Chemosensitivity testing by nuclear damage assay of needle biopsy tissue in 19 patients with advanced pancreatic cancer

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At present, chemotherapy still achieves poor results in advanced pancreatic cancer and sometimes the patient's quality is impaired by useless chemotherapy. To improve this situation, we used the nuclear damage assay (NDA) as a chemosensitivity test for pancreatic cancer. We performed the NDA on percutaneous needle biopsy tissue obtained from 19 patients with inoperable advanced pancreatic cancer, and 16 of them were successfully assayed by the chemosensitivity test. We could get a positive response to the assay in 13 of the 16 patients. The 13 patients with a positive response were treated with the agent selected by the NDA, while 3 patients with no response were treated with 5FU. The outcome of chemotherapy was a complete response in one patient, no change in 5, and progressive disease in 7 of the patients showing tumor sensitivity in the assay. On the other hand, only progressive disease was found in the patients with no response in the assay. The survival rate of the former group was better than the latter ($P < 0.05$), and the median survival time was 23.4 and 11.1 weeks, respectively. We also report in detail the patient with a complete response to VP-16, which is rarely effective against pancreatic cancer. These results suggest that NDA of percutaneous needle biopsy tissue might improve the efficacy of chemotherapy for advanced pancreatic cancer.

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Pancreatic cancer is generally detected at an advanced stage so that about two third of the patients are unsuitable for surgical resection¹⁾.

Although chemotherapy has an important role in the treatment of advanced pancreatic cancer, it is not yet very reliable. At present, there are not many effective anticancer agents and tumor sensitivity varies with the type of cancer and even changes during treatment. If it was possible to reliably select an effective anticancer agent on the basis of chemosensitivity testing, the results of chemotherapy would be greatly improved.

Accordingly, we investigated chemosensitivity testing by the nuclear damage assay (NDA) in patients with advanced pancreatic cancer. The tumor tissue for assay was obtained by percutaneous needle biopsy. Our results indicated that chemosensitivity testing of needle biopsy tissue was useful for determining the appropriate chemotherapy for advanced pancreatic cancer.

Materials and methods

Patients

From June 1991 to March 1994, this study was carried out in 19 patients with advanced pancreatic cancer treated at Chiba University Hospital. The patients were 11 men and 8 women aged from 49 to 78 years (mean : 63.8 years). Chemotherapy based on the test results was performed in 16 patients. A definitive histological diagnosis was made by percutaneous biopsy in all patients. Inoperability was determined from the finding of invasion into neighboring organs including major vessels and/or distant metastasis on imaging diagnosis (US, CT and angiography).

Tumor biopsy

We collected tumor tissue samples by percutaneous needle biopsy under ultrasonic guidance²⁾ using a 21 gauge biopsy needle that was 6.8 mm in diameter and 170 mm long (Sonopsy C1, Hakko Co., Ltd.). The amount of tissue collected was approximately 0.8 mg in dry weight, or about 10,000 cells on average (5,000~20,000) as counted by flowcytometry.

Biopsy was usually performed three times in one session. One of the specimens was used for histological examination and the other two were used for the chemosensitivity assay. The tissue was immediately suspended in RPMI 1640 medium (adjusted to pH 7.2

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Fig. 1 Procedure of nuclear damage assay (NDA) on needle biopsy tissue

Table 1 List of anticancer agents and their concentration *in vitro*

Agents	(abbreviated)	Concentration($\mu\text{g/ml}$)
Group A		
Mitomycin C	(MMC)	2
Ranimustine	(NCNU)	20
Ifosfamide	(Ifos)	100
Nimustine	(ACNU)	20
Vindesin	(VDS)	1
Vincristine	(VCL)	1
Vinblastine	(VLB)	1
Group B		
5-Fluorouracil	(5-Fu)	100
Adriamycin	(ADM)	3
Epirubicin	(EPI)	4
THP-adriamycin	(THP)	3
Neocarzinostatin	(NCS)	1.5
Cisplatin	(CDDP)	5
Carboplatin	(JM8)	40
Etoposide	(VP16)	30
CPT-11	(CPT)	30

Fig. 2 Tumor nuclei shows no change(a), destruction (b) and condensation (c) after incubation with anticancer agent

with NaOH) supplemented with 10% fetal bovine serum and was stored at 0~4°C until assay within 24 hours after biopsy.

Informed consent was obtained from all the patients before biopsy.

Nuclear damage assay

The assay was carried out according to method of Tokita^{3,4}. The tumor tissues were minced into pieces with a blade, and then suspended in the medium and filtered through a mesh to remove mesenchymal components and to obtain micro cell aggregates. The filtered material was then centrifuged at 1,000 rpm for 5 minutes to eliminate fatty and necrotic tissue components in the supernatant. The micro cell aggregates were next suspended in medium containing anticancer agents at the speci-

fied concentrations and incubated for 4~8 hours. Other cell aggregates were incubated in medium with no anticancer agents as a control. Then the aggregates were repeatedly pumped through a needle injector to disperse them, and cells deposited at the bottom were placed on glass slides (Fig.1). More than 100 cells per slide were observed. When destruction, disintegration, or condensation of the tumor cell nuclei were noted, the cells were regarded as sensitive to the chemotherapy agent being tested (Fig.2). The number of cells which showed chemosensitivity was compared with the control by the X^2 test or Fisher's test, and $P < 0.05$ was taken to indicate a significant difference. The anticancer agents were divided into group A and B according to the nuclear changes produced. Group A agents caused nuclear disintegration or destruction and group B agents caused

Table 2 Chemosensitivity test results, actual chemotherapy, and prognosis

Patient No.	Anticancer agents tested	Active agents	Agent used	Response	Survival (weeks)
1	CDDP, MCNU, 5FU, Epi VP16, MMC, CPT	VP16	VP16	CR	92.7
2	JM8, NCS, 5FU, VP16 MMC, Ifos, ACNU, VDS	VDS	VDS	NC	39.8
3	CDDP, JM8, MCNU, THP, ADM NCS, 5FC, Epi, VP16, MMC CPT	CDDP, NCS CPT	CDDP	NC	18.9
4	JM8, 5FU, Ifos, ACR MMC, MCNU	JM8	JM8	NC	18.6
5	VDS, JM8, NCS, 5FU, VP16 MMC, Ifos, THP, ACNU	THP	THP	NC	17.2
6	CDDP, 5FU, Epi, VP16 MMC, CPT	CDDP	CDDP	NC	12.3
7	CDDP, ADM, 5FU, Epi VP16, MMC	MMC	MMC	PD	22.3
8	ADM, 5FU, MMC	MMC	MMC	PD	21.4
9	CDDP, VDS, JM8, MCNU, THP NCS, 5FU, VP16, MMC, CPT	CDDP	CDDP	PD	17.6
10	VDS, JM8, THP, 5FU Ifos, MMC	5FU	5FU	PD	16.3
11	CDDP, JM8, MCNU, NCS 5FU, MMC, Ifos, CPT	JM8	JM8	PD	14.7
12	JM8, ADM, NCS, 5FU Epi, MMC, CPT, ACR	ADM, NCS	ADM	PD	14.1
13	CDDP, MCNU, THP, ADM, NCS 5FU, Epi, VP16, MMC, CPT	Epi	Epi	PD	13.4
14	CDDP, 5FU, EPI, VP16 MMC, CPT	(None)	5FU	PD	19.0
15	CDDP, 5FU, Epi, MMC	(None)	5FU	PD	14.9
16	JM8, 5FU, Epi, VP16 MMC, ACNU	(None)	5FU	PD	10.7

condensation. Table 1 shows the anticancer agents and the concentration of each agent used in the assays. Group A consisted mainly of alkylating agents and vinka alkaloids, while group B consisted of antibiotics and antimetabolites. The *in vitro* concentrations were adjusted to correspond to the maximum blood level of each agent in clinical use.

Chemotherapy

Active anticancer agents were selected from the results of the assay and patients were treated with these agents. When there were more than 2 or more active agents, we chose the one causing the highest level of nuclear damage and gave it intravenously at the standard dosage. Patients whose tumors showed no response to any anticancer agents were treated with 5FU at a dose of 500 mg/m² for 5 days.

The response to chemotherapy was evaluated every 4 weeks according to the general rules for chemotherapy of solid tumors developed by the Japan Society of Cancer

Therapy⁵⁾. Survival time was determined according to the Kaplan-Meier method and differences in survival were assessed by the generalized Wilcoxon test.

Results

Chemosensitivity assay

The assay was technically successful in 16 of 19 patients (84.2%). Because of an insufficient amount of tissue, we could not judge tumor sensitivity in other 3 patients. On an average, 6.7 anticancer agents were successfully tested in one assay. We obtained a positive response in 13 of 16 patients and there were 1.2 active agents on average (Table 2). The active rate for each agent is shown in Table 3. CDDP and JM8 showed relatively higher rates than the other agents.

Chemotherapy

In the patients treated with anticancer agents selected as active by the assay, the following results were obtained :

Table 3 The activity of each anticancer agent in the chemosensitivity assay

Agent	Active/Total(%)
CDDP	1/8 (37.5)
VDS	1/3 (33)
JM8	2/9 (22)
MCNU	1/5 (20)
THP	1/6 (16.7)
ADM	1/6 (16.7)
NCS	1/7 (14.3)
5-FU	2/16 (12.5)
Epi	1/9 (11)
VP16	1/10 (10)
MMC	1/16 (6.3)
Ifos	0/5
ACNU	0/3
CPT	0/7
ACR	0/3

complete response (CR) in one, no change (NC) in 5 and progressive disease (PD) in 7. On the other hand, all of the patients with no active agents detected by the test showed PD. The survival rate of the group with sensitive tumors was significantly better than that of the group without sensitive tumors ($P < 0.05$)(Fig.3). The median survival time was 23.4 and 11.1 weeks, respectively.

A Casa report : advanced pancreatic cancer with a complete response (CR)

The patient was a 75-year-old man who presented to hospital because of upper abdominal pain. Ultra-sonography detected a low-echogenicity tumor (30 mm in diameter) in the body of the pancreas and also multiple small tumors in the liver. Needle biopsy proved that the tumor was a moderately differentiated adenocarcinoma. The patient was also found to be unsuitable for surgical resection. Chemosensitivity testing showed that VP-16 was active against the tumor, and we treated him with this agent at a dose of 80 mg/m² for 2 courses. After this intravenous chemotherapy, the patient also took the agent orally at a daily dose of 25 mg.

After 4 months of treatment, both the primary and metastatic lesions had disappeared on abdominal CT scans and CR was confirmed (Fig. 4). The serum levels of CA19-9 and SPAN-1 decreased markedly from 1,820 to 44 and from 390 to 35, respectively (Fig.5). After treatment, he survived for one year and 10 months, but finally died of malnutrition that was probably due to tumor regrowth.

Discussion

Various kinds of anticancer agents have been used for the treatment of inoperable advanced pancreatic cancer, although the response rate (CR plus PR) still remains

Fig. 3 Survival of patients with the positive group (A) and the negative group (B) in the sensitivity according to chemotherapy.

under 20% at present^{6,7)}. Thus, more effective anticancer agents need to be developed. Adverse effects due to the administration of anticancer agents are a serious problem, since the quality of life of cancer patients can be greatly reduced by the use of chemotherapy with no therapeutic effect. In this situation, chemosensitivity testing is clinically important to prevent the inappropriate use of such agents.

Although various chemosensitivity tests have been reported previously⁸⁻¹¹⁾, only a few of them can be used clinically. A chemosensitivity test applicable to clinical use should have the following features : ① simple and reliable technique, ② a high success rate, ③ rapid results, and ④ a good correlation with therapeutic responses such as tumor regression and long survival. Current tests also require a large amount of cancer tissue to be surgically resected and so have been used mainly for adjuvant therapy accompanied by operative intervention¹²⁾. Accordingly, we generally have to undertake chemotherapy without sensitivity testing in patients with inoperable pancreatic cancer.

The nuclear damage assay (NDA) used in the present study requires only a small amount of cancer tissue, and is a simple and reliable technique. Assessment can be completed in a relatively short period of less than 48 hours. Moreover, it has been shown that the NDA is more closely correlated with the nude mouse method than either the MTT assay or colony formation inhibition assay¹³⁾.

In this study, we performed the NDA on a small amount of cancer tissue obtained by percutaneous needle biopsy and proved that it could be successfully used in daily clinical practice. According to the results of the NDA, we selected an appropriate anticancer agent for chemotherapy. As a result, the cancers sensitive to a certain agents tended to show a better response to therapy. It was noteworthy that CR was achieved in a case of advanced pancreatic cancer by using VP-16, which is rarely effective for this type of cancer¹⁴⁾. Although 5FU is generally used as the first-line anticancer agent, the

Fig. 4 The case of complete response

a : Dynamic X-ray CT scan before treatment demonstrates that the low density tumor in the body of the pancreas (arrow) accompanied by dilated main pancreatic duct and the metastatic liver tumors.
b : 4 months after the treatment, both the pancreas and the liver tumors disappeared. Only dilated main pancreatic duct is noted (arrow head).

Fig. 5 Change of the tumor markers before and after the treatment

sensitivity rate was only 12.5% in the NDA assay. This means that we can expect better results when an appropriate anticancer agent is given based on the NDA assay than when 5FU is selected at random. In addition, NDA of percutaneous needle biopsy tissue might possibly improve the efficacy of chemotherapy in the treatment of advanced pancreatic cancer, which usually requires

laparotomy for the collection of tissue.

However, there is a limitation to successful performance of the test, because a single needle biopsy cannot obtain enough tissue to test many kinds of anticancer agents and fibrosis secondary to the pancreatic cancer may make it difficult to collect any tissue. Thus, a better technique of collecting cancer tissue needs to be devised. In conclusion, the usefulness of the test should be confirmed in a large series of a lot of patients with advanced pancreatic cancer.

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Chemosensitivity testing by nuclear damage assay of needle biopsy tissue in 19 patients with advanced pancreatic cancer
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進行膵癌の治療において抗癌剤を用いた化学療法は重要な位置を占めるが、現在のところその治療成績はあまり期待できない。筆者らは進行膵癌治療において抗癌剤感受性試験を応用し、化学療法の成績向上を試みた。

〔対象と方法〕 切除不能な進行膵癌19例（男11例，女8例，平均年齢64歳）に対し，経皮的針生検によって得られた組織を用いて抗癌剤感受性試験を行った。感受性試験は時田等によって開発された，核の形態変化を応用したnuclear damage assay (NDA)によって行った。組織採取は21G組織生検針を用いて超音波映像下に経皮的に行い，得られた癌組織を細切遠心分離し，一定濃度の抗癌剤を溶解した培養液中で4～8時間 incubationした。核の変化は断裂，破砕あるいは濃縮を陽性所見とし，その出現数を対照との間で統計的に5%以下の危険率で判定した。感受性試験の成績により陽性薬剤のある場合はもっとも核変化の大きい薬剤を1剤，また陽性薬剤のない場合は5FUをそれぞれ単独で投与し，その効果を比較検討した。

〔成績〕 19例中16例において感受性試験の判定が可能であった。1例当たりの判定可能薬剤数は平均6.7剤で，16例中13例に陽性薬剤が得られ，その平均は1.2剤であった。CDDP, JM8が比較的陽性率の高い薬剤であった。固形癌化学療法効果判定基準による評価では，陽性薬剤治療群13例で Complete Response(CR)1例，No Change (NC)5例，Progressive Disease (PD)7例であったのに対し，5FU治療群3例全例がPDであった。Generalized Wilcoxon 検定では陽性薬剤治療群のほうが5FU治療群にくらべ，有意に生存期間の延長が認められ，また，それぞれの平均生存期間は23.4週間と11.1週間であった。CRの得られた症例は75歳の男性で，診断時すでに肝転移がみられた。感受性試験によりVP16が陽性と判定され，経静脈的に80mg/m²を2クール行った結果，画像上原発巣および転移巣とも消失し，1年10ヵ月長期生存した。

〔考察〕 癌は同一組織に発生したものであっても，抗癌剤に対する感受性は各々の症例で異なることが知られている。症例ごとに適切な薬剤を選択し，化学療法の成績を向上させようとする目的で，様々な感受性試験が考案されているが，必ずしも臨床的に応用されているとはいえない。今回，切除不能な進行膵癌に対し，経皮的針生検により開腹することなく組織採取し，感受性試験が施行可能なことを明らかにした。また，陽性薬剤治療群の成績が良好なことを示した。さらに，従来膵癌に対しほとんど無効と報告されているVP16によりCRが得られたことは，感受性試験により適切な薬剤選択が行いければ，治療成績の向上に結びつくことを示唆するものと考えられた。

Evaluation of argyrophilic nucleolar organizer regions (AgNOR) in breast cancer
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〔目的〕 AgNORは腫瘍増殖活性を反映するとされているが，癌腫における予後因子としての有用性は報告により異なる。今回，乳癌におけるAgNOR染色像と臨床病理学的因子との関連および予後因子としての有用性を検討した。

〔対象と方法〕 乳癌157例（平均観察期間82ヵ月），対照として乳腺線維腫瘍9例，硬化性腺症9例，正常乳腺15例を用い比較検討した。AgNOR染色はCrocker法に準じて行い，1症例100核の核内dot数の平均値を算出し，AgNOR scoreとした。

〔結果〕 AgNOR scoreは，正常乳腺（1.97±0.22），硬化性腺症（2.19±0.26），線維腺腫（2.49±0.51）に比し，癌腫（3.95±1.17）にて有意に高値であった（ $P<0.001$ ）。乳癌においてAgNOR scoreは腫瘍径，リンパ節転移との関連がみられたが，閉経の有無，組織型，エストロゲンレセプターとの関連はみられなかった。乳癌症例を平均AgNOR score3.95を境としてAgNOR高値群と低値群の2群に分け比較すると，高値群の健存率は低値群に比し有意に不良であったが（ $P<0.05$ ），生存率に有意差は認められなかった。多変量解析では，AgNOR scoreの独立した予後因子としての有用性は認められなかった。

〔考察〕 AgNOR scoreは，乳癌においても腫瘍増殖活性を反映すると考えられた。AgNOR scoreは独立した予後因子としての有用性を認めないものの，高値群と低値群間の健存率に有意差が認められることより，リンパ節転移など他の予後因子が不明な症例においては，再発予測因子として活用できると考えられた。