UNIVERSITY OF MEDICINE AND PHARMACY OF TÎRGU MUREŞ
DOCTORAL SCHOOL

EVALUATION OF NEW DRUG FORMULATIONS FOR TOPICAL USE IN ACNE PATHOLOGY. DERMATO-COSMETIC PROCEDURES AND DIETARY FACTORS AS NON-PHARMACOLOGICAL ADJUVANT METHODS

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THESIS SUMMARY

ABSTRACT OF PhD THESIS

OBJECTIVES:

Given the interdisciplinary character of the thesis, three distinct research directions have been approached, aiming to achieve the proposed objectives. Thus, the first research direction was oriented towards the development of new semisolid dermatological preparations containing either a combination of clindamycin phosphate and adapalen (CLD/ADP) or nadifloxacin and adapalen (NDX/ADP), along with glycolic acid (AG), associated both as a skin penetration enhancer and for its own anti-acne effect. An experimental study was carried out, having the following main objectives: formulation and preparation of the semisolid products; evaluation of the pharmaceutical quality, as well as the tolerance and sensory effects on skin application of the newly formulated products; validation of the analytical methods for HPLC quantification of the drugs in the studied products (CLD/ADP and NDX/ADP, respectively); assessment of *in vitro* release and permeation processes through membranes of the three drugs (CLD, NDX and ADP), from newly developed products.

The second direction of research was oriented towards the evaluation of dermato-cosmetic procedures as non-pharmacological adjunctive acne therapy and comparison with a standard anti-acne topical treatment, having as objectives: assessment of the efficacy and tolerability of 20% TCA (trichloroacetic acid) peel versus 15% topical azelaic acid gel; evaluation of the patient's satisfaction degree after the treatment, as well as the subjects' perception on issues related to the role of personal hygiene, the use of appropriate cosmetic products, the need of a specialized approach to establish an appropriate treatment for lesions' relieving and aesthetic appearance improvement, in accordance with individual etiopatology.

A third line of research aimed to evaluate the effects of practices and attitude towards lifestyle in a high school students' community of Tîrgu Mureş, as potential risk or protective factors, for both acne development and severity, having as objectives: determination of acne prevalence in the studied community; identification of the risk and protective factors regarding genetic, demographic and other factors derived from the adolescents lifestyle, as well as identification of the potential risk or protective dietary factors associated with acne vulgaris.

MATHERIAL AND METHODS:

Development and evaluation of new semisolid dermatological preparations containing either a combination of clindamycin phosphate and adapalen (CLD/ADP) or nadifloxacin and adapalen (NDX/ADP) - was an experimental study structured in two parts: The first part targeted the development and pharmaceutical quality evaluation (appearance, homogeneity, pH and rheological characteristics) of the newly formulated semisolid products containig a combination of 1% antibiotic (CLD or NDX), 0.1% ADP and 2% AG; the tolerance and sensory effects on skin application were assessed through a clinical observational study performed on healthy volunteers. The second part of the study reffered to *in vitro* release/permeation of the drugs through membranes (synthetic membrane and human heat separated epidermis, respectively), performed with a system consisting of 6 stationary Franz diffusion cells, with a receptor volume of 14 mL, membrane surface area of 2.833 cm^2, using two types of receptor fluid, namely a mixture of PBS (Phosphate Buffer Solution) pH 7.4:ethanol (50:50, v/v) and a mixture of PBS pH 5.5:ethanol (50:50, v/v), respectively.

Evaluation of dermato-cosmetic procedures as non-pharmacological acne therapy and identification of potential risk or protective factors in acne vulgaris – consisted of two studies: The first one, a clinical observational study was carried out with the Ethics Committee of UMF Tîrgu Mureş approvel no. 99/2014, as well as with the patients's consent, on a group of 51 patients aged between 16-40 years, diagnosed with mild and moderate acne. *Inclusion criteria:* age between 16-40 years, diagnosis of mild acne (less than 30 acne lesions on the entire face, open or closed comedones) or moderate acne (31-50 acne lesions, open and closed comedones, papules, inflammatory pustules), given by the dermatologist*, Exclusion criteria:* patients with severe acne (over 51 acne lesions including macro-cysts and nodules), oral contraceptives or other systemic or topical anti-acne medications (isotretinoin, antibiotics, topical preparations). The patients were randomized into two groups: group A (n=27) were treated with topical applications of 15% azelaic acid gel twice daily, whereas the patients belonging to group B (n=24) were performed 4 sessions of 20% TCA peels at 14 days interval. The study was conducted over a period of 8 weeks.

The second study was a cross-sectional survey based on a self-reported questionnaire, conducted through direct interviews of the participants by the investigator. The targeted group consisted of 148 high school students aged between 16-20 years, belonging to a high school community in Tîrgu Mureş. Each student was clinically evaluated and diagnosed by the dermatologist and investigator based on the presence or absence of inflammatory and non-inflammatory facial acne lesions. The subjects were then divided into two groups: the control group (without acne lesions) and the acne group (mild acne and moderate / severe acne, as subgroups).

CONCLUSIONS:

Development and evaluation of new semisolid dermatological preparations containing either a combination of clindamycin phosphate and adapalen (CLD/ADP) or nadifloxacin and adapalen (NDX/ADP)

Six washable semisolid products containing a combination of 1% antibiotic (CLD or NDX), 0.1% ADP, 2% AG and 3 antibiotic free products (with 0.1% retinoid) were developed and prepared, differing by the nature and

composition of the semisolid base: two hydrogels (2.5% hydroxypropyl methylcellulose - HPMC and 3% hydroxyethyl cellulose - HEC) and an oil-in-water (O/W) cream. All 9 products comply with the pharmaceutical quality requirements imposed on the appearance, homogeneity and pH, being well tolerated and the sensory effects on skin application found to be positive in the hedonic scale.

Rheological behavior provides the premises for a possible use in the topical treatment of acne vulgaris in a differentiated manner, depending on the skin typology associated with the acne pathology, specific to each individual, namely: 2.5% HPMC (CLD or NDX) gels have a soft, easily deformable structure, being suitable for topical application in patients with acne lesions associated with sensitive and painful skin, providing a refreshing and soothing effect. Due to the slightly astringent effect shown, 3% HEC hydrogels (CLD or NDX) may be indicated for acne associated with cutaneous seborrhea, helping in removing the excess of sebum from the skin surface. The O/A cream with CLD/ADP combination, which shows a high potential for softening effect, may be indicated in acne types associated with dehydrated skin caused by the prolonged use of astringent cosmeceuticals. Non-antibiotic products containing 0.1% retinoid show favorable premises for a possible recommendation as maintenance treatment between the pharmacological therapies.

The two analytical methods proposed for HPLC analysis of the combinations of active pharmaceutical ingredients (CLD/ADP and NDX/ADP, respectively) are linear, precise and accurate, particularly sensitive to the wavelengths used, and can be applied for the determination of clindamycin phosphate, nadifloxacin and adapalene from various types of samples.

The *in vitro* release/permeation processes of the drugs (CLD, NDX, ADP) through both selected membrane types (synthetic membrane and human heat separated epidermis membrane) are significantly influenced by the antibiotic type, the type of dispersed system formed by the approched preparation method (solution / suspension / emulsion) and the pH of the dissolving medium (a pH of 7.4 is more favorable than pH of 5.5 from this point of view). The antibiotics release rate (CLD or NDX) is significantly higher for the two hydrogels, the cumulative released amounts being double or even triple compared with those obtained for the O/W cream. Between the two antibiotics, CLD showed higher values of permeation parameters (cumulative amount, permeation flow, permeability coefficient) than NDX, probably due to increased solubility and facile release from the semisolid formulations, as well as the diffusing capacity through the hydrophilic environment of viable epidermis. The cumulative amounts of ADP permeated through membranes were very low for all six preparations, suggesting a low risk of systemic adverse effects by penetration into the bloodstream, apparently ADP remaining unreleased from the semisolid formulation at the skin surface, or retained in the epidermis membrane layers. ADP release was significantly influenced by the receptor medium pH and the antibiotic type associated in the composition (CLD or NDX), possibly due to the ionized or hydrolysed/unhydrolyzed states of the antibiotic provided by the medium pH, the cumulative amounts of ADP released from the products containing CLD in composition being significantly superior to those obtained for NDX formulations. Among the newly developed products, the CLD/ADP combination showed superior results than NDX/ADP combination, 3% HEC hydrogel being the most advantageous in this regard. The release kinetics of CLD and ADP from the two hydrogels suggest a super-case II diffusion mechanism, due to both the diffusion of the ingredients through the hydrophilic synthetic membrane and the relaxation/dissolution of the hydrophilic polymer, whereas the release kinetics of NDX suspended in the hydrogels suggest diffusion mechanisms preceded by the fickian relaxation of the hydrophilic polymer, in which the release rate of the drug decreases proportionally with its initial concentration. The release kinetics of the drugs from the O/W creams suggest processes resulting from different mechanisms, correlated with the different solubility of the drugs in the semisolid formulation.

Evaluation of dermato-cosmetic procedures as non-pharmacological acne therapy and identification of potential risk or protective factors in acne vulgaris

Both 20% TCA peel and 15% topical azelaic acid gel are effective methods for the treatment of mild and moderate acne vulgaris, with better tolerability for azelaic acid, TCA peel showing a higher incidence of patient discomfort on the cutaneous application, but at the same time, proved a greater efficacy, leading to a faster improvement of acne lesions, as well as texture and aesthetic appearance of the skin.

Acne prevalence in the studied community (average age 17.7 ± 0.8 years) was of 47.3%, of which 45.71% were mild acne cases, 30% moderate acne and 24.29% severe acne cases.

Acne family history, smoking, excessive intake of dietary fats, sweets, carbonated drinks and white bread could be considered potential risk factors in the development and aggravation of acne vulgaris, an overweight / obesity status being a potential risk factor for acne's severity. An increased weekly intake of fish, vegetables and fruits could have a protective effect on the onset or acne's severity.

The need for a more effective awareness of the population is highlighted, especially amongst adolescents, about the long-term implications of inappropriate approaches of acne symptoms, in terms of hygiene, food and lifestyle behavior. Patient evaluation by specialized healthcare professionals and the establishment of appropriate treatment since the onset of the disease, in conjunction with effective information on the availability of anti-acne cosmeceutical products and dermato-cosmetic procedures, as well as the implementation of nutritional education programes, are extremely important issues for maintaining and extending the results of any pharmacological anti-acne treatment.

Keywords: acne vulgaris, topical treatment, TCA peel, acne and diet, skin penetration