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Randomized trial of fenretinide (4-HPR) to prevent recurrences, new localizations and carcinomas in patients operated on for oral leukoplakia: Long-term results

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We assessed the efficacy of fenretinide at preventing relapses, new lesions and carcinomas after surgical excision of oral leukoplakia. In a controlled multicenter study, 170 patients operated on for oral leukoplakias with benign postoperative histology were randomized to 200 mg fenretinide daily for 1 year *vs.* no intervention. Preliminary analysis indicated that fenretinide had good tolerability and was effective at preventing relapses and new lesions during treatment. Analysis after 5-year follow-up suggested that fenretinide protected against relapses and new lesions up to 19 months after randomization, with both limits of the 95% hazard ratio CI for fenretinide *vs.* control below 1 for 7 months after randomization. There was also a protective effect against all first events, including cancer, for 25 months, with both limits of the 95% CI below 1 up to 11 months after randomization. Subsequently, risk ratio estimates were unstable. Fenretinide was well tolerated and effective at preventing relapses and new leukoplakias during treatment and after. The trial had to be stopped prematurely for very low recruitment and had insufficient power to reveal any protective effect against oral carcinoma; nevertheless, continuing studies on this promising chemopreventive are justified.

Key words: chemoprevention; retinoid; fenretinide; oral leukoplakia; clinical trial

Over the last 2 decades, the locoregional control of head-and-neck cancers has improved, but mortality remains high due mainly to the development of second primaries in relation to continuing exposure of the epithelial surface of the oral cavity, oropharynx and larynx to carcinogens, chiefly tobacco and alcohol.[1] Primary prevention (stopping exposure to carcinogens) would be the best approach to such cancers; however, once started, the multistep carcinogenic process appears to continue even in the absence of continued exposure to known carcinogens, or perhaps because of continuing exposure to unrecognized endogenous and exogenous carcinogenic agents. Oral leukoplakia is a precancerous mucosal condition with a probability of transforming into oral carcinoma estimated at about 10% over 20 years.[2–6] Patients whose leukoplakias are removed surgically frequently develop local relapses or new leukoplakias;[2] they are also at increased risk of developing oral carcinoma even when the leukoplakia is hyperkeratinotic and not dysplastic.[2] Such patients are excellent candidates for trials on chemopreventive agents,[3,4,7] particularly since oral cavity accessibility allows convenient histologic and photographic evaluation of intervention efficacy.

Retinoids are promising chemopreventive agents. They exert a beneficial effect on epithelial differentiation and can inhibit malignant transformation and suppress tumor promotion.[8–10] Many clinical studies have sought to determine whether natural and synthetic retinoids have a primary cancer-preventive action. Some have found that these substances may have such an action by promoting the disappearance of oral leukoplakias.[11–15] In other studies, retinoids had an adjuvant chemopreventive effect by reducing the incidence of new head-and-neck carcinomas.[16–19] However, no studies have been conducted on the use of retinoids for adjuvant chemoprevention of new head-and-neck carcinomas after removal of oral leukoplakia.

In September 1988, we began a phase III multicenter adjuvant chemoprevention trial to assess the efficacy of a 1-year intervention with the synthetic retinoid fenretinide (4-HPR) *vs.* no treatment at preventing relapses, new localizations and carcinomas in patients treated surgically for oral leukoplakia. We closed patient entry prematurely in June 1994 because the accrual rate had become very low, so all patients completed the planned intervention by June 1995. We published preliminary results on 137 patients in 1993.[20,21] We now present the long-term results.

Material and methods

Patients operated on for oral leukoplakia with a postoperative histologic diagnosis of hyperparakeratosis or dysplasia were recruited from 4 Italian centers (Istituto Nazionale dei Tumori, Milan; ENT Department, Aosta Hospital; ENT Department, Pordenone Hospital; Oral Pathology Department, S. Paolo Hospital, University of Milan). Carcinoma was excluded. The study was coordinated by the Istituto Nazionale dei Tumori. The European Institute of Oncology joined the study later, taking over the follow-up of some patients. The study protocol was approved by the scientific and ethical committees of the involved institutes, and written informed consent was obtained from all patients.

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TABLE I – PROGNOSTIC CHARACTERISTICS IN THE TWO STUDY ARMS

	4-HPR ($n = 84$)	Control ($n = 86$)	Total ($n = 170$)
Age (years)			
<45	28 (33%)	14 (16%)	42
46–55	20 (24%)	34 (40%)	54
56–65	21 (25%)	27 (31%)	48
66–75	15 (18%)	11 (13%)	26
Sex			
Male	60 (71%)	61 (71%)	121
Female	24 (29%)	25 (29%)	49
Smoking			
Nonsmokers	17 (20%)	21 (24%)	38
Smokers	41 (49%)	45 (52%)	86
Ex-smokers	26 (31%)	20 (24%)	46
Alcohol consumption			
Nondrinkers	16 (19%)	20 (23%)	36
Drinkers	63 (75%)	61 (71%)	124
Ex-drinkers	5 (6%)	5 (6%)	10
Number of leukoplakia lesions			
1	53 (63%)	53 (61%)	106
2	30 (36%)	29 (34%)	59
3	1 (1%)	4 (5%)	5
Type of leukoplakia			
Homogeneous	60 (71%)	63 (73%)	123
Nonhomogeneous	24 (29%)	23 (27%)	47
Leukoplakia histology			
Hyperparakeratosis	82 (97%)	83 (96%)	165
Moderate dysplasia	2 (3%)	3 (4%)	5

Study design

The study design was described in detail elsewhere.[20,21] Briefly, eligible patients were <75 years old; operated on for a previously untreated homogeneous or nonhomogeneous oral leukoplakia, with postoperative histology that did not include *in situ,* mini-invasive or invasive carcinoma; with normal white cell, erythrocyte and platelet counts; and metabolic, renal and liver function tests within 1.5 times upper normal limits.[22,23]

Patients were randomized to either no treatment or 4-HPR orally at a dose of 200 mg/day (2 capsules of 100 mg) for 1 year. To allocate a patient, the investigator called the data manager at the Milan coordinating center, who checked inclusion and exclusion criteria and allocated to one of the groups using a randomization list stratified by center. The investigator could not know the allocation previously.[20]

Placebo was not given to controls because of the long duration of the intervention, large size of the capsules and objective nature of the main end points (recurrences and new localizations of oral leukoplakias and oral carcinomas). Patients were instructed to take one capsule with the midday meal and one with the evening meal, to ensure optimal absorption.[24] However, because fenretinide lowers plasma retinol levels,[22,25] a 3-day drug holiday at the end of each month was prescribed to minimize diminished adaptation to darkness.

Intervention

Therapy began on the day of randomization. Patients randomized to intervention were given sufficient capsules to last until the next checkup. Treatment continued for a year or until the appearance of an end point or adverse reaction. In the event of mild toxicity,[20] the dose was reduced by 50%. If moderate toxicity occurred, treatment was discontinued and could be restarted at 50% of the original dose after recovery. Treatment was permanently withdrawn if severe toxicity or an adverse reaction occurred.[20]

All patients were followed identically. There were 2 parts to each follow-up examination. First, a head-and-neck surgeon blinded to treatment allocation performed a clinical examination, checking for new lesions and recurrences. Second, the patient was interviewed by the person who performed the randomization, to check for side effects, ascertain compliance and review metabolic, liver and renal function test results. When toxicity occurred, patients were checked at monthly intervals. All suspect lesions were photographed and biopsied. Leukoplakias developing >2 cm from the first were considered new localizations. Time of appearance was calculated from date of randomization. Patients with local relapses and new localizations received CO_2 laser resection (same as initial treatment). If carcinoma developed, treatment was according to established procedures at each center. Patients were checked every 2, 3 and 4 months during the first, second and third years, respectively, and every 6 months thereafter.

Statistical analysis

Data on a consecutive series of patients surgically treated for an oral leukoplakia before the trial indicated that the chance of developing a carcinoma within 4 years of surgery was 5.3%.[2] The original trial was designed to detect a 5% difference between the 2 arms in the incidence of oral cavity carcinomas over 4 years and a 15% difference in the incidence of recurrences or new leukoplakias over 3 years ($\alpha = 5\%$, $\beta = 20\%$, 2-tailed test), assuming proportional hazards.[26] The sample size required was 300 patients.

However, the trial was stopped prematurely as recruitment had slowed to almost zero. It was not possible to perform the planned statistical analyses. Particularly because of the low number of oral cavity carcinomas, we examined only the first unfavorable event, evaluating disease-free survival with the end points cancer (anywhere) and new or recurrent leukoplakia and relapse-free survival (end point new or recurrent leukoplakia). These approaches—and their results—must therefore be considered as explorative.

Disease-free survival curves were estimated by the Kaplan-Meier method. The effect of the treatment, evaluated according to the intention-to-treat principle, was assessed by the Cox regression model. Follow-up was from date of randomization and curtailed at 5 years since patients did not attend checkups regularly after this point. The proportional hazards assumption was investigated by examining plots of Schoenfeld residuals.[27] When plots of residuals had a nonlinear pattern, we added a time-dependent term to the Cox model based on a 3-knot restricted cubic spline time function.[28] In the latter case, the additional contribution of the nonlinear term for time was evaluated by the likelihood ratio test. Plots of estimated hazards (with corresponding confidence intervals, CIs) as a function of follow-up were used to explore the effect of treatment.

For relapse-free survival, crude cumulative incidence curves were estimated by a competing risks approach.[29] The effect of treatment was tested by a semiparametric regression model based on subdistribution hazards;[30] the proportional hazards assumption was investigated in the same way as for disease-free survival. The effect of treatment was also evaluated, adjusting for patient age, and included in the regression models as a continuous variable.

Results

A total of 316 patients were operated on for oral leukoplakia at the study centers from November 1988 to study closure in December 1998. Only 174 were randomized: 85 to 4-HPR and 89 to no treatment. There were 4 protocol violations (3 had abnormal laboratory tests prior to recruitment and one had previous breast cancer); thus, there were 170 evaluable patients: 84 treated, 86 controls. Table I shows the distribution of risk factors (age, sex, smoking, drinking and leukoplakia histology) in the 2 arms. Histology was overwhelmingly hyperparakeratosis, moderate dysplasia being present in 3–4% of cases. The arms were well balanced for all factors except age.

Compliance was assessed as number of pills taken over number envisaged by the protocol: 62/84 (73.8%) had a compliance of ≥80%; 13/84 (15.5%) had a compliance of <50%. Forty-three patients completed treatment at full dosage (most with compliance ≥90%); 9/43 patients had mild side effects that did not necessitate withdrawal from the study. Forty-one patients withdrew, 14 for

TABLE II – FIRST UNFAVORABLE EVENTS

	Total	Year			
		1	2	3–5	>5
Recurrence/new leukoplakia	44	23	6	12	3
4-HPR	19	7	2	9	1
Control	25	16	4	3	2
Cancers (total)	5	1	1	1	2
4-HPR	2	0	0	0	2[1]
Control	3	1[2]	1[3]	1[4]	0

[1]One of prostate and one of oral cavity.–[2]Lung.–[3]Tonsil.–[4]Oral cavity.

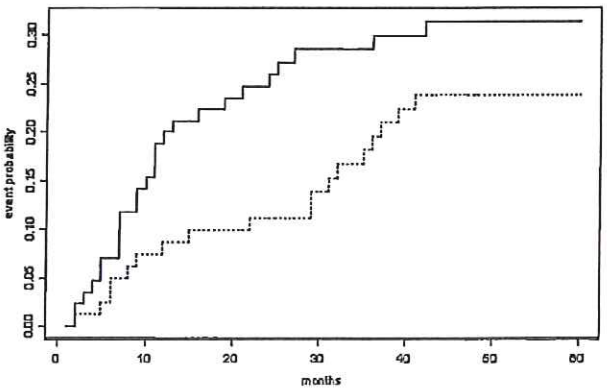

FIGURE 1 – First-event incidence curves after randomization for the 4-HPR arm (dotted line) and control arm (solid line). There were 23 first events in controls and 18 in the 4-HPR group during the first 5 years. Events thereafter are not included.

treatment-related side effects: hematologic toxicity in 7, cutaneous toxicity in 6 and gastric toxicity in one. Hematologic toxicity was moderately increased blood triglycerides and γ-glutamyltransferase in 4 patients, increased triglycerides in association with cardiac problems (one myocardial infarction) in 2 and moderately high bilirubin in one. Skin toxicity was dermatitis (1 mild, 3 severe cases) and skin dryness (2 cases). The frequency of abnormal laboratory results was similar in the 2 groups; dermatitis and skin/mucosal dryness occurred only in the treatment group.

Median follow-up was 78 months; 116 patients were followed regularly for over 5 years; 8/170 patients died, all of non-neoplastic causes; 8/170 patients were lost to follow-up, while 38 refused to continue presenting for checkups 2–3 years after the end of the intervention but agreed we could phone them periodically for up to 5 years from randomization. Two of these patients developed oral cancer and were treated.

First unfavorable events are shown in Table II. Thirty patients had leukoplakia recurrences: 15 in the control group (12 in the first year, 2 at 2–5 years, one after 5 years) and 15 in the 4-HPR group (6 in the first year, 8 at 2–5 years, one after 5 years). Fourteen patients had new leukoplakias: 10 in the control group (4 in the first year, 5 at 2–5 years, one after 5 years) and 4 in the 4-HPR group (one in the first year, 3 at 2–5 years, none after 5 years). Five patients developed primary cancers: 3 in the oral cavity (one in the 4-HPR group at 103 months and 2 in the control group at 24 and 42 months), one lung cancer after 10 months in the control group and one prostate cancer at 81 months in the 4-HPR group. An additional 6 primary oral cavity cancers were observed as second events after leukoplakia relapse (at 29, 91 and 115 months in the 4-HPR group; at 46, 132 and 133 months in the control group).

Incidence curves for first unfavorable event based on 5-year follow-up (after which follow-up was no longer systematic) are shown in Figure 1. The estimated incidence of first unfavorable events was greater in the control than the 4-HPR arm, but the difference decreased with follow-up. Residuals analysis on the Cox model suggested a nonlinear, time-dependent effect of the treatment; however, the contribution of this nonlinear term was not significant ($p = 0.35$). The coefficient of the linear, time-dependent effect in the model was significantly different from 0 (Table III), and this was retained in the model. From the point estimates of hazard ratios (HRs) obtained from the time-dependent Cox model of first unfavorable events, 4-HPR had a protective effect for about 25 months from randomization, when the reference value (HR = 1) was crossed. However, the 95% CIs, which were dependent on sample size, did not include the reference value until about 11 months of follow-up. The results were closely similar when patient age was included in the regression model. Age-adjusted regression coefficient estimates and respective p values were –1.3470 ($p = 0.025$) for 4-HPR *vs.* control and 0.0559 ($p = 0.043$) for the time-dependent effect.

The estimated crude cumulative incidence of local recurrences and new leukoplakias was greater in controls than in the 4-HPR arm, but again the difference decreased with the length of follow-up. Residuals analysis suggested a nonlinear, time-dependent effect of treatment; but the contribution of the nonlinear term in the Fine and Gray regression model was not significant ($p = 0.14$) and was not retained in the model. The coefficient of the linear, time-dependent effect was significantly different from 0 (Table IV) and was retained.

Estimates of the subdistribution hazards from the Fine and Gray regression model indicated a protective effect of 4-HPR on crude cumulative incidence lasting for 19 months after randomization; however, the confidence limits included reference at 7 months of follow-up and beyond. The results were closely similar when patient age was included in the Fine and Gray regression model. Age-adjusted estimated regression coefficients and p values were –1.4610 ($p = 0.018$) for 4-HPR *vs.* control and 0.0744 ($p = 0.011$) for the time-dependent effect.

Discussion

This appears to be the only randomized trial on adjuvant chemoprevention in oral leukoplakias, which are considered precancerous lesions.[1,2,31] Our findings suggest that fenretinide administered for 1 year after surgery for oral leukoplakia is able to prevent recurrences and new occurrences. Although we registered 316 patients, we were unable to randomize the planned 300 over 4 years because of ineligibility (109 patients) and a sharp decrease in the number of patients with leukoplakia presenting for treatment. We are unsure of the reasons for this decrease. Possibilities are greater use of laser devices in private dental clinics to remove lesions and greater use of retinoids and vitamin A–containing dietary supplements. Preparations containing retinoids (and labeled as protective agents) have been available in Italian pharmacies and supermarkets since the early 1990s, though the doses are considerably lower than those prescribed to our treated patients.[32–34]

The modest age imbalance between the 2 arms affected only those <55 years and occurred because the trial was closed prematurely. Imbalances manifesting during recruitment usually resolve as the study proceeds to completion but can be corrected if necessary in the final stages. For the age group at greatest risk (>55 years), the 2 arms were age-balanced. Statistical analysis with age adjustment produced results closely similar to those obtained without age adjustment.

Attendance for checkups during the treatment year was good but fell off thereafter. We kept in telephone contact with the 38 patients who refused to continue checkups, resulting in 2 cases of oral cancer being diagnosed and treated. Smoking and drinking habits did not change.

The intervention was well tolerated in that side effects requiring treatment discontinuation occurred in only 14 patients. Two of

TABLE III – RESULTS OF COX REGRESSION ANALYSIS OF FIRST UNFAVORABLE EVENT (RECURRENCE, NEW LEUKOPLAKIA, CANCER)

	b[1]	SE	Wald[2]	*p*
4-HPR *vs.* control	−1.3700	0.5975	5.2573	0.0219
Time-dependent effect	0.05434	0.0274	3.9331	0.0473

[1]Regression coefficient estimate.–[2]Wald statistic.

TABLE IV – RESULTS OF FINE AND GRAY REGRESSION MODEL OF RELAPSE (EVENTS: LOCAL RECURRENCE, NEW LOCALIZATION)

	b[1]	SE	Wald[2]	*p*
4-HPR *vs.* control	−1.4830	0.6158	5.7997	0.0160
Time-dependent effect	0.0730	0.0288	6.4373	0.0112

[1]Regression coefficient estimate.–[2]Wald statistic.

these had high blood triglycerides and cardiopathy (one myocardial infarction) during the intervention, not considered related to retinoid consumption by a cardiologist. However, only 43/84 patients completed the 1-year treatment.

No patient complained of vision problems, whereas in other 4-HPR interventions to prevent breast and bladder cancer, dark adaptation problems were reported fairly frequently.[25,35–37]

Three previous studies used retinoids to prevent second primaries in patients treated for head-and-neck cancer.[16,18,19] Hong *et al.*[16] studied 103 patients who took isotretinoin for 1 year and found a significant reduction in the occurrence of new carcinomas but no difference in occurrence of relapses or metastases. Bolla *et al.*[18] performed a multicenter study on 316 patients treated for head-and-neck cancer randomized to etretinate for 24 months *vs.* no preventive treatment. No differences between the 2 arms were found. The EUROSCAN trial tested the efficacy of retinol palmitate and *N*-acetylcysteine in a multicenter study on 2,595 people treated for stage I/II head-and-neck or lung cancer; no difference between the arms was found.[19]

Previous studies on retinoids as primary chemopreventives (to reverse oral leukoplakias) showed that the protective effect persisted while the drug was being taken but that leukoplakias recurred when it was stopped.[11–15] How long the effect lasted after drug withdrawal was not assessed. We found that the protective effect of 4-HPR against recurrences and new leukoplakias lasted for 7 months after drug withdrawal, though CIs were dependent on the size of the sample, both because of the original sample size and the fact that it reduced with follow-up. Formelli *et al.*[37] found that 4-HPR and its metabolite 4-MPR were still present (at the limits of detection) 12 months after withdrawal in a chemoprevention study but were not detected 24 months after withdrawal. This persistence might explain the prolonged protective effect.

Three patients developed oral cancer as a first event: one in the 4-HPR group >5 years after randomization and 2 in the control group <5 years after randomization. Six other oral cavity cancers occurred, all after a first event (leukoplakia recurrence). The low number of cancers is related to the low number of patients recruited following premature closure of accrual. The study is therefore underpowered and provides no indication as to whether 4-HPR protects against oral cancers in this high-risk population.

The incidence of oral cancer in our series over the 5 years of follow-up appears lower than our previous series might suggest.[2] However, we assessed the overall cancer incidence in the previous series irrespective of whether the cancer manifested as a first or second/subsequent event after leukoplakia treatment; in the present study, we considered only first-event cancers. Indeed, proportionately more cancers developed in the present than the earlier series, but the observation period was longer (>5 *vs.* 3 years). The distribution of lesion histology did not differ between the present and previous series: in both, hyperparakeratosis was by far the most common histotype.

Although our study is inconclusive regarding its main end point (a protective effect of 4-HPR against oral cancer), we feel that further studies on patients at risk for cancer are justified, *e.g.*, those successfully treated for head-and-neck cancer. Modification of retinoid administration schedules may improve effectiveness. For example, taking pills for 12 months and then alternating with 6-month drug holidays with 6 months of administration could allow administration for longer periods while reducing side effects. Furthermore, biologic and molecular assessment[38–40]—not feasible in the 1980s when the study was designed—may provide useful intermediate end points for evaluating new potential cancer prevention drugs and intervention schedules over shorter periods.

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