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Fenretinide (4-HPR) in Chemoprevention of Oral Leukoplakia

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Abstract A controlled clinical trial has been underway at the Istituto Nazionale Tumori (INT) of Milan since 1988. The goal of the trial is to evaluate the effectiveness of fenretinide (4-HPR) in preventing relapses, new localizations, and carcinomas in patients with benign postoperative diagnoses who have been surgically treated for oral leukoplakias. This paper presents the design and the preliminary results of this study. To date, 137 patients have been randomized, following surgical excision of oral leukoplakia, to receive either 200 mg 4-HPR daily for 52 weeks or no intervention. Twenty local relapses or new localizations have occurred so far in the control group and 9 in the 4-HPR group. Seven patients have interrupted the intervention because of toxicity. No impaired dark adaptation has been observed. We conclude that 4-HPR is well-tolerated and appears to be effective in preventing relapses and new localizations during the treatment period.

Key words: chemoprevention, fenretinide, oral leukoplakias, clinical trials

Based on the beneficial effect that vitamin A and its synthetic derivatives have on epithelial differentiation, and on the observation of low retinol levels in blood and tissues of animals and humans with preneoplastic and neoplastic lesions [1,2,3], many studies over the last ten years have sought to determine whether these drugs have a cancer chemopreventive action. In several nonrandomized clinical studies, retinoids have sometimes proven efficacious in the treatment of precancerous lesions of the oral cavity [4–7]. Two recent controlled trials suggest that retinoids have therapeutic activity in advanced squamous cell carcinoma of the head and neck, and that they can prevent cancer recurrences or new carcinomas [8].

Oral leukoplakia is a mucosal disease with a high cancerization rate [1–8]. Many patients whose lesions are surgically removed later develop local relapses, new leukoplakias, or squamous cell carcinomas [9]. These considerations justify chemopreventive trials, and the accessibility of the oral cavity allows convenient histo-

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logical, photographic, and lesion size evaluation to assess intervention efficacy.

In 1988 a randomized chemoprevention trial began at the Istituto Nazionale Tumori of Milan (INT). Its purpose was to evaluate, after 3 years, the effectiveness of fenretinide (4-HPR) in preventing relapses and new localizations and, after 4 years, in preventing carcinomas in patients surgically treated for oral leukoplakias. Recent work has shown that retinoids are effective while they are being taken [2,4,6,7] and it seems reasonable to expect that their putative efficacy in preventing recurrences and new localizations would be evident during the treatment period of this trial. This paper therefore presents the rationale, design, accrual history, and preliminary results of this study.

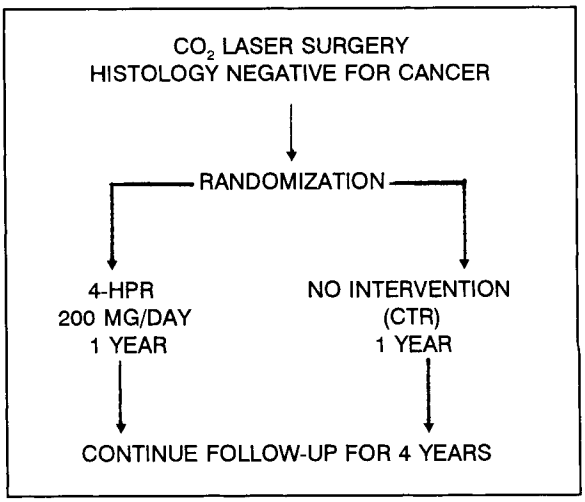

Fig. 1. Study Plan.

MATERIALS AND METHODS

The study began in September, 1988. The study protocol was approved by the Scientific and Ethical Committee and written informed consent was obtained from all patients. Study design is shown in Figure 1.

Study Population

Criteria for eligibility. Patients eligible for entry have been operated on for previously untreated homogeneous or non-homogeneous oral leukoplakias, and have benign post-operative histology. Patients must have normal WBC, RBC and platelet counts as well as metabolic, renal, and liver function tests within 1.5 times the upper normal limit [10,11].

Criteria for exclusion. The criteria for exclusion are: age >75 years, serious cardiovascular disease, neuropsychiatric difficulties, expected difficulties with follow-up, unwillingness to enter the study, plans to have children, inaccessibility of the lesion to CO_2 laser surgery, AIDS, other prior or synchronous malignancies (except adequately treated basal cell carcinoma of the skin or intraepithelial neoplasia of the uterine cervix), concurrent consumption of high doses of vitamin A (greater than 30,000 IU/day), or a family history of tapetoretinal degeneration, and participation in other studies which might interfere with the present study.

Oral leukoplakia classification: (a) Homogeneous leukoplakia; white patch, without infiltration, well-defined margins; (b) Non-homogeneous leukoplakia: (I) Speckled leukoplakia; red and white patch without infiltration; (II) Leuko-erythroplakia; red and white patch without infiltration, sometimes ulcerated, margins not well-defined.

Diagnostic procedure. Procedures include: (a) photographing the lesion; (b) careful oral examination and dental mapping. (Where the lesion is apparently related to badly fitted dentures or broken teeth, patients are advised to seek dental treatment); (c) biopsy of suspicious lesions and areas staining with toluidine blue; (d) metabolic, liver function, and renal function tests, blood work-up (WBC, RBC, and platelet count), and chest x-ray.

Treatment

Surgical treatment. Lesions are stained with toluidine blue before surgery to define the margins. Laser resection is performed under local anesthesia using the laser in the continuous wave mode (9–12 W power output) coupled to an operating microscope of 200 mm focal length. The lesion is excised with at least 0.5 cm margins (in depth and laterally) of normal tissue, providing a specimen for histopathologic examination. The wound is left open; patients are checked weekly until complete re-epithelialization.

Drug intake. Therapy begins on the day of randomization. Patients randomized to intervention receive 4-HPR (200 mg/day) for a maximum of 52 weeks. It was noted during Phase I studies that 4-HPR causes a reversible reduction of plasma retinol levels; therefore a 3-day holiday at the end of each month is prescribed for all patients to avoid adverse effects related to prolonged lowering of serum retinol [11]. Patients receive a sufficient number of 4-HPR capsules to last until the next check-up and are advised to take the capsule after meals (one after lunch and one after dinner) as absorption is more efficient. Treatment continues until the end of the study period or until the appearance of a recurrence, new localization or carcinoma, or the occurrence of adverse reactions such as those specified below. In the event of mild toxicity, the dose is reduced by 50%. If moderate toxicity occurs, the treatment is discontinued and may be restarted at 50% of the original dose after recovery. Therapy must be permanently suspended whenever severe toxicity or an adverse reaction after rechallenge occurs.

Assessment

All patients are checked every two months. Check-up includes clinical examination and metabolic, liver, and renal function laboratory tests. When toxicity occurs, patients are checked at monthly intervals. All suspected lesions are photographed, biopsied, and evaluated by a head and neck surgeon blinded as to the treatment. New lesions separated by more than 2 cm from the first-treated leukoplakia are considered new localizations. Time of appearance is calculated from the date of randomization. Patients with local relapses and new localizations are again treated by CO_2 laser exeresis. If squamous cell carcinoma develops, patients are treated according to established INT therapeu-

TABLE I. Groups According to Age, Sex, Smoking, and Alcohol Consumption

	CTR (70 pts)	4-HPR (67 pts)	Total (137 pts)
Age (years)			
<55	37 (53%)	42 (63%)	79
56–65	24 (34%)	14 (21%)	38
66–75	9 (13%)	11 ((16%)	20
Sex			
male	48 (69%)	45 (67%)	93
female	22 (31%)	22 (33%)	44
Smoking			
non–smokers	17 (24%)	15 (22%)	23
smokers	36 (52%)	30 (45%)	66
ex–smokers	17 (24%)	22 (33%)	39
Alcohol consumption			
non–drinkers	14 (20%)	16 (24%)	30
drinkers	52 (74%)	47 (70%)	99
ex–drinkers	4 (6%)	4 (6%)	8

tic procedures. Control group patients are followed in the same manner as those in the 4-HPR group. Patients completing the one-year study will continue to be checked every three months during the following year, every 4 months the next year, and every 6 months thereafter. Baseline plasma levels of retinol are also evaluated. Retinol, 4-HPR, and its metabolite, *N*-(4-methoxyphenyl)-retinamide, are checked at 4 months, at the end of treatment, and once a year during follow-up. The interval (in hours) between last drug intake and blood sampling is recorded. Plasma concentrations are determined by high-performance liquid chromatography [10,11] and these data will be related to the outcome of disease, drug toxicity and activity. The study will last 5 years.

Toxicity [10,11] is evaluated on the basis of subjective and objective symptoms and by assessment of blood parameters, including bilirubin, cholesterol, triglycerides, γGT, SGOT and SGPT. Mild toxicity is defined as an increase in laboratory values by 1.5–2 times the accepted upper normal limit; moderate toxicity is defined by laboratory values 2–3 times above the upper normal limit; severe toxicity is defined as values more than 3 times the upper normal limit. Evaluation of the severity of signs and symptoms is left to the clinician. Dermatitis, photodermatosis, or impaired night vision with positive electroretinography are considered severe toxicity.

Sample Size

Data on a consecutive series of patients treated surgically for leukoplakia before the present trial indicate that the chance of developing a carcinoma within 4 years of surgery is 5.3%. A trial involving approximately 300 patients (randomized to 4-HPR or no intervention) will be able to detect a difference of 5% in the probability of new carcinomas over a period of 4 years ($\alpha = 5\%$, $\beta = 20\%$, two-tailed test) according to the Freedman method [12]. In a previous study [9] we found that in operated patients the chance of developing relapses or new localizations after three years in operated patients was 40%. Thus, a 3-year study with 300 patients should detect at least a 15% difference in the probability of developing these events ($\alpha = 5\%$, $\beta = 20\%$, two-tailed test) between the two arms. Previous INT experience shows that the probability of developing relapses and new localizations within 1 year of surgery is 23%. Assuming that the 15% difference between the two arms is concentrated in the first year after surgery,

TABLE II. Causes of Interruption

Drug interruption	**18**
Toxicity	**7**
–dermatitis	3
–skin dryness	1
–dermatitis + increase in γGT	1
–increase in triglycerides	1
–increase in triglycerides + γGT	1
Refusal	**5**
Unfavorable event	**4**
Intercurrent event	**2**

190 patients will be needed to detect this difference (α = 5%, β = 20%, two-tailed test)[13].

Statistical Evaluation

Statistical analysis will be performed on an "intention to treat" basis. The comparison between the two arms (intervention and control) in terms of (1) carcinoma-free period within 4 years; (2) relapse- and new localization-free period within 3 years; (3) relapse-and new localization-free period during the therapy period (1 year) will be performed by the Log-rank test [12]. There is no *a priori* stratification; all possible prognostic factors (histology, smoking, alcohol consumption, oral hygiene, and dental status) will be entered in a multiple regression analysis to adjust the comparison between the two arms [12].

Randomization

After surgery, eligible patients are invited by a physician to enter the study; those willing to participate are asked to sign the informed consent form and are randomized by calling the Data Center. To ensure a good balance between the number of patients in the two arms, a permutated blocks randomization list was prepared for each participating center. All randomized patients are urged to improve oral hygiene, have dental treatment if necessary, stop drinking alcohol, and stop smoking tobacco.

RESULTS

As of December, 1992, of the 159 patients who had been operated on for oral leukoplakias, 137 were randomized into the trial (67 in the 4-HPR arm, and 70 in the control arm). The distribution of the series according to risk factors (age, sex, smoking, and alcohol habits) shows that the two arms are well balanced for all considered factors except age (Table I). By December, 1992, 34 patients had completed the treatment at full dosage, 7 are continuing at full dosage, 5 are continuing at reduced dosage because of mild toxicity, 3 patients have interrupted after half-dose reductions, and 18 have stopped taking the drug.

The causes of interruption are given in Table II. Of the 18 who have definitively stopped the treatment, 7 showed evidence of toxicity, most frequently cutaneous toxicity (3 developed dermatitis and 1 patient developed severe skin dryness). Other reasons for definitive suspension were increased blood triglycerides and γGT (3 cases) occurring in patients with histories of high blood triglycerides and γGT.

Unfavorable events are reported in Table III. Fifteen patients had recurrences (8 in control group and 7 in 4-HPR group) and 14 had new localizations (12 in control group and 2 in 4-HPR group). In addition, one patient developed lung cancer 10 months after randomization.

Figure 2 shows the risk of recurrence and new localizations in the two groups who completed the intervention: 6% in the 4-HPR group and 30% in the control group.

DISCUSSION

This randomized study evaluates the efficacy of 4-HPR given as adjuvant therapy after resection of oral leukoplakias in preventing relapses,

TABLE III. First Unfavorable Event

	No.	CTR	4-HPR	At months
Recurrence	15	8	7	2,2,2,4,5,6,6,6,6,9, 10,15,28,31,42
New occurrence	14	12	2	2,4,4,5,6,8,9,10,11, 12,15,18,21,35
New Primary (Lung carcinoma)	1	1	–	10

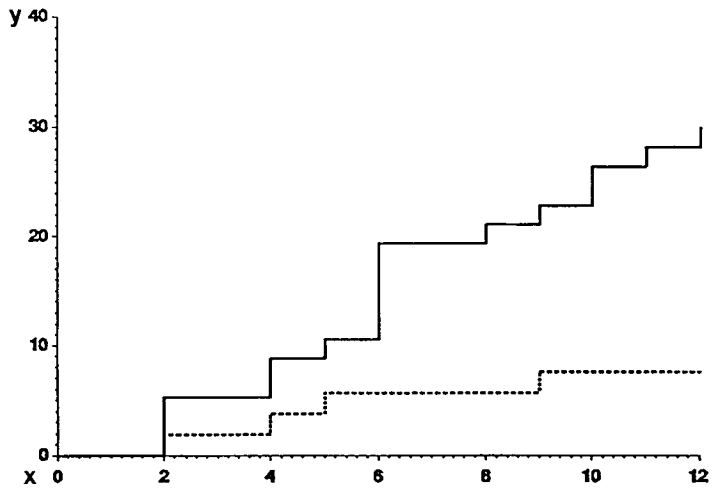

Fig. 2. Risk of unfavorable events during treatment.
x = Months
y = Risk

_____ Control group
- - - - - - - - - 4-HPR group

TABLE IV. Number of Abnormal Laboratory Values and Complaints Observed During Follow-up

	CTR	4-HPR
No. of patients with at least one check-up	68	63
Laboratory Tests		
No. of checks	291	268
-SGOT, SGPT, γGT, Bilirubin	*44/291 (15%)	39/268 (14%)
-Cholesterol, Triglycerides	15/291 (6%)	11/268 (4%)
Complaints		
No. of checks	305	302
Dermatitis	0/305	11/302 (4%)
Skin-Mucosal Dryness	2/305 (1%)	8/302 (3%)
Dyspeptic Syndrome	0/305	3/302 (1%)

* Two patients with Gilbert's syndrome

new localizations, and squamous cell carcinomas. The second aim is to evaluate the toxicity of the drug. Patients were checked every 2 months in the absence of side effects or monthly when problems were observed; providing detailed information on toxicity and failure.

Studies on women with breast cancer have shown that 4-HPR is less toxic than other synthetic retinoids and is well tolerated by patients [10]. Our results confirm those findings in males with oral leukoplakias, including some with impaired liver function [14]. Abnormal metabolic and liver function parameters were recorded in both the control and therapy groups (Table IV), and statistical evaluation of toxicity showed no difference in blood parameters between these groups. Two patients in the 4-HPR group developed high blood triglyceride levels during the intervention, although these had returned to normal when rechecked 1 month after suspending 4-HPR. Fifteen of the 47 patients (32%) completing the year of intervention had some side effects (subjective or objective), but only 7/47 (15%) did not complete the intervention due to toxicity.

The preliminary oncological results of this study are very encouraging. The difference between the two groups in terms of total unfavorable events shows a trend in favor of 4-HPR intervention, particularly for the occurrence of new leukoplakias. This trend is consistent with the hypothesis that 4-HPR can prevent mucosal abnormalities, and supports Dr. Hong's observation [8] of a significant reduction in the occurrence of new carcinomas, but no difference in the development of relapses and metastases in treated carcinomas.

These results are only preliminary, however, and they refer to 1 year of follow-up after surgical removal of oral leukoplakias. It is our experience that carcinomas are to be expected after the second year of follow-up. If the trend is confirmed with a larger number of patients (still to be recruited) and over a longer follow-up period, this would argue for routine use of 4-HPR in chemoprevention.

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