TITLE: Histological findings after intralesional methotrexate treatment in cutaneous squamous cell carcinoma

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AUTHOR CONTRIBUTION STATEMENT:

All named authors meet the International Committee of Medical Journal Editors (ICMJE) criteria for authorship for this article, take responsibility for the integrity of the work as a whole, and have given their approval for this version to be published.

M.B.S. designed the research, collected the data, analyzed the results and wrote the paper. V.P.B analyzed the histological samples and the results. A.P.P, LM.N.B, AM.R.D. and R.S.F contributed to the collection of the data and the analysis of the results.

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ABSTRACT:

Background: Intralesional methotrexate (il-MTX) has been reported as a useful therapy in keratoacanthoma (KA) and cutaneous squamous cell carcinoma (cSCC). However, the data available on the histological changes induced by this therapy are very scarce.

Methods: We conducted a single center, prospective study that included 65 cases of cSCC treated with il-MTX before surgical treatment. Two histological studies were conducted in all patients: before intralesional treatment and after surgical removal. Lesions were assessed longitudinally both clinically and histologically.

Results: 60 patients (92,3%) responded to il-MTX treatment. There were no differences regarding aggressive histological features of the cSCC between responder and non-responder patients. All cases showed a chronic inflammatory infiltrate after il-MTX. Intratumoral necrosis areas were frequently observed. All cases showed local fibrosis with fine thickening of collagen bundles.

Conclusion: Il-MTX induces a chronic lymphohistiocytic inflammatory reaction in both clinical responder and non-responder patients. Tumor involution after il-MTX is followed by a fine fibrosis that explains the great cosmetic results and improves the accuracy of the follow up.

MAIN TEXT:

Introduction

Intralesional agents have a role in the management of some cutaneous malignancies, since they allow for local delivery of chemotherapeutic drugs avoiding systemic toxicity [1,2]. Methotrexate is one of the better reported options for intralesional infiltration in keratoacanthoma (KA) and cutaneous squamous

cell carcinoma (cSCC), with response rates above 90% and an excellent safety profile[3,4]. Although scientific literature provides many studies about its intralesional use, only few reports have shallowly described the histological changes induced by this therapy [5,6].

Material and methods

Under institutional review board approval, we conducted a prospective longitudinal study that included 65 consecutive cSCC patients attended in our Dermatology Service from January to December 2018. All patients were scheduled for conventional surgical treatment at the time of diagnosis. During the waiting period until the scheduled surgery, two doses of 20mg of intralesional methotrexate (il-MTX) were administered one week apart.

Clinical responder patients were considered when both measurements of major and minor diameters were reduced after il-MTX treatment. This group included complete responders (no clinical lesion remaining) and partial responders (remaining lesion). Clinical non-responder patients were considered when one or both diameters of the tumor increased or stayed the same despite il-MTX.

Two histological studies were conducted in all patients: before intralesional treatment and after surgical removal. In complete clinical responders, an incisional biopsy was performed in the area where the cSCC had settled. Tumor features (differentiation degree, perivascular and/or perineural invasion, aggressive patterns) and histological changes after il-MTX were studied. All samples were analyzed by the same dermatopathologist (V.P.B.).

Statistical analysis was performed using IBM SPSS version 23 software (Chicago, Illinois, USA), considering $p<0,05$ statistically significant for all results. For comparisons of continuous cuantitative data (expressed as means±standard deviations), ANOVA or Student´s t-test were applied. For comparing proportions, chi-squared test or Fisher´s test were used.

Results

In 60 cases (92,3%) the size of the cSCC was reduced after il-MTX (clinical responder patients). 38 Cases (58,5%) showed no remaining displasic cells in

the final histological study. Only 5 patients (7,7%) did not achieve a tumor size reduction after intralesional treatment (clinical non-responder patients).

Data about clinical and histological findings are collected in Table 1. All cases showed a chronic inflammatory reaction after il-MTX: 26% mild, 62% moderate and 12% intense. The inflammatory infiltrate was mainly composed of lymphocytes and histiocytes, with frequent plasma cells *(Fig. 1A,1B)*. In cases with residual cSCC (partial responders) intratumoral necrosis areas were observed in 58% of cases, although no neutrophilic infiltration, abscessification nor other signs of acute inflammation were found *(Fig. 2)*. There was also common finding of giant foreign body cells, related to tumor regression phenomena triggered by il-MTX. There was no statistically significant difference between responder and non-responder patients regarding the inflammatory reaction degree or the detection of necrosis areas.

All cases of our series showed local fibrosis with fine thickening of collagen bundles. No collagen sclerosis or foreign body granulomas were detected.

Discussion

We comparatively analyzed the histological characteristics of cSCC in clinical responder patients versus non-responders. The studied variables included the differentiation degree, perivascular and/or perineural invasion and other aggressive features (infiltrative or acantholytic patterns). None of these variables showed significant differences between both groups. Therefore, our results do not allow to establish any histological predictor factor of response, although the small number of non-responder patients is a limitation in this regard. Rossi *et al.* studied potential histological factors that may be associated with a poor response to il-MTX therapy in a series of 14 keratoacanthomas [7]. They found aggressive features (poor differentiation, perineural invasion and/or infiltrative pattern) in their four non-responder patients. However, the small sample of their study did not allow to establish any statistically significant conclusion.

All cases of our series showed a chronic lymphohistiocytic inflammatory reaction after il-MTX treatment. Similar changes have been described in previous reports

[5,6]. Although this inflammatory infiltrate has been present in all cases of our series, its intensity has been variable. However, we were unable to demonstrate any relationship between the inflammation degree and that of therapeutic response.

Another histological finding in all cases of our series has been a local fibrosis with fine thickening of collagen bundles, in the context of il-MTX induced tumor regression. To our knowledge, this description has not been reported previously. Fibrosis was more evident in cases with complete tumor involution, although it also occurred in tumors with partial response and even in non-responder cases. Fibrosis related to il-MTX administration has some specific features: it is "assimilable" to dermis, without hyperplasia or collagen sclerosis and without foreign body reactions. In this way, it differs from fibrosis secondary to surgical procedures (with collagen hyperplasia and frequent foreign body granulomas) and from that associated with radiotherapeutic treatments (with marked sclerosis) [8,9]. These histological data support clinical findings: no cases with complete resolution showed scarring or local atrophy, so that the skin on which the tumor had settled was almost indistinguishable from adjacent skin *(Fig.3)*. The excellent cosmetic results after il-MTX treatment obtained in our study agree with previous reported cases [10,11].

Il-MTX induces a fine fibrosis that tends to remodel more easily than that associated with surgical or radiotherapeutic procedures. This implies some advantages: from a clinical view, the absence of scarring facilitates the monitoring of these patients, improving the accuracy of relapses detection; from a cosmetic view, the result of complete tumor regression after il-MTX is better than that of any surgical scar or radiation treatment (which induces skin sclerosis in the radiated area). Finally, from an anatomopathological view, it facilitates the recognition of relapses or tumor persistences by avoiding confusion factors such as sclerosis or collagen hyperplasia.

The review of the histological samples after il-MTX treatment has revealed an eye-catching fact: all non-responder cases showed similar lymphocytic inflammation and necrosis areas as responder cases. This implies a histological

response to il-MTX treatment even without a clinical response. In this way, the five clinical non-responder patients of our series could be considered “histological partial responders”. This phenomenon is consistent with our clinical observation of initial reduction of the tumor size followed by a subsequent regrowth, shown by all non-responder patients. Since clinical non-responder cases correspond to the larger tumors of our series, we consider that this lack of clinical response could be justified by the lower dose per cm^2 received by these patients. This observation could imply that an intensification of il-MTX treatment would increase the response rate in larger tumors.

Conclusions

Our study agrees with the previous descriptions of the chronic inflammatory reaction induced by il-MTX in a larger case series. It adds the description of a characteristic fine fibrosis that explains the great cosmetic result obtained with this therapy. Optimal healing after il-MTX treatment improves the accuracy of clinical follow up since it facilitates the early detection of relapses. The presence of histological response signs in clinically non-responder cases suggests that intensifying il-MTX treatment could be a useful strategy to increase the response rate to this therapy.

ETHICAL STATEMENT:

Authors state that they have received the approval from their institutional Ethics in Investigation Committee review board.

This study was performed in accordance with the Helsinki Declaration of 1964. All subjects provided informed consent to participate in the study and for publication.

CONFLICT OF INTEREST:

The authors have no conflicts of interest to declare.

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DATA AVAILABILITY STATEMENT

Data are available on request from the authors

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TABLES:

Table 1. Clinical and histological features (responder vs non-responder patients)

Il-MTX: intralesional methotrexate

		Clinical responder patients (n=60)	Clinical non-responder patients (n=5)	p value
Pre il-MTX treatment	**n**	60	5	
	Tumor surface cm^2 mean (SD)	1,69(1,41)	7,72(2,80)	<0,001
	Differentiation degree %	**Good** 55% **Moderate** 45% **Poor** 0%	**Good** 51% **Moderate** 48% **Poor** 0%	>0,05 >0,05 >0,05
	Infiltrative/acantholytic patterns %	3,3%	0%	>0,05
Post il-MTX treatment	**Tumor surface** cm^2 mean (SD)	0,42(0,68)	9,83(3,39)	<0,001
	Complete histological response	58,5%	0%	<0,001
	Inflammatory infiltrate %	**Mild** 52,3% **Moderate** 45,5% **Intense** 2,2%	**Mild** 49,5% **Moderate** 47,6% **Intense** 2,9%	>0,05 >0,05 >0,05
	Necrosis areas	36,6% (100% in partial responders)	100%	>0,05
	Perineural invasion %	0%	0%	>0,05

FIGURE LEGENDS:

Fig 1. Chronic inflammatory infiltrate after il-MTX treatment

Fig 2. Necrosis areas after il-MTX treatment

Fig 3. Clinical healing and histological fibrosis after il-MTX (a) cSCC before il-MTX treatment; (b) complete clinical resolution after il-MTX treatment; (c) histological fibrosis after complete resolution