COMPARISONS OF PLACEBO, PYRIDOXINE, AND TOPICAL THIOTEPA IN PREVENTING RECURRENCE OF STAGE I BLADDER CANCER*

DAVID BYAR, M.D.
CLYDE BLACKARD, M.D.
THE VETERANS ADMINISTRATION COOPERATIVE UROLOGICAL RESEARCH GROUP†

ABSTRACT — Animal studies have shown that metabolites of tryptophan can cause bladder cancer, and human observations reveal an appreciable incidence of abnormalities of tryptophan metabolism in patients with bladder cancer. It has been suggested that pyridoxine (vitamin B_6) may correct this abnormality and prevent recurrences of superficial bladder cancers. Intravesical instillation of thiotepa has been used for more than fifteen years in the treatment of superficial bladder cancer, but no controlled trials have been done. We report here a prospective clinical trial of 121 patients with Stage I bladder cancer randomized to placebo, pyridoxine, or intravesical thiotepa. The percentages of patients with recurrences over the period of study were 60.4, 46.9, and 47.4 for the three groups, respectively, and did not differ significantly. However, if patients having recurrences during the first ten months or followed up less than ten months were excluded, pyridoxine was significantly better than placebo ($P = 0.03$). Thiotepa significantly reduced the recurrence rate compared with placebo ($P = 0.016$) or pyridoxine ($P = 0.015$). These results suggest that a new trial of pyridoxine should be undertaken in which the tryptophan metabolites are measured and that further study of intravesical instillation of chemotherapeutic agents is warranted.

Noninfiltrative or subepithelial infiltrative papillary bladder tumors (Jewett's Stages O and A) comprise a large percentage of admissions for bladder cancer to any urologic service. According to the TNM classification[1] these tumors would be Tl-Nx-MO and are often referred to as Stage I bladder carcinoma. Usually these tumors are treated by transurethral resection (TUR) and fulguration alone. However, in many patients these tumors recur repeatedly and sometimes show a higher degree of malignancy[2] and may even progress to invasive carcinoma in as many as 10 per cent of the cases.[3] The purpose of this study was to examine by randomized treatment comparisons two therapies given in addition to TUR.

The first method is prophylactic administration of pyridoxine (vitamin B_6). Bryan, Brown, and Price[4] showed that direct intravesical implantation of pellets containing tryptophan

*Work was supported in part by National Institutes of Health Grant No. R-10-CA-16499. Most of these results will appear in Federation Proceedings as part of the report from the Second International Meeting on Tryptophan Metabolism.

†Members of the group who participated in this study were: Clyde Blackard, M.D., Minneapolis, Minn., Prince D. Beach, M.D., Houston, Tex., William W. Christeson, M.D., Little Rock, Ark., James S. Elliot, M.D., Palo Alto, Calif., Maurice J. Gonder, M.D., Buffalo, N.Y., John D. Jefferies, M.D., Newington, Conn., Willis P. Jordon, M.D., Memphis, Tenn., David F. Paulson, M.D., Durham, N.C., R. E. H. Puntenney, M.D., Des Moines, Ia., Andrew Sporer, M.D., East Orange, N.J., David P. Byar, M.D., Bethesda, Md., and Raymond Yesner, M.D., West Haven, Conn.

TABLE I. *Recurrences by treatment*

Clinical Data	Treatment			
	Placebo	Pyridoxine	Thiotepa	Totals
No. of patients	50	33	38	121
No. without follow-up	2	1	0	3
Evaluable patients	48	32	38	118
No. without recurrences	19	17	20	56
No. with recurrences	29	15	18	62
Per cent with recurrences	60.4	46.9	47.4	52.5
Total no. of recurrences	84	57	44	185
Total months of follow-up	1,510	983	1,169	3,662
Recurrence rate per 100 patient-months	5.56	5.80	3.76	5.05

metabolites produced bladder cancer in mice. Price and Brown[5] have demonstrated an increased urinary excretion of some of the same tryptophan metabolites in nearly 50 per cent of patients with bladder cancer in a study in Wisconsin, even though many were free of cancer at the time of study, and showed that patients with abnormal tryptophan metabolism had a higher incidence of recurrent bladder tumors. However, geographic variations in the percentage of bladder cancer patients with abnormal tryptophan metabolism have been reported. Brown *et al.*[6] demonstrated that daily oral administration of 25 mg. of pyridoxine to patients with abnormal tryptophan metabolites in their urine would restore the urinary levels to normal. More recently Yoshida, Brown, and Bryan[7] studied tryptophan metabolism in 38 patients with low-stage bladder tumors. Follow-up studies of 18 patients with abnormal tryptophan metabolism disclosed that all had one or more recurrences within five years, but among 20 patients with normal tryptophan metabolism only 12 patients had recurrences within five years. To our knowledge no clinical trials of pyridoxine have been conducted in patients with bladder cancer, therefore, one of our goals was to evaluate this therapy.

Another proposed method of preventing or reducing the number of recurrences in Stage I bladder cancer is the periodic instillation of thiotepa, a cytotoxic alkylating agent, into the bladder. This treatment was first reported by Jones and Swinney in 1961[8] and by Veenema *et al.* in 1962.[9] Recently Staquet[10] cited nine nonrandomized studies including the two just mentioned in which intracavitary thiotepa had been used with success rates ranging from 24 to 100 per cent. Schulman *et al.*[11] observed that although this therapy has been used for fifteen years or more, its true effectiveness remains to be demonstrated since there have been no randomized controlled trials. The second goal of our study was to compare this treatment used prophylactically to pyridoxine and placebo in a randomized fashion.

Material and Methods

One hundred-twenty one patients from ten Veterans Administration hospitals were admitted to this study between November, 1971, and August, 1976. Patients were assigned at random to one of three treatment groups: placebo, 1 tablet per day; oral pyridoxine, one 25 mg. tablet per day; or thiotepa, 60 mg. in 60 ml. of water instilled in the bladder for two hours once a week for four weeks and then once a month. Treatments were to be compared for a period of two years. All patients with Stage I bladder cancer were eligible for this study whether the tumors were new or recurrent. Patients with papillomatosis could be included if the tumors could be completely removed by TUR. Specifically excluded were patients in poor physical condition such that the study would endanger life, patients having received previous radiotherapy or chemotherapy for bladder cancer, patients with bladder carcinoma invading the muscularis or beyond (Stages II, III, IV), carcinoma arising completely with a diverticulum, bladder papillomatosis which could not be completely resected by TUR, and tumors of the bladder other than carcinoma.

The average follow-up information available for study was about thirty-one months in all three treatment groups, but some patients have been followed up as long as five years. Actuarial

curves were used to estimate the time till first recurrence,[12] and differences between curves were tested by the Mantel-Haenszel chi-square.[13] Since by hypothesis the two active treatments were expected to decrease the recurrence rates, one-tailed significance tests have been used throughout. Comparison of rates of recurrence (number of recurrences per patient months of observation) was performed using an F-test.[14] Responses which could be treated as percentages (per cent that recurred, per cent that progressed, and so on) were compared using the chi-square for two by two tables.

Results

The number of patients in the three treatment groups are shown in Table I. Although treatments were assigned at random with equal probability, the numbers in the three treatment groups are not identical. However, since randomization was carried out separately at each of the nine hospitals, such a result could easily arise by chance. Patients in the three treatment groups were comparable with respect to age, grade of tumors at diagnosis, number of tumors at diagnosis, and number of previous bladder tumor resections before going on study.

The numbers of patients who have had recurrences of bladder cancer are also shown in Table I. In this discussion the word "recurrence" will refer to a visit at which one or more tumors have reappeared in the bladder after having been removed previously by TUR. This term is not to be confused with the numbers of tumors present at a single visit. In the upper part of the table we see that the percentages which have shown recurrence are 60.4 for placebo, 46.9 for pyridoxine, and 47.4 for thiotepa. Although there are somewhat fewer recurrences for the two active treatments, these differences are not significant when we ignore the times at which they occurred. In the lower part of the table recurrence rates are determined for each treatment by dividing the total patient-months of follow-up for all patients in a treatment group into the total number of visits for all patients at which recurrences of tumors were present. Under the assumption that the recurrence rate for any one treatment group is an exponential process governed by a single rate constant, we may compare these rates using an F-test. Such comparisons reveal that placebo and pyridoxine do not differ significantly, but thiotepa differs significantly from placebo ($P = 0.016$) and from pyridoxine ($P = 0.015$).

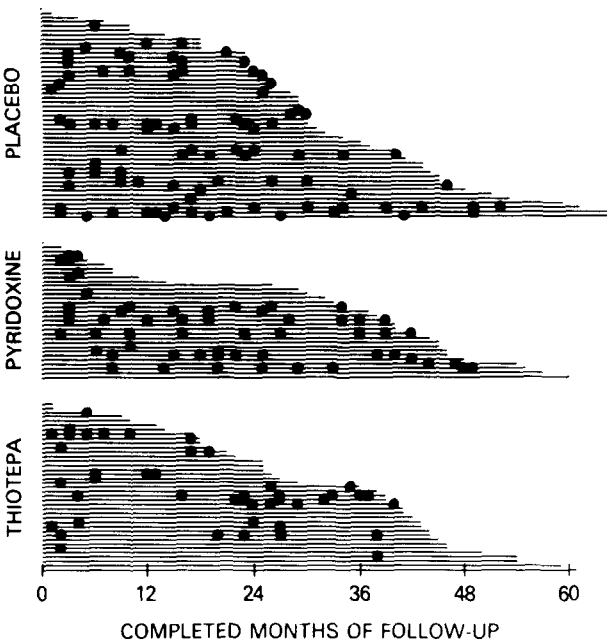

FIGURE 1. *Circles represent recurrent tumors; lengths of lines represent duration of follow-up.*

The basic data for this study are illustrated in Figure 1. The lengths of the lines represent the period of follow-up for individual patients. The circles represent the visits at which tumor recurrence was present. Not all patients stayed on their assigned treatment for the full period of the study either because of progression or for various administrative reasons. However, all analyses reported here were done using the full follow-up period to avoid biased conclusions, but parallel analyses were done using just the portion of time the patients were on their assigned treatment. The conclusions which could be drawn from these parallel analyses are essentially similar to those reported. It is evident from examining Figure 1 that many patients, even those receiving thiotepa, have had multiple recurrences.

Since the goal of this study was to prevent recurrences, the first recurrence for any patient is of greater interest than any other single event. Actuarial curves for the time until first recurrence are shown in Figure 2 for the three treatment groups. Although there have been more recurrences in the placebo group, the differences between these actuarial curves are not significant for any of the three pair-wise comparisons. However, the pattern for pyri-

TABLE II. *Progression versus treatment**

Treatment	No.	Increase in No. of Tumors	Development of Papillomatosis	Increase in Tumor Grade
Placebo	48	22 (46)	6 (13)	13 (27)
Pyridoxine	32	7 (22)	5 (16)	8 (25)
Thiotepa	38	5 (13)	2 (5)	7 (18)

*Figures in parentheses represent per cent.

TABLE III. *Numbers by treatment group of patients with possible complications at any time during study**

Treatment	White Blood Cells < 3,500	Platelets < 100,000	Numerous White Blood Cells in Urine	Positive Urine Culture†
Placebo	5 (10.0)	1 (2.0)	7 (14.0)	10 (20.0)
Pyridoxine	2 (6.1)	1 (3.0)	2 (6.1)	5 (15.2)
Thiotepa	5 (13.2)	5 (13.2)	8 (21.1)	8 (21.1)
TOTALS	12 (9.9)	7 (5.8)	17 (14.0)	23 (19.0)

*Figures in parentheses represent per cent.
†Excluding patients with positive cultures before beginning treatment.

doxine suggests that after an initial recurrence rate similar to that for placebo, tumors do not recur as frequently. This impression would be consistent with the idea that some time is required for pyridoxine to take effect. If we exclude all patients who were followed up less than ten months and those who had recurrences during the first ten months, the comparison of pyridoxine with placebo is significant (P = 0.03, one-tailed); but thiotepa does not differ significantly from placebo, nor does pyridoxine differ significantly from thiotepa.

Information about progression of the disease by treatment group is given in Table II. An increase in the number of tumors seen at a single visit compared with the number present originally was more common in the placebo-treated group than in those receiving pyridoxine (P = 0.026) or thiotepa (P = 0.001). The difference between pyridoxine and thiotepa was not significant. Comparison of the treatments with respect to the development of papillomatosis or an increase in tumor grade did not reveal any significant differences.

Possible complications of therapy are presented in Table III. Both WBC < 3,500/mm.^{3} and platelet count < 100,000/mm.^{3} were more frequently observed in patients treated with thiotepa. However, hematologic toxicity due to thiotepa was easily managed by discontinuing the drug until recovery. The incidence of pyuria was also somewhat greater in the group treated with thiotepa and might be caused by chemical cystitis. The percentages showing

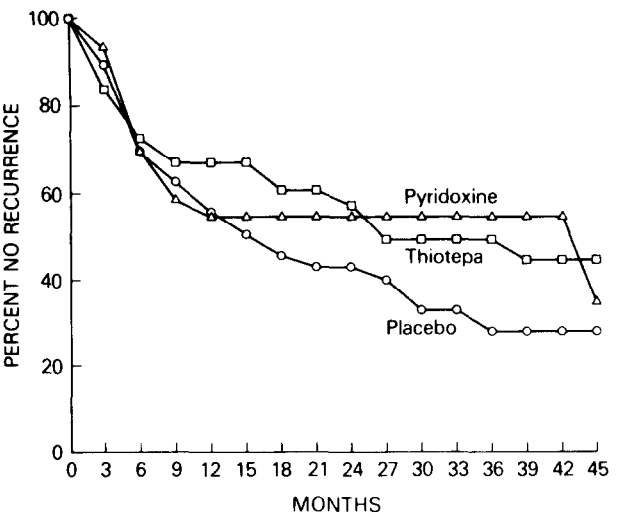

FIGURE 2. *Actuarial curves for time until first recurrence.*

positive urine cultures at some time during the study among patients whose urine was sterile before treatment were about the same in the three treatment groups. If patients are included who had positive cultures before treatment, the percentages for positive cultures sometime during the study were 42.0, 42.4, and 39.5, respectively, for placebo, pyridoxine, and thiotepa. This high incidence of bacteriuria cannot be ascribed to the instillation of thiotepa since it is about the same in all the treatment groups and must therefore be attributed to other causes, such as outlet obstruction and the frequent instrumentation necessitated by the bladder tumors themselves.

At the time of this analysis 30 of the 121 patients had died. Only 2 of these deaths were attributed to cancer of the bladder, 1 in the placebo group, and the other in the thiotepa group.

Comment

Despite the fact that the rationale for using pyridoxine in this study was based on animal experiments and human observations suggesting that abnormal tryptophan metabolites might be responsible for the development of bladder tumors in some patients, tryptophan metabolites were not measured in this study. The decision to omit these studies was based on the desire to see first if there was any evidence that pyridoxine might be beneficial before undertaking a more detailed study which would be logistically difficult in a multicenter cooperative trial, since tryptophan load tests would be required with collection of twenty-four-hour urine specimens which would have to be sent to a central laboratory.

The findings of this study suggest that a more detailed study should be undertaken. Recently Leklem and Brown[15] studied tryptophan metabolism in a family in which 2 members had a history of bladder cancer. Three of 5 family members studied had abnormal tryptophan metabolism characterized by high excretion levels of kynurenine and 3-hydroxykynurenine. None had a pyridoxine deficiency. These findings suggest that kynureninase activity may be defective in these patients and that abnormal tryptophan metabolism may be partly responsible for bladder cancers. However, abnormalities of tryptophan metabolism are known to occur in a variety of other clinical states including Hodgkin's disease, rheumatoid arthritis, and breast cancer. Some workers have questioned the theory that abnormalities of tryptophan metabolism are related to the development of bladder cancer,[16,17] and Brown *et al.*[18] have demonstrated significant geographic differences in the incidence of abnormal tryptophan metabolism among bladder cancer patients. In a study in the Boston area only 17 per cent of bladder cancer patients showed abnormalities compared with 47 per cent in a study in Wisconsin. Although the authors could not rule out differences in selective factors, such as age and racial origin, they suggested that possibly there were different etiologic factors involved in the two study areas.

In our study the most interesting point was the significant difference between patients treated with placebo and pyridoxine after ten months of observation. We may speculate that abnormal tryptophan metabolism would have been found only in about half of our patients. If the same half were those who did not have recurrences after ten months, the results would be highly significant indeed. Only further studies will resolve this point. It is possible that the clinical benefit of pyridoxine is not related to tryptophan metabolism but works by some other means. Recently Romas *et al.*[19] have shown that bladder cancer patients with an abnormality of tryptophan metabolism showed a greater degree of unreactivity to cutaneous delayed hypersensitivity testing. A similar relationship has been demonstrated in Hodgkin's disease.

Our results show that while thiotepa did not decrease the number of patients who had recurrent bladder tumors, it did decrease significantly the frequency of recurrences and the frequency of an increase in the number of tumors compared with the number present at the original visit. Since this was a randomized trial, we may take these results as definite evidence that prophylactic use of intravesical thiotepa has clinical benefit in reducing the frequency of recurrences in patients with bladder cancer. This result agrees with the observation of Veenema, Romas, and Fingerhut[20] that five reports comparing recurrences before and after prophylactic use of thiotepa appear to indicate a beneficial effect. Intravesical instillation of other agents such as cytosine arabinoside and mitomycin C have recently been reported from Japan, but in these nonrandomized studies the drugs were used therapeutically rather than prophylactically.[21-23] Another drug, VM-26, a new epipodophyllotoxin has also shown some possible activity in noncontrolled trials.[24] The idea of treating superficial bladder tumors, or at least preventing their recurrence by the intravesical instillation of chemotherapeutic agents, deserves further study. Even if we should eventually learn that patients with abnormalities of tryptophan metabolism can be treated successfully with pyridoxine, other treatments will be needed for patients not falling in this category.

Landow Building-Room C-509
Bethesda, Maryland 20014
(DR. BYAR)

References

1. UICC (International Union Against Cancer): TNM Classification of Malignant Tumours, 2nd ed., Geneva, Imprimerie G. de Buren S.A., 1974, p. 79.

2. Marshall VF: Current clinical problems regarding bladder tumors, in Bladder Tumors, A Symposium, Philadelphia, J. B. Lippincott Company, 1956, p. 2.

3. Greene LF, Hanash KA, and Farrow GM: Benign papilloma or papillary carcinoma of the bladder? J. Urol. **110:** 205 (1973).

4. Bryan GT, Brown RR, and Price JM: Mouse bladder carcinogenicity of certain tryptophan metabolites and other aromatic nitrogen compounds suspended in cholesterol, Cancer Res. **24:** 596 (1964).

5. Price JM, and Brown RR: Studies on the etiology of carcinoma of the urinary bladder, Acta Un. Int. Cancer **18:** 684 (1962).

6. Brown RR, Price JM, Satter EJ, and Wear JB: The metabolism of tryptophan in patients with bladder cancer, *ibid.* **16:** 299 (1960).

7. Yoshida O, Brown RR, and Bryan GT: Relationship between tryptophan metabolism and heterotopic recurrences of human urinary bladder tumors, Cancer **25:** 773 (1970).

8. Jones HC, and Swinney J: Thio-TEPA in the treatment of tumors of the bladder, Lancet **2:** 615 (1961).

9. Veenema RJ, *et al.*: Bladder carcinoma treated by direct instillation of thio-TEPA, J. Urol. **88:** 60 (1962).

10. Staquet M: The randomized clinical trial: a prerequisite for rational therapy, Eur. Urol. **2:** 265 (1976).

11. Schulman CC, *et al.*: EORTC randomized trial for the adjuvant therapy of T1 bladder carcinoma, *ibid.* **2:** 271 (1976).

12. Cutler SJ, and Ederer F: Maximum utilization of the life table method in analyzing survival, J. Chronic Dis. **8:** 699 (1958).

13. Mantel N: Evaluation of survival data and two new rank order statistics arising in its consideration, Cancer Chemother. Rep. **50:** 163 (1966).

14. Gehan EA: Statistical methods for survival time studies, in Staquet, M. J., Ed.: Cancer Therapy: Prognostic Factors and Criteria of Response, New York, Raven Press, 1975, p. 25.

15. Leklem JE, and Brown RR: Abnormal tryptophan metabolism in a family with a history of bladder cancer, J. Natl. Cancer Inst. **56:** 1101 (1976).

16. Gailani S, *et al.*: Studies on tryptophan metabolism in patients with bladder cancer, Cancer Res. **33:** 1071 (1973).

17. Benassi CA, Perissinotto B, and Allegri G: The metabolism of tryptophan in patients with bladder cancer and other urological diseases, Clin. Chim. Acta **8:** 822 (1963).

18. Brown RR, Price JM, Friedell GH, and Burney SW: Tryptophan metabolism in patients with bladder cancer: geographical differences, J. Natl. Cancer Inst. **43:** 295 (1969).

19. Romas NA, *et al.*: Anergy and tryptophan metabolism in bladder cancer, J. Urol. **115:** 387 (1976).

20. Veenema RJ, Romas NA, and Fingerhut B: Chemotherapy for bladder cancer, Urology **3:** 135 (1974).

21. Ogisu F, Ishikawa F, and Kabei T: Intravesical infusion with cystosine arabinoside (kiloside) for tumors of the bladder, Shin'yaku to Rinsho **25:** 111 (1976).

22. Ooi Y: Antineoplastic effects of intracystic administration of cancer chemotherapeutic agents, Nishinichi Hinya **38:** 233 (1976).

23. Mishina T, *et al.*: Mitomycin C bladder instillation therapy for bladder tumors, J. Urol. **114:** 217 (1975).

24. Pavone-Macaluso M, Caramia G, Rizzo RP, and Messana V: Preliminary evaluation of VM26, a new epipodophyllotoxin derivative in the treatment of urogenital tumours, Eur. Urol. **1:** 53 (1975).