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Research Article

EVALUATION OF CUTANEOUS MICROCIRCULATION OF THREE COSMACEUTICALS CONTAINING DIFFERENT PERCENTAGE OF ACTIVE (BIOTROPICS®FD101): AN OPEN, SINGLE CENTRIC, PLACEBO CONTROL, INTRA-INDIVIDUAL STUDY IN HEALTHY VOLUNTEERS

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ABSTRACT

A large number of non-invasive methods are available for investigating the skin, ranging from those that permit the visualisation of microvessels, to those that monitor blood flow or one of its derivatives (e.g., skin temperature and transcutaneous oxygen). The cutaneous microcirculation or skin blood flow is an important parameter for assessment of drug delivery by a cosmaceutical to the vasculature of superficial and deep horizontal plexuses. Laser Doppler fluxmetry and photoplethysmography provide an estimate of blood flow through the skin. In this study, a non-invasive method for measuring cutaneous blood flow using laser Doppler flowmetry (LDF) is presented. The MC Gel is an herbal formulation containing, herbal active named biotropics®FD101 derived from extract of ficus deltoidea. The present study was planned to evaluate the cutaneous microcirculation of MC Gel for cosmaceutical use. This study was simple blind, intra-individual clinical trial conducted at the Ellead skin research centre, Korea from January 20 to February 23, 2011. Twenty (20) female healthy volunteers, aged 23 to 64 with specific phenotype I, II and III were included in the study. Subject below 18 years of age, subject already on medication or therapy, subjects with preexisting severe skin diseases or cutaneous pathology on the studied zone, subjects with microcirculatory or cardiovascular disorders, subjects having treatment of pulmonary, cardio, circulatory problems or undergone surgery since less than one month, subjects with excessive consumption of alcohol, tea or coffee and those who refused to give informed consent were excluded from the study. Pregnant or lactating women and women having change, started or stopped oral contraceptive or hormonal therapy since less than 1.5 months were also excluded from the study. The predefined primary outcome was measurement of cutaneous microcirculation with Laser Doppler. The predefined secondary outcome measures were incidence of adverse events and compliance to the treatment. Statistical analysis was done on the values obtained before and at the different times of kinetics after treatment. Data were analyzed with a paired t-test. The included female volunteers had their microcirculation assessed by a laser Doppler probe being placed on their forearm with previously defined four zones by standardized application of 2 µl/cm^2 of each studied product. The assessment of capillary flux was carried out at 15, 30, 60, 90 and 120 minutes after product application. A significant difference was observed in perfusion unit for product containing 2.5% biotropics®FD101 (*ficus deltodeia* extract; pending patent no. PCT/MY2011/000008) compared with placebo which induced a higher increase in the cutaneous microcirculation (+1.46 P.U). The Product containing 1.0% biotropics®FD101 induced a tendency of microcirculatory flow, which further responded to the vascular challenge, resulting in increased flow changes at 30 minutes with a increase in capillary flux of +10%, measured in 59% of the subjects. The product containing 0.5% biotropics®FD101 was insignificant for the capillary flux at each time point after its single application. There were no clinically significant adverse reactions (either reported or observed), during the entire period of study and excellent patient compliance to MC Gel was also observed. Based on these observations, it may be concluded that the product containing 1.0% and 2.5% biotropics®FD101 induced a tendency (non-significant) to an increase in microcirculation 30 minutes and 15 minutes respectively after its single standardized application. It is also clinically effective and safe for external usage.

Keywords: Laser Doppler, Microcirculation, capillary flux, Clinical, LDF, Gel etc.

INTRODUCTION

The cutaneous microcirculation or skin blood flow is an important parameter for assessment of drug delivery by a cosmaceutical to the vasculature of superficial and deep horizontal plexuses[1]. Different methods exist to measure cutaneous microcirculation. Among those, Laser Doppler® measurement done *in-vivo*, have the advantage of being non-invasive and direct. Doppler echoes sent by the Laser Doppler are sent back by a flux of red corpuscles at the dermal blood vessel level. The obtained signal is proportional to the quantity of the red corpuscles in the blood vessel and their speed, reflecting as well as the action of cosmetic or pharmaceutical products on superficial cutaneous microcirculation. Laser Doppler fluxmetry and photoplethysmography provide an estimate of blood flow through the skin[1]. The microcirculation measurement using Laser Doppler is based on the "optic doppler effect". A Laser light beam is guided by a fibre optic to the measurement zone and diffused in a small volume of the tissue. A part of this luminescent energy is absorbed by the tissue and the other part is reflected by fixed or mobile structures. The light reflected by the moving structures, principally red corpuscles, undergoes a frequency of shift according to the spatial Doppler principle or the "Doppler Effect". Inversely, light reflected by the fixed structures does not undergo the "Doppler Effect" (Fig 1).

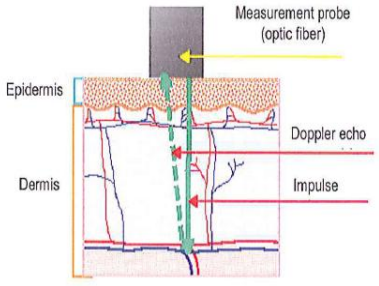


Figure1: Principle of Laser Doppler®

The magnitude and the change of the probe length are proportional to the number and speed of the red corpuscles, although not related to the direction of the movement. The information gathered by the reception fiber is converted into an electronic signal that is treated and analyzed. Measurements are expressed in perfusion units (PU) and are calculated according to the formula;

Perfusion (orcapillary flux) = Number of moving cells × Average speed

Laser Doppler has been used in a variety of studies owing to its relative ease of use and can be used on any region of the skin[2]. It is relatively cheap; it very easy to use and does not require specialist training, which means it is relatively free of operator bias[2-4]. The penetration of tissue by laser is good, but its depth is limited by its wavelength (penetration is better with longer wavelengths[1]. This, therefore, means that its use in other regions beyond the skin is limited. The use of laser Doppler is more suited to acute studies rather than longer term intervention trials[2]. Herbs have been used for many purposes, including medication, nutrition, flavouring, beverages, fragrance and cosmetics. Much of the early interest in functional foods, nutraceuticals and cosmaceutical was based on the medicinal uses of herbs. The MC Gel (Table 1) is an herbal formulation containing, herbal active named biotropics®FD101 derived from extract of ficus deltoidea (pending patent no. PCT/MY2011/000008). The present study was planned to evaluate the cutaneous microcirculation of MC Gel for cosmaceutical use.

Table 1: Composition of MC Gel Formulations

S.No.	INCI Name	Trade Name	Function	Content A (%)	Content B (%)	Content C (%)	Content D (%)
1	Water	Amigel	Thickner	qs	qs	qs	qs
2	Sclerotium Gum		Humectant	2.00	2.00	2.00	2.00
3	Glycerin			3.00	3.00	3.00	3.00
4	Active	biotropics®FD101		0.50	1.00	2.50	-
5	Water			5.00	10.00	25.00	25.00
6	Phenoxyethanol(and) Methylisothiazolinone	Neolone PE	Preservative	0.50	0.50	0.50	0.50

Legends: A= MC Gel Formulation P11/FP001; B= MC Gel Formulation P11/FP002; C= MC Gel Formulation P11/FP003; D= MC Gel Formulation P11/FP004; biotropics®FD101= *ficus deltodeia* extract

MATERIALS AND METHODS

Aim of the study

This study was aimed to evaluate the effect on the cutaneous microcirculation of three cosmetic products "MC Gel batch no: P11/FP001", "MC Gel batch no: P11/FP002" and "MC Gel batch no: P11/FP003" containing 0.5%, 1.0% and 2.5% of active (biotropics®FD101) respectively after 15, 30, 60, 90 and 120 minutes of their single standardized application and versus placebo "MC Gel batch no: P11/FP004".

Study design

This study was simple blind, intra-individual clinical trial conducted at the Ellead skin research centre, Ellead Co., Ltd. 272-1, Sehyneo-dong, Boondang-gu, Seongnam-si, Gyeonggi-do, Korea from January 20 to February 23, 2011, as per the ethical guidelines of the Declaration of Helsinki. The study protocol, case report forms (CRFs), regulatory clearance documents, product-related information and informed consent forms were submitted to the Institutional Ethics Committee and approved by the same.

Inclusion criteria

Twenty (20) female healthy volunteers, aged 23 to 64 with specific phenotype I, II and III were included in the study, conducted at the Ellead skin research centre, Ellead Co., Ltd. 272-1, Sehyneo-dong, Boondang-gu, Seongnam-si, Gyeonggi-do, Korea from January 20 to February 23, 2011. A written informed consent was obtained from all patients.

Exclusion criteria

Subject below 18 years of age, subject already on medication or therapy, subjects with preexisting severe skin diseases or cutaneous pathology on the studied zone, subjects with microcirculatory or cardiovascular disorders, subjects having treatment of pulmonary, cardio, circulatory problems or undergone surgery since less than one month, subjects with excessive consumption of alcohol, tea or coffee and those who refused to give informed consent were excluded from the study. Pregnant or lactating women and women having change, started or stopped oral contraceptive or hormonal therapy since less than 1.5 months were also excluded from the study.

Study procedures

A baseline history was obtained in order to determine the patient's eligibility for enrolment in the trial. At day 0, subjects came to the laboratory without having applied any product to the forearms since the previous evening. The informed consent procedure was conducted and subjects received a copy. Verification of inclusion and exclusion criteria was carried out with clinical examination. The subject's forearms were defined with four zones: one zone for each product. A measurement of the cutaneous microcirculation with the Laser Doppler on the previously defined zone was carried out. Further a standardized application (2µl/cm^2) of each studied product was applied to the predefined zones (Fig 2). The assessment of capillary flux was carried out at 15, 30, 60, 90 and 120 minutes after product application.

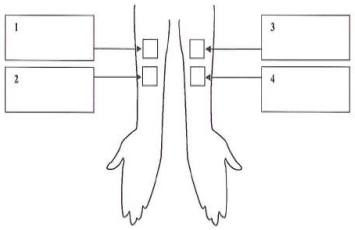


Figure 2: Demonstration of standardized application to predefined zones

Primary and secondary outcome measures

The predefined primary outcome was measurement of cutaneous microcirculation with Laser Doppler. The predefined secondary outcome measures were incidence of adverse events and compliance to the treatment.

Adverse events

All local and systemic adverse events, reported or observed by patients were recorded with information about severity, time of onset, duration and action taken regarding the study drug. The severity/intensity of adverse events was graded on a three-point scale; Mild or Grade 1: discomfort noted, but does not disturb normal daily activities; Moderate or Grade 2: discomfort sufficient to reduce or affect normal daily activities; Severe or grade 3: inability to work or have normal daily activities. Patients were allowed to voluntarily withdraw from the study, if they had experienced serious discomfort during the study or sustained serious clinical events requiring specific treatment. For patients withdrawing from the study, efforts were made to ascertain the reason for dropout.

Statistical analysis

Statistical analysis determined the significance of the measurement variations obtained under the effect of the tested product. The comparison was on the values obtained before and at the different times of kinetics after treatment. Data were analyzed with a paired t-test. Before carrying out a test, a type I error of 5% was chosen. The minimum level of significance was fixed at 99% confidence limit and a 2-sided p value of ≤0.05 was considered significant.

RESULTS

Twenty (20) female healthy volunteers, aged 23 to 64 with specific phenotype I, II and III were included in the study and no patients were lost to follow up. In comparison with the initial state, product "MC Gel P11/FP001" did not induce any significant variation in the capillary flux, 15, 30, 60, 90 and 120 minutes after its single standardized application (Table 2). In addition no significant difference in the capillary flux was measured in comparison to placebo "MC Gel P11/FP004".

In comparison with the initial state, product ""MC Gel P11/FP002" did not induce any significant variation in the capillary flux, 15, 30, 60, 90 and 120 minutes after its single standardized application. However a tendency to an increase in the capillary flux was measured 30 minutes after the single standardized application (+10% on average), effect measured in 59% of the subjects (Table 3). No significant difference concerning the capillary flux between product and placebo was measured (Table 2).

In comparison with the initial state, product "MC Gel P11/FP003" did not induced any significant variation in the capillary flux, 15, 30, 60, 90 and 120 minutes after its single standardized application (Graph 1). However a tendency to an increase in the capillary flux was measured during the kinetics; +11%, +13%, +12%, +13% on an average in 56%, 56%, 53%, and 58% of the subjects at t_{15}, t_{60}, t_{90} and t_{120} respectively (Table 3). A significant difference (+1.46 P.U. on average) was measured at t_{15} min in favour of product MC Gel F11/FP003 compared to placebo which induced a higher increase in the cutaneous microcirculation (Table 4).

Table 2: Variations of the capillary flux (in Perfusion Units), after a single standardized application of MC Gel P11/FP001, MC Gel P11/FP002 and MC Gel P11/FP003 in comparison with the placebo

Kinetic(min)	Δ (Mean ± SEM)			
	MC Gel P11/FP001	MC Gel P11/FP002	MC Gel P11/FP003	MC Gel P11/FP004
Δt_0-t_{15}	-0.38±0.63	+0.33±0.64	+0.33±0.64	+1.10±0.64
Δt_0-t_{30}	-0.79±0.74	+1.04±0.64	+1.04±0.64	+0.64±0.52
Δt_0-t_{60}	+0.01±0.67	+0.31±0.42	+0.31±0.42	+0.90±0.71
Δt_0-t_{90}	-0.41±0.77	+0.60±0.57	+0.60±0.57	+0.99±0.87
Δt_0-t_{120}	-0.69±0.77	+0.24±0.64	+0.24±0.64	+1.06±0.61

Data expressed in Mean ± SEM for *n* = 20

Table 3: Variations of the capillary flux (in Perfusion Units) after a single standardized application of MC Gel P11/FP001, MC Gel P11/FP002 and MC Gel P11/FP003 in comparison with placebo with respect to effect measured in percentage of subjects

Kinetic (min)	MC Gel P11/FP001		MC Gel P11/FP002		MC Gel P11/FP003		MC Gel P11/FP004	
	Average Δ%	% of subjects with positive effect	Average Δ%	% of subjects with positive effect	Average Δ%	% of subjects with positive effect	Average Δ%	% of subjects with positive effect
Δt_0-t_{15}	-4%	26%	+3%	44%	+11%	56%	-4%	32%
Δt_0-t_{30}	-11%	39%	+10%	59%	+6%	41%	-1%	50%
Δt_0-t_{60}	0%	42%	+1%	59%	+13%	56%	-8%	37%
Δt_0-t_{90}	-4%	42%	+8%	47%	+12%	53%	-11%	33%
Δt_0-t_{120}	-7%	42%	+3%	47%	+13%	58%	-4%	37%

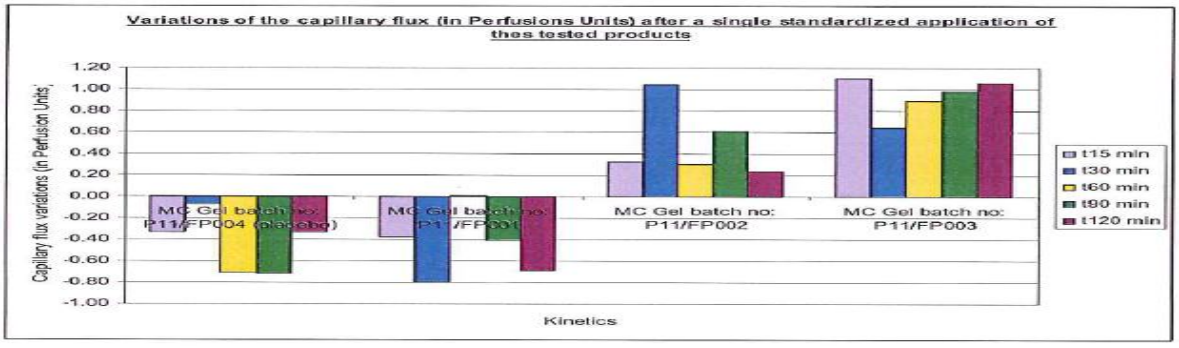
Table 4: Variations of the capillary flux (in Perfusion Units) after a single standardized application of MC Gel P11/FP001, MC Gel P11/FP002 and MC Gel P11/FP003 in comparison with the initial state and comparison product/placebo

Kinetic (min)	Δ (Mean ± SEM) Comparison MC Gel P11/FP001/Placebo	Comparison MC Gel P11/FP002/Placebo	Comparison MC Gel P11/FP001/Placebo
$\Delta\Delta t_0$-t_{15}	-0.04±0.76	+0.44±0.64	+1.46±0.66*
$\Delta\Delta t_0$-t_{30}	-0.45±0.83	+1.46±1.03	+0.87±0.93
$\Delta\Delta t_0$-t_{60}	+0.72±0.97	+0.54±0.72	+1.26±1.06
$\Delta\Delta t_0$-t_{90}	+0.46±0.99	+1.21±0.86	+1.72±1.14
$\Delta\Delta t_0$-t_{120}	-0.35±0.95	+0.57±0.80	+1.39±0.88

Data expressed in Mean ± SEM for *n* = 20

***Significantly higher cutaneous microcirculation (p≤0.05)**

Graph 1: Variation of the capillary flux (in Perfusion Units) after a single standardized application of MC Gel P11/FP001, MC Gel P11/FP002, MC Gel P11/FP003 and MC Gel P11/FP004 (Placebo).



There were no clinically significant short and long-term adverse reactions (either reported or observed), during the study.

DISCUSSION AND CONCLUSION

The skin is a large focal for research with interest from the cosmetics industry [5,6]. Indeed, it is commonly investigated, as it is easily accessible (as compared with other circulations) and often afflicted by disease[4]. There are a huge range of methods and dynamic tests for studying the microcirculation of the skin and therefore the present technique (LDF) was used for the non-invasive approaches employed in clinical investigation. The arterioles, venules, capillaries and arteriovenous anastomosis make-up the vasculature of the microcirculation[7]; in the skin, the vasculature is arranged into superficial and deep horizontal plexuses. The superficial plexus (lying in the papillary dermis) branches to form the dermal papillary loops (comprised of capillaries and primary sites) for oxygen and nutrient exchange[4]. The capillary flow is recorded via LDF and it demonstrates compartment flow and, dependent on the region under investigation, between 5% and 10% [4]. The present investigation was aimed to evaluate the effect on the cutaneous microcirculation of three cosmetic products "MC Gel batch no: P11/FP001", "MC Gel batch no: P11/FP002" and "MC Gel batch no: P11/FP003" containing 0.5%, 1.0% and 2.5% of active (biotropics®FD101) respectively after 15, 30, 60, 90 and 120 minutes of their single standardized application and versus placebo "MC Gel batch no: P11/FP004". This study observed significant difference in favor of product containing 2.5% biotropics®FD101 (MC Gel P11/FP003) compared with placebo. It induced a higher increase in the cutaneous microcirculation. The product containing 0.5% biotropics®FD101 (MC Gel P11/FP001) did not induce any significant variation and remained non effective. However product containing 1.0% biotropics®FD101 (MC Gel P11/FP002) induced a tendency to an increase in microcirculation 30 minutes after the application. In addition product containing 2.5% biotropics®FD101 also induced a tendency to increase in microcirculation from 15 to 120 minutes after standardized application. Furthermore, a significant difference was measured at 15 minutes in favour of product containing 2.5% biotropics®FD101 compared to placebo which induced a higher increase in cutaneous microcirculation. The products containing 0.5% and 1.0% biotropics®FD101 did not induced any statistically different results of those obtained under placebo. Under this investigation, the cutaneous microcirculation or skin blood flow is confirmed by product containing 2.5% biotropics®FD101 and detail the drug delivery by this cosmaceutical to the vasculature of superficial and deep horizontal plexuses.

There were no clinically significant short and long-term adverse reactions (either reported or observed), during the entire period of study and excellent patient compliance to MC Gel was also observed. This favorable increase in the microcirculation in skin by product containing 2.5% biotropics®FD101 might be due to synergistic actions of its ingredients or compositions. Based on these observations, it may be concluded that the product containing 2.5% biotropics®FD101 confirmed drug delivery via microcirculation. It is also clinically effective and safe for external usage as cosmaceutical.

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