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TOPICAL APPLICATION OF EOSIN 2% with CLOROXILENOL 0.3%, PROPYLENE GLYCOL 30% (NEOMERCUROCROMO) AND COLLOIDAL SILVER: A NEW TOPICAL TREATMENT FOR LICHEN SCLEROSUS.

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ABSTRACT

Our aim is to evaluate safety and efficacy of topical Eosin 2% + Chloroxylenol 0.3% + Propylene glycol 30% (Neo mercurocromo) and Colloidal Silver to improve Lichen Sclerosus (LS) symptoms.We postulated that the disinfectant, anti-moist, anti-dystrophic, antifibrotic and cytoprotective activity of NEOMercurocromomight be of help in improving LS symptoms. Forty-four LS patients were randomised into two groups: 22 patients received the active drugs (Group 1) and 22 received the placebo (NaCl 0.9%) for 4 weeks (Group 2). The active drugs were used for two weeks each: NEOMercurocromo first and subsequently Colloidal Silver. Active drugs and placebo were rubbed with a sterile gauze on the LS lesions twice a day. Three clinical controls using a photo archive (baseline, and one and three months after active drug or placebo delivery) were carried out on each patient using a specific LS scale score, and the Dermatology Life Quality Index (DLQI) score. One and three months after active drugs or placebo delivery, the patients defined their post-treatment condition using the Patient Global Impression of Improvement (PGI-I) scale. The scores were compared within and between groups using non parametric tests, and the side effects were compared between the groups using the Chi-squared test (χ^2). The LS and the DLQI scores were similar between the groups at baseline. Both scores significantly improved in Group 1 to a larger extent than in Group 2 at one and three months. The PGI-I score in Group 1 was higher than in Group 2 at one and three months. No side effects emerged in either group. NEOMercurocromo + Colloidal Silver is a safe and efficient therapy for LS.

KEYWORDS: ***Chloroxylenol, NEOMercurocromo, colloidal silver, topical treatment, lichen sclerosus.***

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INTRODUCTION

Lichen sclerosus (LS) is an inflammatory, sclerotic skin disease. Lichen Sclerosus can affect all age groups but, for the most part, involves male and the anogenital region. The major subjective complaints are severe pruritus, dysuria and painful defecation. Clinically, LS is characterised by porcelain-white sclerotic plaques[1]. Many findings obtained in recent years point more and more towards an autoimmune-induced disease in genetically predisposed patients. Preceding infections, trauma and occlusive moisture may play a provocative role[2]. Topical corticosteroids still head the therapeutic armamentarium. Topical calcineurin inhibitors, topical and systemic retinoids and phototherapy may be therapeutic options in cases refractory to corticosteroid treatment. Surgery is restricted to scarring processes leading to functional impairment. An elevated rate of relapse (60-80%) has been described [2]. Polydeoxyribonucleotide (PDRN), a healing and anti-dystrophic drug with anti-inflammatory effects, has recently shown excellent tolerability and therapeutic efficacy [3]. The Authors postulated that the disinfectant, anti-moist and anti-dystrophic properties of NEOMercurocromo and Colloidal Silver [4], the antifibrotic and cytoprotective activity of eosin (a component of NEOMercurocromo)[5], the cytokine release induced by *Chloroxylenol* (a component of NEOMercurocromo) and the subsequent inactivation of fibroblasts [6] might be of help in improving LS symptoms. Thus, the authors began the current prospective controlled study to test the hypothesis that the topical administration of NEOMercurocromo + Colloidal Silver to LS patients improved LS symptoms and the quality of life of LS patients to a larger extent than a control substance.

MATERIALS AND METHODS

The study was authorised by the Institutional Review Board (IRB) of Gynepro, and the trial was registered with number 2017-1 in the International Standard Randomised Controlled Trial Number (ISRCTN) registry at the following website http://www.controlled-trials.com. The following is the link to reach the trial: http://www.isrctn.com/ISRCTN27941065. Patient recruitment began on 2 January 2017 and finished on 2 December 2017. Written informed consent was obtained from each patient. This was a multicentre, blind, randomised, control substance (NaCl 0.9%) controlled study.

Patients

Each male patient referred to our centres with a history of LS of the genitalia was considered as a candidate. The diagnosis of LS was reached on the basis of medical history and objective signs; the diagnosis was confirmed in all patients with a biopsy [7]. The histological features included orthokeratotic hyperkeratosis, vacuolar degeneration of the basal layer, oedematous and sclerotic papillary dermis as well as lymphohistiocytic infiltrates in the mid-dermis [8, 9]. Patients were excluded if any of the following were present: refusal to participate in the study (5 patients); phimosis (1 patient); skin conditions at baseline that would interfere with LS evaluation (3 patients); immunosuppression (1 patient); malignancies (no patient), or bacterial, viral or fungal infection of the genitalia within 2 months prior to the beginning of the study (6 patients); any prior genital intervention or surgical therapy (cryotherapy, laser, curettage, electrocautery, excision or immunomodulators) (3 patients) or any prior medical or surgical intervention for LS (8 patients). The patients were randomly assigned to one of the two groups to receive the active drugs (NEOMercurocromo and Colloidal Silver) or a placebo (NaCl 0.9%). The IRB reviewed the data for patient safety; there were no interim analyses for efficacy. A two group randomisation was carried out using an on-line randomiser: https://www.randomizer.org; the Group 1 patients received active drugs and the Group 2 patients received a control substance. A power analysis was carried out to estimate the number of observations needed in order to have a reliable chance of detecting the effect sought. There are no formal standards for power (π); the power of our tests used π = 0.90 as a standard for adequacy [10]. The calculations were carried out using the G*Power3 program[11].

LS assessment

Four clinical controls using a photo archive (baseline, and one week, one and three months after active drug or placebo delivery) were carried out on each patient. The LS was evaluated in each patient by the investigator using a specific LS scale (0: no disease—no inflammatory sign; 1: mild disease— mild erythema, infiltration, lichenification and excoriation; 2: moderate disease—moderate erythema, infiltration, lichenification and excoriation; 3: severe disease—severe erythema, infiltration, lichenification and excoriation)[3] and the Dermatology Life Quality Index (DLQI)[12] validated for the Italian language [13-14] (Table 1). The specific LS scale and the DLQI were administered to each patient before, and one week, one and three months after the end of the active drug or placebo administration. One week, one and three months after the active drug or placebo delivery, the patients were invited to define their post-treatment condition using the Patient Global Impression of Improvement (PGI-I) seven grade scale: 1: very much better, 2: much better; 3: slightly better, 4: no change, 5: slightly worse; 6: much worse, 7: very much worse [3]. The scores of each scale were collected and the side effects were also recorded.

Interventions

The patients were instructed to carefully cleanse glans and prepuce with an oil-free cleanser before using active drugs or placebo.

Active drugs

The patients were instructed to carefully apply for a sterile gauze soaked with NEOMercurocromo (Laboratorio Farmaceutico SIT, SRL, Mede (Pavia, Italy)) to the affected area(s) for five minutes twice a day for two weeks. The composition of the NEOMercurocromo was Eosin 2%, Chloroxylenol 0.3% and Propylene glycol 30%. The patients were later instructed to apply a sterile gauze soaked with Colloidal Silver (Argento Colloidale Puro, Hydromed, Schio (Vicenza-Italy)) to the affected areas for five minutes twice a day for two weeks after using the NEOMercurocromo.

Control substance
Sterile physiological solution (NaCl 0.9%) was used as a control substance. The patients were instructed to carefully apply a sterile gauze soaked with sterile NaCl 0.9% to the affected area(s) for five minutes twice a day for four weeks .

Endpoints
With respect to the efficacy and the safety of NEOMercurocromo and Colloidal Silver, the primary endpoints were an improvement in the scores of the specific LS scale and the DLQI, and in the side effects. The PGI-I score was an additional primary endpoint.

Statistical analysis of the data
The differences between the unmatched groups were assessed using the Mann-Whitney Rank test, and the differences between before and after (matched groups) therapy were assessed using the Wilcoxon Signed Rank test. The side effects were compared between the two groups using the chi^2 test [10].

Procedures to ensure blindness
The active drugs and the placebo were delivered in anonymous dark-glass bottles, with color-coded. labels. The active drugs and the control substance were delivered by nurses, who were blinded to the colour code of the labels. The physicians were blinded as well. All the study personnel and participants were blinded to the treatment assignment for the duration of the study. Only the Gynepro Institutional Review Board (IRB) aware of the unblinded data in order to alert physicians in case of major side effects, but this was never necessary, and no one from the IRB had any contact with the study participants. The colour and the number codes were disclosed at the end of the study[15].

RESULTS

Forty-nine patients were eligible for participation in the study: 24 were randomly assigned to receive the active drugs (Group 1) and 25 the control substance (Group 2). Two patients in Group 1 violated the protocol, and 3 dropped out from Group 2. A phone call indicated that, in three cases, an insufficient therapeutic effect occurred. Thus, 22 patients treated with the active drugs and 22 treated with the control substance were studied. Table 2 shows the baseline demographic and clinical characteristics of each group. No significant differences emerged between the groups even when the baseline specific LS scale scores and the baseline DLQI scores were compared (Table 3). Table 3 summarises the results of each study group, i.e. the scores of specific LS scale and the DLQI at baseline, and one week, one and three months after active drug or placebo application. The score of the PGI-I one week, one and three months after active drug or placebo application has been reported as well. The median scores of the specific LS scale and the DLQI significantly improved in both groups one week, one month after the end of the application of the active drugs and of the placebo, but a significantly higher improvement occurred in Group 1 rather than in Group 2. The median score of the specific LS scale and the DLQI of Group 1 at three months was not significantly different from the 1 month score whereas the scores of Group 2 at three months were similar to the baseline scores. The PGI-I median score of Group 1 was significantly higher than that of Group 2 at one week, one and three months. The PGI-I score of Group 2 one week, one month after the end of the placebo delivery was significantly higher than the three month score. Side effects were absent in both groups.

Table 1
Dermatology Life Quality Index (DLQI): Items and dimensions evaluated by the version validated for the Italian Language.

Items / Questions*	Latent dimensions
1. During the last week, how much was your skin affected due to itching, inflammation, pain or burning?	Symptoms and feelings
2. During the last week, how much embarrassment or other kind of limitation was caused by your skin?	
3. During the last week, how much did your skin interfere with your activities of shopping or riding in a vehicle, at home or in public places?	Daily activities
4. During the last week, up to what point did your skin interfere with the clothes you normally wear?	
5. During the last week, how much did your skin affect one or more of your social or leisure activities?	Leisure
6. During the last week, how difficult was it for you to practice sports?	
7. During the last week, did your skin prevent you from working or studying?**	Work and school
8. During the last week, did your skin problem create any difficulty with your partner, close friends or relatives?	Relationships
9. During the last week, up to what point did your skin present difficulties for your sexual life?	
10. During the last week, up to what point did your dermatological treatment present problems for you?	Treatment

*Possible answers: 3 (very much), 2 (a lot), 1 (a little), 0 (not at all or irrelevant); **Possible answers: 3 (very much) or 0 (not at all or irrelevant). If the answer was No: did your skin create problems for you at work or at school? possible answers: 2 (a lot) or 0 (not at all or irrelevant).

Table 2
Baseline demographic and clinical characteristics of each group.

	Group 1 Neomercurocromo + Colloidal Silver (n. 22).	Group 2 Control substance (physiological solution) (n 22)
Age (years)	53 (42–62)	55 (40–63)
Duration of Lichen Sclerosus (months)	8 (2-13)	7 (2-12)

Data are shown as median values, and the ranges are in parentheses. No differences between the comparisons were significant when examined using the Mann-Whitney Rank test.

These scales were administered before and one week, one and three months after active drug or placebo delivery. Comparison among the scores of the Patient Global Impression of Improvement (PGI-I) scale after one and three months of active drug or placebo delivery. Data are shown as median values, and the ranges are in parentheses. The differences between the unmatched groups were assessed using the Mann-Whitney test, and the differences between before and after (matched groups) therapy were assessed using the Wilcoxon test.

Active drugs

Topical Neomercurocromo (Laboratorio Farmaceutico SIT, SRL, Mede (Pavia, Italy)). The neomercurocromo composition was Eosin 2%, Cloroxilenol 0.3% and Propilenglicol 30%. Two applications/day for two weeks. And subsequently topical Colloidal Silver (Argento Colloidale Puro, Hydromed, Schio [Vicenza-Italy]), two applications/day, for two weeks. Control substance. Topical physiological solution (NaCl 0.9%) two applications/day for four weeks. vs = versus.

Table 3
Comparisons among the scores of the specific Lichen Sclerosus (LS) scale and of the Dermatology Life Quality Index (DLQI).

	Before active drug or placebo use		One week after the end of active drug or placebo use		One month after the end of active drug or placebo use		Three months after the end of active drug or placebo use	
	Group 1: (22 patients treated with active drugs) [1]	Group 2: (22 patients treated with a placebo) [2]	Group 1: (22 patients treated with active drugs) [3]	Group 2: (22 patients treated with a placebo) [4]	Group 1: (22 patients treated with active drugs) [5]	Group 2: (22 patients treated with a placebo) [6]	Group 1: (22 patients treated with active drugs) [7]	Group 2: (22 patients treated with a placebo) [8]
Specific LS scale score. Median (range: min-max)	2 (2-3)	2 (2-3)	0 (0-1)	1 (0-2)	0 (0-1)	2 (2-3)	0 (0-1)	2 (2-3)
DQLI score Median (range: min-max)	22 (26-16)	21 (27-15)	7 (4-10)	11 (7-12)	6 (3-10)	12 (7-17)	7 (4-11)	20(26-15)
PGI-I score Median (range: min-max)			1 (1-3)	2 (1-4)	1 (1-3)	2 (1-4)	1 (1-3)	4 (3-5)

Comparisons

Comparisons were made between columns which were identified with progressive numbers between square brackets

17 Specific LS scale score: 1 vs. 2: p = 62.72%; 1 vs. 3: p = 0.12%; 1 vs. 5: p 0.13%; 1 vs 7: p = 0.14%; 2 vs. 4: p = 0.96%; 2 vs. 6: p = 26.97%; 2 vs 8: p = 42.3%

18 DLQI score: 1 vs. 2: p = 27.32%; 1 vs 3: p = 0.2% 1 vs. 5: p = 0.1%; 1 vs. 7: p = 0.02%; 2 vs 4: p = 0.7%; 2 vs. 6: p = 0.6%; 2 vs 8: p = 31.61%.

19 PGI-I score: 2 vs. 4 vs. 6: p = 34.8%; 3 vs. 4: p = 0.94%; 5 vs. 6: p = 0.92%; 7 vs. 8: p = 0.01%; 4 vs. 6: p = 21.7%; 6 vs. 8: p = 0.05%.

DISCUSSIONS

These data showed that the topical application of NEOMercurocromo and subsequently of Colloidal Silver was a safe therapy which improved LS symptoms to a larger extent and more durably than a placebo. Recurrence appeared in all patients treated with the placebo within three months whereas no recurrence emerged in the group of patients treated with the active drugs. A potential bias of the present study was the two branch structure whereas a four branch research structure (1st branch: NEOMercurocromo + Colloidal Silver; 2nd branch: NEOMercurocromo + placebo; 3rd branch: placebo + Colloidal Silver; 4th branch: placebo) would have been more suitable for our purposes; however, a first attempt at carrying out a four branch prospective research structure failed due to its being extremely complicated. An additional potential bias was the fact that NEOMercurocromo made the mucosa and the cutis reddish. However, the subsequent application of colloidal silver cancelled that colour, and the authors could not identify the treatment group when inspecting the genitalia in the course of the programmed clinical controls. The follow-up was limited to three months since longer times were associated with a high rate of dropouts which would have invalidated our research. A longer follow-up will be a subject of an additional and more extensive research. However, the fact that no LS recurrence occurred in the treatment group supported the hypothesis that NEOMercurocromo associated with Colloidal Silver could be effective in resolving LS symptoms, at least in part. The aetiology of LS is still unclear and a number of factors have been sustained to be the cause; therefore, a multifactorial aetiology of LS is considered to be a reliable hypothesis [2]. The Authors chose to use NEOMercurocromo and Colloidal Silver because their properties fit with the postulated aetiology of LS (see the Introduction), even though, at present, the reasons which make the sequence of NEOMercurocromo first and Colloidal Silver second are unknown. However, previous preparation tests which had been performed on four groups of 5 LS patients each indicated that the topical application of NEOMercurocromo twice a day for two weeks and subsequently of Colloidal Silver twice a day for two weeks gave the best results. In these tests, the treatment groups were as follows: Group a) Colloidal Silver twice a day for two weeks; Group b) Colloidal Silver twice a day for two weeks and subsequently NEOMercurocromo twice a day for two weeks; Group c) NEOMercurocromo twice a day for two weeks and Group d) NEOMercurocromo twice a day for two weeks and subsequently Colloidal Silver twice a day for two weeks. Furthermore, two more treatment groups had been studied in preparation tests. Each group was made up of 5 LS patients. Group 1 used the active drugs only by touch, and the second group carefully, but briskly, applied a sterile gauze soaked with the active drugs to the affected areas. The second group obtained much better results than the first group. Briskly applying the active drugs to the LS lesions was critical in improving the LS symptoms; even briskly applying the placebo improved the LS symptoms. Brisk application cleaned the LS surface from tissue debris and serum, i.e. it removed factors which determined/worsened LS progression[2]. Moreover, it could be presumed that chronic tissue stress induced by brisk application improved the immune response [16].The literature showed side effects from NEOMercurocromo and Colloidal Silver only after ingestion and when large mucous areas were treated [4]; these situations did not occur in our study and no side effects were observed in our study.

CONCLUSION

In conclusion, the brisk application of NEOMercurocromo + Colloidal Silver is safe and effective for LS symptoms whose long term recurrence will be studied in a further research subsequent study.

AUTHORS CONTRIBUTION STATEMENT

Carlo Maretti invented the research, edited the manuscript, and treated 27 patients; Giorgio Cavallini prepared the research protocol, analyzed the data, wrote the manuscript and treated 17 patients.

CONFLICT OF INTEREST

Conflict of interest declared none.

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