RESEARCH COMMUNICATION

Clinical Application of the Adenosine Triphosphate-based Response Assay in Intravesical Chemotherapy for Superficial Bladder Cancer

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Abstract

Objective: To investigate correlations between adenosine triphosphate chemotherapy response assay (ATP-CRA) and clinical outcomes after ATP-CRA-based chemotherapy for drug selection in patients receiving intravesical chemotherapy to prevent recurrence of superficial bladder cancer after surgery. Methods: The chemosensitivities of 12 anticancer drugs were evaluated, including 5-Fu ADM, and EPI, using ATP-CRA and primary tumor cell culture in 54 patients. In addition, a further 58 patients were treated according to clinical experience. Differences in post–chemotherapeutical effects between drug sensitivity assay and experience groups were compared. Results: The evaluable rate of the test was 96.3%, the clinical effective rate was 80.8%, the sensitivity rate was 97.6% (41/42), the specificity was 20%, the total predicting accuracy was 74.3%, the positive predictive value was 83.7% (41/49), the negative predictive value was 66.7% (2/3); in the drug sensitivity test group, the clinical effective rate was 80.8%, the experience group response rate was 63.8%, with a significant difference in clinical effects between the ATP-based sensitivity and experience groups (χ^2 =7.0153, P<0.01). Conclusion: ATP-CRA is a stable, accurate and potentially practical chemosensitivity test providing a predictor of chemotherapeutic response in patients with superficial bladder cancer.

Keywords: Adenosine triphosphate - chemotherapy response assay - superficial bladder cancer

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Introduction

As we all known, the transitional cell carcinoma of the bladder is the most common malignant tumour of urinary system (Riedl et al., 2001; Jichlinski et al., 2003), and its incidence is gradually increasing in the Republic of China.

At present, its main therapeutic approach is early operation combined with postoperative chemotherapy of bladder irrigation. However, neither operation nor chemotherapy could solve the problems on high recurrence and progression (Gasión & Cruz, 2006). Related study reported that the recurrence rate of transitional cell carcinoma was 10-67% after transurethral resection (Sylvester et al., 2006). This is because the biological behaviors of bladder cancer are comp1icated and diversify, and the chemotherapy of malignancies always is based on physicians' empirical judgement. Based on this, the most therapeutic approaches is limited to kill malignant tumour. Therefore, the resistance or sensitivity of chemical treatment is immense importance for prevention of tumor recurrence.

As a detection tool, the tumor chemosensitivity assay (TCA) such as Human tumor clonogenic assay (HTCA), Methylthiazoletetrazolium (MTT), Fluorescent Cytoprint Assay (FCA) and Dye Exclusion Assay (DEA) has been used to decide which anti-cancer drugs are more likely to work well enough on their patients in the past few decades (Kornmann et al., 2003). However, the above TCAs are still limitations on drug sensitivity and specificity (Yamaue et al., 1991; Huh et al., 2009). Recent years, the analysis of endogenous ATP was reported to be the most predictive of toxicity testing methods (Ekwall & Sussman, 2000). Several studies also reported that adenosine triphosphate chemotherapy response assay (ATP-CRA) results could predict the chemosensitivity of drugs in patients with ovarian cancer or gastrointestinal cancer (Cree et a1., 2007; Moon et al., 2007). However, the ATP-CRA research about urinary system tumors is rarely reported. This study was to investigate the clinical applicability and accuracy of ATP-CRA as a predictor of chemotherapeutic response in patients with superficial bladder cancer.

Materials and Methods

Clinical characteristics of patients

From January 2007 to December 2007, a total of 112 patients with superficial bladder cancer confirmed by histology or cytology at Department of Urinary Surgery,

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the First Affiliated hospital of Soochow University. All patients had no previous chemo-or radiotherapy. 54 patients with post-operation received ATP-TCA directed chemotherapy regimens and intravesical instillation according to the optimal protocol as indicated by the results of drug sensitivity test, control group. 58 cases were given intravesical instillation basing on the experience of doctors. There were no significant differences in pretreatment parameters such as age, grade, and stage between two groups. This clinical trial was approved by the appropriate Institutional Review Board and all patients in the study gave written informed consent.

Preparation of drugs

The drugs tested were paclitaxel, gemcitabine, doxorubicin, cisplatin, pirarubicin, hydroxycamptothecin(HCPT), epirubicin, mitomycin. The drugs were purchased commercially. They were the most frequently used for Intravesical Therapy of Superficial bladder Cancer. Drug concentrations (TDC) values were determined by pharmacokinetic/clinical information and empirical clinical evaluation. TDCs correspond with the plasma concentrations achievable in vivo following a standard dose of each drug tested and allow the identification of dose response effects.

Six different concentrations for each drug used were 200%, 100%, 50%, 25%, 12.5%, 6.25% TDC.

Cancer cells isolation

The method in ATP-TCA followed manufacturer instructions (DCS Innovative Diagnostika-Systeme, Hamburg, Germany). First of all,fresh tumor tissues were immediately placed into sterile containers containing RPMI1640 with penicillin, streptomycin and gentamicin as additives. These were transported to arrive in the laboratory within 3 h. These specimens were first washed, quantified, and minced 0.5~1mm3 pieces, then isolated by enzymatic dissociation using Collagenase TypeIIat a concentration of 0.75 mg/mL in a centrifuge tube at 37°C for 1.5-3 h. Cells were harvested using 200 mesh cell strainer. To eliminate red blood cells and dead cells,the cell suspensions were subjected to Ficoll gradient centrifugation at 400g for 10 minutes. The viability of isolated cells was tested using Trypan blue exclusion. Finally Separated tumor cells were seeded at the density of $2\sim4\times10^4$ cells per well of 96-well plates, which restricted the growth of normal cells such as fibroblasts, then six different TDCs of the chemotherapeutic agents in triplicate was added to seeded cells. For quality control purposes. The control groups was set, one was positive control that the cells were incubated with ATP inhibitor for minimal viability, the other was negative control that the cells were received only the medium without any drug were used for maximum viability.

ATP measurement

The ATP content of each well was measured after 5-7 days incubation(5%CO_2, 37°Cand 100% humidity) by the addition of luciferin-luciferase to an aliquot of the lysed cells in a OrionII luminometer (MPLX, Berthold Diagnostic Systems Hamburg, Germany) and analyzed with custom software to provide both numerical and graphical results. Luminescence measurements are directly related to ATP levels and allow measurement of the percentage inhibition by referenceto untreated control wells included with each plate. The tumor growth inhibition(TGI)formula: TGI=1.0-(Test -MI)/(MO-MI)×100% (Test: mean luminescence in drug treated group; MI: mean luminescence in positive control; MO: mean luminescence in negative control).

Statistical analysis

In this study, we divided the chemotherapeuic drug or drug combination into four ranks, they are strong sensitivity, partial sensitivity, weak sensitivity and resistance. l tatistical analysis were carried out using the SPSS Windows program, v.11.5 (SPSS, Chicago, III). P value of less than 0.05 was considered to be statistically significant.

Results

Evaluability rate

We failed to culture cancer cells from two of the 54 atients. One of the samples because of bacterial contamination, other ample did not yield an adequate number of cells. Thus, the valuability rate of the ATP assay using tru-cut biopsy specimens was 93.0% (40 out of 43). The evaluable rate of was 96.3%, the clinical effective rate was 80.8%, the sensitivity was 97.6% (41/42), the specificity was 20%, the total predicting accuracy was 74.29%, positive predictive value was 83.7% (41/49), negative predictive value was 66.7% (2/3); in the drug sensitivity test group, the clinical effective rate was 80.8%, the experience group response rate was 63.8%, there was a significant difference of the clinical effects between the ATP-based sensitivity group and the experience group (χ^2=7.0153, P=0.0081).

In vitro drug sensitivity

The results showed that the chemosensitivities of 12 anticancer drugs have different in vitro of bladder cancer patients (Figure 1). The average inhibition rate of DDP, ADM, CBP, HCPT, EPI is relatively high. The single-factor analysis of variance between the groups was no significant difference (p>0.05). MTX, VCR, Gemzar showed a relative resistance of bladder cancer. A relatively

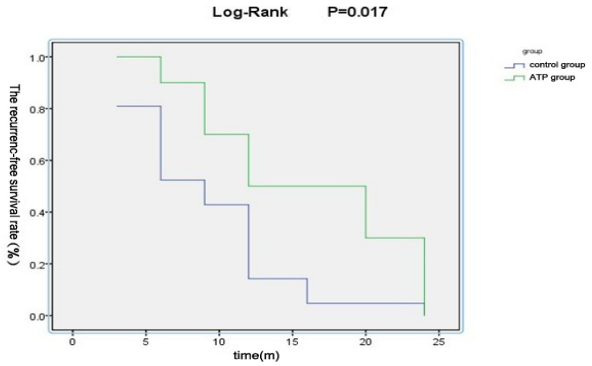

Figure 1. The Kaplan-Meier of Tumor Recurrence Time Between ATP-TCA-based Chemotherapy Group and Experienced Chemotherapy Group

Table 1. The Inhibition Rate of 12 Drugs

	Sensitivity				Average inhibition rate	cv
	Weak	Partial	Strong	Overall		
5-FU	23.9%	15.2%	6.52%	45.7%	30.0±25.8%	0.86
MTX	31.9%	6.38%	0.00%	38.3%	22.2±19.7%	0.891
ADM	31.3%	29.2%	22.9%	83.3%	50.2±24.1%	0.481
EPI	37.5%	16.7%	16.7%	70.8%	43.3±24.3%	0.56
THP	17.7%	33.3%	9.80%	60.8%	39.1±27.7%	0.708
PYM	29.0%	3.23%	3.23%	35.5%	19.8±21.5%	1.082
MMC	24.0%	24.0%	12.0%	60.0%	38.5±25.1%	0.651
VCR	20.4%	12.2%	2.04%	34.7%	22.1±22.1%	1.001
Gemzar	21.1%	7.89%	2.63%	31.6%	23.8±19.8%	0.833
HCPT	32.0%	42.0%	8.00%	82.0%	45.9±19.7%	0.429
DDP	12.5%	41.7%	31.3%	85.4%	57.6±23.9%	0.416
CBP	11.6%	27.9%	30.2%	69.8%	48.6±28.0%	0.576

small dispersion of 12 chemotherapeutic drugs is DDP, HCPT, ADM, were less than 50%,indicating the relative stability of the inhibition rate of bladder cancer.

Sequence of weak sensitivity: EPI > HCPT > MTX > ADM; Sequence of partial sensitivity: HCPT > DDP > THP > ADM; Sequence of strong sensitivity: DDP > CBP > ADM > EPI.

Relationship between TGI and histological grade

The relation between TGI and histological grade is shown (Table 2). Although high-grade bladder cancers tend to be less sensitive to drugs and high-grade renal cell cancers to be more sensitive, while the other drugs showed no significant relationship between histological grade and TGI. The inhibition rate of MMC, PYM show an increase in tumor suppressor with the classification rate has dropped (P= 0.002, P = 0.019).

Clinical Response

The follow-up lasted for 24.12±4.74 months, 10 patients recurrence in sensitivity group, while 21 patients in experience group. A significant difference was observed according to the recurrenc rate. 10 patients recurrence in sensitivity group, 6-months follow up found in 1 patient, 9-months in 2, 12-months in 2, 24-months in 2 and 24-months in 3, while 21 patients recurrence in experience group, 3-months follow up found in 4 patient, 6-months in 6, 9-months in 2, 12-months in 6, 24-months in 2 and 24-months in 1. The recurrence-free survival rate evaluated according to Kaplan-Meier was significantly better in sensitive group than in insensitive group.

Discussion

In vitro chemosensitivity assay is an attractive method for knowing about responses of a tumor treatment and assess the best dose in the patient with cancer. The assays refer to any laboratory analysis that is performed specifically to evaluate whether or not tumor growth is inhibited by various chemotherapy drugs. Currently there are a number of in vitro chemosensitivity assays.

Although some in vitro assays guided therapy seems to be ideal,in actuality this therapy is not widely used in clinical practice because of various technical problems encountered with this assay, including the requirement of a high technical skill level, the large number of required

Table 2. Relationship Between TGI and Histological Grade

drugs	G1	G2	G3	P
5-FU	38.9±29.9	40.2±22.3	41.5±9.91	0.986
MTX	32.7±12.4	31.7±16.7	39.6±12.7	0.784
ADM	58.6±16.9	50.5±22.0	46.4±17.1	0.39
EPI	49.3±23.0	46.8±23.3	35.2±10.9	0.481
THP	51.2±23.1	49.8±22.8	57.4±7.77	0.742
PYM	46.9±6.46	32.6±12.2	28.7±14.4	0.019
MMC	56.1±16.1	33.8±15.0	19.0±9.87	0.002
VCR	53.5±19.6	36.8±15.5	27.6±4.16	0.077
Gemzar	34.9±10.2	32.3±18.5	27.9±6.11	0.697
HCPT	52.4±15.5	45.8±17.8	44.9±22.2	0.492
DDP	62.3±20.1	60.6±20.1	51.1±17.2	0.493
CBP	57.1±21.2	55.3±24.5	40.2±28.6	0.238

tumor cells, and the excessive amount of time required (Cree & Kurbacher, 1997).

The Adenosine Triphosphate-based chemotherapy response assay (ATP-TCA) was first reported by Kangas in 1984 (Kangas et al., 1984). The assay is technologically more advanced due to its luminescence-based methodology and enabled the evaluation of chemosensitivity more rapidly, simply and accurately (Cree & Andreotti, 1997). The basic principle of assay: Cellular ATP represents the most important chemical energy reservoir. When cell death, the ATP level decreases dramatically (Garcia & Massieu, 2003). ATP is one of the most sensitive end points in measuring cell viability. The ATP assay is based on the reaction of luciferin to oxyluciferin catalyzed by the enzyme luciferase in the presence of Mg^{2+} and ATP yielding a luminescent signal. A linear relationship exists between the intensity of the luminescent signal and the ATP concentration (Mueller et al., 2004). Determination of fluorescence intensity can be calculated the number of living cells.The success rate was more than 90%. The clinical efficacy of chemotherapy has been shown to have a strong correlation with ATP-TCA data in various kinds of solid tumor (O'Meara & Sevin, 2001; Kurbacher et al., 2003). However,there have been few reports on its use in urological cancer.

In our study, we found that to assure the success of the tumor cell primary culture and chemosensitivity test, it is better that the volume of the tumor sample would no smaller than 0.5cm ×0.5cm×0.5cm,the quality of cell suspension is directly influence the test result. Mechanical Separation way such as quick Separation, glassy needle grinding can produce high survival rate single cell suspension quickly. It is very important for the experiment's success about the time of drug and cell culture. The cells were cultured together with drugs for 72h which is a time point often used in the literature (Ulukaya et al., 2008). Compared with inhibition rate of cancer cells which are cultured 12h, 24h, 48h, 60h, 72h, 84h, 96h, 108h, 120h and 132h, we have found that inhibition rate of cancer cell is upward with the time being longer. The inhibition rate has reached the highest at the point of 120h. Then it will decline. Therefore it will reach much more meaningful results that has a higher compliance in clinic if you have made the drug time of 120h and extended the culture time for some time-depended drugs, such as 5 Fu or VLB.

We valued inhibition rate of the 12 drugs In different individual patients, and found that it was obviously different. The results showed that the sensitivities of 12 anticancer drugs have different in vitro of bladder cancer patients, which is the reason why based on physicians' empirical judgment has much limitation. The average inhibition rate of DDP, ADM, CBP, HCPT, EPI is relatively higher (Table 1). According to the clinical practice, ADM, EPI, THP, CDDP were considered to be the most effective drugs, which was correlatively consistent with the test result. In addition to MCC PYM, no correlation has been found between the growth inhibition with the pathological grade, stage, which was consistent with the Schmittgen et a1. (1991) result.

Most of the patients in experience group recurrence within one year, and would likely incline to be invasive tumor. We chose the most sensitive drugs as the intravesical chemotherapy, the recurrence rate was 36.2% in experience group, while only 19.2% in sensitivity group. Meanwhile the recurrence time in sensitivity group was comparable later than in experience group. Gemzar was a newly used intravesical chemotherapy drug recently. It was used by Serretta et al. (2005) to treat with 27 remnant bladder cancer patients, 7 patients have clinical complete remission, 2 have partial remission, no recurrent and metastatic tumor was discovered, the patients entire toleration were good. But inhibition rate in our test was a little lower, it may related to the limited sample.

According to the former report and our research the Intravesical Therapy for Superficial bladder Cancer based on the ATP-CRA result should be effective. It can improve therapeutic effect; reduce the ineffective drugs usage, and comparable decrease recurrence rate than in experience group.

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