

Original Article

IMPROVING TRANSDERMAL DRUG DELIVERY SYSTEM FOR MEDROXYPROGESTERONE ACETATE BY OLIVE OIL AND DIMETHYLSULFOXIDE (DMSO) AS PENETRATION ENHANCERS: *IN VITRO* PENETRATION STUDY

CAMELIA DWI PUTRI MASRIJAL¹, HARMITA HARMITA¹, ISKANDARSYAH ISKANDARSYAH^{1*}

¹Laboratory of Physical Pharmacy, Faculty of Pharmacy, Universitas Indonesia, Depok, 16424, Indonesia
Email: iskandarsyah@farmasi.ui.ac.id

Received: 03 Jan 2020, Revised and Accepted: 18 Feb 2020

ABSTRACT

Objective: Medroxyprogesterone Acetate (MPA) using a transdermal drug delivery system for contraception by passive diffusion is limited by the skin barrier properties. Penetration enhancers such as olive oil (fatty acid permeation enhancer) and DMSO (chemical enhancer) can be used. The objective of this study was to overcome MPA penetration problem by using olive oil and DMSO.

Methods: An *in vitro* penetration study using the Franz diffusion cells was performed. The first penetration study used MPA in olive oil (O) and MPA in coconut oil (C) with the concentration 100 µg/ml to each sample and MPA suspension as a control with the same concentration. The second study used MPA in olive oil with the concentration 200.0 µg/ml (A), MPA in olive oil with 0.5% DMSO with the concentration 200.0 µg/ml (B), and MPA in olive oil with 1% DMSO with the concentration 200 µg/ml (C).

Results: MPA penetration test for olive oil+0.5% DMSO had flux value 4.24 ± 0.074 µg/cm². hr and it was not significantly different (t-test, P>0.05) with olive oil+1% DMSO. While the MPA penetration test in only Olive oil had flux value 0.90 ± 0.0087 µg/cm². hr.

Conclusion: This research concluded that olive oil and 0.5% DMSO could improve the penetration of MPA into skin membrane by 4.5 times more than olive oil alone.

Keywords: Medroxyprogesterone acetate, Olive oil, DMSO, Franz diffusion cells, Subcutaneous penetration, Transdermal drug delivery system

© 2020 The Authors. Published by Innovare Academic Sciences Pvt Ltd. This is an open access article under the CC BY license (<http://creativecommons.org/licenses/by/4.0/>) DOI: <http://dx.doi.org/10.22159/ijpps.2020v12i4.36762>. Journal homepage: <https://innovareacademics.in/journals/index.php/ijpps>

INTRODUCTION

Medroxyprogesterone acetate is a synthetic progestin which is used as long-acting hormonal contraceptive and anabolic steroid. The effectiveness of MPA as a contraceptive is for 3 mo when used as 150 mg intramuscularly or 104 mg subcutaneously [1].

Using a transdermal drug delivery system for contraception presents some clinical advantages over conventional oral contraception [2]. The once-weekly administration is more convenient for women than once-daily administration with oral contraception and should also improve efficacy by decreasing the degree to which it is dependent on user compliance [3]. From a pharmacokinetic perspective, transdermal delivery of contraceptive hormones eliminates variability in gastrointestinal (GI) absorption, due to factors such as stomach pH, stomach emptying rate, GI motility, and GI transit time. The drug is delivered directly into the systemic circulation, avoiding the hepatic first-pass metabolism experienced with oral contraception and maintaining constant drug concentrations in the circulation by eliminating peaks and troughs in serum concentrations climatic that occur with oral administration [4].

The number of drugs that can be delivered by passive diffusion is limited by the barrier properties of the skin. Only low molecular-weight substances (<1000 Da) with the log p-value of <5 can be delivered effectively through the skin. The drug molecule itself needs to be potent because of the amount that can be delivered [5].

Penetration enhancers are the substances that functions by lowering the potential of the barrier characteristics of skin so that it turns more permeable for the drug molecules to traverse the skin rapidly. The drug diffusivity in the stratum corneum (SC) can be increased by these substances by liquefying the skin lipids or by denaturing skin proteins [6].

Olive oil is natural penetration enhancer extracted from the fruits of *Olea europaea* tree. The main constituents are tripalmitin, triolein,

tristearate, trilinolein, triarachidi, squalene, monostearate, tocopherol and b-sitosterol. Olive oil is a very potent fatty acid permeation enhancer [7] while DMSO is a chemical enhancer that act by disruption of the highly ordered lipids of the stratum corneum that can make enhancement drug across the skin mucosa [8].

In this study, the research question was the potential of using olive oil and DMSO as an improvement for MPA penetration. *In vitro* penetration study using Franz diffusion cells was performed to answer this question. The suspension of MPA was made as a control.

MATERIALS AND METHODS

Chemicals and animals

The materials that were used in this study were: medroxyprogesterone acetate (Sigma Aldrich), olive oil, virgin coconut oil, hydroxypropyl methylcellulose, phosphate buffer pH 7.4, dimethylsulfoxide were from Merck (Darmstadt, German), methanol, double-distilled water, formic acid and acetonitrile were of an analytical grade that purchased from Jakarta, Indonesia. The animals in this study were white female rats (*Sprague Dawley* strain), aged 2-3 mo and weight about 150-200 g were provided by Bogor Agricultural Institute. All of the methods for sacrificing the animals have been approved by the ethics committee from Cipto Mangunkusumo Hospital, Faculty of Medicine, Universitas Indonesia (approval no: KET-943/UN2. F1/ETIK/PPM.00.02/2019).

Preparation of a calibration curve of a standard solution of MPA

A calibration curve of a standard solution of MPA in methanol was made. The concentration of the standard solution was 1000 µg/ml. It was diluted to obtain six different concentrations ranged from 10 to 60 µg/ml. Each standard solution then analyzed by chromatography at a wavelength of 240 nm using a High-Performance Liquid Chromatography HPLC with the mobile phase was composed of 0.1% formic acid solution and acetonitrile; flow rate of 1.0 ml/min; and the injection volume was 20 µl.

In vitro penetration test

At the first study, MPA suspension was made as a control. HPMC was dispersed in distilled water then mixed with the gel whilst stirring. Solution of MPA was added and the concentration of suspension was 100.0 µg/ml (S). While MPA in olive oil (O) and coconut oil (C) was also made with the concentration 100.0 µg/ml.

At the second study, MPA in olive oil was made with the concentration 200.0 µg/ml (A), MPA in Olive oil with 0.5% DMSO with the concentration 200 µg/ml (B), and MPA in Olive oil with 1% DMSO the concentration 200 µg/ml (C).

In vitro Penetration Test: The abdomen skin of female *Sprague Dawley* rats aged 2-3 mo was used as a membrane in the test. Permeation studies were performed by using a Franz diffusion cell apparatus with a receptor compartment capacity of 16 ml (area 3.14 cm²) [9]. The excised dorsal rat abdominal skin was mounted between the donor and receptor compartment of the diffusion cell. The rat skin was adjusted with the stratum corneum layer facing

into the donor compartment, while the dermis faced the receptor compartment. Phosphate-buffered saline (pH 7.4) were used as the receptor solution for penetration studies of all formulations prepared (temp 37 °C±2 °C) 12 h [10]. The 1.0 ml sample solution (O), (C) and (S) were placed over the skin with stratum corneum with fat (F) and non-fat (NF) facing the donor compartment. The samples (2 ml) were withdrawn at different intervals (1,2,3,4,5,6,8,10 and 12 h) and analyzed for drug content by HPLC analysis. The experiment was conducted in triplicate and with measurement of means. The amount of cumulative release MPA per square centimeter of the sample was plotted against time to obtain the flux value.

RESULTS

Preparation of a calibration curve of a standard solution of MPA

Calibration curve of MPA was linear with the correlation coefficient (r²= 0.998) in the concentration range from 10 to 60 µg/ml as shown in fig. 1.

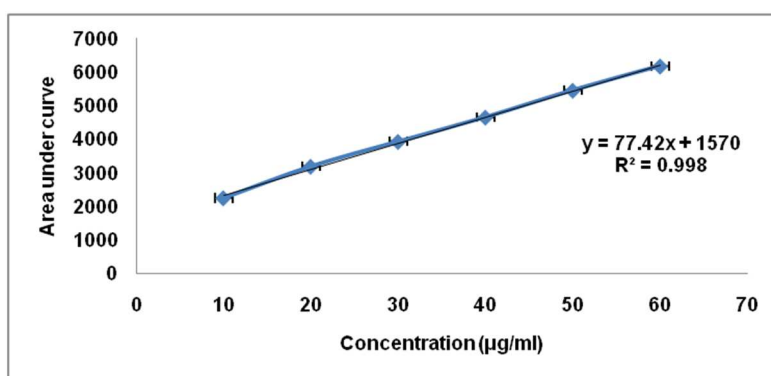


Fig. 1: Calibration curve of MPA

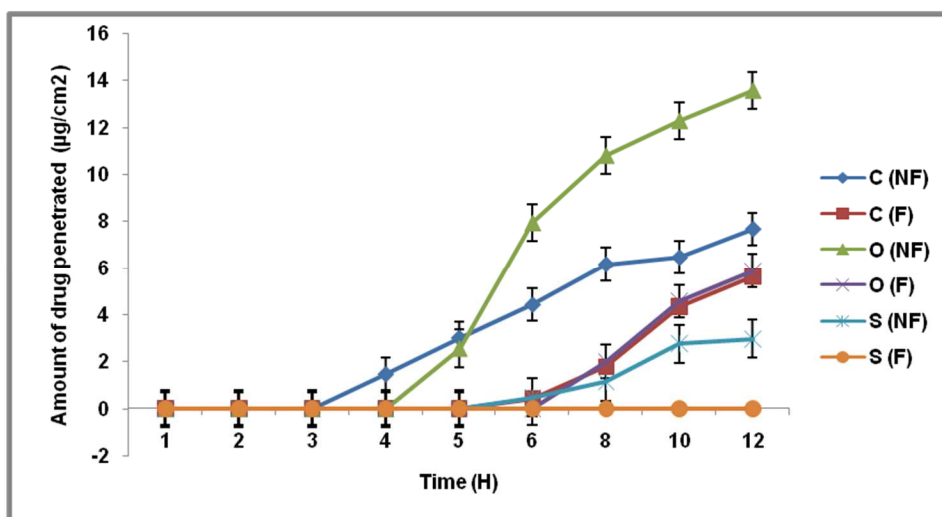


Fig. 2: Cumulative of total MPA penetrated, all values were represented as mean±SD (n=3). NF: Non-Fat, F: Fat

At the first study, MPA penetration test was conducted in coconut oil and olive oil as natural penetration enhancer with MPA suspension as control and amount of penetrated as shown in fig. 2.

The MPA penetration test with non-fat skin rat in olive oil had the highest cumulative percentage release of 13.56±0.23% and was started to appear after 4 h, while with fat skin rat the result at 5.87±0.11% after 6 h. The MPA penetration test with non-fat skin rat in coconut oil had the percentage of release 7.67±0.21% and

began to appear after 3 h, while with fat skin rat at 5.64±0.14% after 6 h.

The MPA suspension gave a percentage of release at 2.96±0.04% and began to appear after 6 h, while with fat skin rat did not had any release until 12 h.

The result showed in fig. 3, the flux value of O(NF) had the highest flux value was 0.64±0.027 µg/cm². hr. C(NF), O(F), C(F), and S(NF) were 0.346±0.034 µg/cm². h, 0.218±0.031 µg/cm². h, 0.210±0.028 µg/cm². h and 0.121±0.018µg/cm². h, respectively.

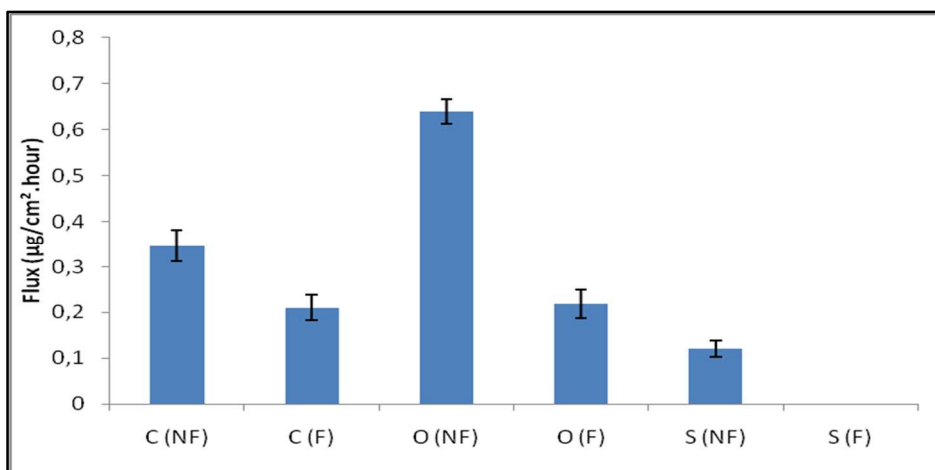


Fig. 3: Average penetration flux of O(NF) C(NF), O(F), C(F),S(NF), and S(F) (mean±SD, n=3)

At second MPA penetration test, the MPA penetration test with fat skin rat in Olive oil+0.5% DMSO had the cumulative percentage release 29.95±% and began to appear after 2 h, with the cumulative amount of MPA penetrated was 9.66±0.12µg/cm². The MPA penetration test with fat skin rat in Olive oil+1% DMSO had a percentage of release 30.19±% and also began to appear after 2 h

with the amount of MPA penetrated was 9.71±0.25µg/cm². Both of 0.5% and 1% DMSO were not significantly different (t-test, P>0.05) While the MPA penetration test with fat skin rat in Olive oil had the percentage of release 12.72±% and began to appear after 6 h, with the amount of MPA penetrated was 4.06±0.016 µg/cm², as shown in fig. 4.

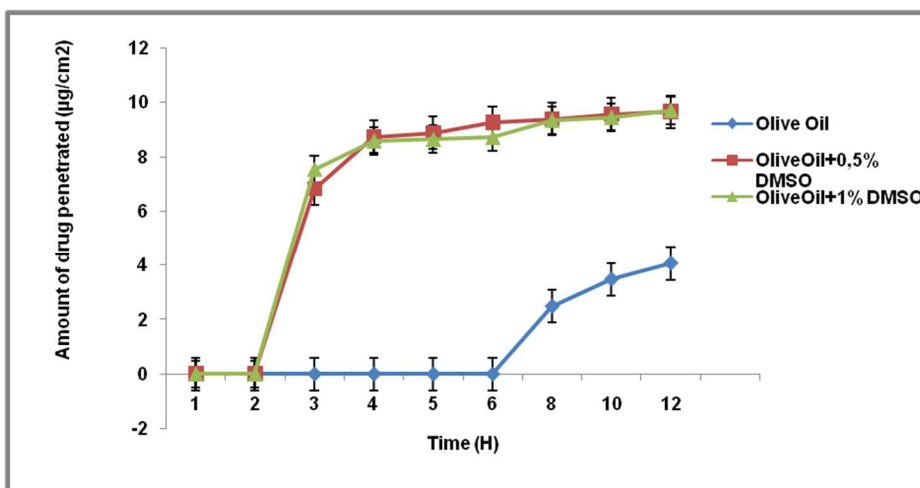


Fig. 4: Cummulative of total MPA penetrated, all values were represented as mean±SD (n=3)

The result showed in fig. 5, the flux value of Olive oil+0.5% DMSO, Olive oil+1% DMSO and Olive oil were 4.24±0.074

µg/cm². h, 4.20±0.036µg/cm². h, and 0.90±0.0087 µg/cm². h, respectively

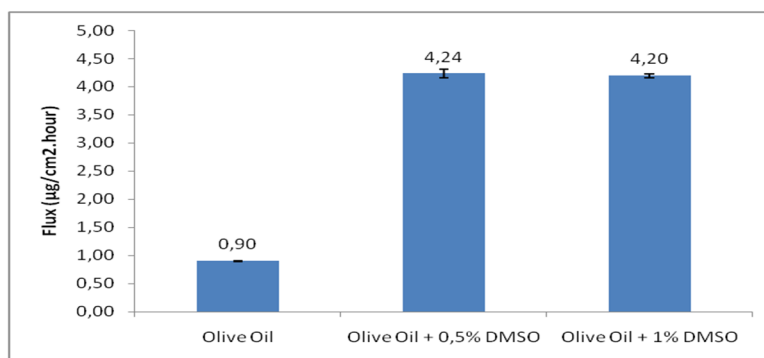


Fig. 5: Average penetration flux of olive oil+0,5% DMSO, olive oil+1% DMSO and olive oil) (mean±SD, n=3)

DISCUSSION

For the first MPA penetration test, olive oil in the non-fat skin rat gave the highest percentage of release at $13.56 \pm 0.23\%$ and began to appear after 4 h, while with fat skin rat $5.87 \pm 0.11\%$ after 6 h. This indicated that MPA was deposited in fat for 2 h with Olive oil enhancer. The MPA penetration test with nonfat skin rat in coconut oil had the percentage of release at $7.67 \pm 0.21\%$ and began to appear 3 h, while with fat skin rat $5.64 \pm 0.14\%$ after 6 h. This indicated that MPA was deposited in fat for 3 h with a coconut oil enhancer.

The MPA suspension had percentage of release at $2.96 \pm 0.04\%$ and began to appear after 6 h, while with fat skin rat until 12 h did not have any release. From this penetration test, MPA with suspension as a control had difficulty to traverse the skin without any enhancer while olive oil had a higher percentage of release than coconut oil.

The flux value of O(NF) had the highest flux value $0.64 \pm 0.027 \mu\text{g}/\text{cm}^2 \cdot \text{hr}$. The flux value obtained from the comparison between the cumulative numbers of penetration to time [11].

Natural oils are complex lipid mixtures containing various ratios of saturated and unsaturated Fats. Fatty acids (FAs) from natural oils interact with the skin as a mixture of skin penetration enhancers and are also affected by the presence of other components. Olive oil was mainly composed of FAs: C16:0 (palmitic), C18:0 (stearic), C18:1 (oleic) and C18:2 (linoleic). Coconut oil was mainly composed of saturated short-chain C8:0 (caprylic), C10:0 (capric), C12:0 (lauric), C14:0 (myristic) and lower amounts of C16–C18 fatty acids [12]. Lipid protein partitioning theory explains the interaction between individual

FAs and the skin barrier [13]. According to this theory, FAs containing C18 chain and at least one double bond have the potential to form a “link” structure and can disrupt the ordered skin lipids. Oleic acid was used as a model FA to prove the theory since it is a C18 FA and it contains a cis double bond at C9 position [14]. H. Tanojo *et al.* evaluated FAs structure differences, resulting in the perturbation of skin lipids and changes in penetration enhancement, suggesting that C18 FAs with more than one double bond should increase the fluidity of skin lipids even more [15]. This indicates, that C18 linoleic acid-containing two unsaturated double bonds should disturb the skin lipids more than oleic acid [16]. In this study, it can be concluded that MPA had deposition in fat before released to blood as in non fat skin rat MPA after 2 h earlier than fat skin rat.

For the second MPA penetration test, Olive oil+0.5% DMSO in fat skin rat gave percentage of release at $29.95 \pm \%$ and began to appear after 2 h. The MPA penetration test with fat skin rat in olive oil+1% DMSO gave the percentage of release at $30.19 \pm \%$ and began to appear after 2 h. Both 0.5% and 1% DMSO were not significantly different (t-test, $P > 0.05$). While the MPA penetration test with fat skin rat in Olive oil had the percentage of release at $12.72 \pm \%$ and began to appear after 6 h. The highest flux value was Olive oil+0.5% DMSO with $4.24 \pm 0.074 \mu\text{g}/\text{cm}^2 \cdot \text{hr}$ but was not significantly different (t-test, $P > 0.05$) with olive oil+1% DMSO.

DMSO can change keratine conformation of *stratum corneum* from α -helical conformation to β -sheet conformation [17]. DMSO increased flux of the drug with its interaction with lipid in *stratum corneum* and changed the peptide structure, and then caused a change of the partition coefficient (8). These changes indicated that DMSO can be a chemical enhancer that penetrated MPA into the skin membrane through the diffusion process.

CONCLUSION

This research concluded that olive oil and 0.5% DMSO could improve the penetration of MPA into the skin membrane by 4.5 times more than olive oil alone. Considering that DMSO can cause skin damage in high concentration, using as low as possible DMSO as enhancer with olive oil avoid skin damage but still give enhancer effect and could improve MPA subcutaneous penetration.

ACKNOWLEDGMENT

The authors gratefully acknowledge to Directorate of Research and Community Engagements of Universitas Indonesia for financial support: Hibah Q1Q2 No: NKB-0199/UN2. R3.1/HKP.05.00/2019.

FUNDING

Nil

AUTHORS CONTRIBUTIONS

All 3 authors participated in the practical work and writing of the manuscript. Iskandarsyah acted as the corresponding author, Camelia Dwi Putri Masrijal acted as first author and Harmita as the second author.

CONFLICT OF INTERESTS

The authors declared that they have no conflict of interest

REFERENCES

- Vera Halpern, SL Combes, LJ Dorflinger, DH Weiner, DF Archer. Pharmacokinetics of subcutaneous depot medroxyprogesterone acetate injected in the upper arm. *Contraception* 2014;89:31–35.
- Benson Heather AE, Watkinson Adam C. Topical and transdermal drug delivery: principal and practice. New Jersey: John Wiley and Sons; 2012. p. 16-27.
- Potts RO, Lobo RA. Transdermal drug delivery: clinical considerations for obstetrician-gynecologist. *Obstet Gynecol* 2005;105:953–61.
- Jaydatt KJ, Sreenivas SA. Review on chemical permeation enhancer used in Transdermal drug delivery system. *Int J Sci Innovations Discoveries* 2012;2:204-17.
- CA Lipinski, F Lombardo, BW Dominy, PJ Feeney. Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. *Adv Drug Delivery Rev* 2001;46:3–26.
- Suchika Sharma, Geeta Aggarwal, Sanju Dhawan. Design and evaluation of olanzapine transdermal patches containing vegetable oils as permeation enhancers. *Scholars Research Library. Pharm Lett* 2010;2 Suppl 6:84-98.
- Hussain A, Khan GM, Jan SU, Shah S, Shah K, Akhlaq M, Rahim N, *et al.* Effect of olive oil on transdermal penetration of flurbiprofen from topical gel as an enhancer. *Pak J Pharma Sci* 2012;25 Suppl 2:365-9.
- Kumar S, Jain SK, Prajapati SK. Chemical penetration enhancer dmsco in in vitro skin permeation of acyclovir transdermal microencapsulation formulation. *Int J Drug Delivery* 2011;3:83–94.
- Prasanthi D, Lakshmi PK. Vesicles—mechanism of transdermal permeation: a review. *Asian J Pharm Clin Res* 2012;5:18-25.
- Anwar E, Utami TD, Ramadan D. Transfersomal gel containing green tea (*Camelia sinensis* L. K) leaves extract: increasing *in vitro* penetration. *Asian J Pharm Clin Res* 2017;10:294-8.
- Sinko P, Singh Y, Martin A. Martin's physical pharmacy and pharmaceutical sciences. Philadelphia Pa. Wolter Kluwer/lippincott Williams and Wilkins; 2011. p. 25-37.
- Dubois V, Breton S, Linder M, Fanni J, Parmentier M. Fatty acid profiles of 80 vegetable oils with regard to their nutritional potential. *Eur J Lipid Sci Technol* 2007;109:710–32.
- Barry BW. Action of skin penetration enhancers—the lipid-protein partitioning theory. *Int J Cosmet Sci* 1988;10:281–93.
- Tanojo H, Bouwstra JA, Junginger HE, Bodde HE. *In vitro* human skin barrier modulation by fatty acids: skin permeation and thermal analysis studies. *Pharm Res* 1997;14:42–9.
- Kim MJ, Doh HJ, Choi MK, Chung SJ, Shim CK, Kim DD. Skin permeation enhancement of diclofenac by fatty acids. *Drug Delivery* 2008;15:373–9.
- Trommer H, Neubert RHH. Overcoming the stratum corneum: the modulation of skin penetration. *Skin Pharmacol Physiol* 2006;19:106–21
- Shembale AI, Borole DK, Lohiya RT. Useful permeation enhancers for transdermal drug delivery: a review. *Int J Pharm Res Dev* 2012;1:2-7.