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Prevention of Local Relapses and New Localisations of Oral Leukoplakias with the Synthetic Retinoid Fenretinide (4-HPR). Preliminary Results

F. Chiesa, N. Tradati, M. Marazza, N. Rossi, P. Boracchi, L. Mariani, M. Clerici, F. Formelli, L. Barzan, A. Carrassi, A. Pastorini, T. Camerini, R. Giardini, S. Zurrida, F.L. Minn, A. Costa, G. De Palo and U. Veronesi

This paper analyses preliminary results of a randomised chemoprevention trial in patients surgically treated for oral leukoplakia started in 1988 at the Istituto Nazionale Tumori of Milan with the synthetic retinoid *N*-(4-hydroxyphenyl)-retinamide (4-HPR). To date 115 patients have been randomised, after surgical excision of oral leukoplakia, to receive 200 mg 4-HPR daily for 52 weeks versus no intervention. 80 patients completed the 1-year intervention, 41 in the control group and 39 in the 4-HPR group. During this period 12 local relapses or new lesions occurred in the control group and three in the 4-HPR group. Only 5 patients interrupted the intervention because of toxicity. No impaired dark adaptation was observed. It is concluded that 4-HPR is well tolerated and seems efficacious in preventing relapses and new localisations during the treatment period. This promising trend needs further confirmation.
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INTRODUCTION

ORAL LEUKOPLAKIA is a mucosal disease with a high canceration rate—estimated to range between 0% and 20% over 20 years [1–8]. Surgical removal is considered the best therapy [9–11]. However many patients operated on for oral leukoplakia later develop local relapses, new leukoplakias or squamous cell carcinomas. This finding is consistent with the field cancerisation concept introduced by Slaughter in 1953 for head and neck cancers: a whole tissue region repeatedly exposed to carcinogenic insult (tobacco, alcohol) is at increased risk for developing multiple independent foci of malignant lesions [12]. These considerations justify chemopreventive trials and the accessibility to the oral cavity allows convenient histological, photographic and size evaluation to assess intervention efficacy.

Over the past 10 years several studies have indicated that vitamin A and its derivatives are effective in the treatment of oral leukoplakias [13–18], although the mechanisms of this action are not completely understood. These compounds have also been shown to be effective in modulating the growth of premalignant cells and suppressing their progression to neoplasia [19, 20] and in a recent randomised study it was shown that 13-*cis*-retinoic acid is efficacious in preventing the development of second primary tumours in patients disease-free after local therapy for squamous cell carcinoma of the head and neck [21].

In general therapeutic doses of these retinoids cause severe side effects (skin dryness, cheilitis, hypertriglyceridemia and conjunctivitis) and many patients are unable to continue therapy [14, 16, 19]. 4-HPR has proved to be safer and less teratogenic than other retinoids and is effective in preventing tumours in various organs in rodents [22, 23].

In 1988 a randomised chemoprevention trial began at the Istituto Nazionale Tumori of Milan (INT). Its purpose was to evaluate, after 3 years, the effectiveness of 4-HPR in preventing relapses and new localisations and, after 4 years, in preventing carcinomas in patients surgically treated for oral leukoplakias. Recent work has shown, however, that retinoids are effective during the assumption period [14, 15, 17, 18] and it seems resonable to expect that their efficacy in preventing recurrences and new localisations would be evident during the treatment period of this trial. This paper therefore analyses preliminary results on relapses, new leukoplakias and complaints in 80 patients who have completed 1 year of the study.

PATIENTS AND METHODS

The study began at the INT in September 1988; in January 1989 the Odontostomatology Dept of the University of Milan, S. Paolo Hospital, joined the study, and in January 1990 the ENT Departments of the Aosta and of the Pordenone

Correspondence to F. Chiesa, N. Tradati, M. Marazza, N. Rossi, P. Boracchi, L. Mariani, M. Clerici, F. Formelli, T. Camerini, R. Giardini, S. Zurrida, A. Costa, G. De Palo and U. Veronesi are at the Head and Neck Surgical Oncology Department, Istituto Nazionale Tumori, Via Venezian 1, 20133 Milano, Italy; L. Barzan is at the ENT Department, Pordenone Hospital; A. Carrassi is at the Oral Pathology Department, University of Milano; A. Pastorini is at the ENT Department, Aosta Hospital, Italy; and F.L. Minn is at the Johnson Pharmaceutical Research Institute, Spring House, Pennsylvania, U.S.A.
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Hospitals also joined. The study protocol is approved by each Institution's Scientific and Ethical Committee and written informed consent is obtained from all patients. Study design is reported in Fig. 1.

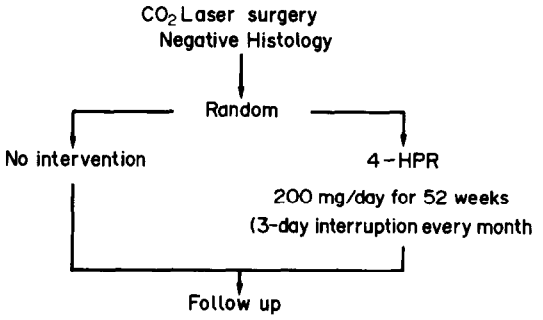

Fig. 1. Plan of the study.

Study population

Criteria for eligibility. Patients eligible for entry have been operated on for previously untreated homogeneous or non-homogeneous oral leukoplakias with benign post-operative histology. They must return metabolic, renal and liver function within 1.5 × upper normal limit and have normal white blood cell (WBC), red blood cell (RBC) and platelet counts [22, 23].

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

Oral leukoplakia classification. (a) Homogeneous leukoplakia: white patch, without infiltration, well defined margins; (b) non-homogeneous leukoplakia; red and white patch without infiltration, sometimes ulcerated, margins not well-defined.

Diagnostic procedure includes: (a) photograph of lesion; (b) careful oral examination and dental map. Where the lesion is apparently related to badly-fitted dentures or broken teeth, patients are advised to seek dental treatment; (c) suspicious lesions and areas staining with toluidine blue are biopsied; (d) metabolic, liver function and renal function tests; WBC, RBC and platelet count; chest X-ray.

Treatment

Surgical treatment. The lesions are stained with toluidine blue before surgery to show up their margins. Laser resection is performed under local anaesthesia using the laser in 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) in normal tissue, thus a specimen is available for histopathological examination. The wound is left open; patients are checked weekly until complete re-epitelialisation.

Drug intake. Therapy begins on the day of randomisation. Patients randomised 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 from the drug at the end of each month is prescribed for all patients to avoid adverse effects possibly related to prolonged lowering of serum retinol [24]. Patients receive sufficient 4-HPR capsules to last to the next check-up and are advised to take the capsule after a meal (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 localisation or carcinoma, or occurrence of adverse reactions as specified below. In the event of mild toxicity, the dose is reduced by 50% of the original dose after recovery. Therapy must be permanently suspended whenever severe toxicity or adverse reaction after rechallenge occurs.

Assessment

All patients are checked every 2 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 who does not know to which arm of the study the patient has been randomised. New lesions separated from the first-treated leukoplakias by more than 2 cm are considered new localisations [25]. Time of appearance is calculated from the date of randomisation. Patients with local relapses and new localisations are treated again by CO_2 laser exeresis. If squamous cell carcinoma develops patients are treated according to established INT therapeutic procedure. Control group patients are followed in the same way as those of the 4-HPR group. Patients completing the one-year study will be checked every 3 months during the subsequent year, every 4 months the year after and subsequently every 6 months. Also evaluated are plasma levels of retinol at baseline and of retinol, 4-HPR and its metabolite *N*-(4-methoxyphenyl)-retinamide 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 [24] and these data will be related to outcome of disease, drug toxicity and activity. The study lasts for 5 years.

Toxicity [22, 23] is evaluated on the basis of subjective and objective symptoms and by assessment of blood parameters: bilirubin, cholesterol, triglycerides, Gamma GT, GOT and GPT. Mild toxicity is defined as increase in laboratory parameters by 1.5–2 times the upper normal limit as notified by the laboratory. 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

From data on patients consecutively treated by surgery before the trial, the chance of developing a carcinoma within 4 years from surgery is estimated as 5.33%. A trial involving about 300 patients (randomised to 4-HPR or no intervention) will be able to detect a difference of 5% in the probability of new carcinomas after 4 years ($\alpha = 5\%$, $\beta = 20\%$, 2-tailed test) according to the Friedman method [26]. In a previous study [8] we found that the chance of developing relapses and new localisations at 3 years in operated patients was 40%. Thus, recruiting 300 patients will allow detection of at least a 15% difference, between the two arms of the trial at 3 years, in the probability of developing these events ($\alpha = 5\%$, $\beta = 20\%$, 2-tailed test). Previous INT experience shows that the probability of developing relapses and new localisations within 1 year of surgery is 23%. Assuming that the 15% difference between the two arms is concentrated in the first year after surgery, 190 patients will be needed to detect this difference ($\alpha = 5\%$, $\beta = 20\%$, 2-tailed test).

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 (I) carcinoma-free period within 4 years; (II) relapse- and new localisation-free period within 3 years (RFS); (III) relapse and new localisation-free period during the therapy period (1 year) will be performed by the Log-rank test [27]. There is no "*a priori*" stratification and 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 [28].

Randomisation

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 randomised by calling the Data Center. To ensure a good balance between the number of patients in the two arms, a permutated blocks randomisation list was prepared for each participating centre. All randomised patients are urged to improve oral hygiene, have dental treatment if necessary, stop drinking alcohol and stop smoking tobacco.

RESULTS

115 patients have entered the study to date. 80 were operated on at least 1 year ago: their characteristics are shown in Table 1, according to intervention and control group; the two groups are well-balanced for all considered factors except age, being slightly imbalanced in the <45 and 56–65 classes.

With regard to retinol plasma levels, blood samples were available from 44 patients at baseline and after 1 year. Mean retinol plasma concentrations in control and 4-HPR patients were similar at baseline, levels in males were higher than in females in both groups. No change in control subject retinol levels was observed after 1 year. Retinol levels in the 4-HPR groups were reduced by 64% and 46% of baseline in males and females, respectively, as noted previously in breast cancer patients [24] (Table 2).

The two arms remain well-balanced with regard to follow up (Table 3) and the majority of patients are regularly checked. 2 patients have died; 1 from lung cancer diagnosed 10 months after randomisation, the other from a ruptured oesophageal varicosity 15 months after randomisation.

Drug intake in 4-HPR patients is shown in Table 4; 20/39 patients finished the 1-year intervention without drug reduction or interruption. 3 patients refused to continue taking the drug and 3 permanently stopped drug assumption because of severe intercurrent disease (myocardial infarction), local relapse and new leukoplakia, respectively. No impaired dark adaptation was observed.

Table 1. Characteristics of 80 patients who completed intervention

	Control (41 patients) *n* (%)	4-HPR (39 patients) *n* (%)	Total (80 patients) *n* (%)
Sex			
Males	30 (73.2)	28 (71.8)	58 (72.5)
Females	11 (26.8)	11 (28.2)	22 (27.5)
Age			
<45	6 (14.6)	13 (33.3)	19 (23.8)
46–55	18 (43.9)	13 (33.3)	31 (38.8)
56–65	13 (31.7)	6 (15.4)	19 (23.7)
66–75	4 (9.8)	7 (18.0)	11 (13.7)
Smokers			
No	10 (24.4)	9 (23.1)	19 (23.8)
Yes	21 (51.2)	17 (43.6)	38 (47.5)
Ex	10 (24.4)	13 (33.3)	23 (28.7)
Drinkers			
No	7 (17.1)	10 (25.6)	17 (21.3)
Yes	31 (65.6)	26 (66.7)	57 (71.2)
Ex	3 (7.3)	3 (7.7)	6 (7.5)
Type of lesion			
Homogeneous	31 (75.6)	25 (64.1)	56 (70.0)
Non-homogeneous	10 (24.4)	14 (35.6)	24 (30.0)
Number of lesions			
1	26 (63.4)	27 (69.2)	53 (66.3)
>1	15 (36.6)	12 (30.8)	27 (33.7)

Table 2. Plasma retinol concentrations [ng/ml (SD)] according to study group

	Control			4-HPR	
	n	Baseline	After 1 year	*n*	Baseline	After 1 year
Males	15	715 (141.12)	692 (167.29)	14	617 (147.94)	225 (141.06)
Females	8	615 (50.85)	605 (95.85)	7	545 (75.53)	294 (146.87)

Table 3. Follow-up according to study group

	Control (41 patients)	4-HPR (39 patients)
Dead patients	2	—
Alive patients	39	39
Regular follow-up	35	33
Irregular follow-up*	3	4
Lost to follow-up	1	2

*Follow-up not as foreseen in protocol.

Table 4. Drug intake in treatment group

	n	*n*
One year treatment full dose		20
Dose reduction		5
Skin dryness	2	
Increase in triglycerides	1	
Increase in bilirubin	2	
Dose reduction—interruption		4
Dermatitis	1	
Increase in triglycerides—intercurrent disease	1	
Skin dryness—increase in bilirubin	1	
Dyspeptic syndrome	1	
Interruption		10
Refuse to continue	3	
Dermatitis	1	
Increase in triglycerides	1	
Increase in γ-GT	1	
Increase in triglycerides—γ-GT	1	
Intercurrent disease	1	
Unfavourable event	2	
Total		39

Table 5. Clinical signs and symptoms in 39 control patients and 39 4-HPR patients with at least one check

Variable	Group	*a*	*m*	*x*
Dermatological	CTR	2	214 (0.9%)	1
	4-HPR	11	224 (3.9%)	7
Dermatitis	CTR	0	214	0
	4-HPR	4	224	3
Skin/mucosal dryness	CTR	2	214	1
	4-HPR	7	224	4
Other (Gastralgia)	CTR	0	214 (0%)	0
	4-HPR	3	224 (1.3%)	3

a = number of signs and symptoms observed during all checks.
m = total number of checks performed.
x = number of patients with signs and symptoms.

Table 5 reports clinical signs and symptoms: in 214 checks there were two complaints of dermatological signs or symptoms in the control group (0.9%) versus 11/224 (4.9%) of the 4-HPR group. Dermatitis was only observed in the 4-HPR group. Gastralgia was noted three times (1.3%) in the 4-HPR group and 0/224 in the control group.

Table 6. Abnormal laboratory values in patients with at least one check

Variable	Group	*a*	*m*	*x*	*n*	*P*
BIL	CTR	1	199	1	38	
	4-HPR	12	179	4	33	0.125
GOT	CTR	0	199	0	38	
	4-HPR	1	181	1	33	0.351
GPT	CTR	9	199	3	38	
	4-HPR	2	181	2	33	0.722
GGT	CTR	18	195	9	37	
	4-HPR	19	178	5	33	0.348
CHOL	CTR	1	197	1	37	
	4-HPR	0	180	0	33	0.273
TRY	CTR	7	197	3	38	
	4-HPR	8	180	4	33	0.664

a = number of abnormal laboratory values.
m = number of assessments with the upper normal limit available.
n = total number of subjects considered.
x = number of subjects with abnormal values.
P = significance levels with Mantel–Haenszel test.

The blood levels of total bilirubin (BIL), glutamic oxaloacetic transaminase (GOT), glutamic pyruvic transminase (GPT), γ-glutamyl transpeptidase (GGT), total cholesterol (CHOL) and triglycerides (TRY) were recorded as abnormal when above 1.5 times the upper normal limit, and normal otherwise [22, 23]. Table 6 reports the number of abnormal values for data where the upper normal limit was provided by the laboratory, the number of patients with at least one abnormal result and the *P* values obtained by the Mantel–Haenszel test by which the frequency of patients with abnormal values was compared between groups, after stratifying by the number of assessments available for each patient [29].

Although not significant at the 5% level, the number of abnormal values of bilirubin was substantially higher in the 4-HPR group. However, 10 of the 12 abnormalities reported in

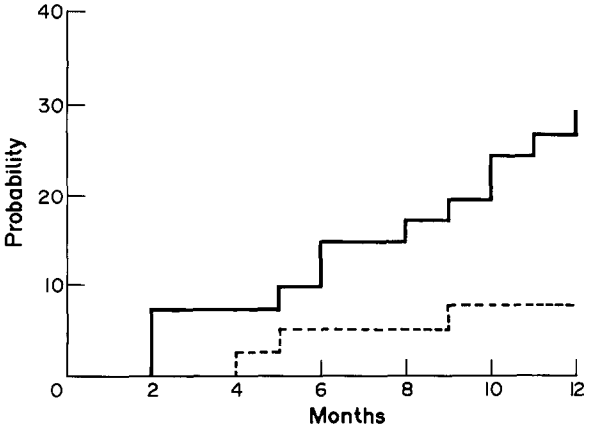

Fig. 2. Probability of developing local relapses and new leukoplakias. —— = control group, – – – = 4-HPR group.

Table 7. Local relapses and new localizations according to study group

	CTR (*n*)	4-HPR (*n*)
Local relapses	5/41	2/39
New localisations	7/41	1/39
Total	12/41	3/39

this group occurred in 2 subjects, both of whom suffered from Gilbert's syndrome (congenital hyperbilirubinemia) and hence were not related to the intervention.

Although not foreseen in the protocol, the present interim analysis was performed to compare recurrences and new localizations during the 1-year intervention in the two arms. The approach suggested by Lan and Wittes [30] was adopted to calculate the conditional power of rejecting the null hypothesis of no difference, in occurrence of relapses and new leukoplakias, between the two arms on completion of the study from results obtained at the time of the interim analysis and assuming that the data still to be gathered will follow the same pattern in the two arms (null trend).

For the total of 15 events reported on 80 patients by 29 February, 1992, log rank statistics gave a Z-value of 2.418 ($P=0.002$). The total expected events (by calculation according to sample size) was 30; therefore the observed events were half those expected. For the null trend the conditional probability of observing a final Z-value exceeding 1.96 (i.e. the probability of the null hypothesis of no difference in probability of recurrences and new leukoplakias between the two arms) was 35%. Thus, the result obtained on the data so far must be interpreted cautiously, since the possibility of the opposite result on the whole sample cannot be excluded. Figure 2 shows the probability of recurrences and new leukoplakias after 1-year in both groups. Failures are given in Table 7 according to intervention group; no oral carcinomas were observed and the groups differ in the number of new localisations.

DISCUSSION

The aim of this randomised study is to evaluate the efficacy of 4-HPR, given as adjuvant intervention after resection of oral leukoplakias, in preventing local relapses, new localisations and squamous cell carcinomas; the second end point is to evaluate the toxicity of the drug. The total study period is long (expected recruitment period 4 years; treatment period 1 year; follow-up 4 years; total = 9 years). Because of the high toxicity observed in similar studies employing retinoids [14, 16, 19], we have carried out this preliminary evaluation of toxicity and of efficacy to assess progress and the need for any design modifications.

Patients were seen every 2 months in the absence of side effects and every month when complaints were observed, thus detailed information on toxicity and failure was available. Studies mainly on women have shown that 4-HPR is less toxic than other synthetic retinoids and is well-tolerated by patients [22–24]. This study confirms those findings in males with oral leukoplakias and with impaired liver function. It is noteworthy that abnormal laboratory values of metabolic and liver function were registered in both groups. In the control group 8 patients had abnormal laboratory results, 1 patient registering very high γ-GT levels. 2 patients (1 in the 4-HPR group and 1 in the control group) suffered myocardial infarction during the treatment period. A total of 18/32 (54.3%) patients had some complaint during this time but only 5/32 (15.6%) did not complete the intervention because of toxicity. These findings are lower than those observed by other authors using synthetic retinoids (isotretinoin or etretinate) [14, 16, 19]. Statistical evaluation of toxicity, to be published elsewhere, shows no difference in blood parameters between the two groups. Although 3 patients in the 4-HPR group developed high blood triglyceride levels during the period, they returned similar values when rechecked 1 month after suspending 4-HPR. Furthermore, 2 patients in the 4-HPR group had Gilbert's disease, explaining the higher median bilirubin levels observed in this group. It may be concluded that 4-HPR is relatively well-tolerated and that dermatitis is the only side effect to be expected.

The preliminary oncological results of this study are encouraging. The difference between the two groups in terms of unfavourable events shows a promising trend in favour of 4-HPR intervention particularly for the occurrence of new leukoplakias. This trend is consistent with the hypothesis that 4-HPR is useful in preventing mucosal abnormalities and supports Hong's observation of a significant reduction in the occurrence of new carcinomas but no difference in the development of relapses and metastases in treated carcinomas [21]. These results are only preliminary however and there is a 35% probability that the null hypothesis of equivalence between the two arms will be respected at the end of the study. These data refer to patients after 1 year: it is our experience that carcinomas are to be expected after the second year of follow-up. If, however, this trend is confirmed on 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.

It is concluded that the study should continue as initially designed and that additional centres should be involved in order to complete recruitment as quickly as possible.

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