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ORIGINAL ARTICLE

Oral lichen planus treated with 13-*cis*-retinoic acid (isotretinoin): effects on the apoptotic process

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Abstract The aim of the present study is to verify the efficacy of isotretinoin in oral lichen planus (OLP). In a double-blind study, ten patients with biopsy-proven OLP were treated for 4 months with 0.1% isotretinoin gel and another ten patients with placebo. At the end of the first period of observation, the patients who had been given the placebo were given isotretinoin for a further 4 months. A complete response was defined as the disappearance of the lesions as assessed by inspection, whereas a partial response was defined as a 50% or more reduction in the size of the lesions. All patients treated with isotretinoin showed a significant improvement of the oral lesions, whereas in the patients who were given the placebo, the size of the lesions remained the same. The patients who were given isotretinoin after the placebo showed a reduction in lesions. In total, there were ten complete and ten partial responses. Lesions were analysed histologically and immunohistochemically with antibodies against bcl-2 and Ki-67. Ki-67 and bcl-2 have statistical significant increased values from before to after treatment, whereas apoptotic bodies decreased one. All these facts could have contributed to the partial or complete regression of OLP lesions. The increase in Ki-67 positive cells show that the epithelium requires for enhanced proliferation and healing. The present results revealed a disturbed cell death programme in OLP that could underline an abnormal epithelial differentiation. The results of this pilot study show that the topical use of isotretinoin is effective in treating OLP.

Keywords Apoptosis · bcl-2 · 13-*cis*-retinoic acid · Isotretinoin · Ki-67 · Oral lichen planus

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Introduction

Oral lichen planus (OLP) is a disease of middle age with a prevalence of 0.5–2% in the general population that affects men and women in nearly equal numbers, children being rarely affected [13]. Several types of OLP have been described: The most common type is the reticular form, involving the buccal mucosa with symmetrical lesions, characterised by numerous interlacing white keratotic lines or striae (so-called Wickham's striae) and few symptoms. The plaque form of lichen planus resembles leukoplakia but has a multifocal distribution. The atrophic form presents as red patches with fine white striae, causing burning and discomfort, and often associated with reticular or erosive variants. In the erosive form, the central area of the lesion is ulcerated and a fibrinous plaque or pseudomembrane covers the ulcer. Keratotic striae, peripheral to the site of

erosion, and erythema are usually present, and the pattern of involvement may change from week to week. The bullous variant is rare, with bullae or vesicles, usually in the buccal mucosa, ranging from a few millimetres to centimetres in diameter, generally short lived and extremely uncomfortable.

Recently, topical therapy with 13-*cis*-retinoic acid (isotretinoin) has been shown to be effective in OLP, especially in the active atrophic–erosive forms [15]. 13-*cis*-Retinoic acid (isotretinoin) induces also remission of oral leukoplakia, prevents the development of cancer in patients with leukoplakia and inhibits the development of second primary tumours in patients with previous head and neck cancer [10, 16, 21]. As retinoid, the drug has an important role in regulating cellular proliferation and differentiation of the epithelium [6], by inhibiting growth and inducing cell differentiation in many normal and neoplastic types of cell, even if the specific mechanism of this action is not completely understood [6, 10, 16, 21]. OLP is a lymphocyte-mediated immunological disorder in which the basal cells seem to be targeted by T lymphocytes [5, 7, 8, 17, 19, 22, 23]. Different degrees of increased epithelial proliferation, apoptosis and expression of major histocompatibility complex class II antigen human leucocyte antigen-DR and decreased epithelial thickness have been observed in OLP [1, 7]. It has been suggested that the death of basal cells in OLP may be to the result of apoptosis [2, 17].

Our aims in the present study were to evaluate the effects of topical application of an isotretinoin gel in patients with OLP and to study whether the therapy induces changes of cell proliferation and apoptosis by analysing the Ki-67 and bcl-2 immunoreactivity and the presence of apoptotic bodies in tissues to highlight the mechanisms of action of the retinoids in this disease.

Materials and methods

Twenty patients (ten men and ten women) with biopsy-proven OLP were enrolled in the present study (Table 1). The protocol was approved by the Ethics Committee of the University of Chieti–Pescara. Informed consent was

Table 1 Clinical data of the 20 patients with oral lichen planus enrolled in this study and outcome after the topical treatment with isotretinoin gel

Patient	Age (years)	Sex (M/F)	Site	Clinical aspect	Mean duration of lesions	Symptoms	4 Months follow-up	3 Years follow-up
1	44	F	Tongue	Atrophic	~3 months	Burning; pain	Improved	Healed
2	64	M	Cheek and skin)	Reticular	~1 year	Burning	Healed	Healed
3	31	M	Vestibular mucosa	Reticular	~6 months	Asymptomatic	Healed	Healed
4	49	F	Cheek	Reticular	~1 year	Burning	Improved	Healed
5	40	F	Gingiva	Plaque	~6 months	Asymptomatic	Improved	Relapse
6	36	M	Cheek	Plaque	~6 months	Asymptomatic	Improved	Relapse
7	69	F	Tongue	Plaque	~1 year	Asymptomatic	Improved	Relapse
8	75	M	Tongue	Erosive	~1 year	Burning	Improved	Lost to follow-up
9	71	F	Cheek	Reticular	~1 year	Asymptomatic	Healed	Healed
10	38	M	Cheek	Reticular	~6 months	Asymptomatic	Healed	Healed
11	58	M	Tongue	Reticular	~1 year	Asymptomatic	Healed	Lost to follow-up
12	28	F	Cheek	Plaque	~6 months	Asymptomatic	Healed	Lost to follow-up
13	56	F	Cheek	Reticular	~1 year	Asymptomatic	Healed	Healed
14	37	F	Cheek	Reticular	~6 months	Burning	Healed	Healed
15	43	M	Gingiva	Reticular	~1 year	Asymptomatic	Healed	Healed
16	73	M	Cheek	Plaque	~1 year	Asymptomatic	Improved	Lost to follow-up
17	62	F	Tongue	Atrophic	~6 months	Burning	Improved	Relapse
18	70	F	Vestibular mucosa	Erosive	~6 months	Burning	Improved	Relapse
19	51	M	Tongue	Plaque	~6 months	Asymptomatic	Improved	Relapse
20	46	M	Cheek (and skin)	Reticular	~1 year	Asymptomatic	Healed	Lost to follow-up

obtained from all patients, and women of childbearing age were excluded. Any topical or systemic medication was stopped for at least 8 weeks before the start of our study. The isotretinoin used was supplied in soft capsules. An adhesive gel was prepared with carboxymethylcellulose, water, glycerol, methylhydrobenzoate and mint; ten capsules (10 mg each) were added to this gel to obtain a concentration of isotretinoin of 0.1%. The patients were divided into two groups of ten patients each, which were homogeneous for sex (five men and five women), site (five lesions in the cheek, three in the tongue, one in the vestibular mucosa, one in the gingiva) and in the clinical aspect (five reticular, three plaque, one atrophic, one erosive). Patients in one group were given topical 0.1% isotretinoin gel (Roaccutan, Roche) and the other group plain gel, which appeared identical to the active medication. Patients used the gel topically on the affected mucosal surfaces three times daily for 4 months. All patients were evaluated after 1, 2, 3 and 4 months, and at each visit, the lesions were photographed. Serum concentrations of cholesterol, tryglycerides, aspartate aminotransferase, alanine aminotransferase and alkaline phosphatase were measured before and during the treatment. At the end of 4 months, patients who had been given the placebo gel were switched to the active medication for 4 months. All 20 patients completed the treatment. A complete response was defined as the disappearance of the lesion as assessed by inspection, whereas a partial response was defined as a 50% or more reduction in the size of the lesions [21]. Three years from the end of the study, all 20 patients were recalled, but only 15 came to the clinic. Two biopsy specimens were taken from each patient, one before the beginning of the treatment and the other after 4 months. As controls, specimens retrieved during extraction of mandibular wisdom teeth were used. All specimens were routinely fixed in 10% buffered formalin for 24–48 h, dehydrated in graded

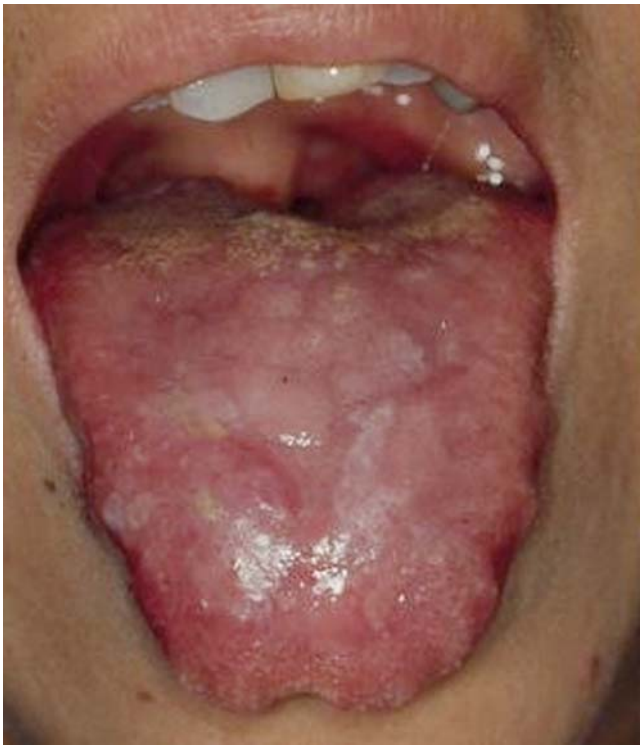

Fig. 2 After 4 months treatment with isotretinoin: A clinical improvement of the lesion is evident

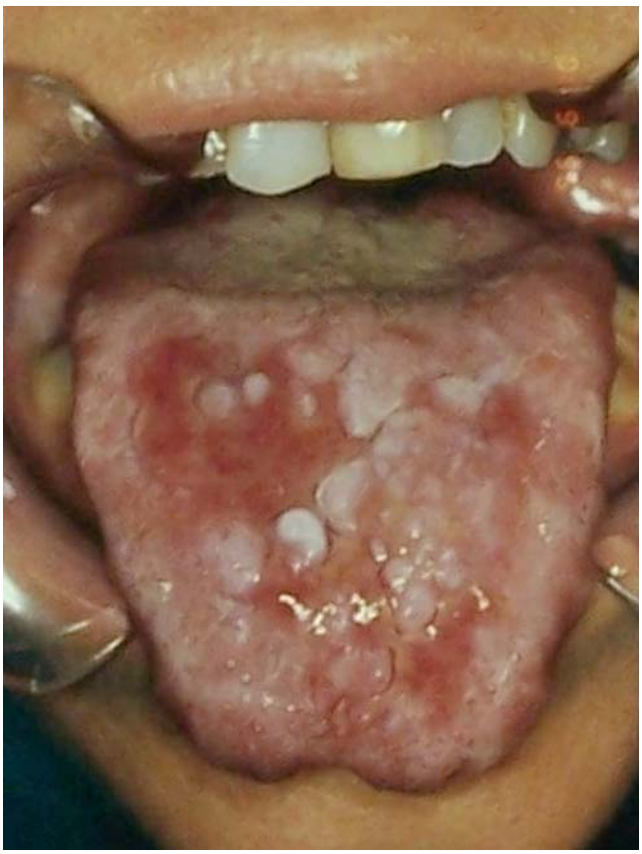

Fig. 1 Atrophic OLP of the tongue

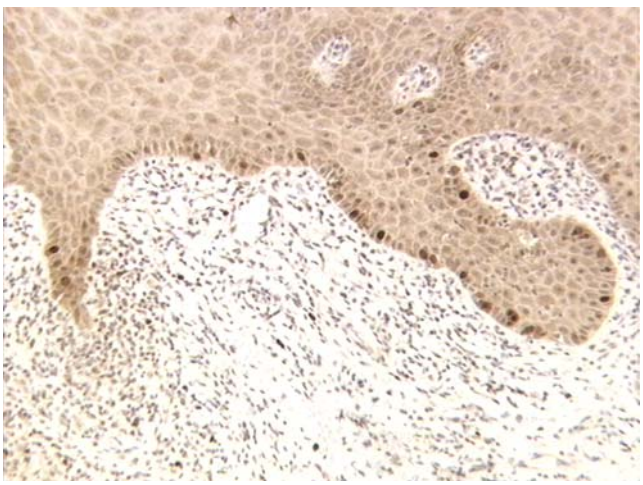

Fig. 3 Ki-67 expression before treatment showed staining in the basal layers (×200)

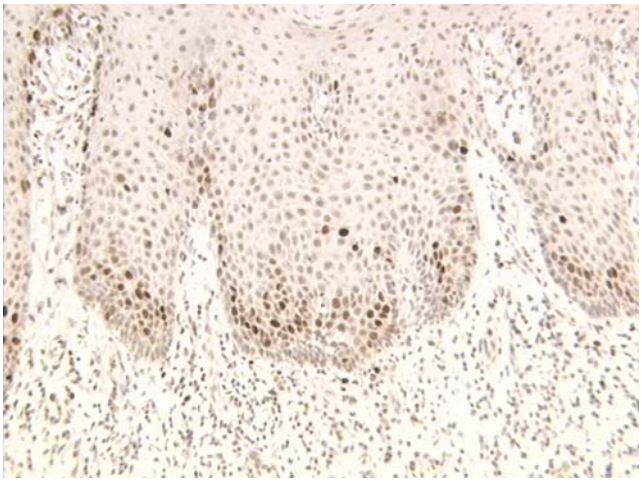

Fig. 4 Ki-67 expression after treatment; in all specimens, there was an increase in the Ki-67 positivity of basal and parabasal cells (×200)

alcohols, cleared in xylene and embedded in paraffin. Sections were stained with haematoxylin and eosin, and a selection of the slides was made for immunohistochemical staining for Ki-67 and bcl-2. The stained cells were evaluated from a minimum of 1,000 cells in each case. The cells were evaluated in the basal and parabasal layers.

The number of apoptotic bodies was recorded in a random fashion in the basal and parabasal layers in ten high power fields.

Results

All 20 patients completed the study. There were no alterations in serum concentrations of cholesterol, tryglycerides, aspartate aminotransferase, alanine aminotransferase and alkaline phosphatase levels. Of the ten patients given isotretinoin gel, four showed almost complete healing of the lesions, whereas the other six showed improvement of the lesions (Figs. 1 and 2). The patients given with placebo showed virtually no change. When the patients who had initially received the placebo were given isotretinoin gel for 4 months, in six cases, there was complete healing of the lesions, whereas the other four showed an improvement. At the 3-year follow-up, there was complete healing in 9 of the 15 patients (60%), whereas in 6 (40%), there had been a recurrence of the lesion. All the reticular types and one atrophic type had completely healed, whereas the plaque, erosive and one atrophic lesion had recurred. All the lesions that had improved showed a reduction in volume and a change in colour from white to pink.

Histologically, the specimens of patients who had been treated showed the presence of hyperkeratosis and an absence of parakeratosis.In normal healthy control mucosa, the mean value of the Ki-67 expression was 45.0% and of the bcl-2 expression was 6.0%, and the mean number of apoptotic bodies was 0.5/10 HPF. Immunohistochemical analysis showed a low staining for Ki-67 (Fig. 3) and bcl-2 in the basal layers, with some focal staining in parabasal cells in OLP biopsy specimens before treatment. After treatment, there was an increase in staining of basal and parabasal cells in all OLP specimens for Ki-67 (Fig. 4) and in 7 out of 20 OLP specimens for bcl-2 (Table 2). Only a few apoptotic bodies were seen in the specimens: A reduction in the number of apoptotic bodies, with fragmented and pyknotic nuclei and eosinophilic cytoplasm, was seen after treatment in 14 of 20 cases in the basal layers (Table 2). In only two samples were the apoptotic bodies in a parabasal or superficial location. In one case, a cell in the process of phagocytosing an apoptotic body was seen. Statistical analysis using Student's *t* test showed that the differences of Ki67 and bcl2 expression and of the number of apoptotic bodies between the two groups, before and after treatment, were significant (respectively, $p=0.029$; $p=0.045$; $p=0.0093$).

Discussion

OLP is generally considered to be an immunologically mediated process that microscopically resembles a hypersensitivity reaction. It is characterised by an intense infiltrate of T cells (cluster of differentiation 4+ [CD4+] and particularly CD8+ cells) located at the epithelial–connective tissue interface, but the factor that initiates this condition is unknown.

A balance between cell proliferation and cell death plays an important part in the homeostasis of normal tissues [6],

Table 2 Proliferative activity, bcl-2 expression and apoptotic index (A.I.) in oral lichen planus treated with isotretinoin

Clinical diagnosis	Mean Ki-67 before treatment	Mean Ki-67 after treatment	Mean Bcl-2 before treatment	Mean Bcl-2 after treatment	Mean A.I. before treatment	Mean A.I. after treatment
Reticular	18.6	40.4	6.2	8.2	2.0	1.0
Plaque	22.3	32.8	6.5	8.0	1.6	1.0
Atrophic	18.0	31.0	0.0	6.0	1.0	1.0
Erosive	19.0	33.0	0.0	0.0	1.0	0.0
Total cases	19.7	36.4	6.3	7.4	1.8	1.1

and a disarray of this balance is probably present in diseases like OLP. In normal squamous and columnar epithelium, bcl-2 immunoreactivity is seen in the basal cell layers [3, 14, 18, 20]. Bcl-2 has an important role in preserving stem cells of keratinocytes [4] and protects epithelial cells from death [11]. Its absence in the suprabasal layers shows that bcl-2 is not required for completion of the process of differentiation. In OLP, bcl-2 expression by the lymphocytes and macrophages of the infiltrate may contribute to the longevity of these inflammatory cells and, therefore, to chronicity of the lesion [2]. Apoptotic bodies have been found in the epithelial basal cell layers in OLP [2, 23]. However, other researchers have not reported increased apoptosis and have found only weak staining of bcl-2 in oral keratinocytes [7].

Our immunohistochemical data showed that, after a 4-month period of treatment with topical isotretinoin, there was a significant increase in the percentage of bcl-2 stained cells (from 6.3 to 7.4%). This was accompanied by a significant reduction in the number of apoptotic bodies. It could be important that, after treatment, there was also a significant increase in the number of Ki-67 positive cells [6]. We think therefore that the retinoid could have determined the partial or complete regression of the clinical OLP lesions through a restoration of cell proliferation and block of apoptosis in the squamous epithelium. The observed increase in Ki67 and bcl-2 expression could indicate that the healing process require an enhanced proliferation and a block of cell death programme [7].

In a study on cervical carcinoma cells [12], fenretinide [*N*-(4-hydroxyphenyl)retinamide (HPR)]-induced apoptosis was not mediated by suppression of bcl-2. It has been suggested that this synthetic retinoid HPR induces apoptosis by a mechanism that is independent of nuclear retinoid receptors [12]. It is possible that, with the local application of a substance, it is possible to obtain a higher concentration of the substance directly to the surface of the target tissue [9]. Moreover, the local application of 13-*cis*-retinoic acid avoided the side effects associated with oral treatment, as no patient reported any discomfort from the use of the drug.

In conclusion, the data obtained from this pilot study confirm that good results can be obtained in OLP with the topical use of 13-*cis*-retinoic acid.

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References

1. Da Silva Fonseca M, Do Carmo MAV (2001) Identification of the AgNORs, PCNA and CK16 proteins in oral lichen planus lesions. Oral Dis 7:344–348
2. Dekker NP, Lozada-Nur F, Lagenaur LA, MacPhail LA, Bloom CY, Regezi JA (1997) Apoptosis-associated markers in oral lichen planus. J Oral Pathol Med 26:170–175
3. Flohil CC, Janssen PA, Bosman FT (1996) Expression of bcl-2 protein in hyperplastic polyps, adenomas, and carcinomas of the colon. J Pathol 178:393–397
4. Harada H, Mitsuyasu T, Seta Y, Maruoka Y, Toyoshima K, Yasumoto S (1998) Overexpression of bcl-2 protein inhibits terminal differentiation of oral keratinocytes in vitro. J Oral Pathol Med 27:11–17
5. Hirota M, Ito T, Okudela K, Kawabe R, Yazawa T, Hayashi H, Nakatani Y, Fujita K, Kitamura H (2002) Cell proliferation activity and the expression of cell cycle regulatory proteins in oral lichen planus. J Oral Pathol Med 31:204–212
6. Kalemkerian GP, Ramrath N (1996) Retinoids and apoptosis in cancer therapy. Apoptosis 1:11–24
7. Karatsaidis A, Schreurs O, Helgeland K, Axell T, Schenk K (2003) Erythematous and reticular forms of oral lichen planus and oral lichenoid reactions differ in pathological features related to disease activity. J Oral Pathol Med 32:275–281
8. Kawamura E, Nakamura S, Sasaki M, Ohyama Y, Kadena T, Kumamaru W, Shirasuna K (2003) Accumulation of oligoclonal T cells in the infiltrating lymphocytes in oral lichen planus. J Oral Pathol Med 32:282–289
9. Lo Muzio L, della Valle A, Mignogna MD, Pannone G, Bucci P, Bucci E, Sciubba J (2001) The treatment of oral aphthous ulceration or erosive lichen planus with topical clobetasol propionate in three preparations: a clinical and pilot study on 54 patients. J Oral Pathol Med 30:611–617
10. Lotan R, Xu XC, Lippman SM, RO JY, Lee JS, Lee JJ, Hong WK (1995) Suppression of retinoic acid receptor-β in premalignant oral lesions and its up-regulation by isotretinoin. N Engl J Med 332:1405–1410
11. Lu QL, Abel P, Foster CS, Lalani EN (1996) Bcl-2 role in epithelial differentiation and oncogenesis. Hum Pathol 27:102–110
12. Oridate N, Suzuki S, Higuchi M, Mitchell MF, Ong WK, Lotan R (1997) Involvement of reactive oxygen species in *N*-(4-hydroxyphenyl) retinamide-induced apoptosis in cervical carcinoma cells. J Natl Cancer Inst 89:1191–1198
13. Petruzzi M, De Benedittis M, Grassi R, Cassano N, Vena G, Serpico R (2002) Oral lichen planus: a preliminary clinical study on treatment with tazarotene. Oral Dis 8:291–295
14. Ramsay JA, From L, Kahn HJ (1995) Bcl-2 protein expression in melanocytic neoplasms of the skin. Mod Pathol 8:150–154
15. Scardina GA, Messina P, Carini F, Maresi E (2006) A randomized trial assessing the effectiveness of different concentrations of isotretinoin in the management of lichen planus. Int J Oral Maxillofac Surg 35:67–71
16. Shalinsky DR, Bischoff ED, Gregory ML, Gottardis MM, Hayes JS, Lamph WW, Heyman RA, Shirley MA, Cooke TA, Davies PJ et al (1995) Retinoid induced suppression of squamous cell differentiation in human oral squamous cell carcinoma xenografts (line 1483) in athymic nude mice. Cancer Res 55:3183–3191
17. Sklavounou A, Chrysomali E, Scorilas A, Karameris A (2000) TNF-alpha expression and apoptosis-regulating proteins in oral lichen planus: a comparative immunohistochemical evaluation. J Oral Pathol Med 29:370–375
18. Ter Hamsel B, Smedts F, Kuijpers J, Jeunink M, Trimbos B, Ramaekers F (1996) bcl-2 Immunoreactivity increases with severity of CIN: a study of normal cervical epithelia, CIN, and cervical carcinoma. J Pathol 179:26–30
19. Thongprasom K, Luengvisut P, Wongwatanakij A, Boonjatturus C (2003) Clinical evaluation in treatment of oral lichen planus with topical fluocinolone acetonide: a 2-year follow-up. J Oral Pathol Med 32:315–322

20. Tjalma W, Weyler J, Goovaerts G, De Pooter C, Van Marck E, Van Dam P (1997) Prognostic value of bcl-2 expression in patients with operable carcinoma of the uterine cervix. J Clin Pathol 50:33–36
21. Toma S, Mangiante PE, Margarino G, Nicolò G, Palumbo R (1992) Progressive 13-*cis*-retinoic acid dosage in the treatment of oral leukoplakia. Eur J Cancer B Oral Oncol 28B:121–123
22. Villaroel Dorrego M, Correnti M, Delgado R, Tapia FJ (2002) Oral lichen planus: immunohistology of mucosal lesions. J Oral Pathol Med 31:410–414
23. Zhao ZZ, Savage NW, Sugerman PB, Walsh LJ (2002) Mast cell/T cell interactions in oral lichen planus. J Oral Pathol Med 31:189–195