were positive. The reliability of the SRCA was discussed, and it was concluded that the assay is a promising predictive method for individualizing chemotherapy.*

INTRODUCTION

THE RESPONSE rate of the squamous cell carcinomas of the female genital tract to chemotherapy is poor, e.g. for cervical cancer only 20–30% [1], and there is an urgent need of predictive methods for optimizing chemotherapy. The clonogenic stem cell assay developed by Salmon [2] has aroused optimism in this respect, but the clinical usefulness of the assay has been criticized [3]. Recently, Bogden *et al.* [4] have described an *in vivo* assay called the subrenal capsule assay (SRCA) using normal immunocompetent mice for testing the sensitivity of solid malignant tumors to chemotherapy.

The current study was conducted to investigate how applicable the SRCA is for determining the response of vulvar and cervical cancers to single and combination chemotherapy.

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MATERIALS AND METHODS

From 1982 to 1984, 31 tumor samples were taken from 19 patients with vulvar cancer and from five patients with cervical cancer treated at the Department of Obstetrics and Gynecology, University Central Hospital of Turku. Nineteen samples were of untreated tumors, eight of tumors treated with chemotherapy and four of tumors treated with chemo-, radio- and surgical therapy. The samples were taken before and after therapy from six patients and twice in the course of therapy from one patient. All tumors were of squamous cell origin.

The samples for the SRCA and histological examination were taken from the same areas of the tumors. The tumor samples for the SRCA were immediately transferred to sterile test tubes containing culture medium 199 and were transported to the laboratory of the Farmos Group Research Center, where the assay was performed.

The technique of the SRCA has been described in detail by Bogden *et al.* [4]. The samples were cut into 1-mm^3 pieces in the laboratory and implanted under the renal capsule of normal, immunocompetent female mice. The time between sample excision and implanting under the renal capsule did not exceed 24 hr. The exact

size of the transplant [(length + width)/2] was measured by a stereomicroscope fitted with an ocular micrometer. The size was expressed in terms of ocular micrometer units (omu), where 10 omu = 1 mm. One group of five mice served as control and 3–5 groups of five mice received different cytostatic drugs and drug combinations. The animals were treated on a daily schedule for 5 days. On day 6 the animals were killed and the final tumor sizes were measured. The difference between the initial and the final tumor size (ΔTS) was used as the indicator of tumor growth or growth inhibition.

The initial and final body weights of the mice were measured. Toxicity was considered tolerable when weight loss was less than 20% [4]. If weight loss in a treated group exceeded 20%, this group was not considered.

An individual assay was considered evaluable if the tumor grafts in the control group increased in size. A tumor was considered sensitive (S) to a drug or drug combination if the mean tumor size decreased ≥1 omu; intermediately sensitive (I) if the mean tumor size decreased <1 omu, or increased less than a third of that of the control group and resistant (R) if the mean ΔTS was equal to or more than a third of the ΔTS in the control group (Fig. 1).

The drugs, their abbreviations, doses and routes of administration used in the SRCA appear in Table 1. The doses were adjusted to reach maximum effect with tolerable toxicity.

The statistical analyses of the frequency tables were performed by the Fisher's exact test (Tables 2, 3 and 4) and by the χ^2 test (Table 5).

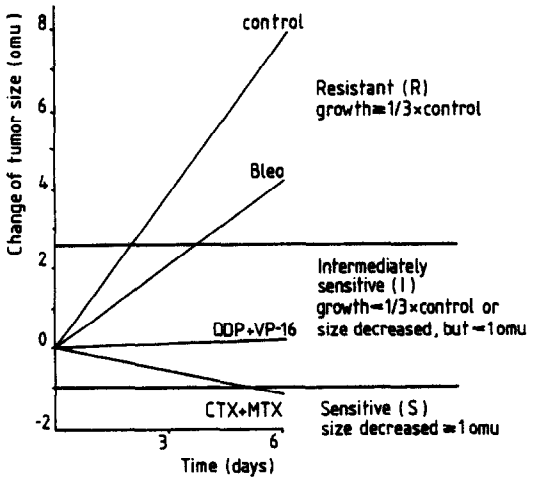

Fig. 1. The response of an untreated moderately differentiated vulvar cancer (patient No. 14) to bleomycin (Bleo), cisplatin (DDP) + etoposide (VP-16) and cyclophosphamide (CTX) + methotrexate (MTX) in the SRCA.

RESULTS

Of the 31 tumor samples, 25 (81%) produced evaluable assays. The inevaluable tumors were five vulvar cancers and one cervical cancer. The failures seemed to be caused rather by bacterial contamination than by low growth capacity of the tumors. In the 15 evaluable assays of untreated tumors the growth in the control groups was 3.5 ± 3.5 omu (mean ± S.D.), and in ten evaluable assays of treated tumors, the growth in the control groups was 3.4 ± 2.5 omu (Tables 2 and 3).

As a whole, seven (28%) of the tumors were sensitive, 12 (48%) were intermediately sensitive and six (24%) were resistant to chemotherapy (Table 4).

Five (33%) of the untreated tumors were sensitive, seven (47%) were intermediately sensitive and the rest (20%) resistant (Tables 2 and 4). One of the seven highly differentiated tumors was sensitive vs three of the five grade II tumors. All three poorly differentiated tumors were cervical cancers; of these one was sensitive.

Two (20%) of the previously treated tumors were sensitive, five (50%) were intermediately sensitive and the rest (30%) were resistant (Tables 3 and 4). Four tumors were highly, four moderately and two poorly differentiated. Two sensitive tumors were of grades II and III.

The differences in the responsiveness of the tumors as related to prior treatment status and the differentiation grade were not statistically significant.

The SRCA results for the drugs and drug combinations successfully tested against at least five tumors are presented in Table 5. The drugs tested most often were the combination of cisplatin and VP-16, and bleomycin as a single agent. The combination was significantly superior to bleomycin ($P < 0.01$). Of the other drugs tested, the combination of mitomycin C and tegafur was the least effective one, 0/5 tumors being sensitive. Two of eight tumors were sensitive to CTX + MTX, and 2/5 to A + CTX + DDP. Moreover, there was one previously treated vulvar cancer which was sensitive (ΔTS -1.3 omu) to DDP + CTX and one untreated vulvar cancer which was sensitive (ΔTS -1.0 omu) to cisplatin alone.

The number of clinical comparisons is small because most patients were treated according to conventional regimens regardless of the assay results. Three illustrative case reports are presented.

Case 1

Patient No. 6 had a partly exophytic, partly ulcerative cancer involving the whole right and the upper half of the left sides of the vulva,

Table 1. Cytotoxic drugs tested and the dosages used in the SRCA

Drug	Abbreviation	Manufacturer	Daily dosage (mg/kg) Single	Combination	Route of administration*
Aclarubicin	Acla	All Union Research Institute, Moscow, U.S.S.R.	6	-	s.c. or i.p.
Adriamycin	A	Farmitalia Carlo Erba Milano, Italy	4	3	s.c. or i.p.
Bleomycin	Bleo	H. Lundbeck & Co. A/S, Copenhagen, Denmark	30	30	s.c. or i.p.
Chlorambucil	Chlor	Farmos, Turku, Finland	-	6	p.o.
Cisplatin	DDP	Farmos, Turku, Finland	2	1	s.c. or i.p.
Cyclophosphamide	CTX	Farmos, Turku, Finland	-	30	s.c. or i.p.
Dibromodulcitol	DBD	Chinoin, Budapest, Hungary	180	150	p.o.
Etoposide	VP-16	Farmos, Turku, Finland	32	20	s.c. or i.p.
Ftorafur (tegafur)	Ft	Farmos, Turku, Finland	-	250	p.o.
Methotrexate	MTX	Farmos, Turku, Finland	-	4	s.c. or i.p.
Mitomycin	MMC	Bristol Myers, New York, NY, U.S.A.	-	0.5	s.c. or i.p.
Vincristine	V	Lilly, Indianapolis, IN, U.S.A.	-	0.5	s.c. or i.p.

*s.c., subcutaneous; i.p. intraperitoneal; p.o., peroral.

Table 2. The study population without prior treatment, the histological grade of the tumors, the control growth and the response to chemotherapy in the SRCA (1 omu = *ocular micrometer unit* = 0.1 mm)

Patient	Age (yr)	Histological grade	Control growth in the SRCA (omu)	The drugs tested*	The best response to chemotherapy in SRCA†
Vulvar cancer					
1. A.B.	84	I	13.3	1,3,8,9	I to 1,8,9
2. O.B.	83	II	1.3	1,2,10	I to 1,10
3. N.K.	58	III	negative		
4. H.K.	83	I	2.2	1,2,5	R to all
5. N.M.	75	II	1.0	1,2,3,4,17	R to all
6. A.N.	72	II	1.25	1,3,4,8,9	S to 4
7. K.N.	63	II	2.85	1,2,13	S to all
8. I.O.	81	I	1.0	1,3,11,12,14	S to 3
9. L.O	65	I	3.4	1,2,5	R to all
10. A.P.	II	negative			
11. E.R.	II	negative			
12. A.S.	77	I	2.85	1,2,7	I to all
13. B.S.	85	I	8.3	1,2,18	I to 1,18
14. T.V.	56	II	7.9	1,2,3	S to 3
15. A.V.	77	I	1.0	1,3,11,12,14	I to all
Cervical cancer					
16. R.H.	31	III	2.45	1,4,6,15	I to all
17. K.J.	72	III	1.95	1,4,6,19	S to 1,4
18. A.U.	68	III	2.25	1,2,3,20	I to 2,3
19. R.K.	38	III	negative		

*The codes for drugs: 1, DDP + VP-16; 2, Bleo; 3, CTX + MTX; 4, A + CTX + DDP; 5, MMC + Ft; 6, A + CTX; 7, A + DBD; 8, A; 9, Acla; 10, CTX + DDP; 11, DDP + VP-16 + Bleo; 12, DDP + VP-16 + A; 13, DDP; 14, Chlor + MTX; 15, V + MMC + Bleo + MTX; 16, VP-16; 17, MMC + V; 18, CTX + Ft; 19, V + MMC + MTX; 20, DBD.
†S, sensitive; I, intermediately sensitive; R, resistant.

extending near the anus on the right. According to the SRCA, the tumor was intermediately sensitive to DDP + VP-16 (ΔTS -0.60). After three courses of therapy with DDP and VP-16 the tumor involvement on the left side had almost disappeared and clearly diminished on the right side. At this point an electroexcision was performed.

Case 2

Patient No. 18 had a FIGO St IIIa cervical cancer with a 2 × 3-cm ulcerative process in the lower third of the vagina. According to the SRCA the tumor was intermediately sensitive to CTX + MTX (ΔTS -0.20). After one course of treatment with this combination the carcinomatous ulceration had completely disappeared. Unfortunately, chemotherapy had to be discontinued because of the poor general condition of the patient, and she was lost to follow-up thereafter.

Case 3

Patient No. 13 had a 2 × 3-cm exophytic cancer on the left side of the vulva and a 2 × 3-cm

Table 3. The study population with prior treatment, the histological grade of the tumors, the control growth and the response to chemotherapy in the SRCA (1 omu = *ocular micrometer unit* = 0.1 mm)

Patient	Age (yr)	Histological grade	Prior treatment	Control growth in SRCA (omu)	The drugs tested*	The best response to therapy in SRCA†
Vulvar cancer						
2. O.B.	83	II	DDP + VP-16	2.85	1,2,10	S to 10
3. N.K.	58	III	Bleo	4.8	1,13,16	S to 1
4. H.K.	83	I	Bleo	1.8	1,2,5	R to all
9. L.O.	65	I	Bleo	1.3	1,2,5	R to all
11. E.R.	81	II	Bleo	4.0	1,2,7	I to 1
12. A.S.	77	I	Bleo, DDP + VP-16	negative		
20. A.L.	77	I	Bleo	8.8	1,2	R to all
			Bleo, VP-16 + surgery + radiation	1.2	1,2,3	I to all
21. H.L.	47	III	Bleo + surgery + radiation	1.6	1,2,7	I to 7
22. S.N.	70	II	Bleo + surgery + radiation	6.15	1,2,5,6	I to 1,5,6
23. E.L.	65	II	Bleo + surgery + radiation	negative		
Cervical cancer						
24. A.N.	75	II	DDP + VP-16 + surgery + radiation	1.45	1,4,6,15	I to 4

*,†See Table 2.

Table 4. The response of the squamous cell carcinomas to chemotherapy

	n	Response in the SRCA* S	I	R
Untreated tumors	15	5 (33%)	7 (47%)	3 (20%)
Previously treated tumors	10	2 (20%)	5 (50%)	3 (30%)
Total	25	7 (28%)	12 (48%)	6 (24%)

*S, I, R; see Table 2.

Table 5. Response of squamous cell carcinomas of the female genital tract to bleomycin and to cytostatic drug combinations in the SRCA

Drugs	No. of evaluable SRCAs	Response* S	I	R	
1. DDP + VP-16	25	3 (12%)	13 (52%)	9 (36%)	†
2. Bleo	17	1 (6%)	3 (18%)	13 (76%)	
3. CTX + MTX	8	2	4	2	
4. A + CTX + DDP	5	2	2	1	
5. MMC + Teg	5	0	1	4	

*S, I, R; see Table 2.
†$P < 0.01$.

ipsilateral inguinal lymph node metastasis. According to the SRCA the tumor was intermediately sensitive to CTX + tegafur (ΔTS +2.1). After one course of treatment with CTX + tegafur the primary tumor was electroexcised. The patient was treated with two additional courses. After 8 months the vulva was disease-free, and the lymph node metastasis had remained stable.

DISCUSSION

Nude mice were originally used as recipients in the SRCA [5]. The subrenal capsule implants in the nude mice were allowed to grow for 11 days. Subsequently, normal immunocompetent mice were found to be suitable as recipients provided the assay was restricted to 6 days [6]. However, the use of normal mice without a histological control

has been criticized based on inflammatory reactions induced by the grafts [7], which may lead to false-positive ΔTSs in the controls or to an overestimation of drug effect if the drug is immunosuppressive. These shortcomings are theoretically relevant, but from a practical point of view a routine histological survey of all grafts limits the usefulness of the assay. Dumont and co-workers have suggested that a histological confirmation should be done only in selected cases [8].

We have also made histological examinations of the grafted tumors in seven cases of ovarian cancer [9]. Although inflammatory reactions were found in the controls, the cytotoxic effect of a drug combination (A + CTX + DDP) was clear and similar both histologically and 'macroscopically', as measured by the ΔTS.

A mean growth rate of more than 3 omu in the control groups in the evaluable assays reflects a marked growth potential of the tumor samples, and indicates that those specimens were indeed viable and, for instance, not necrotic.

An evaluability of 81% of the assays in the current work agrees well with other studies; Hunter and co-workers reached a figure of 77% (20/26) and Griffin and co-workers one of 90% (52/58) of cervical cancers [10, 11]. To our knowledge, vulvar cancer has not previously been tested for chemosensitivity in the SRCA. Because bacterial contamination appears to be the main cause of failures, antimicrobial pretreatment and aseptic conditions during tissue sampling are important for proper testing of tumors in the lower female genital tract. In addition, since the current study was completed, we have begun to add gentamycin at a concentration of 15 μg/ml to the culture medium.

The SRCA as a method for testing the sensitivity of tumors to chemotherapy is new and the interpretation of the results is still somewhat tentative. In Bogden's original method, three criteria for drug response have been used: (1) a decrease in xenograft size of more than 0.5 omu [12]; or (2) a decrease of more than 1 omu [12]; and (3) a xenograft regression of ≥25% [10]. In these studies the value of the control ΔTS was not considered when tumor response was determined. In the current study a decrease in xenograft size of at least 1 omu was used as the criterion of drug response. However, in clinical practice a spectrum of drug response is seen ranging from complete response to progressive disease. Therefore we have found it appropriate to rank tumors as sensitive, intermediately sensitive or resistant. Based on experiments with rat tumors, the upper limit of intermediate sensitivity was defined as growth of less than half of that in controls; this takes into account the growth properties of each individual tumor [13]. For slower growing human tumors the limit was set at growth of less than one-third of that in controls. Our clinical comparisons support a three-grade interpretation of drug effect, as do our studies with ovarian cancer (to be published).

Chemosensitivity assays have thus far been performed with single agents [2, 4, 10–12]. For combination therapy, two or three of the most active drugs have been chosen, which does not take into account possible antagonism or synergism of the drugs. Therefore we have mostly tested drug combinations instead of single agents, except in the case of bleomycin, which in vulvar cancer is used clinically as a single agent. According to the above-mentioned experiments with rat tumors, the SRCA is suitable for predicting response to combination chemotherapy [13]. Our experiences in testing the effect of combined cytostatic drugs on ovarian cancer have also been encouraging [9].

The low number of sensitive tumors in the current study (28%) is in agreement with clinical experience regarding the squamous cell carcinomas of the female genital tract [1]. Bleomycin has been used in the treatment of vulvar cancer either alone or combined with radiotherapy [14]. The present findings suggest that the combination of cisplatin and VP-16 may be more effective than bleomycin in treating vulvar cancer.

The previously treated tumors and the highly differentiated tumors tended to be the ones most unresponsive to chemotherapy. Lack of statistical significance may be caused by the rather limited material. It is of interest that there were two tumors that were more sensitive after therapy than before therapy (patient Nos. 2 and 20). An explanation would be that therapy generated a more aggressive cell population with higher mitotic activity and, hence, greater sensitivity to chemotherapy.

There were three patients (Nos. 6, 13 and 18) in whom chemotherapy was prospectively based on the SRCA results. The tumor xenografts from two patients (Nos. 6 and 18) decreased in size in the assay as response to chemotherapy. The patients also showed a clear clinical drug response. The tumor xenograft from the third patient (No. 13) grew in the SRCA in spite of chemotherapy, although markedly less than in the control group. The patient showed a stagnation of the disease. These case reports illustrate the usefulness of the SRCA in selecting individualized, possibly unorthodox but still effective chemotherapy, e.g. CTX + MTX, for cervical cancer.

It can be concluded that the SRCA appears to be useful for selecting active drugs for individual

patients with vulvar and cervical cancer, but its final place as a tool for aiding decision of optimal therapy cannot be stated until more extensive prospective and controlled studies are performed.

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