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# Chemotherapy for Bladder Cancer with Neocarzinostatin: Evaluation of Systemic Administration*

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**Abstract**—*Thirty patients with newly diagnosed bladder cancer were treated with i.v. neocarzinostatin. In* 2 *patients* (6.7%), *the tumors disappeared completely. In* 16 *patients* (53.3%), *a reduction to less than half of the initial size of the tumor was observed. In another* 5 *patients* (16.7%), *the tumor was moderately reduced in size. In* 6 *patients* (20%), *no effect was observed cystoscopically. Tumor growth was seen in* 1 *patient* (3.3%). *Anorexia, general fatigue, slight weight loss and leukopenia appeared in most patients. Slight increase of S-GOT level was detected in* 3 *patients. However, these side effects were reversible and disappeared with the interruption of chemotherapy. All patients underwent transurethral resection after chemotherapy. Up to this date, although the periods of observation after treatment were* 12–46 months, 5 *recurrences* (16.7%) *have occurred. The recurrence rate was* 3.3% (1/30) *within* 6 months, 6.7% (2/30) *within* 1 yr *and* 26.3% (5/19) *within* 2 yr. *These results suggest that neocarzinostatin may be of value in treatment of bladder cancer.*

## INTRODUCTION

NEOCARZINOSTATIN (NCS), a new protein antitumor antibiotic [1], is an acidic single chain polypeptide with a mol. wt. of about 10,700 [2]. The beneficial effects of NCS on leukemia [3], gastric cancer [4] and pancreatic cancer [5] in man have been reported, but its efficacy on bladder cancer is unknown, although preliminary work has been reported [6].

Maeda and associates [7] investigated the metabolism of [$^{14}$C] succinyl NCS given to rats i.v. and found very high radioactivities in the bladder and the kidney tissues as well as in urine. The drug distribution in the bladder and the kidney were about 10–100 times more than in the other 26 organs tested. More recently, we have investigated the mechanism for accumulation of NCS in the bladder of rabbits by measuring both biological activity and radioactivity [8] and have shown that the drug concentration in the bladder tissue in a control group was twice as high as that in the ureterostomized group ($P<0.001$). Furthermore, the radioactive component instilled into the bladder cavity was excreted in urine from the ureter of the ureterostomized rabbits. These studies, thus, verified that the high level of the drug in the bladder tissue was derived not only from the supply via the iliac arteries (blood) but also from the absorption via the bladder epithelium [8]. Furthermore, renal clearance of the drug was very high despite its molecular size [7, 8]. About half of the drug given systemically was excreted in the urine within 20 min. About 60% of the total urinary excretion was recovered in the urine within the initial 5 min. The biological activity of the excreted NCS increased about 2-fold during incubation *in vivo* perhaps through the mechanism of limited-proteolysis of the drug [8, 9].

These experimental studies implied the therapeutic usefulness of NCS on bladder cancer. We have accumulated 30 cases of bladder cancer since this time which have been treated in this manner. The first clinical evaluation of the efficacy of NCS therapy is presented here.

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## MATERIALS AND METHODS

*Patients*

Thirty male patients with bladder cancer having a median age of 60.0 (range 32–76) were chosen in the present series between April 1975 and April 1978. The ages are shown in Table 1. None had been previously treated and all agreed to try the NCS therapy. In the present series, clinically advanced carcinoma were excluded, but in patients 3 and 23, the tumors were at the invasive stage C and B2 as revealed after an operation, and were misdiagnosed as a low stage before NCS therapy.

*Preoperative evaluation of bladder tumor*

When the patients were admitted, cystoscopy was carefully performed and a transurethral biopsy was carried out to evaluate the grading of the tumor in all patients before NCS therapy. Grading of the bladder cancer was determined according to the grading system by Broders [10]. The initial size and incidence of multiplicity of the tumors are shown in Table 1. The clinical staging was assessed by cystoscopy, bimanual palpation under anesthesia and roentgenographic examinations including cystography and pelvic angiography. However, it is difficult to diagnose the stage accurately before an operation. The staging was, therefore, determined by the histological findings of operated tumor specimens [by transurethral resection (TUR) of the tumor base]. Staging system by Jewett and Strong was used in the present series [11]. The grade and stage are also shown in Table 1. Since in case 10, complete disappearance of the tumor was observed after chemotherapy, the stage was assessed by the clinical staging procedures at admission.

*Drug and schedule of therapy*

NCS, a product of Kayaku Antibiotic Research Laboratories, Tokyo, was dissolved in normal saline at 1 mg/ml as supplied in dark-brown ampoules and stored in a refrigerator until use. The drug was used within a year from the date of preparation and was found to be stable [12]. A course of NCS therapy consisted of i.v. injections of 1 mg daily for 7 days, followed by intermission for 7 days. The same dose of NCS was given to all patients even though the body weight varied from 38.0 to 76.5 kg because of simplicity in practice. When the white blood cell counts dropped to about 3000/mm$^3$, the administration of NCS was interrupted or ceased, which occurred mostly after 3 or 4 courses of therapy.

*Routine examinations during NCS therapy*

Routine examinations were carried out on the general status of the patients, including a hemogram, blood chemistry analysis and study of liver and pulmonary functions. Cystoscopy, transurethral biopsies, if necessary, and roentgenographic examinations were periodically performed.

*Surgical therapy*

After NCS therapy, TUR of the bladder tumor was performed in all patients. Even when the tumors disappeared from the primary tumor sites, TUR was still performed for histological examinations. In 2 patients, a radical cystectomy was performed after TUR, because in patient 3, the tumor was found to be at stage C, and in patient 5, the tumor implantation caused by TUR was observed cystoscopically.

*Postoperative therapy*

Intravesical instillation with 5 mg of NCS (cases 1–17) or 10 mg of mitomycin C (cases 18–30) in 20 ml of normal saline was performed for 15 successive weeks after TUR. The reason for changing the drugs in the instillation (NCS to MMC) was primarily economical. A $\beta$-glucuronidase inhibitor (2, 5-di-*O*-acetyl-D-glucaro-1, 4–6, 3-dilactone, Chugai Pharmaceutical Co., Tokyo), at a daily dose of 1.5 g and vitamin B6 at a daily dose of 90 mg, were also given orally to normalize high $\beta$-glucuronidase activity and abnormal tryptophan metabolism, if any, during the entire observation period [13, 14].

*Follow-up*

A cystoscopy was carried out every 3 months up to now in all patients, and hematological examinations were also performed to predict adverse effect.

## RESULTS

*Response to chemotherapy*

The total doses of NCS administered, the number of courses, follow-up periods after operation and the therapeutic effects, are also shown in Table 1.

Two cases of complete disappearance of the tumors (6.7%) were observed. In patient 10,

*Table 1. Effects of NCS on papillary transitional cell carcinoma of the bladder*

| Case | Age (yr) | Size* (cm) | Multiplicity | Grade and stage† | Total dose NCS (No. of courses) | Follow-up‡ (months) | Effect§ |
|---|---|---|---|---|---|---|---|
| 1 | 61 | 3.0 | | 2–B1 | 24 (4) | 46 | ++‖ |
| 2 | 67 | 2.5 | | 2–B1 | 28 (4) | 46 | ++ |
| 3 | 58 | 3.0 | Multiple¶ | 3–C | 29 (4) | 18 | ++** |
| 4 | 68 | 3.5 | | 2–B1 | 17 (3) | 44 | ++ |
| 5 | 32 | 4.0 | Multiple | 2–B1 | 24 (4) | 43 | +†† |
| 6 | 59 | 1.5 | Multiple¶ | II–A | 28 (4) | 42 | ++ |
| 7 | 58 | 2.5 | Multiple | 2–B1 | 21 (3) | 40 | ± |
| 8 | 47 | 2.5 | Multiple¶ | 2–B1 | 22 (4) | 27 | ++‡‡ |
| 9 | 42 | 2.0 | | 2–A | 7 (1) | 35 | ++ |
| 10 | 38 | 2.5 | | 3–B1 | 26 (4) | 35 | +++ |
| 11 | 55 | 2.5 | | 2–B1 | 25 (4) | 35 | ++ |
| 12 | 61 | 2.5 | | 3–B1 | 21 (3) | 34 | ± |
| 13 | 40 | 3.0 | | 2–B1 | 26 (4) | 31 | ± |
| 14 | 69 | 1.5 | | 2–A | 28 (4) | 31 | ++ |
| 15 | 57 | 2.0 | | 2–B1 | 28 (4) | 30 | + |
| 16 | 49 | 2.5 | | 2–A | 28 (4) | 26 | ++ |
| 17 | 76 | 2.5 | Multiple | 3–B1 | 26 (4) | 26 | +++ |
| 18 | 67 | 1.0 | Multiple | 2–A | 14 (3) | 24 | ± |
| 19 | 66 | 3.0 | Multiple¶ | 2–B1 | 7 (1) | 25 | ++ |
| 20 | 66 | 3.0 | Multiple | 2–B1 | 21 (3) | 23 | ++ |
| 21 | 57 | 3.5 | | 2–B1 | 12 (2) | 23 | ++§§ |
| 22 | 46 | 1.5 | | 2–B1 | 21 (3) | 22 | + |
| 23 | 67 | 3.5 | | 3–B2 | 21 (3) | 21 | + |
| 24 | 71 | 1.5 | Multiple¶ | 2–A | 21 (3) | 21 | ++ |
| 25 | 66 | 3.0 | | 2–B1 | 13 (2) | 21 | + |
| 26 | 72 | 3.5 | | 2–B1 | 19 (3) | 19 | − |
| 27 | 72 | 3.0 | | 2–B1 | 16 (3) | 16 | ++ |
| 28 | 71 | 1.5 | | 3–B1 | 28 (4) | 14 | ++ |
| 29 | 74 | 2.0 | Multiple | 2–B1 | 14 (3) | 14 | ± |
| 30 | 67 | 1.0 | | 3–B1 | 5 (1) | 12 | ± |

*Approximate diameter of tumor in cm. Since the tumor size was estimated by cystoscopy, the accurate measurement was difficult to calculate. In the case of multiple tumors, only the largest mass of the tumors was shown.

†Grade was assessed by the transurethral biopsy before chemotherapy and stage was determined by the histological findings during the operation (TUR of the tumor base). In case 10, complete disappearance was observed cystoscopically and histologically after chemotherapy. The stage was, therefore, assessed by the clinical staging procedure at admission.

‡Follow-up period after operation (months).

§Therapeutic effect by cystoscopy. (+++): Disappearance, (++): Marked reduction in size to less than half of the initial size, (+): Moderate reduction in size, (±): No growth or change, (−): Tumor growth observed during chemotherapy.

‖The case of contralateral recurrence.

¶Some of the multiple tumors disappeared as judged by cystoscopy.

**The case of lung metastasis.

††The case of total cystectomy due to tumor implantation after TUR.

‡‡The case of skin metastasis without any evidence of relapse in the bladder.

§§The case of ipsilateral relapse.

whose tumor was 2.5 cm in diameter and located at trigone of the bladder initially, a complete disappearance of the papillary tumor was observed cystoscopically as well as histologically after 4 courses of treatment. In patient 17, multiple tumors, between 1 and 2.5 cm in diameter, completely disappeared after three courses of treatment as judged by cystoscopy. In 16 patients (53.3%), tumors were reduced to less than half of the initial size (diameter), and some of the multiple tumors also disappeared in 5 patients (16.7%) in this group. In another 5 patients (16.7%) the size reduction was moderate. In the remaining 7 patients (23.3%) an obvious antitumor effect was not observed. Macroscopic tumor growth was, however, suppressed in 6 of these patients (20.0%) and tumor growth during NCS therapy was observed in only 1 patient (3.3%).

The relationship between grade and response to chemotherapy is summarized in

Table 2. Fourteen of 23 patients (60.9%) with a grade 2 tumor showed a marked anticancer effect while for 5 patients (21.7%) it was ineffective. Tumor growth during NCS therapy was observed in one of these patients, and the remaining 4 tumors were stabilized in size cystoscopically. In grade 3 tumors, 4/7 patients (57.1%) showed a marked effect. In 2 of these 4 patients, a complete disappearance of the tumors was observed. Two patients had no tumor regression, but tumor growth was suppressed in these patients during and after chemotherapy.

In some patients, acdema and necrosis of tumor could be seen endoscopically after 2 or 3 courses of treatment (Fig. 1, a–d). In patient 9 and 19, the white blood cell count dropped to 3000/mm$^3$ after 1 course and the administration was ceased. Although the amounts of NCS were small, the anticancer effect was clearly observed in these two patients.

Roentgenographic examinations have confirmed the diminution in size of the filling defect due to the tumor after the treatment. Bladder deformity and lack of distensibility decreased after the treatment in some patients (Fig. 2, a–d).

Histological examinations revealed degeneration of tumor cells with marked pyknosis of nuclei, swelling of cells, vacuolations of cytoplasma, proliferation of interstitial tissues, deposits of hyalinous substance and enhanced lymphoid reactions (Fig. 3, a–d). Moreover in patient 10, the residue of cancer cells was not observed in the thin sections of TUR of the primary site

*Side effect of chemotherapy*

Anorexia and general fatigue were observed 3–7 days after the beginning of the treatment in most cases. These symptoms disappeared by an interruption of 3–4 days and did not delay the next course. Other side effects such as fever, vomiting, diarrhea, lethargy, and hypo- and hyperlipidemia were not observed during or after treatment. There was no apparent difference in the side effects among the patients whose body weight ranged from 38 to 76.5 kg, although the total dose either per day or per course was the same. Hematopoietic suppressions manifested by pancytopenia were observed through examinations of bone marrow and peripheral blood in most cases (Fig. 4). The administration of NCS was discontinued in 4 courses when white blood cell count dropped to about 3000/mm$^3$. In some patients (patients 3, 9, 19 and 30), leukopenia was so severe that NCS therapy was unable to resume after one course.

In 3 patients, a slight increase of the S-GOT (serum glutamic oxaloacetic transaminase) level was detected, which rapidly returned to the normal level after cessation of the treatment. In other laboratory examinations including renal function tests, pulmonary function tests (DLco), blood chemistry and other liver function tests, no significant change was observed during and after NCS therapy.

*Follow-up*

The period of postoperative observation was between 12 and 46 months. Five recurrences (16.7%) have been observed to the present. In patient 1, a contralateral relapse was recognized cystoscopically 13 months after TUR and re-TUR was performed after 4 courses of NCS therapy. In patient 3 whose tumor was at stage C and grade III, a lung metastasis was found 11 months after the total cystectomy. In patient 5, TUR was performed after 4 courses of NCS therapy and disseminated tumor implantations caused by TUR were recognized cystoscopically 3 weeks after TUR. Total cystectomy was, therefore, carried out. In patient 8, skin metastasis without any evidence of recurrent findings in the bladder was found 24 months after TUR. In patient 21, an ipsilateral relapse, perhaps due to the cancer cell residue of an earlier operation, was observed 13 months after TUR and re-TUR was carried out.

To recapitulate the prognosis, the recurrence rate of the bladder cancer after NCS +TUR therapy including 2 cases of cystectomy was 3.3% (1/30 patients) within 6 months, 6.7% (2/30 patients) within a yr and 27.0% (5/19 patients) within 2 yr respectively.

In patient 23, whose tumor was 3.5 cm in diameter initially, tumor size reduced moderately and TUR was carried out successfully. Histological examinations revealed that tumor was at stage B2 and grade III. The period of postoperative observation was 18 months. Recurrence was not observed cystoscopically to the present.

## DISCUSSION

Many anticancer agents have been tried for the treatment of bladder cancer, but little beneficial chemotherapy is available. The other alternative widely used is the radical

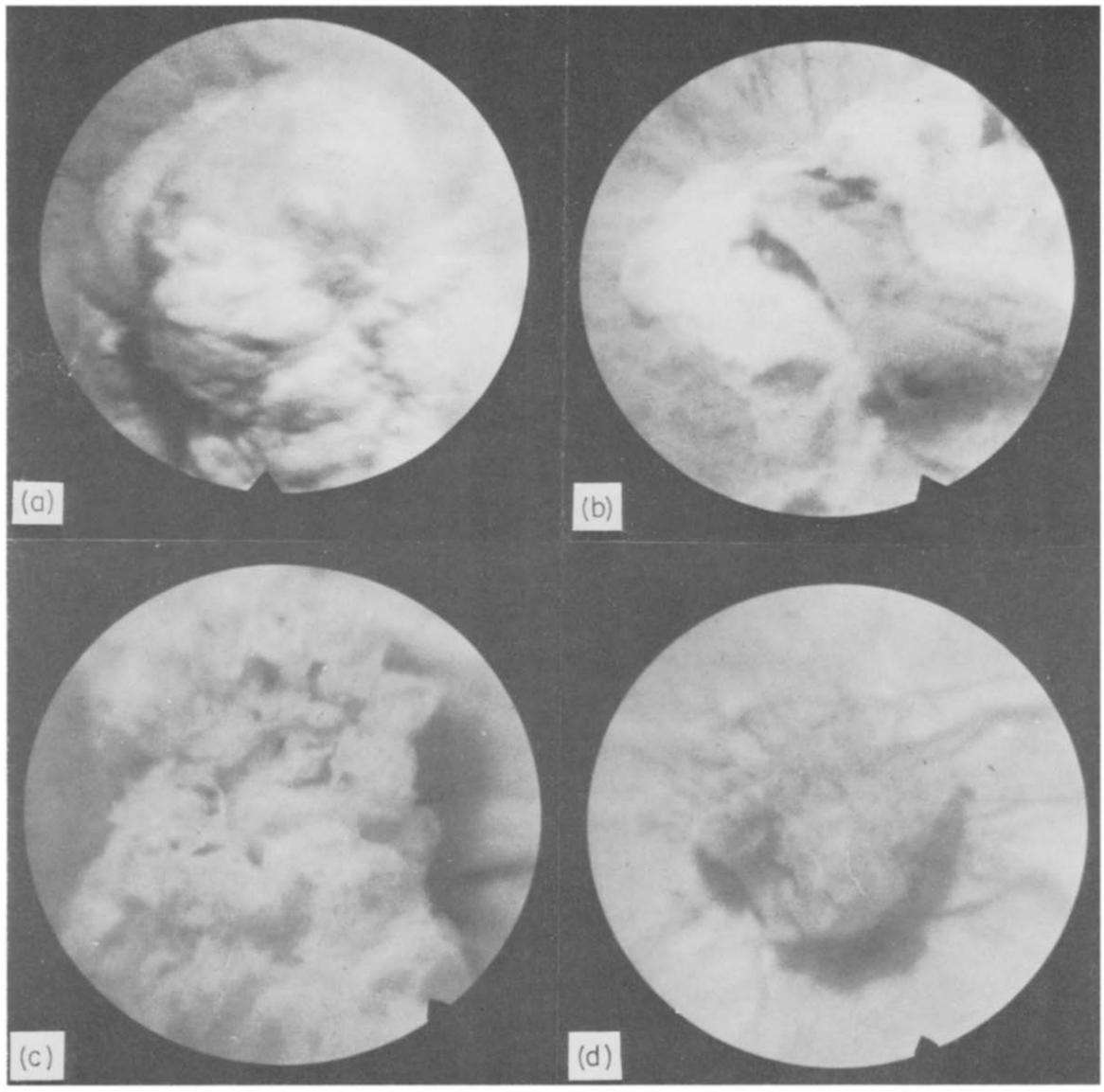


*Fig.* 1. *Cystoscopic findings in patient* 2 (*a and b*) *and in patient* 4 (*c and d*). (*a*) *Before treatment. Tumor was* 2.5 cm *in diameter in size.* (*b*) *After treatment* (*NCS* 28 mg). *Tumor decreased to* 0.5 cm *in diameter in size and became anemic and necrotic.* (*c*) *Before treatment. Tumor was* 3.5 cm *in diameter in size.* (*d*) *After treatment.* (*NCS* 17 mg). *Tumor decreased to* 0.5 cm *in diameter in size and became edematous and anemic.*

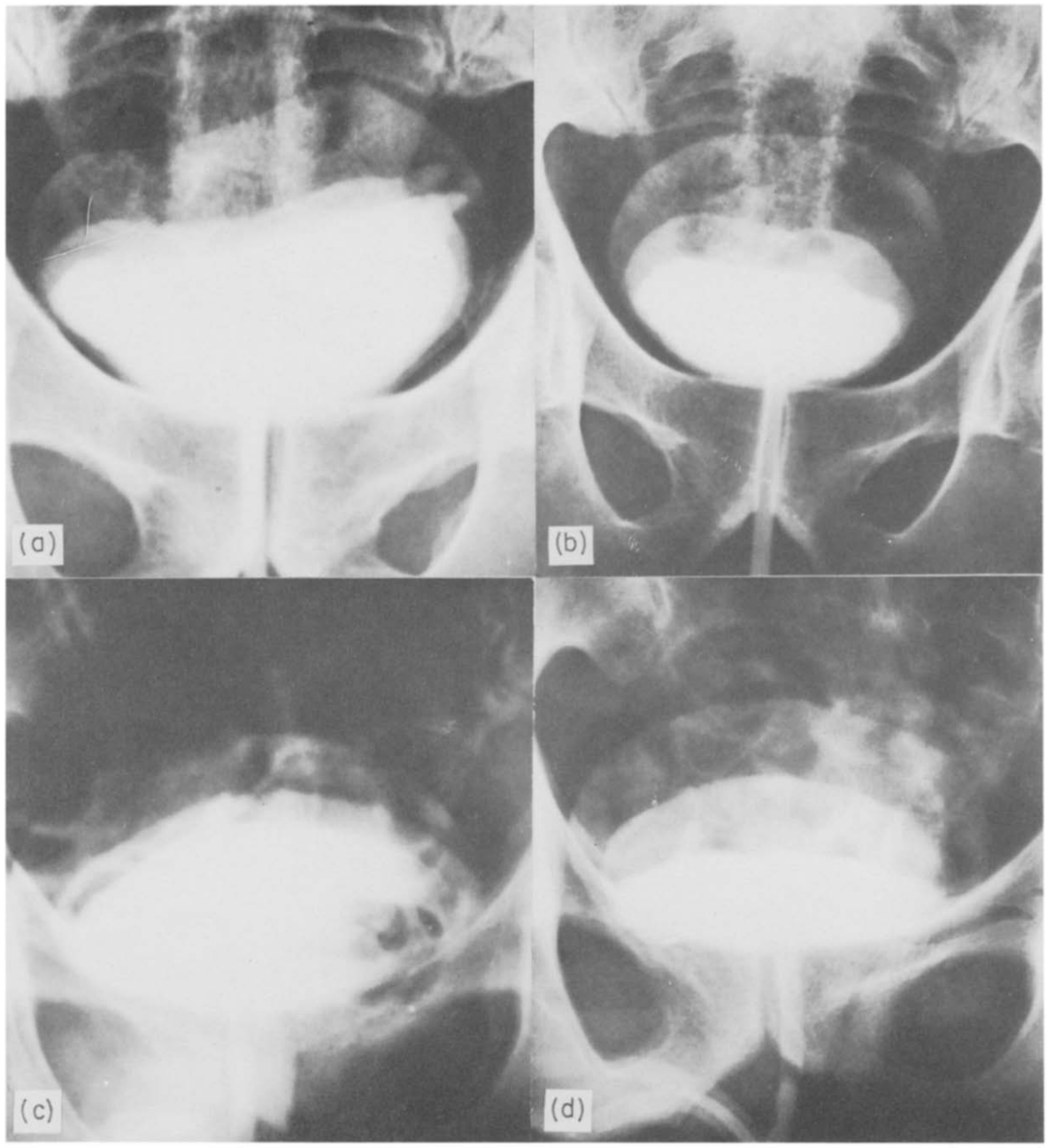


*Fig. 2. Fractionated cystograms in patient 2 (a and b) and patient 4 (c and d). (a) Before treatment. Note lack of distensibility at the left lateral wall of the bladder. (b) After treatment. (NCS 28 mg). Note normal elasticity of the bladder wall. (c) Before treatment. Large filling defect at the left side of the bladder with lack of distensibility was observed. (d) After treatment (NCS 17 mg). Less marked filling defect was seen with normal elasticity of the wall.*

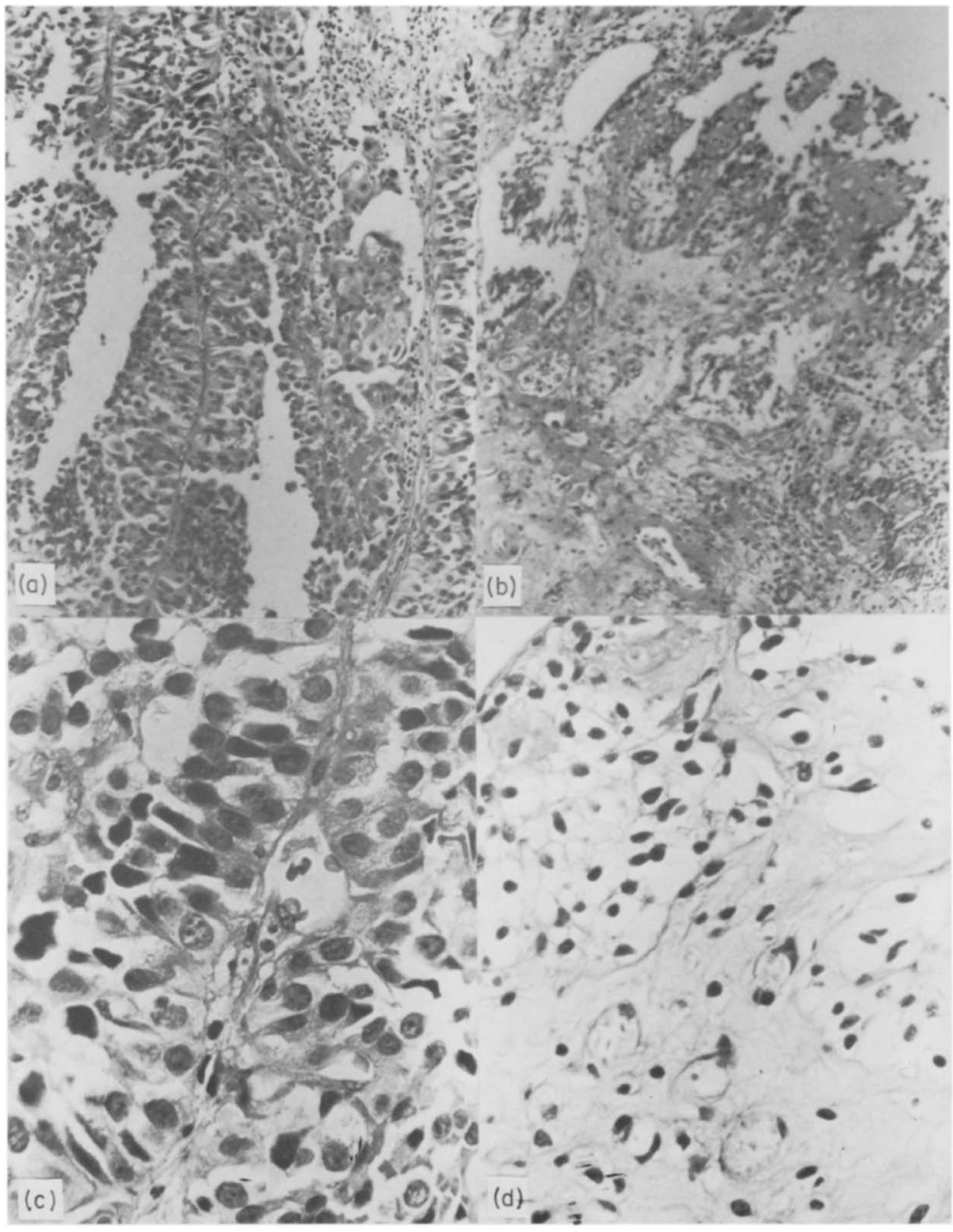


*Fig. 3. Histological findings (H and E) in patient 2. (a) Before treatment (×150). (b) After treatment (NCS 28 mg). Note degenerative changes of tumor cells (×150). (c) Before treatment (×600). (d) After treatment. Note the swelling of cells, vacuolation of cytoplasm and pyknosis of nuclei (×600).*

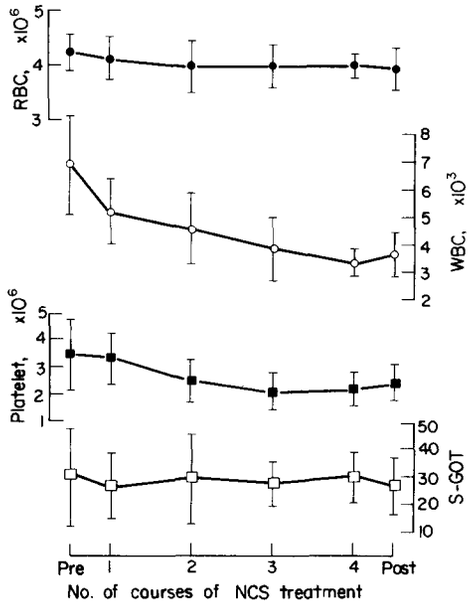


*Fig. 4. Effect on hemogram and S-GOT during NCS therapy. Mean value and standard error of 33 cases.* (●). *Red blood cell* counts/mm³. (○). *White blood cell* counts/mm³. (■). *Platelet* counts/mm³. (□). *S-GOT, normal range below* 40 Karmen *unit.*

cystectomy although with accompanying inconvenience. The effective methods of chemotherapy on bladder cancer which have been known administered locally. Jones and Swinney [15] reported favorable results by the instillation of Thio-TEPA into the bladder cavity but the effect of this treatment was limited to a localized area. Ogata and associates [16] treated bladder cancer by the continuous infusion of MMC via the internal iliac artery and obtained good results. Recently, Matsumoto and Seto reported 10 cases of bladder cancer treated by the infusion of NCS into the common iliac artery with excellent results without any side effects.* In such administrations, although via different routes, the efficacy of NCS on bladder cancer appeared much better than other drugs so far reported. However, these techniques involve some disadvantages such as a considerable difficulty in performance.

It is a well known fact that even in early stages at the clinical level, bladder cancer frequently had micrometastasis at the histological level [17] and the stage of the tumor is difficult to diagnose accurately before radical treatment. The systemic administration of anticancer drugs, if effective, is not only a simple and feasible method but also it is an advantageous one to control and prevent micrometastasis. Schabel emphasized that the cell-cycle specific agents like NCS should be used for this purpose as a counter-measure against micrometastasis [18]. NCS was shown previously to block late $G_2$ [19, 20]. Furthermore, in our previous study, we have found another unique characteristic of NCS which accumulated predominantly in the bladder and the kidney tissues when given i.v. These findings strongly suggested the potential applicability of NCS on bladder cancer by systemic administration [1, 7, 8]. The present method offers one more advantage: simplicity.

In 2 patients, papillary tumors completely disappeared through NCS therapy as observed by cystoscopy. One of these 2 cases was revealed to be histologically tumor free. In most other cases, although a complete disappearance of the tumors was not observed, the tumor size was reduced markedly. We want to emphasize that these results made TUR of the tumors much easier to perform than for subjects without NCS therapy. Furthermore, even when a total cystectomy had been the method choice for multiple tumors, the disappearance of some of the tumors could replace cystectomy with TUR after NCS therapy in some cases. At present in our clinic, the number of patients undergoing a total cystectomy has declined and those undergoing TUR have increased accordingly.

Complete disappearance was observed in 2 patients (28.6%) with grade III tumors. However, in cases with grade II tumors, no complete cure of the 23 patients was observed and tumor growth during chemotherapy was recognized in one patient (Table 2). These results may suggest that anticancer effects may be greater in high grade than in low grade tumors.

Many reports concerning the relapse of the tumor after TUR have been described. Wescot reported the high incidence of relapse in low stage bladder cancer [21]. In his results, 45 of 60 patients have had previous TUR and 67% (30/45 patients) recurred within a year. A similar recurrence rate was observed in Japan, Hiramatsu *et al.*, who described one of the lowest recurrence rates so far, reported that the recurrence rate after TUR was 16.3% within 6 months and 25.4% within a year [22]. Saitoh *et al.* [23] reported a 6-month recurrence rate of 60.5% (25/43 patients) and 69.1% (18/26 patients) recurred within a year after TUR. Furthermore, within 2 yr, most of the tumors (24/26 patients,

*Unpublished data, Proceedings of the Japanese Cancer Association, The 34th Annual Meeting, Abstract No. 291, Osaka, Japan, 1975.

*Table 2. Grade of the papillary transitional cell carcinoma and response to chemotherapy*

| Patients | | Anticancer effect | | | | |
|---|---|---|---|---|---|---|
| Grade of tumor | Total Nos. | (−) | (±) | (+) | (++) | (+++) |
| II | 23 | 1 (4.3) | 4 (17.4) | 4 (17.4) | 14 (60.9) | 0 (0) |
| III | 7 | 0 (0) | 2 (28.6) | 1 (14.3) | 2 (28.6) | 2 (28.6) |
| Total | 30 | 1 (3.3) | 6 (20) | 5 (16.7) | 16 (53.3) | 2 (6.7) |

Antitumor effect; See Table 1. Numbers in parenthesis are percentage of the total numbers of cases.

92.3%) recurred. The recurrence rate in patients with NCS+TUR therapy was lower than other recurrence rates mentioned above. We utilized adjuvant chemotherapy, i.e. intravesical instillation of MMC or NCS and oral administration of SLA and B6, postoperatively. Therefore, this decrease of relapse may not be an effect of NCS therapy only. While a recent report suggests that SLA and B6 did not contribute any significant effect in preventing the recurrence [24].

No degeneration of normal cells was apparent histologically. This may be explained by the evidence that an uptake of fluorescein labeled NCS into tumor cells obtained from bladder cancer was higher than that into normal epithelial cells. This fact may be a possible selectivity of this drug to tumor cells and less cytotoxicity to normal tissues [25].

Hematopoietic suppression and gastrointestinal symptoms were observed as side effects in most cases. Among this, the suppression of white blood cells was the most marked side effect as already mentioned. These suppressions were transitory in all cases. Severe adverse effects on liver, renal and pulmonary function were not observed. In four patients, neither anticancer effects nor any side effects were observed during and after therapy. Side effects and anticancer effects were paralleled in most cases. It is a well-known fact that the therapeutic efficacy of a proteinaceous drug is reduced by the presence of circulating antibodies. Since NCS is a protein, antibody formation is of great concern. We have attempted to detect the antibody in animal and patient sera using several techniques, but have been unable to detect it experimentally or clinically [26]. However, serum obtained from cases showing no response to treatment appeared to degrade NCS molecules, in a study employing the fluorescence polarisation method using fluorescein labelled NCS [26, 27]. It may be that degradation of NCS by such sera is related to clinical ineffectiveness.

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