Assessment of Transdermal Vasodilatatory Effect of a Combined Panthenol, Amlodipine, Isosorbide Dinitrate and Betahistine HCl on Peripheral Vessels

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Abstract

Peripheral vascular diseases are group of disorders characterized by stenozing peripheral circulation as a complication of primary disease like diabetes mellitus. Peripheral vascular diseases affect 202 million around the world. Peripheral vascular complications of diabetes mellitus are common which affect 30 million over the world and may give rise to infectious necrotizing sequalae called diabetic foot. In a trial of assessment for transdermally applied vasodilator drugs, 30 individual had participated in a case controlled study. Test group (N =11) and control group (N =19) were assessed for the signs of vasodilatation over the dorsum of the hand and figure blood perfusion detection. There was significant increase in perfusion index (from 7 to 11) induced by the test vasodilator as compared with control (from 7 to 8) $P < 0.05$ and parallel results were obtained in induction of redness (redness ratio of 7 with C.I. over 0.95 and thermal increase in degrees of (C) over the dorsum of the hand in comparison with the control group. From the overall results the combined vasodilatatory transdermal formula caused a significant peripheral vasodilatation which could be a candidate therapeutic effect in diabetic foot.

Key words: *PVD; combined transdermal formula; peripheral blood perfusion; thermal effect; nitrate; RGB.*

Introduction

Peripheral vascular disease (PVD) is the abnormality of vessels located outside of the heart and brain, mainly leg vessels characterized by complete or partial blockage and impairment of perfusion. PVD could affect both arteries (peripheral arterial disease PAD) and veins. PVD is one of the significant health challenges that affects up to 20% after the age of 60 year [(1,2)] and 202 million patients around the world [(3,4)].

Different etiologic and pathogenic factors might share common impacts on peripheral circulation. Of the most critical form of PVD presentation is the angiopathic complications of diabetes mellitus[(5-7)].

However, Burger disease and Raynaud phenomenon are also characterized by impaired peripheral circulation [(8,9)]. Impaired peripheral circulation in uncontrolled diabetes is characterized by different pathological processes including occlusive ischemia [(10-12)], impaired immune response and peripheral neuropathy [(13,14)]. Impairment of circulation and immune system in addition to hyperglycemia and neuropathy can severely deteriorate any skin lesion in the foot. That lesion may be refractory to treatment due to lacking of pharmacokinetic opportunity of drugs treatment to diffuse to extreme tissues of the lower limbs. This pathogenic fact make foot prone for untreatable infections that even cause ascending cellulitis, tissue gangrene and septicemia which is frequently necessates amputation of the foot or lower limb[(15)].

A potent systemic vasodilatatory approach like nefidipine, nicorandil can cause intolerable adverse effects like palpitation, throbbing headache and edema due to the need for extensive vasodilatation [(16-18)]. Transdermal route of application gives opportunity for rational option of applying combined efficacious

vasodilators just proximal to the site of lesion that bypassing systemic impacts [19-21].

Different methods are used to assess peripheral circulation. These include: Doppler ultrasound, angiographic techniques in addition to the clinical assessment of peripheral pulsation. However, other methods like infrared detection of blood flow. Quantitative assessment of peripheral perfusion is detected by the perfusion index PI which ranges between 0.02% to 20% according to site whereas blood flow is 5000 ml/min[22,23]. Tissue blood flow rate varies also according to type of tissue. Thermographic camera images are also reliable noninvasive methods for long term follow up of circulatory perfusion [24-26].

In the present work, the effect of transdermally applied combination of panthenol, mineral oil base, amlodipine, pH buffer, isosorbide dinitrate, stabilizer and betahistine with peripheral perfusion rate and thermographic analysis were assessed.

Sample, Materials and Method

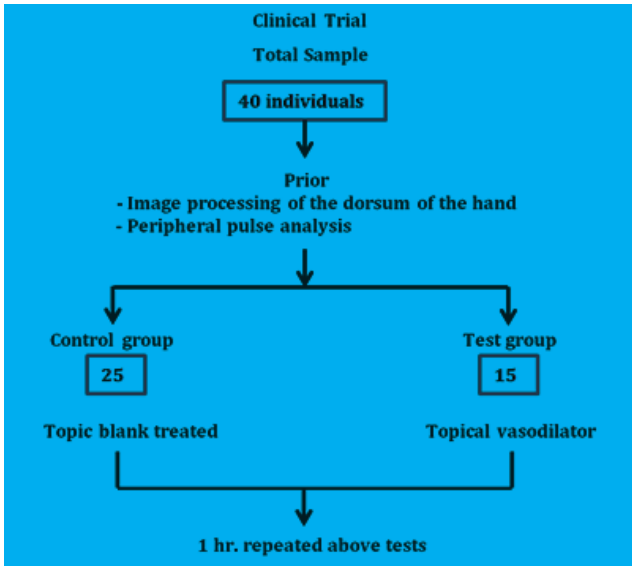


Figure (1) A diagram of the main practical steps of conducting this study.

A 40 individuals (20f/20m) aged 20-24 years (body weight 50-65 kg) have participated in clinical assessment of the effects for the designed formula.

Groups of the study:

1- Test group (N = 15: 8 f/ 7 m).

2- Control group (N = 25: 12 f/ 13 m).

All persons had no chronic medical illnesses.

Study design

The study was arranged in a pilot clinical trial design to determine the effects of test formula so that one group had transdermally applied vasodilators and the other had only the blank application.

Medical and Research ethics requisites

These considerations were fulfilling Geneva requisites guide for medical ethics. All of the participants were informed about the study design and the expected topical adverse effects of the agents used.

Free autonomy, drugs safety, rights reservation, individual consents and drugs benefits were considered and insured.

Materials used

All the materials used were processed, filtered, and confirmed with Fourier UV/V range 200-700 nm based on British Pharmacopia 2007 drugs standard spectrophotometry.

Drug dosages were weighed for base of transdermal formula design.

- Amlodipine (Bristo, UK, production vs expiry:PD-ED: 2013-2016)

7000 mg package. Used in 3% within transdermal design.

- Isosorbide dinitrate (Epico, Egypt, PD-ED: 2013-2015)

100 mg package. Used in 0.05%.

- Panthenol (Zynova, Oman, PD-ED: 2014-15). Used in dosage of 0.3%.

- Betahistine HCL (Aleppo, Syria, PD-ED; 2013-2015). Used in 0.02%.

- Hydrocarbone base vehicle.

Mineral laurate, stearate and solid excipients were prepared for 20 ml per dosage form.

Treatment Mode

Objective vasodilatation parameters were measured prior to application of both blank and test formula as a

zero reading. Then 10 minutes after application of the blank and the control formula another record of RGB, thermograph and PI were measured after careful topical message over the dorsum of the right hands only for standardization.

Methods of assessment of vasodilatation

Peripheral blood perfusion was measured with Beijing Safe Heart Technology. Transducer was connected to PERFUSION-Kufa program of analysis developed by Dr. Hussein Abdulkadhim on Mathwork 2013a blockset for estimating model formula of perfusion in response to the transdermal test treatment.

Perfusion index PI is readily detected and calculated by the computer from which another measurement could be estimated which is the perfusion range is determined with in unit of time to calculate the rate of perfusion in ml/min and compared for the control and observational control group. The fixed level of the hand and index figure was carefully considered because it is important confounder.

Another assessment method for vasodilatatory activity of the test formula includes thermographic correlative analysis detected by combined spot tissue thermal camera

Since temperature correlates proportionally with rate of peripheral blood perfusion, a rise in temperature of the dorsum of the hand correlates with a parallel rise in the rate of perfusion (parameters detailed in guidelines manual).

Image processing program was used to analyze RGB shift as an indicator of redness associated with vasodilatation by the test combination.

Lab techniques quality confirmation:

Accuracy of Safe Heart Technology was X +/- 0.2 PI. Efficiency of the thermographic camera was insured by software processing and calibration.

UV/V (Cecil, UK, Programmed wavelength, accuracy: X +/- 0.5%). Sensitive Balance (mini digital, China, accuracy: X +/- 0.01 mg). PHELECT, USA computerized pH measuring electrode is used to assess adjustment of formula pH around 7.5.

The Sample Size, Statistical Processing and Analysis

The sample size for this study was calculated based on the Cochran formula n= z^2 * p*q/e^2 where (e) is the margin error, p and q are the complimentary proportions and z is the score at confidence level 95% equals to 1.96. Although the margin error will be wide, however it's preferred to reduce the sample size as much as it's statistically possible in a pilot clinical trial in order to fulfill FDA approval guidelines where a sample size of 10 individuals is considered while conducting a phase zero trial.

Mathwork 2013a model interpolation was used to verify the perfusion curve. Perfustion index PI modification ratio was estimated with C.I. of 0.24 and a cnfidence level of 95%. Sample size was determined based on Cochran formula. Paired t test with Minitab 2014 at P <0.05.

Results

Calculation of the rate of perfusion.

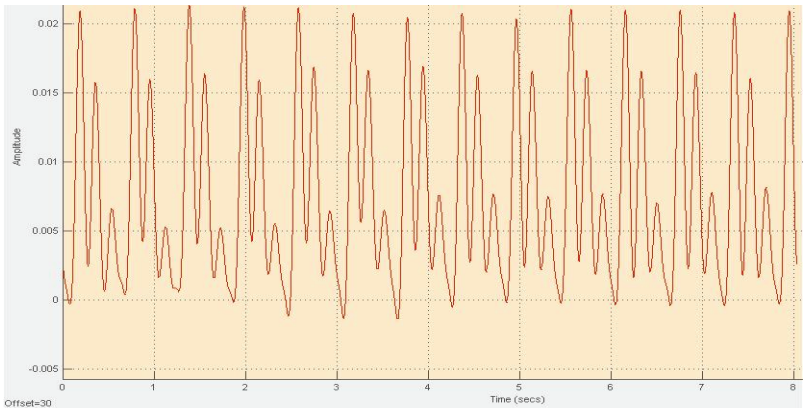


Figure (2): Matlab analysis of peripheral perfusion rate by model analysis and rate determination. The amplitude axis was calibrated to obtain perfusion rate in mL/min.

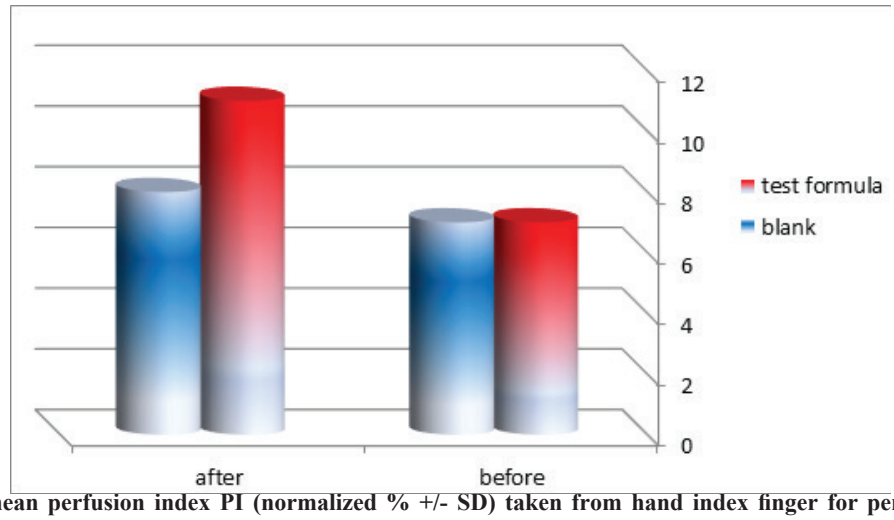
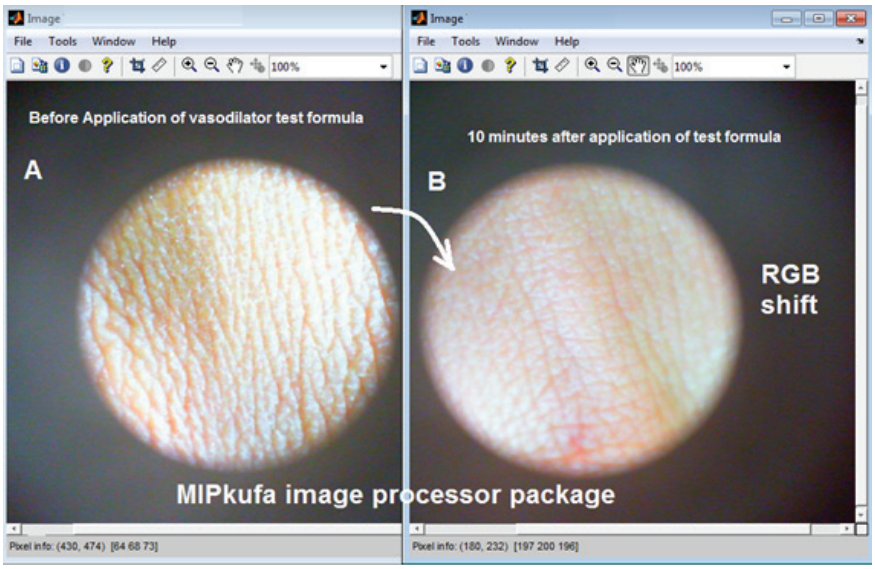


Figure (3): The mean perfusion index PI (normalized % +/- SD) taken from hand index finger for persons taking the test transdermal formula (raised from 7 +/- 2 to 11 +/- 2) as compared with the control (7 +/- 2 to 8 +/- 2 PI).



Topical RGB findings for assessment of redness induction as an indicator of vasodilatatory effect.

Figure (4): The objectively analyzed RGB to determine level of redness induced on the dorsum of the hand by the combined vasodilator formula after 10 minutes from topically being applied as a quantitative indicator of vasodilatatory effect.

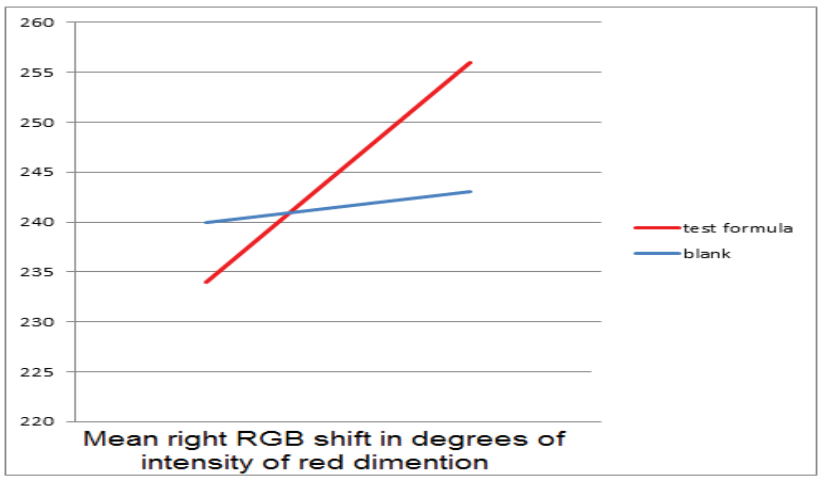


Figure (5): The effect of the test formula on the RGB right shift of red intensity in comparison with the control. Red intensity shift was from 234 to 256 as compared to control which caused just a shift from 240 to 243.

Discussion

The sample of this study represented healthy nondiabetic population. It was taken as a model to assess the vasodilatation at the upper extremities where temperature, RGB and peripheral perfusion were measured from.

That sample was beneficial to estimate the vasodilatatory prior to recommendation in diabetic patients' sample since this was in agreement with FDA phases of drug evaluation.

Transdermal approach is a promising way of treatment since many advanced techniques had made this route more reliable and superior to conventional systemic administration of drugs. Of these advance techniques are the nanoparticulate reservoir of active drug, sonophoresis, electrophoresis, microarray needle and matrix patches [27].

Transdermal application of drugs has many advantages like convenience, safety and it is easily controlled.

The results showed that a significant increase in perfusion index (PI) was obtained with the test formula (from 7 to 11%) in comparison with the blank base (from 7 to 8%) with ratio of increase 1.3 in PI. In one study, amlodipine showed a significant induction of increased forearm blood flow. Results of some studies concerned assessment of vasoliatatory effect of transdermal amlodipine monotherapy have agreed with this current study [28]. Panthenol in combination with other additives was used as a topical wounds healing enhancer [29]. Betahistine effects in some applications agreed with this study [30], however there was no improvement of cold regional pain syndrome by using transdermal isosorbide dinitrate in a small controlled trial [31,32].

Redness parameters have confirmed and went parallel to data of perfusion rate increment. The mean RGB was raised from 234 to 256 in test formula group as compared with a mild raise from 240 to 243 in blank taken group. This indicates a redness ratio of 7 with C.I. at 0.95 [33].

Thermographic results revealed parallel findings with redness image processing outcomes. Hand temperature was raised from 36.0 to 37.8 °C in vasodilatatory test group in comparison with 35.5 to 36.1 °C in the control. The temperature raising activity was 3 and C.I. at 0.95.

Conclusion

From the overall effects, the combined transdermal formula panthenol, mineral oil base, amlodipine, pH buffer, isosorbide dinitrate, stabilizer and betahistine showed a significant vasodilatatory effects on the peripheral circulation. There was a significant increase of thermal effect due to the topical combination of vasodilators in comparison with the control

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

Conflict of Interest: The authors declare that they have no conflict of interest.

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