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Comparison of the Subrenal Capsule Assay and Succinate Dehydrogenase Inhibition Test as Drug Sensitivity Tests for Cancer

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Abstract

The same chemotherapeutic agents were tested against fresh surgical explants of solid tumors obtained from 50 patients using the in vivo subrenal capsule (SRC) assay and the in vitro succinate dehydrogenase inhibition (SDI) test in comparison. Control growth adequate to meet evaluable assay criteria was obtained in 36 of the 50 tumors tested in the SRC assay (72.0%). In the SDI test, 46 of 50 tumors were evaluable (92.0%). Correlations between the two test systems were dependent upon the activity criteria established for each system. With activity criteria set at a change of less than or equal to -2.0 in the drug sensitivity score for the SRC assay and greater than or equal to 50.0% inhibition of succinate dehydrogenase activity for the SDI test, 12.5% of the drugs tested were active in the SRC assay and 22.3% were active in the SDI test. Correlations of tumor response between the two test systems were 31.7% for sensitivity (13/41) and 95.1% for resistance (98/103). In spite of the fundamental difference between the SRC assay and SDI test, meaningful correlations between the test results and clinical tumor responses in both test systems were obtained. This fact suggests that the two methods are complementary to each other.

KEYWORDS: drug sensitivity test, subrenal capsule assay, succinate dehydrogenase inhibition test

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The same chemotherapeutic agents were tested against fresh surgical explants of solid tumors obtained from 50 patients using the *in vivo* subrenal capsule (SRC) assay and the *in vitro* succinate dehydrogenase inhibition (SDI) test in comparison. Control growth adequate to meet evaluable assay criteria was obtained in 36 of the 50 tumors tested in the SRC assay (72.0%). In the SDI test, 46 of 50 tumors were evaluable (92.0%). Correlations between the two test systems were dependent upon the activity criteria established for each system. With activity criteria set at a change of $\leqq -2.0$ in the drug sensitivity score for the SRC assay and $\geqq 50.0\%$ inhibition of succinate dehydrogenase activity for the SDI test, 12.5% of the drugs tested were active in the SRC assay and 22.3% were active in the SDI test. Correlations of tumor response between the two test systems were 31.7% for sensitivity (13/41) and 95.1% for resistance (98/103). In spite of the fundamental difference between the SRC assay and SDI test, meaningful correlations between the test results and clinical tumor responses in both test systems were obtained. This fact suggests that the two methods are complementary to each other.

Key words : drug sensitivity test, subrenal capsule assay, succinate dehydrogenase inhibition test

Out of the fair number of *in vitro* drug sensitivity tests which have been introduced in recent years to predict the response of an individual patient's tumor to a particular chemotherapeutic agent, the succinate dehydrogenase inhibition (SDI) test has received the most attention (1-12). In the SDI test, a tumor cell suspension is assayed for succinate dehydrogenase activity using a tetrazolium salt (TTC) as a hydrogen acceptor. This rapid colorimetric test is based upon the degree of inactivation of the cells. Recently, the *in vivo* subrenal capsule (SRC) assay was developed by Bogden *et al.* in response to the need for a rapid *in vivo* test system for screening drugs against human tumors (13-21). The SDI test attempts to disperse solid tumors into cell suspensions by physical means and to determine the drug sensitivity of tumors by using a color reaction as a measure of the viability of tumor cells. In contrast, the SRC assay attempts

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to maintain cell membrane integrity, cell-to-cell contact and the spatial relationship of heterogeneous tumor cell populations by utilizing tumor fragments as xenografts for subcapsular implantation into normal immunocompetent mice (13-21). Thus, drug sensitivity in the SDI test is measured by the response of tumor cell suspensions, and, in the SRC assay, as a net response of tumor fragments. The present report describes a comparative study of the SDI test and the SRC assay in which the same samples of human tumors were tested.

Materials and Methods

Solid tumor specimens from 50 patients transported in our department were divided into 2 portions, one for the SDI test, and the other for the SRC assay. These specimens were from tumors of the stomach, colon, rectum, breast, lung, liver and kidney.

The *in vitro* SDI test utilized in this study was performed in the following manner under sterile conditions: 1. Fresh surgical tumor specimens were cut and minced with scissors and suspended in phosphate-buffered saline. 2. Tumor cells suspended in 3.0 ml of phosphate-buffered saline were exposed to four anticancer chemotherapeutic agents at five or ten times the peak plasma concentration (Table 1). 3. After this tumor suspension was incubated at 37°C for 1 h, 0.3 ml of a solution containing 0.03 g of 2, 3, 5-triphenyltetrazolium chloride (TTC) and 2.7 g of sodium succinate in 100 ml of 1/15 M phosphate buffered saline were added, and the mixture was incubated further for 24 h. 4. For titration, 3.0 ml of ethyl acetate containing 0.5% of trichloroacetic acid was added to each tumor cell suspension. 5. Formazan formed from TTC, a hydrogen acceptor, was transferred to the ethyl acetate layer by shaking and centrifugation. 6. Finally, the quantity of formazan was measured spectrophotometrically at 480nm, and the inhibition of succinate dehydrogenase activity was calculated by the following formula: Inhibition $= (a-p)/a$, where "p" is the value obtained from the tumor cell suspension with an anticancer drug and TTC solution and "a" is that without a drug but with solution.

The *in vivo* SRC assay was carried out as previously described by Bogden *et al.* (13-16). Four different drugs, Mitomycin-C (MMC), 5-fluorouracil (5-FU), Adriamycin (ADR) and Cis-DD platinum (DDP) were tested. MMC, 5-FU and DDP were subcutaneously injected and ADR was injected intravenously. The doses are shown in Table 1. Drug treatment was initiated on the day following tumor implantation and repeated daily for four consecutive days. On day 5, one day after the last anticancer drug treatment, animals were killed by cervical dislocation. Each tumor-bearing kidney was fixed in 10% formalin and subsequently sectioned at the margin of the xenograft and embeded in paraffin. The section with the largest tumor depth was selected for staining with hematoxylin-eosin. The following four parameters, C (cancer area ratio), N (necro-

Table 1 Dose and route of anticancer drugs in the subrenal capsule (SRC) assay and concentration of drugs in the succinate dehydrogenase inhibition (SDI) test

Anticancer drug	SRC assay (mg/kg)	SDI test (μg/ml)
Mitomycin-C	0.7 (s. c.)	10
5-Fluorouracil	50.0 (s. c.)	100
Adriamycin	4.0 (i. v.)	4
Cis-DD Platinum	2.0 (s. c.)	5

Table 2 Drug sensitivity score (S) of the subrenal capsule assay (modified PAPAN score)

C:	Cancer area ratio (%) 4: >60, 3: 41-60, 2: 21-40, 1: 1-20, 0: 0
P:	Pathological effect of cancer cell 4: no effect, 3: cell damage, 2: cell damage+cancer cell nest destruction, 1: no viable cell, 0: no cancer cell
M:	Mitosis amount 2: >4, 1: 1-4, 0: 0
N:	Necrosis area ratio (%) 4: >60, 3: 41-60, 2: 21-40, 1: 1-20, 0: 0
S:	S = C+P+M−N

sis area ratio), P (pathological effect of cancer cells) and M (amount of mitoses) were scored in a semiquantitative fashion from 0 to 2 or 4 as shown in Table 2. The areas occupied by cancer cells and necrosis were identified histologically and calculated by means of a computer image analyzer (CIA-102, Olympus Corp., Tokyo, Japan). To estimate the overall quality of each xenograft, we modified the PAPAN score (21) and devised a drug sensitivity score for the SRC assay (22). Our drug sensitivity score (S) is C+M+P−N; this score varied from −4 to +10. Differences (ΔS) in the drug sensitivity score were calculated as follows: $\Delta S = S_d - S_c$, where S_d is the mean of scores from 5 anticancer-drug-treated xenografts, and S_c is that of untreated control xenografts. A high drug sensitivity score indicated a good preservation of tumor tissue, *i.e.*, cancer cells were not responsive to the anticancer drugs. When cancer cells were responsive to drugs, cancer cells were damaged by the drugs and the poor preservation of tumor tissue resulted in a low drug sensitivity score. The larger the difference (ΔS) in the drug sensitivity score is, the higher the sensitivity of the cancer cells to the drug is.

In the SDI test, no infection was required for the drug effects to be considered evaluable. Two criteria for judging a drug to be active were compared: an inhibition of succinate dehydrogenase activity of $\geqq 75.0\%$ and an inhibition of $\geqq 50.0\%$. For evaluability in the SRC assay, viable cancer cells were required in control groups on day 5. For purposes of this comparative analysis, activity criteria for the SRC assay ranged from $\leqq -1.0$ to $\leqq -3.0$.

Results

Evaluability rate. There was no infection of 46 of the 50 tumor specimens tested in the SDI test, providing an evaluability rate of 92.0%. On the other hand, adequate growth of control xenografts in the SRC assay was obtained in 36 of the 50 tumors, resulting in an evaluability rate of 72.0%, and the 36 tumors producing adequate growth to meet the criteria for evaluable assay exhibited no infection in the SDI test.

Table 3 Comparative sensitivity of the subrenal capsule (SRC) assay and the succinate dehydrogenase inhibition (SDI) test

Activity criteria	Active tests/ Total tests	% Active
SRC assay[a]		
$\Delta S \leqq -1.0$	45/144	31.3
$\Delta S \leqq -2.0$	18/144	12.5
$\Delta S \leqq -3.0$	7/144	4.9
SDI test[b]		
$\geqq 50.0\%$	41/184	22.3
$\geqq 75.0\%$	8/184	4.3

a: Difference in the sensitivity score.
b: Inhibition of succinate dehydrogenase activity.

Drug sensitivity testing. Although the same drugs were tested in each test system, the greater number of evaluable cases obtained with the SDI test provided a total of 184 (4 drugs×46 evaluable cases) drug tests, as compared to a total of 144 (4×36) drug tests in the SRC assay.

The effect of different criteria for indicating drug activity in the two test systems were compared (Table 3). In both systems, increasing the stringency of the criteria for activity decreased the sensitivity of the test. With activity in the SDI test set at an inhibition of succinate dehydrogenase activity of $\geqq 75.0\%$, there were fewer sensitive responses.

Of the total number of two test systems run in parallel, 36 SRC-SDI correlations of individual drug activity were found. Table 4 illustrates the effect of increasing SRC assay activity criteria on SRC-SDI correlations when activity for the SDI test is set at the most effective level, a $\geqq 50.0\%$ inhibition of succinate dehydrogenase activity. As activity criteria for the SRC assay were made more stringent, SRC-SDI correlations of tumor response for sensitivity decreased. The correlations for tumor resistance, however, increased. The over-

Table 4 Effect of increasing subrenal capsule (SRC) assay activity criteria on SRC-SDI test system correlations with the succinate dehydrogenase inhibition (SDI) test activity criterion set at ≧50.0% inhibition of succinate dehydrogenase activity

Activity criteria		SRC/SDI correlations of tumor response[a]		
SRC	SDI (%)	Sensitive/Sensitive	Resistant/Resistant	Overall
$\Delta S \leqq -1.0$	≧50.0	18/41 (43.9)	76/103 (73.7)	94/144 (65.2)
$\Delta S \leqq -2.0$	≧50.0	13/41 (31.7)	98/103 (95.1)	111/144 (77.1)
$\Delta S \leqq -3.0$	≧50.0	4/41 (9.8)	100/103 (97.3)	104/144 (72.2)

a: Percentage is shown in parentheses.

Table 5 Effect of increasing SRC assay activity criteria on SRC-SDI test system correlations with the SDI test activity criterion set at ≧75.0% inhibition of succinate dehydrogenase activity

Activity criteria		SRC/SDI correlations of tumor response[a]		
SRC	SDI (%)	Sensitive/Sensitive	Resistant/Resistant	Overall
$\Delta S \leqq -1.0$	≧75.0	8/8 (100.0)	99/136 (72.8)	107/144 (74.3)
$\Delta S \leqq -2.0$	≧75.0	7/8 (87.5)	126/136 (92.3)	133/144 (92.4)
$\Delta S \leqq -3.0$	≧75.0	4/8 (50.0)	133/136 (97.8)	137/144 (95.1)

a: Percentage is shown in parentheses.

all SRC-SDI correlations of tumor response were highest when activity criteria were set at $\Delta S \leqq -2.0$ for the SRC assay and at ≧50.0% inhibition of succinate dehydrogenase activity for the SDI test. Using these criteria of activity, SRC-SDI correlations were 31.7% for sensitivity and 95.1% for resistance.

In Table 5, the effect of increasing SRC assay criteria on SRC-SDI correlations when the activity criterion for the SDI test has been set at ≧75.0% inhibition of succinate dehydrogenase activity is shown. At an inhibition of ≧75.0%, there were fewer sensitive responses in the SDI test. With activity in the SRC assay set at $\Delta S \leqq -2.0$, SRC-SDI correlations were 87.5% for sensitive and 97.8% for resistance.

Discussions

For effective cancer chemotherapy, accurate and rapid drug sensitivity tests with high evaluability rates for predicting tumor sensitivity to anticancer agents are required. Few drug sensitivity tests satisfy these requirements. The succinate dehydrogenase inhibition (SDI) test, one of the most common drug sensitivity tests, has a high predictive accuracy of 89% (8, 9) and a high evaluability rate of 91% (10, 11).

However, it is a fact that clinical responses do not always parallel *in vitro* effectiveness. This fact arises from the ability of some drugs, so called masked compound drugs such as cyclophosphamide and FT-207, to show efficacy after undergoing structural changes *in vivo*. Thus, the host response to drugs must also be taken into consideration, which is the main reason why an *in vivo* drug sensitivity test is required.

The SRC assay is a new rapid *in vivo* procedure with a high evaluability rate and high predictive accuracy. Griffin *et al.* reported an 85.8% evaluability rate and 85.5% predictive accuracy in a 6-day assay (17, 18). In these studies, the tumor specimens tested were mostly breast, lung, ovar-

ian cancers and lymphomas, while in the present study, our attention was focused on gastrointestinal tumors. In our study of the SRC assay, a comparatively low evaluability rate of 72% was obtained.

The SRC assay was initially developed for use with nude mice (16), but later publications have stressed the usefulness of normal immunocompetent mice (13-15). The fact that host reactions appear in immunocompetent mice carrying subrenal allografts during that assay period is now well established (19-22). The use of normal mice poses the problem of how to assess the drug sensitivity by tumor size measurement. At present, the histopathologic evaluation of the SRC assay is of importance and indispensable for correct interpretation of drug effects.

The SRC assay has problems to be overcome. However, this *in vivo* assay has the attractive feature that it can maintain cell-to-cell contact by utilizing tumor fragments. It should be also pointed out that *in vivo* activation of chemotherapeutic agents similar to the clinical condition is possible with the SRC assay.

As described above, the evaluability rate of the SRC assay was 72.0% in our study. On the other hand, a 92.0% evaluability rate was obtained in the SDI test. The comparatively low evaluability rate is due to the difficulties in obtaining adequate growth of xenografts caused by the host immune response, either with immunocompetent mice or with nude mice. Delaying chemotherapy for about a week to await assay results, only to obtain an unevaluable assay, is a serious problem of the SRC assay.

It should also be pointed out that the SRC assay can only utilize solid tumors for subcapsular implantation of fragments. On the other hand, the SDI test is best with malignant pleural effusions, ascites which could not be tested with the SRC assay. A method similar to the SRC implant method is needed that permits chemosensitivity testing of non-solid tumors.

Activity criteria for any predictive drug test are generally determined from a retrospective analysis of test data and reflect a balance between the detection of sensitivity and resistance so as to provide a strong correlation between test results and clinical responses. Both sensitivity and specificity are desirable goals, but it is a more serious problem to be sensitive to inactive drugs, *i.e.*, to overpredict clinical usefulness, than to be insensitive to active drugs.

Correlations between predictive test systems are dependent upon the activity criteria established for each test system, which reflect a balance between the detection of sensitivity and that of resistance. In both test systems, as activity criteria were increased, correlations of tumor response for sensitivity decreased and correlations of tumor resistance increased to as high as over 90%.

A workable balance between detection of tumor sensitivity and resistance was obtained for both test systems with the SRC assay having an activity cutoff at a $\Delta S \leqq -2.0$ and the SDI test having an activity cutoff at $\geqq 50.0\%$ inhibition of succinate dehydrogenase activity. Although the highest percentage of SRC-SDI correlations were obtained with the SRC assay criterion set at $\Delta S \leqq -2.0$ and the SDI test set at $\geqq 75.0\%$ inhibition of succinate dehydrogenase activity, setting the inhibition at $\geqq 75.0\%$ reduced the sensitivity of the SDI test unacceptably.

Therefore, the activity criterion was set at $\Delta S \leqq -2.0$ in the SRC assay and at $\geqq 50.0\%$ inhibition of succinate dehydrogenase activity in the SDI test. Using these criteria of activity, 12.5% of the drugs tested were active in the SRC assay and 22.3% were active in the SDI test. The

SRC-SDI correlations of tumor response were 31.7% for sensitivity, 95.1% for resistance and 77.1% overall.

The SRC assay and the SDI test as drug-testing systems differ fundamentally, not only because one evaluates drug activity *in vivo* and the other *in vitro*, but because the time required by each method is different, 5 days for the SRC assay and one day for the SDI test. In spite of conceptual and practical differences, the SRC-SDI correlations were surprisingly good. Drug sensitivity tests for cancer chemotherapy are best when both test systems, *in vivo* and *in vitro*, are used, because the assay results produced by the two systems complement each other.

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References

1. Pechman HV and Runge P: Oxydation der formazylverbindungen. Ber Dtsch Chem Ges (1984) **27**, 2920.
2. Schrek R: A method for counting the viable cells in mormal and malignant cell suspensions. Am J Cancer (1936) **28**, 389-392.
3. Kuhn R and Jerschel D: Uber invertseifen VIII mitterl: Reduktion von tetrazolium durch Bakterien, garende hefe, und keimende samen. Ber Dtsch Chem Ger (1941) **74B**, 949.
4. Straus FH, Cheronis ND and Straus E: Demonstration of reducing enzyme systems in neoplasms and living mammalian tissues by triphenyltetrazolium chloride. Science (1948) **108**, 113.
5. Dallner G: Viability and tetrazolium salt in ascites tumors. Nature (1960) **186**, 176.
6. Kondo T and Moore GE: The limitations and adverse effects of cancer chemotherapy. Surg Forum (1958) **9**, 607-611.
7. Kondo T, Imamura T and Ichihashi H: *In vitro* test for sensitivity of tumor to carcinostatic agents. Gann (1966) **57**, 113.
8. Kondo T and Ohkubo K: *In vitro* test for prediction of side effects of carcinostatic agents. Gann (1967) **58**, 349.
9. Kondo T: Prediction of response of tumor and host to cancer chemotherapy. Natl Cancer Inst Monograph (1971) **34**, 251.
10. Maehara Y, Anai H and Masuda H: *In vitro* chemosensitivity of various human tumors evaluated by SDI (succinate dehydrogenase inhibition) test. Jpn J Cancer Chemother (1985) **12**, 2018-2023 (in Japanese).
11. Anai H, Maehara Y and Masuda H: *In vitro* chemosensitivity of various human tumors evaluated by succinate dehydrogenase inhibition (SDI) test (the second report). Jpn J Cancer Chemother (1986) **13**, 2544-2548 (in Japanese).
12. Mosmann T: Rapid colorimetric assay for cellular growth and survival: Application to proliferation and cytotoxicity assays. J Immunol Methods (1983) **65**, 55-63.
13. Bogden AE, Cobb WR and Le Page DJ: An *in vivo* method for testing chemotherapeutic agents against first transplant generation human tumor xenografts; in Design of Models for Testing Cancer Therapeutic Agents, Fidler and White eds, Van Nostrand Reinhold Company, New York (1982) pp 174-184.
14. Bogden AE, Cobb WR and Le Page DJ: Chemotherapy responsiveness of human tumors as first transplant generation xenografts in the normal mouse: six-day subrenal capsule assay. Cancer (Phila) (1981) **48**, 10-48.
15. Bogden AE, Houchens DP and Ovejera AA: Advances in chemotherapy studies with the nude mouse; in The Nude Mouse in Experimental and Clinical Research, Fogh and Giovanella eds, Vol. 2, Academic Press, Inc., New York (1982) pp 367-400.
16. Bogden AE, Kelton DE and Cobb WR: A rapid screening method for testing chemotherapeutic agents against human tumor xenografts; in Proceeding of the Symposium on the Use of Athymic (Nude) Mice in Cancer Research, Houchens and Ovejera eds, Gustav Fischer, Inc., New York (1978) pp 231-250.
17. Griffin TW, Bogden AE and Reich SD: Clinical update on the subrenal capsule assay as a predictive test for tumor response to chemotherapy. Proc Am Soc Clin Oncol (1982) 1, 17.
18. Griffin TW, Bogden AE and Reich SD: Initial clinical trials of the subrenal capsule assay as a predictor of tumor response to chemotherapy. Cancer (Phila) (1983) **52**, 2185-2192.
19. Edelstein MB, Fiebig HH and Smink T: Comparison between macroscopic and microscopic evaluation of tumor responsiveness using the subrenal capsule assay. Eur J Cancer Clin Oncol (1983) **19**, 995-1009.
20. Edelstein MB, Smink T and Ruiter DL: Improve-

ment and limitation of subrenal capsule assay for determining tumor sensitivity to cytostatic drugs. Eur J Cancer Clin Oncol (1984) **20**, 1549-1556.

21. Levi FA, Blum JP and Lemaigre G: A four-day subrenal capsule assay for testing the effectiveness of anticancer drugs against human tumors. Cancer Res (1984) **44**, 2660-2667.

22. Iwagaki H, Fuchimoto S and Orita K: A subrenal capsule assay for testing the effectiveness of anticancer drugs against human tumors. J Med (1988) **19**, 21-31

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