Urol Res (1999) 27:483–488

ORIGINAL PAPER

Yasuhiro Hirano · Tomomi Ushiyama · Kazuo Suzuki
Kimio Fujita

Clinical application of an in vitro chemosensitivity test, the Histoculture Drug Response Assay, to urological cancers: wide distribution of inhibition rates in bladder cancer and renal cell cancer

Received: 17 July 1998 / Accepted: 25 March 1999

Abstract To investigate the variations in chemosensitivity of individual cancers, we performed an in vitro chemosensitivity test, the Histoculture Drug Response Assay (HDRA), on fresh biopsied or surgical specimens. They were 26 bladder cancers and 19 renal cell cancers. Ten anticancer drugs were tested. By prolonging the drug exposure time to 7 days, we obtained reliable results. The mean inhibition rates (IRs) were higher for bladder cancer than for renal cell cancer, and the difference was significant for cisplatin, carboplatin, vinblastine, mitomycin C, and adriamycin. There was no significant correlation between the histological grade of the tumor and HDRA sensitivity. IR values showed a wide distribution and cancers could be classified into two groups of sensitive and resistant. This was especially true for 4-hydroxy-ifosfamide. Three bladder cancer patients with evaluable lesions were treated with drugs selected on the basis of the results of the HDRA. One patient achieved a complete response and the other patients showed a partial response. Our results suggest that chemosensitivity is independent of the clinicopathological classification of cancer, and that the HDRA may be useful for selecting the effective anticancer drug for patients with urological cancer.

Key words Chemosensitivity test · Histoculture Drug Response Assay · Bladder cancer · Renal cell cancer

Introduction

Renal cell cancer is resistant to various chemotherapeutic agents. Although interferons are widely used for the treatment of renal cancer, the response rate is no more than 20% [1]. Even the combination of interferon-α with another anticancer drug or interferon-γ only achieves about a 30% response rate [2, 13]. On the other hand, bladder cancer is generally thought to be sensitive to chemotherapy, but the results are not satisfactory.

It is known that the sensitivity to anticancer drugs differs between cancers even if they show the same histological findings [4, 15]. There is a risk of tumor progression when anticancer drugs are not effective [6, 7]. Therefore, it may be important to make an effort to select the drug which will be effective, because it may produce a better result and improve the quality of life for individual cancer patients.

The Histoculture Drug Response Assay (HDRA) is an in vitro chemosensitivity test that was reported by Hoffman in 1991 [5]. The clinical efficacy of chemotherapy has been shown to have a strong correlation with HDRA data in various kinds of solid tumor [3, 4, 9]. However, there have been few reports on its use in urological cancer [11, 14]. In order to investigate the variation in chemosensitivity in urological cancers, we tested fresh biopsied or surgical specimens of bladder cancer and renal cell cancer with the HDRA. Chemotherapy was done on the basis of the HDRA results in some of the patients.

Materials and methods

Cancer samples

Cancer tissues were obtained during either surgical removal or biopsy at Hamamatsu University Hospital and related hospitals between August 1997 and March 1998. Informed consent for the chemosensitivity test was obtained from 45 patients before the procedure. Of the 45 specimens, 26 were bladder cancers and 19 were renal cell cancers. Twenty-four specimens of bladder cancer were histologically diagnosed as transitional cell cancer (TCC), of histological grade 1 in five specimens, grade 2 in 12, and grade 3 in seven. The other two specimens were diagnosed as signet ring cell cancer and adenocarcinoma respectively; both arose from the urinary bladder. Renal cell cancers were grade 1 in six specimens, grade 2 in 10, and grade 3 in three.

Y. Hirano (✉) · T. Ushiyama · K. Suzuki · K. Fujita
Department of Urology,
Hamamatsu University School of Medicine,
3600 Handa-cho, Hamamatsu City, 431-3192, Japan

Drugs

The medium used was RPMI 1640 medium (Nissui, Tokyo, Japan) supplemented with 20% heat-inactivated fetal bovine serum, 2 mM L-glutamine, and antibiotics (100 U/ml of penicillin and 100 μg/ml of streptomycin). Ten anticancer drugs were used: cisplatin (CDDP), carboplatin (CBDCA), 4-hydroxy-ifosfamide (4-H-IFO), methotrexate (MTX), vinblastine (VLB), 5-fluorouracil (5-FU), mitomycin C (MMC), adriamycin (ADM), bleomycin (BLM), and etoposide (VP-16). Each drug was diluted in complete medium at 10 times therapeutic peak plasma concentration (10PPC) achieved by intravenous administration of clinical doses [12, 15]. The concentration of each drug was as follows: CDDP 20 μg/ml, CBDCA 250 μg/ml, 4-H-IFO 250 μg/ml, MTX 10 μg/ml, VLB 100 μg/ml, 5-FU 10 μg/ml, MMC 7.5 μg/ml, ADM 15 μg/ml, BLM 7 μg/ml, and VP-16 100 μg/ml.

4-H-IFO, which is the active form of ifosfamide, was kindly provided by Shionogi Pharmaceutical Company and the other nine drugs were purchased commercially. Collagen sponge gels were purchased from Yamanouchi Pharmaceutical (Tokyo, Japan). Although we tried to test all the drugs, this was not possible for all specimens because of the small volume of tissue obtained.

HDRA with MTT assay

The HDRA was performed as reported by Furukawa et al. [4]. Cancer tissue was identified by the naked eye and cut into pieces approximately 2–3 mm in diameter using scissors. After weighing the individual pieces, they were placed on 1 cm cubes of collagen sponge gel in each well of a 24-well plate. Then each tumor piece was immersed in 800 μl of complete medium and 200 μl of an anticancer drug solution, followed by incubation at 37°C in a humidified 5% CO_2 atmosphere for 7 days. Each drug was assessed in triplicate. Three tumor pieces incubated with 1000 μl of complete medium without any anticancer drug were used as controls for cell viability. After 7 days of incubation, 100 μl of RPMI 1640 containing 0.06% collagenase (type 1: Sigma) and 100 μl of 0.2% MTT (Sigma) solution were added to each well. The plate was then incubated for an additional 24 h. The supernatant in each well was aspirated carefully and 500 μl of dimethylsulfoxide (DMSO; Wako Pure Chemicals, Osaka, Japan) was added for solubilizing the MTT-formazan. After another 4 h of incubation, 100 μl of MTT-formazan solution from each well was transferred to the wells of a 96-well microplate and the absorbance of each well was read on a microplate reader (Bio-Tek Instruments, USA) at a test wavelength of 550 nm. The inhibition rate (IR) was calculated as follows:

IR (%) = (1 − mean absorbance for drug-treated tumor per gram/mean absorbance for control tumor per gram) × 100.

Microscopic examination

After 7 days of culture, a piece of each specimen incubated with 1000 μl of complete medium alone was observed under a microscope. Staining was done with hematoxylin-eosin and the findings were compared with those before culture.

Clinical chemotherapy based on the HDRA

Three bladder cancer patients with evaluable lesions received chemotherapy based on the results of the HDRA. Anticancer drugs which showed an IR value of more than 50% were selected. Before chemotherapy we obtained informed consent from all three patients.

Statistical analysis

The significance of differences between the IR values of bladder cancer and renal cell cancer were determined by an unpaired *t*-test. The relationship between the IR values of each anticancer drug and histological tumor grade was analyzed by one-factor ANOVA. A P value of less than 0.05 was considered to be statistically significant.

Results

HDRA assessment of the samples

HDRA was possible for all of the bladder cancers (100%) and 17 of 19 (89.5%) renal cell cancers. Two renal cell cancer samples could not be assessed because they had a low optical density.

Microscopic findings after the HDRA

The microscopic findings of bladder cancer before and after the HDRA are shown in Fig. 1. Cancer cells grew

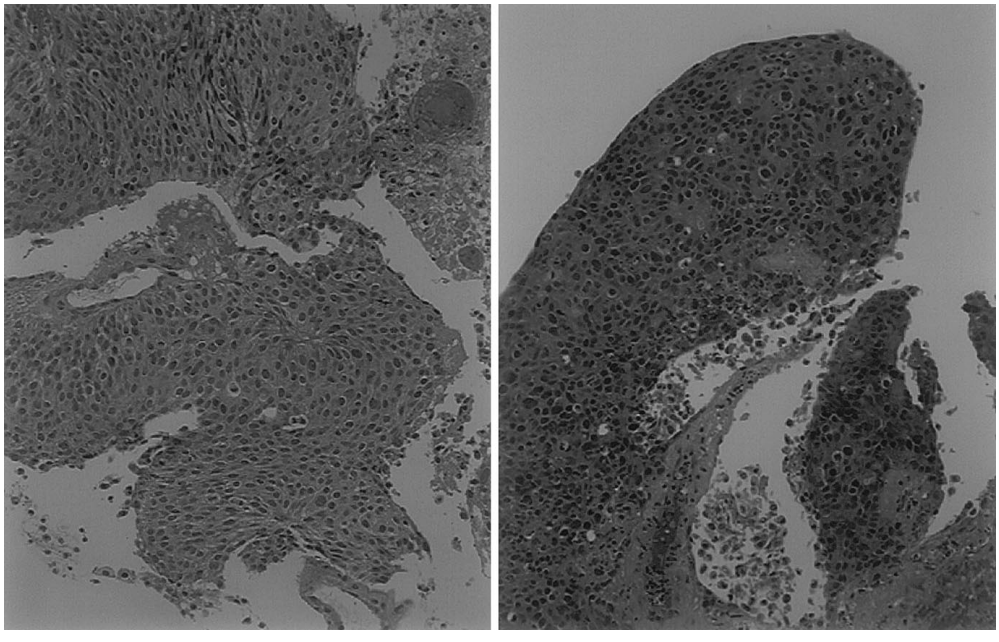

Fig. 1 Histological findings before (*left*) and after (*right*) culture on collagen gels of transitional cell cancer, grade 2. Cancer cells grew three-dimensionally with cell-to-cell contact, and tissue architecture was maintained on the collagen gels even after 7 days of culture

Table 1 Inhibition rate (*IR*) of anticancer drugs: comparison between bladder cancer and renal cell cancer [data are expressed as the mean ± standard error (%)]

Drug	IR of bladder cancer (n = 26)	IR of renal cell cancer (n = 17)	P value
CDDP	83 ± 5	63 ± 7	<0.05
CBDCA	92 ± 6	65 ± 12	<0.05
4-H-IFO	70 ± 12	57 ± 13	NS
MTX	20 ± 4	9 ± 6	NS
VLB	72 ± 6	47 ± 6	<0.05
5-FU	59 ± 7	38 ± 7	<0.05
MMC	80 ± 5	54 ± 9	<0.05
ADM	70 ± 6	45 ± 9	<0.05
BLM	41 ± 16	31 ± 12	NS
VP-16	45 ± 9	27 ± 8	NS

three-dimensionally with cell-to-cell contact, and tissue architecture was maintained on the collagen gels even after 7 days of culture.

Comparison of inhibition rates between bladder cancer and renal cell cancer

The mean IR values of each drug for both cancers are shown in Table 1. The IRs of CDDP, CBDCA, VLB, 5-FU, MMC, and ADM were significantly higher for bladder cancer than renal cell cancer. The IRs of all other drugs were also higher for bladder cancer, although the differences were not statistically significant.

Relation between IR and histological grade

The relation between IR and histological grade is shown for VLB and 5-FU (Fig. 2). Although high-grade bladder cancers tend to be less sensitive to drugs and high-grade renal cell cancers to be more sensitive, there was no significant relationship between histological grade and IR for either drug or either cancer.

Distribution of IR values

The distributions of IR values are shown for MTX, VLB, and 4-H-IFO. Most cancers seemed to be rather resistant to MTX (Fig. 3), while the other drugs showed a wide distribution of IR values. The distribution for VLB is shown in Fig. 3 as an example. On the whole, the chemosensitivity of renal cell cancer was lower than that of bladder cancer. Nevertheless, several renal cell cancer specimens showed a high sensitivity to certain drugs. Regarding the sensitivity to 4-H-IFO, renal cell cancer seems to be divided into sensitive and insensitive cancers (Fig. 3).

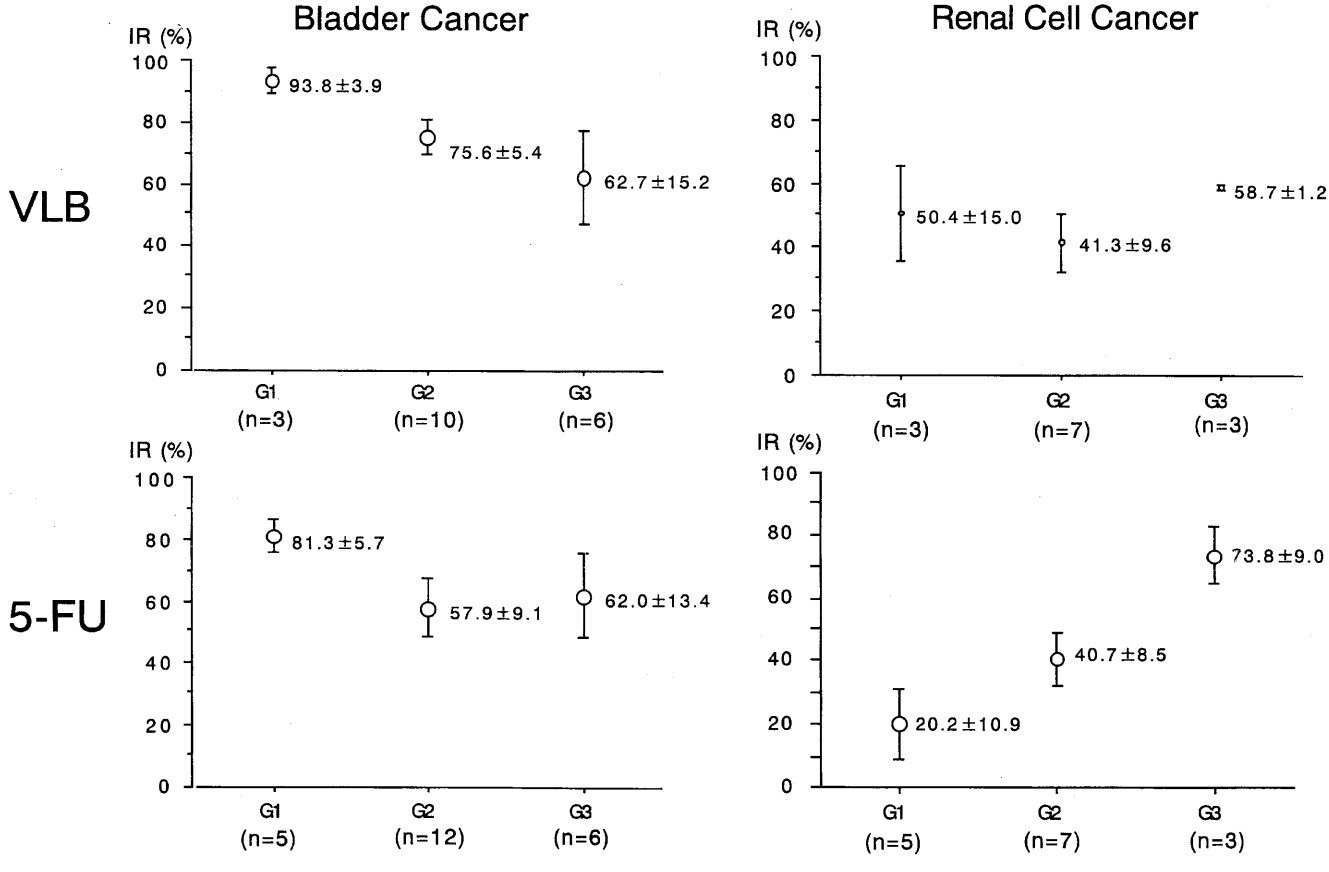

Fig. 2 Relation between inhibition rate (*IR*) and histological grade (*G*) for each cancer following treatment with vinblastine (*VLB*) and 5-flurouracil (*5-FU*). Data on bladder cancer are those for transitional cell cancers. Data are expressed as the mean ± standard error (%)

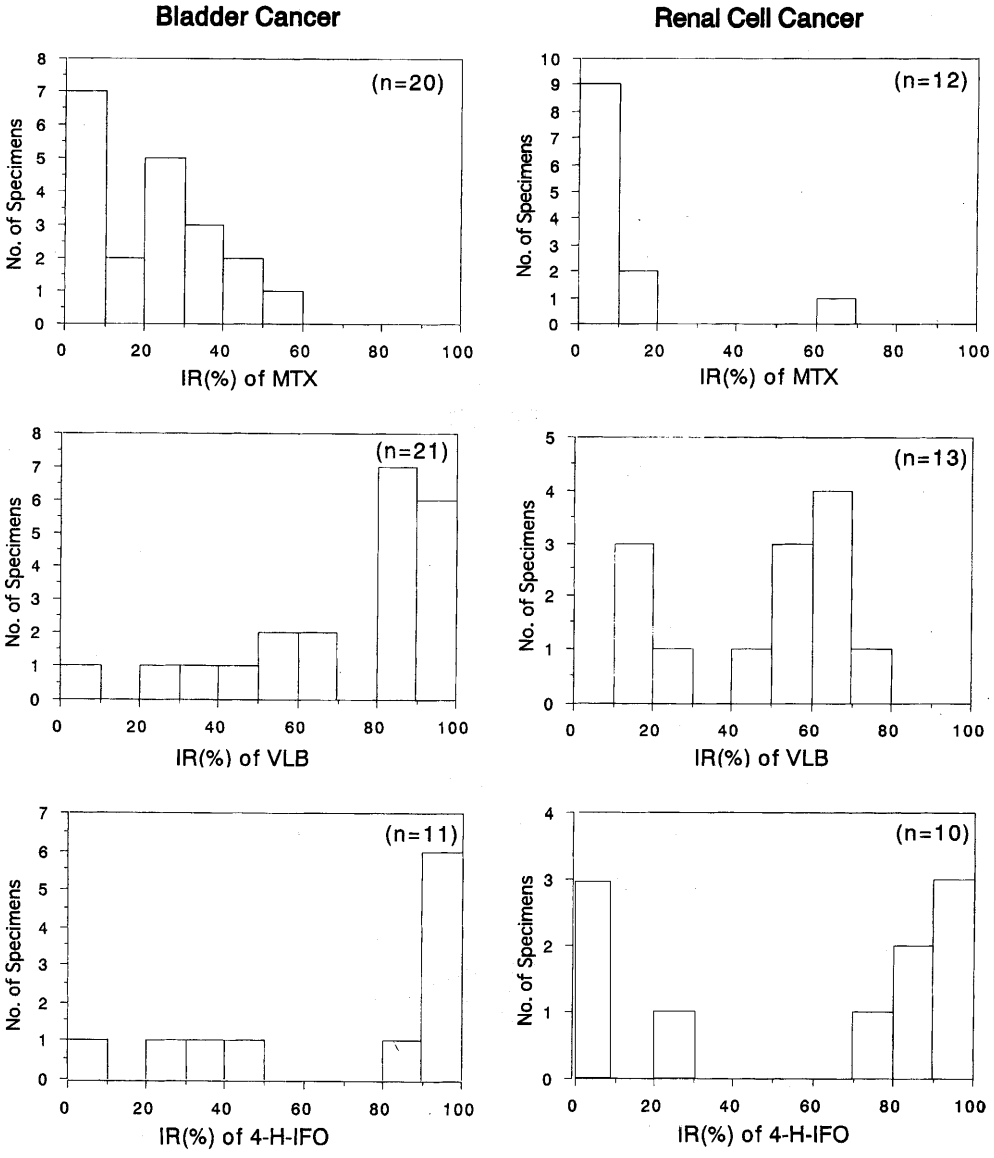

Fig. 3 Distribution of chemosensitivity. *Top*: Most cancers showed a low sensitivity to methotrexate (MTX). *Middle*: Cancers showed a wide distribution of sensitivity to most of the drugs. The data for vinblastine (VLB) are shown here. *Bottom*: Both bladder and renal cell cancers were divided into two groups: sensitive and insensitive to 4-hydroxyifosfamide(4-H-IFO)

Clinical response of bladder cancer treated with drugs selected on the basis of the HDRA

Three bladder cancer patients with evaluable lesions received chemotherapy using drugs selected on the basis of the results of the HDRA. Table 2 shows the clinical outcome of these three patients. Case 1 was a 65-year-old man with signet ring cell cancer. Since the cancer occupied the whole bladder and no distant metastasis was found, we recommended total cystectomy. However, he refused the cystectomy and asked for conservative treatment. We predicted that standard chemotherapy would not be effective, and performed intra-arterial infusion of CBDCA. After three courses of treatment tumor could not be identified. After 12 months of follow-up, no recurrence and/or metastasis has been found.

Case 2 was an 84-year-old woman who presented to our hospital with gross hematuria and severe anemia. Cystoscopy revealed widespread papillary, sessile, grade 2, and T3 transitional cell cancer. We selected bladder-preserving therapy because of her age. Intra-arterial infusion of CBDCA was performed. After three courses, tumor volume was reduced by more than 50%, and the gross hematuria was decreased. Eight months later, however, she died from cardiac failure.

Case 3 was a 70-year-old woman who had grade 2 transitional cell cancer of the bladder with metastases to the pelvic lymph nodes, liver, and lung. Based on the HDRA using the endoscopic biopsy specimen, she received one course of systemic chemotherapy with CDDP and 5-FU as the first-line treatment. She was then given two courses of systemic chemotherapy with CDDP, VLB, and ADR. After the three courses of chemotherapy the metastatic lesions in the lung and pelvic lymph nodes disappeared and her bladder tumor was reduced in size. Twelve months later, she is still alive and has no symptoms.

Table 2 Clinical response in bladder cancer patients to the drugs selected on the basis of the HDRA (Cases 1 and 2 were treated by intra-arterial infusion; case 3 was treated by systemic chemotherapy)

Case no. (age/sex)	Pathological Diagnosis	Evaluable lesions	Chemotherapy (inhibition rate, %)	Response	Survival (months)
1 (65/M)	Signet ring cell carcinoma	Primary lesion	CBDCA (98)	Complete	12
2 (84/F)	Transitional cell carcinoma	Primary lesion	CBDCA (100)	Partial	8; death from cardiac failure
3 (70/F)	Transitional cell carcinoma	Primary lesion, pelvic lymph node, liver, lung	CDDP (100), 5-FU (98), VLB (83), ADR (90)	Partial	12

Discussion

In vitro chemosensitivity tests of solid cancer tissue have been performed using various methods [3–5, 7, 9, 11, 12, 14, 15]. Kondo et al. [7] reported the succinic dehydrogenase inhibition test (SDI method) in 1966. After that, Mossmann reported the MTT assay, which was a simplified version of the SDI method [10]. The MTT assay used the tetrazolium salt, 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT), as an enzymatic receptor, the optical density being measured with a microplate reader [10]. This assay enabled the evaluation of chemosensitivity more rapidly and simply. The success rate was more than 90%. However, in this method the cancer cells are assessed as a single cell suspension and cell-to-cell contact is abolished [4]. Furthermore, most of the cells examined may actually be interstitial cells [4], which are difficult to separate from cancer cells. Yamaue et al. [15] obtained good results for gastrointestinal tract cancer using a modified MTT assay which included a process for removing interstitial cells.

The MTT assay has another weak point. Because drug contact time is only 72–96 h, it is considered to be too short for time-dependent drugs such as 5-FU [16]. Hoffman et al. [5] reported the HDRA utilizing a three-dimensional culture system. In this method, approximately 1 mm^3 of cancer tissue is cultured on a collagen gel without enzymatic tissue digestion. Therefore, cell-to-cell contact is maintained and long-term culture is possible under conditions resembling those in vivo, allowing cancer cells to remain alive for long enough to test 7 days of exposure to anticancer drugs. However, the original HDRA employed autoradiography [4]. In 1992, Furukawa et al. [4] reported an improved HDRA, which used the MTT assay to assess cell viability instead of autoradiography. His HDRA has already been used for gastric cancer, colorectal cancer, and other kinds of solid cancer [3, 4, 5, 9]. The results have confirmed a strong correlation between the assay and the clinical response [3, 4, 5, 9].

For urological cancers, a three-dimensional culture system was tried in the form of the collagen gel matrix assay by Uchibayashi et al. in 1993 [14]. They investigated six types of anticancer drugs in 40 specimens of cancer, including renal cell, urothelial, and prostatic cancer. Ogiwara et al. [11] also employed the collagen gel matrix assay to select the best anticancer drug for intra-arterial infusion chemotherapy in patients with bladder cancer. Although cancer tissues were cultured for 7 days on a collagen gel in both studies, the actual drug exposure time was only 3 days, which seems to be inadequate for predicting the effect of time-dependent drugs such as 5-FU or VLB. Yamaue et al. [16] compared the chemosensitivity of 100 samples of gastric cancer tissue to 5-FU between 72 h of drug exposure using the MTT assay and 7 days of drug exposure using the HDRA [16]. They found that chemosensitivity to 5-FU with 72 h drug exposure has the possibility of a higher false-positive rate [16]. That is the reason why we chose Furukawa's 7-day HDRA.

According to our results, the mean IRs for bladder cancer were higher than those for renal cell cancer, with a significant difference in CDDP, CBDCA, ADM, 5-FU, MMC, and VLB. These results are compatible with the clinical consensus that bladder cancer is comparatively sensitive and renal cell cancer is resistant to chemotherapy.

This study also revealed a wide distribution of chemosensitivity among cancers, even in bladder cancers. Regarding the data for 4-H-IFO, it suggested that, for this particular drug, individual cancers could be divided in two groups: sensitive and insensitive. Drug sensitivity is independent of the histological grade and therefore a chemosensitivity test for individual cancer cells is necessary to enable the choice of the appropriate therapeutic anticancer drug. The HDRA has been used for gastrointestinal cancers and Kubota et al. [9] reported that both the overall and disease-free survival rates of HDRA-sensitive gastric cancer patients were significantly higher than those of the HDRA-resistant group. For bladder cancer, MVAC therapy achieves a comparatively high response rate of approximately 50–60% [8], but the remaining 40–50% patients do not show enough response although they suffer from severe and harmful side effects.

Based on the results of the HDRA, we performed chemotherapy in three patients. One of the patients showed a complete response to intra-arterial therapy with a single effective anticancer drug. The other two patients showed a partial response. Although chemotherapy was not curative, their symptoms improved and quality of life was maintained.

In conclusion, this study has confirmed that renal cell cancer, in general, is refractory to chemotherapy. However, HDRA can find a effective drug even for these

cancers. There is a wide variation in chemosensitivity among cancers of the same histological classification. Therefore, the HDRA might be a feasible and a useful technique for predicting efficacy and selecting the appropraite anticancer drug for individual patients.

Acknowledgements The authors thank Dr Hiroki Yamaue and Miss Mariko Sugano of the 2nd Department of Surgery at Wakayama Medical School for their kind advice on the HDRA technique.

References

1. Aso Y, Umeda T, Niijima T (1989) Alpha interferon therapy for renal cell carcinoma: summary of four collaborative trials. In: Murphy GP, Khoury S (eds) Therapeutic progress in urological cancers. Liss, New York p 635
2. Foon K, Doroshow J, Bonnem E, Ferer A, Graham S, Grosh B, Narayan P, Elias L, Harvey H, Sculof R, Williams R, Rinehart J, Zeken P, Catalona W, Dillman R, Salmon S, Galasso F (1988) A prospective randomized trial of alpha$_{2b}$-interferon/gamma-interferon or the combination in advanced metastatic renal cell carcinoma. J Biol Resp Modifiers 7:540
3. Furukawa T, Kubota T, Watanabe M (1992) High in vitro–in vivo correlation of drug response using sponge-gel-supported three dimensional histoculture and the MTT end point. Int J Cancer 51:489
4. Furukawa T, Kubota T, Hoffman RM (1995) Clinical applications of the histoculture drug response assay. Clin Cancer Res 1:305
5. Hoffman RM (1991) In vitro sensitivity assays in cancer: a review, analysis and prognosis. J Clin Lab Anal 5:133
6. Homma Y, Aso Y (1994) Effect of alpha-interferon alone and combined with other antineoplastic agents on renal cell carcinoma determined by the tetrazolium microculture assay. Eur Urol 25:164
7. Kondo T, Imamura T, Ichihashi H (1966) In vitro test for sensitivity of tumor to carcinostatic agents. Gann 57:113
8. Kotake T, Usami M, Miki T (1983) Combination chemotherapy including adriamycin for advanced transitional cell carcinoma of the urinary tract. Cancer Chemother Pharmacol 11 [Suppl]:38
9. Kubota T, Sasano N, Abe O, Nakao I, Kawamura E, Saito A, Endo M, Kimura K, Demura H, Sasano H, Nagura H, Ogawa N, Hoffman RM, the Chemosensitivity Study Group for the Histoculture Drug-Response Assay (1995) Potential of the histoculture drug-response assay to contribute to cancer patient survival. Clin Cancer Res 1:1537
10. Mossmann T (1983) Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. J Immunol Methods 65:55
11. Ogiwara M, Aikawa K, Ishibashi K, Irisawa C, Shiraiwa Y, Koseki K, Ishiwata H (1996) Efficacy of the chemosensitivity test using collagen gel matrix supported culture system for urogenital tumors. Jpn J Urol 87:740
12. Scheithauer W, Clark GM, Salmon SE, Dorda W, Shoemaker RH, Von Hoff D (1986) Model for estimation of clinically achievable plasma concentrations for investigational anticancer drugs in man. Cancer Treat Rep 70:1379
13. Sella A, Logothetis CJ, Fitz K, Dexeus FH, Amato R, Kilborn R, Wallace S (1992) Phase 2 study of interferon-α and chemotherapy (5-fluorouracil and mitomycin C) in metastatic renal cell cancer. J Urol 147:573
14. Uchibayashi T, Egawa M, Hisazumi H, Hasegawa T, Tanaka T, Sasaki T (1993) In vitro chemosensitivity test for human genitourinary tumors using collagen gel matrix. Eur Urol 24:267
15. Yamaue H, Tanimura H, Nakamori M, Noguchi K, Iwahashi M, Tani M, Hotta T, Murakami K, Ishimoto K (1996) Clinical evaluation of chemosensitivity testing for patients with colorectal cancer using MTT assay. Dis Colon Rectum 4:416
16. Yamaue H, Tanimura H, Sugano M, Tani M, Tsunoda T, Iwahashi M, Hotta T (1998) Histoculture drug response assay in gastric cancer patients. Jpn J Cancer Clin 44:265