The Change of AgNORs Numbers in Arterial Chemotherapy Followed by Open Surgery to Treat the Transitional Cell Carcinoma of Bladder

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Abstract **Objective** Testing the change of the particle numbers in cancer nucleolar AgNORs before and after arterial chemotherapy to assess the significance of preoperative chemotherapy in transitional cell carcinoma (TCC) of urinary bladder. **Methods** 25 cases of patients with bladder TCC were selected randomly, Of them 8 cases were grade Ⅱ, 4 cases were grade Ⅱ~Ⅲ, 8 cases were grade Ⅲ, 1 case was grade Ⅲ~Ⅳ, 4 cases were grade Ⅳ. A silica plastic tube was intubated into common iliac artery via inferior epigastric artery before open surgery. Infused ADM and cisplatin for 1~2 course(s), then performed partial cystoctomy. The pathologic tissues which obtained by cystoscope and operation were stained by AgNORs technique, counted the mean AgNORs particle numbers, did paired t test, and did the follow-up survey. **Results** paired data t=5.230, while t0.01(24)=2.787, t>t', $p<0.01$, thus, there was significant difference between pre- and post- operational tissues. All the 25 cases of patients had no obvious complications. The 3-year recurrence rate was 8.33%; 5-year recurrence rate was 12.5%; while 5-year survival rate was 87.5%. **Conclusions** Pelvic arterial chemotherapy followed by open surgery could decrease the malignancy and the recurrence rate, increase the survival rate of bladder caner. The results suggested that it was an ideal method to treat higher grade TCC of urinary bladder.

Key words Arterial chemotherapy; Transitional cell carcinoma of urinary bladder; AgNORs

Transitional cell carcinoma (TCC) of urinary bladder is the most common malignancy of the urinary system in China [1]. The main therapeutic methods are tumor resection and followed by ntravesical chemotherapy to prevent the recurrence[2]. However, there are about 60%-70% of these patients recur after 1-2 year (s)[1]. Many methods are performed to prevent the recurrence, such as intravenous chemotherapy, but the effects are not good [2]. The intra-arterial approach is known to increase the drug concentration delivered to the tumor with minimal alterations of the systemic pharmacokinetics and less toxicity[3]. During the year of 1976, a new method, preoperative intra-arterial chemotherapy, was used in some higher malignancy patients of TCC of urinary bladder in the 3rd hospital of Harbin Medical University, Harbin, which get better results. In our study, we used the Argyrophilic nucleolar organizer regions (AgNORs) technique to assess the change of AgNORs particle numbers, and did the follow-up survey of recurrence rate and survival rate[1,4].

MATERIALS AND METHODS

Clinical materials 25 patients were selected randomly from April 1986 to August 1998, of these cases, 17 were male, 8 were female, and ages were from 32-71 years old, the average age was 64.5 years old. All patients were diagnosed as TCC of urinary bladder by preoperative cystoscopic biopsy. The tumours were graded as recommended by a high standard pathologist according to the TNM staging system. Of the 25 cases, 8 cases were grade Ⅱ, 4 cases were grade Ⅱ-Ⅲ, 8 cases were grade Ⅲ, 1 case was grade Ⅲ-Ⅳ, 4 cases were grade Ⅳ. All the routine examinations were normal. The patients had not received any chemotherapy or radiology before the diagnosis of TCC of urinary bladder.

Methods

25 patients were performed tran-sinferior epigastric artery incubation before open surgery. A silica plastic tube was incubated into common iliac artery via inferior epigastric artery, and then fixed the

tube. After 1 to 3 days of the incubation, block the femoral artery, and then injected ADM+CDDP solution via the tube, and gave the supporting therapy for 1~2 courses. After 1~2 weeks, the patients were performed partial cystectomy and submucosal injection of anti-cancer drug in the operations routinely.

All the tumour specimens which obtained by cystoscopes and operations were fixed in 10% buffered formalin and embedded in paraffin wax. Sections (5 μm thick) were stained with haematoxylin and eosin, and then dewaxed, desiccation, and rinsed thoroughly with distilled water. Dispensing the working solution using gelatin and silver nitrate, stained by AgNORs technique in the dark room, counted the AgNORs particle numbers in 100 nuclei in every slide under the oil immersion objective randomly, obtained the mean particle number of every specimen, did paired t test, then did the follow-up survey.

RESULTS

Clinical observation Of the 25 patients, 12 cases had continuous hematuria, which were getting better after arterial chemotherapy. The red color or bleeding of the tumors observed in the cystoscopy examinations turned to pale or deep red color that were observed in operations, and the surface of the tumors exfoliated easily. 2 of these patients who were planned to perform radical cystectomy were performed partial cystectomy, they did not recur in 3 years. During the treatment progress, 2 patients present leucocytopenia, 1 patient present infection of incision, who were getting better after appropriate treatment. No severe complications of the heart, lung, liver, kidney were found in the treatment.

The change of AgNORs The pre-, post-opera-

Table 1 The change of AgNORSs particle numbers in pre-, post-operation

Serial number of patients	Contrast of grade of pre- and post- chemotherapy		The mean AgNORs numbers and their differences		
	pre-	post-	pre-	post-	differences
1	Ⅱ	Ⅱ	3.48	3.36	0.12
2	Ⅳ	Ⅳ	5.66	5.67	-0.02
3	Ⅱ-Ⅲ	Ⅱ	3.45	2.38	1.07
4	Ⅲ	Ⅲ	4.25	4.03	0.22
5	Ⅲ	Ⅲ	4.61	4.28	0.33
6	Ⅱ	Ⅱ	3.96	3.52	0.44
7	Ⅱ	Ⅱ	3.65	3.46	0.19
8	Ⅱ	Ⅱ	3.87	3.49	0.38
9	Ⅲ	Ⅱ-Ⅲ	4.14	3.64	0.50
10	Ⅱ	Ⅱ	3.44	3.19	0.25
11	Ⅲ	Ⅱ-Ⅲ	3.42	2.36	1.06
12	Ⅱ	Ⅱ	4.22	3.81	0.41
13	Ⅱ	Ⅰ-Ⅱ	3.40	2.60	0.80
14	Ⅳ	Ⅳ	5.11	5.10	0.01
15	Ⅱ-Ⅲ	Ⅱ-Ⅲ	4.79	4.71	0.08
16	Ⅲ	Ⅲ	3.81	3.26	0.55
17	Ⅲ	Ⅲ	3.94	3.12	0.82
18	Ⅱ~Ⅲ	Ⅱ-Ⅲ	4.08	4.04	0.04
19	Ⅱ	Ⅱ	4.52	3.51	1.01
20	Ⅳ	Ⅳ	5.24	5.22	0.02
21	Ⅲ	Ⅲ	3.52	2.89	0.63
22	Ⅲ~Ⅳ	Ⅲ-Ⅳ	4.34	4.32	0.02
23	Ⅲ	Ⅲ	4.24	3.88	0.36
24	Ⅱ	Ⅱ	3.56	3.59	-0.03
25	Ⅳ	Ⅳ	5.42	5.40	0.02

By the paired t test, paired data t=5.230, while $t_{0.01(24)}$=2.787, t>t′, p<0.01, thus, there was a significant difference of mean AgNORs particle numbers between pre- and post- chemotherapy tissues.

tion mean AgNORs particle numbers and there differences were listed in table 1.

Follow-up survey In a 5-year follow-up survey, 24 patients were got follow-up survey in all of the 25 patients, none of them recurred in 1 year; 2 patients recurred in 3 years (8.33%); 3 patients recurred in 5 years (12.5%); 21 patients still alive in 5 years, the 5-year survival rate was 87.5%. In the 3 dead patients, 2 died of cardiocerebral diseases which were no relationship with TCC. 1 died of general body exhaustion caused by TCC pelvic metastasis.

DISCUSSION

TCC of bladder has the multi-focal and aggressive characters, the simple bladder-reserved operation may lead to recur easily[6]. The main causes of recurrence were: multi-focal genesis, orthotopic recurrence, and abdominal wall implantation[7]. In our opinion, the most important cause is multi-focal genesis, due to the development of carcinoma in situ and atypical hyperplasia of epithelial cells, which are not easily found by naked eyes in the operations[1] . The onset of TCC of bladder is the long-time stimulation of mutagens that exist in the urine[1,4]. The whole urinary epithelial cells are influenced by the carcinogens, which make them abnormal. So, the onset of TCC of urinary bladder is multifocal, only the lesions were at different stages[2].

At present, the treatment of TCC of bladder inclines to reserve the bladder and its function, but to the high malignant diseases, the effects of simple and local dissection of the foci are not good [1-3] . Therefore, it is important to combine pre-, intra-, and post-operative chemotherapy with operation. There are many articles reported that intra-operative submucosal injection and post-operative infusion of anti-cancer agents may decrease the recurrent rate, but these two methods have little effects to the cancer cells in lamina propria, muscle layer, and peripheral tissues[6].

Through more than 20 years´ investigation, we applied a new method of pelvic arterial chemotherapy followed by open surgery. Trans-arterial and local pelvic chemotherapy can make up the deficiencies of the above methods, which can make the urinary bladder and its peripheral tissue get long-time and higher concentration of anticancer agents, which may play an important role to destroy the carcinoma in situ and early carcinomatous change. It can also increase the drug concentration delivered to the tumor with minimal alterations of the systemic pharmacokinetics and less toxicity[3]. In addition, the anticancer agents injected into internal iliac artery may kill the cancer cells that exist in the peripheral tissues of urinary bladder, which is beneficial for the radical dissection of tumors and decrease recurrent rate, increase survival rate[1,7]. It is proved to be an effective way to decrease the recurrent rate[1,4].

In our present study, we randomly selected the archival tissue samples of 25 TCC of urinary bladder patients who were treated by pre-operative chemotherapy, applied AgNORs technique to contrast their pre-, and post- chemotherapeutic mean AgNORs particle numbers. AgNORs are loops of DNA present in metaphase, on D- and G-group acrocentric chromosomes, which increase with increased proliferative activity of the cells. This technique is used in diagnosis of precancerous lesion, differentiation of benign and malignant diseases, staging and grading of tumors, and the predicting of prognosis[5,8,9].

In our investigation, we found that there was a significant difference of the mean AgNORs particle numbers between pre- and post- chemotherapy tissues. Namely, the arterial chemotherapy may decrease the malignancy of transitional cell carcinoma of urinary bladder. Meanwhile, the follow-up survey showed that the recurrent and survival rates were better than that of reported before. In conclusion, we considered that this method was an ideal method to treat the patients with higher grade malignant TCC of urinary bladder. The effects were better than that of simple partial cystectomy followed by intravescical chemotherapy.

REFERENCES

1. Zhao Pixian, Lan Zhitai, Niu Kuiwu, et al. The effect of arterial infusion of anticancer agents combined with intra-operational injection of anticancer agents to treat bladder cancer. Chinese Journal of Urology, 1986, 7(6): 339-341.
2. Solowag MS. Rationale for intensive intravescical chemotherapy for superficial bladder cancer. J Urol, 1980, 123:461.
3. Akaza Hideyuki. Advanced in chemotherapy for invasive bladder cancer. Curr Opinion Urology, 2000, 10(5):453-457.
4. Wang Xiaomin, Zhao Pixian, Miao Yansheng, et al. The observation of clinical, pathological, and ultrastructural

changes in the arterial chemotherapy of TCC of urinary bladder. Chinese Journal of Urology, 1991, 12(4):270–272.

5. Underwood JC, Giri DD. Nuclear organizer regiona as a diagnostic discriminants for malignancy. J Pathology, 1988, 155:95–96.
6. Naito S, Kuroina T, Ueda T, et al. Combination chemotherapy with intra–arterial cisplatin and doxorubicin plus intravenous methotrexate and vincristine for locally advanced bladder cancer. J Urol, 1995, 154(5): 1704–1709.
7. Deng Xin, Wu Yongan, Cong Peisheng, et al. The clinical observation of pre–operational superselective arterial chemotherapy in invasive bladder cancer. Chinese Journal of Surgery, 2000, 38(4):278–279.
8. Misra Vatsala, Mukherjee Jayati, Gupta Suresh, et al. AgNORs in benign, borderline and transitional cell neoplasms of the urinary bladder. APMIS 1998, 106(100): 987–992.
9. Korneyev IA, Mamaev NN, Kozlov VV, et al. Interphase argyrophilic nucleolar organiser regions and nucleolar counts in transitional cell bladder tumours. Molecular Pathology, 2000, 53(3):129–132.