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13-*cis*-Retinoic Acid in Chemoprevention of Superficial Bladder Cancer

George R. Prout, Jr., MD, DSc, FACS and Bruce A. Barton, PhD

for the National Bladder Cancer Group
Urological Service, Massachusetts General Hospital
Harvard Medical School and Maryland Medical Research Institute

Members of the National Bladder Cancer Group who contributed to this study:
G.R. Prout, Jr. (Massachusetts General Hospital), W.W. Koontz, Jr. (Medical College of Virginia), S. Loening (University of Iowa), M.J. Flanagan (Rush-Presbyterian-St. Luke's Medical Center), G. Brannen (Virginia Mason Research Center).

Abstract Animal studies indicate that 13-*cis*-retinoic acid (CRA) inhibits bladder tumor growth and is effective in treating patients with serious dermatologic disorders. A trial of CRA in patients at high risk for recurrent Ta, T1 tumors was initiated at an experimental dose of 0.5 mg/kg/d in three divided doses, increasing to 1 mg/kg/d at four weeks. Treatment of twenty eligible patients lasted for six months with an additional 24 month follow-up period. One patient was later excluded due to toxicity resulting in an early dose reduction.

Eight patients stopped treatment before three months; of these five, had recurrences within three months, one developed pulmonary metastasis, and one developed a T2G3 tumor. Four patients stopped treatment between three and six months; three of them had recurrences before one year and one had no evidence of disease at seven years. Seven patients completed the course; of these three had recurrences within six months, and three more had recurrences at 8, 15, and 45 months, respectively.

Toxicity was nearly universal; cheilosis, conjunctivitis, pruritus, joint and eye pain, flashing lights, and erythrocyte sedimentation rate (ESR) over 60 were all noted. The lack of positive results and the frequency and severity of toxicity led to termination of the study.

Key words: 13-*cis*-retinoic acid, bladder carcinoma, chemoprevention

13-*cis*-Retinoic acid (CRA), an analogue of Vitamin A, was shown to be effective in suppressing or preventing bladder tumor growth in rats fed *N*-4-5-nitro-2-furyl-2-thiazolylformamide (FANFT) or *N*-methyl-*N*-nitrosourea, and in mice fed 4-butyl-4-(4-hydroxybutyl) nitrosamine [1–3]. Many patients with a prior history of superficial bladder tumors (TCC) are at high risk for recurrence. For this reason, members of the National Bladder Cancer Cooperative Group A (NBCCGA, later known as the National Bladder Cancer Group, NBCG) decided, in 1978, to conduct a brief Phase I/II study to examine the influence of CRA on the incidence of recurrence of bladder tumors.

METHODS AND MATERIALS

Eligibility criteria included:

- Histologically documented recurrent Ta or T1, G1–3 TCC within the previous six months.
- Biopsies of pre-selected mucosal sites, transurethral resection, or electrofulguration to insure that the patient was tumor-free at the time of accession.
- Negative cytology and post-resection/fulguration urine/saline washings.
- Exclusion of patients with a history of disease >T1 TCC or Tis.

Experimental protocols [4,5] included:

- Treatment with CRA started within two weeks at an oral dose of 0.5 mg/kg/d in three equal divided doses. After four weeks, if no significant toxic effects were noted, the dose of CRA was increased to 1 mg/kg/d in three divided doses. Treatment was then continued for six months.
- Compliance was measured by pill counting; toxicity was measured at 4, 8, 12, 18 and 23 weeks by liver, renal and thyroid function as well as hematologic studies. Cutaneous, oral, bone, joint and visual disturbances were determined at the indicated intervals.
- Cystoscopy, random biopsies and biopsy of any suspicious areas as well as urine/saline washings for cytology were performed every three months for the 24 month follow-up period.
- The statistical objective of the study was to demonstrate a six month, tumor-free rate of 80% with a 95% probability that the true non-recurrent rate was no lower than 70%. To accomplish this objective, 44 patients were needed. Fifty-five patients were accrued to allow for a 25% dropout rate. Recurrent, biopsy-proven TCC indicated failure.

This study was undertaken at a time when the investigators were under constraints recommended by the Site Review Group to register Protocol patients only, and the Central Pathology Laboratory Director, Gilbert H. Friedell, was to receive only material obtained from patients on an existing Protocol. Therefore, some information prior to accession is absent and much of the histopathology material was reviewed only by the Institution's pathologist. This created a major defect in the availability of data since neither the initial evaluative studies nor subsequent follow-up material was submitted to the Central Pathology Laboratory before the patient was accrued.

RESULTS

Fourteen patients were TaG1 on accession; three were TaG2, one had a single T1G2 tumor, and one was of unknown grade and stage but known to be less than T1G3. Three other patients presented with single tumors, the remainder with multiple tumors.

On recording the results, some patients were evaluated 10 to 30 days after the three and six month periods stipulated in the Protocol. These patients were assumed to have been evaluated at these times. Twenty-two patients were accrued, and 19 were available for analysis; two were ineligible and a third, due to toxicity, had early reduction in CRA (10 mg/day).

The results of treatment are shown in Table I. One of the successful patients had marked exacerbation of tic doloreaux on treatment and at 15 months was found to have a pulmonary tumor of neuroendocrine origin as determined by electron microscopy (Table II). A large TaG1 tumor was simultaneously discovered. Three others who failed had multiple TaG1 tumors.

Of the eight patients treated three or less months, multiple TaG1 tumors were found in five and multiple tumors in a sixth. At 13 months, a G3 tumor recurred in a patient with a previous history of G1 tumors; after an additional three months, a muscle invasive squamous cell carcinoma was discovered (Table II). Another patient without previous history of high grade or T1 tumors had a negative cystoscopy at three months, but was found to have abnormal liver function studies. CRA was discontinued and pulmonary metastases discovered a month later. A third patient in this group developed acceleration of Dupuytren's contracture and shortening of the extensor tendons to his great toe (Table II).

Of the four patients who completed three but not six months of therapy, one had a TaG1 recurrence at 11 months (number of tumors not known), two had TaG1 (multiple) and the fourth, who entered with a single tumor, had no recurrence for over five years.

Toxicity was almost universal. Only three patients were free of side effects. The most commonly encountered complaints were cheilosis, conjunctivitis and pruritus, joint pain, tendon pain, "swollen face," abnormal liver function tests, erythrocyte sedimentation rate (ESR) greater than 60 and eye pain with "shooting lights." These symptoms were severe enough to cause most of those who failed to reach six months to stop the medication if the investigator did not.

TABLE I. Results of CRA Study in 19 Patients [11]

	Patients
1. Completed treatment	**7**
Response	4
Recurrence (8, 15, 45 months)	3
No recurrence	1
No response	3
Recurrence at 3 months	2
Recurrence at 6 months	1
2. Failed to complete 3 months of treatment	**8**
Recurrence at 3 months	6
Recurrence at 30 months	1
Pulmonary metastasis	1
3. Failed to complete 6 months of treatment	**4**
Recurrence (4, 8, 11 months)	3
No recurrence	1

TABLE II. Unusual Events Following CRA Treatment [11]

1. Neuroendocrine pulmonary tumor with metastasis—15 months
2. Marked increase in Dupuytren's contractures, shortening of hallux extensor tendons—2 months
3. T2G3 squamous cell carcinoma—16 months
   CRA stopped at 3 months
4. Pulmonary metastasis—4 months after treatment

STATISTICAL RATIONALE FOR TERMINATION OF CRA STUDY AFTER 19 EVALUABLE PATIENTS

The observed success rate in the first 19 evaluable patients was 21% (4 of 19 patients). The objective for the success rate was 80%. The total anticipated sample size for evaluable patients was 44. For the expected success rate of 80%, 35 successes (or 9 failures) would be expected in the 44. Even if we assume that the 15 observed failures were still within the realm of possibility of a true success rate of 80% (the actual probability of this is $<.0001$ or one

chance in 10,000), this would mean that the next 25 patients would need to be successes. The probability of this happening was <.003, or 3 chances in 1000. Thus, the study had virtually no chance of success after this initial assessment of study results.

DISCUSSION

This group of patients was selected as previously stated. In retrospect, the only additional strategy we might have employed would have been to treat the patients with the cytotoxic agent most widely used at the time, *N,N',N"*-trimethylene phosphoramide (thiotepa). This might have diminished or destroyed the submicroscopic tumors in place in patients with low grade tumors, or, if implantation occurred, viable tumor cells left from previous resections. One other possibility remains—that tumors were not completely resected although the bladders appeared clear. We have recently reviewed longitudinal records of 25 patients followed for a median of five years. These tumors were G1 and G2, and recurrences were at random sites unless the original tumor was ≥2 cm in diameter. In the latter case, recurrence at the original site was occasionally observed, suggesting that the recurrence was actually persistence of a previous tumor [6].

At first glance, patients with bladder tumors would seem to be an excellent population in which to test chemopreventive agents. It is quite likely that the animal models using a particular tumor initiation and promotion protocol are vastly different from human carcinogenesis. It is also quite likely that the group of patients selected already had tumor(s), resulting in a study of chemotherapy rather than chemoprevention. If most of these patients had microscopic tumor(s), whether the products of field change or implantation, there is little evidence that systemic CRA is chemotherapeutic. It is cytotoxic, but not in the bladder.

Tumors recurred in these patients after treatment with an agent shown to be effective in suppressing bladder tumor growth in experimental animals. This raises major issues concerning the applicability of rat and mouse models of bladder carcinogenesis to man, the source of recurrent tumors in humans and their causes, and whether CRA is effective in suppressing tumors. The difficulties in interpreting the results of chemoprevention in animals are epitomized by the experience of Croft *et al.* [7] who used CRA to prevent FANFT-induced tumors in female Fischer 344 rats. CRA failed to inhibit the incidence and severity of FANFT-induced tumors. On the other hand, Sporn *et al.* [1], using *N*-methyl-*N*-nitrosourea in rats and Becci *et al.* [3], using *N*-butyl-*N*-[4-hydroxybutyl)-nitrosamine in B6D2F1 mice, reported inhibition and suppression of tumor growth. Thus, one might view the results of experiments in animals with some reservation.

The experience in human tumors is scant. Favorable responses to CRA therapy in oral leukoplakia and aggressive, recurrent laryngeal papillomatosis have been reported by Lippman *et al.* [8] in a summary of selected studies in humans. These authors also reported a small objective response rate (16%) in patients with advanced refractory squamous cell carcinoma of the head and neck. Peck *et al.* [9] have highlighted what appears to be the major problem associated with the chemical application of retinoids in humans. They treated 12 patients with a total of 270 basal cell carcinomas with 3.1 mg/kg of CRA daily for a mean of eight months. Only 8% underwent complete regression. All patients had moderate to severe toxicities including skeletal disease, and five patients withdrew from treatment.

Thus, while the agents may have great promise, the experience to date suggests that less toxicity and more efficacy may make the agents more acceptable for therapeutic regimens. Hicks *et al.* [10] have concluded that *N*-[4-hydroxyphenyl]retinol (4-HPR) is the most anticarcinogenic retinoid in rodents and is better tolerated in humans than CRA or etretinate. A chemopreventive trial with this agent was started in 1985 at Middlesex Hospital, London. Thus far, no data are available.

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