Permeability Enhancement of Methotrexate Transdermal Gel using Eucalyptus oil, Peppermint Oil and Olive Oil (Conference Paper)

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Abstract

The idea of this study is to improve transdermal permeability of Methotrexate using eucalyptus oil, olive oil and peppermint oil as enhancers.

Eucalyptus oil (2% and 4%), peppermint oil (2% and 4%) and olive oil (2% and 4%) v/v all used as natural enhancers to improve transdermal permeability of Methotrexate via gel formulation. The gel was subjected to many physiochemical properties tests. In-vitro release and permeability studies for the drug were done by Franz cell diffusion across synthetic membrane, kinetic model was studied via korsmeyer-peppas equation.

The results demonstrate that, nonirritant and cause necrosis to rats' skin and stable till 60 days gel was successfully formulated. Methotrexate permeability without enhancer was only about 20%, while with enhancers, it reached to 85%, 99% and 90% with eucalyptus oil 4(v/v %), peppermint oil 4(v/v %) and olive oil 4(v/v %) respectively after 24 hours.

Methotrexate transdermal gel was prepared and evaluated fruitfully in-vitro with a good permeation across semipermeable membrane. The results indicated that using of peppermint oil as enhancer has superiority to enhance the transdermal permeation of the Methotrexate.

Keywords: Permeability, Methotrexate gel, Eucalyptus oil, Peppermint oil and olive oil.

Introduction

Methotrexate (MTX) is an anti-metabolite that is a synthetic chemical analogue of folic acid and is commonly prescribed to treat psoriasis. Although it is authorized for the treatment of mild/moderate psoriasis with oral and parenteral dosing, long-term usage causes diarrhea, mucosal ulcers, stomatitis, bone marrow suppression, hepatic fibrosis, and cirrhosis. As a result, patients might benefit from topical MTX treatment to prevent its serious side effects. Unfortunately, current commercial topical MTX formulations have limited penetration into the stratum corneum, which has been linked to its hydrophilicity and significant dissociation at physiological pH (1,2).

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Transdermal medication delivery is a method of delivering a drug formula via the skin for systemic absorption. It is completely painless technique that begins with drug penetration from the surface of the skin (stratum corneum) to the deeper layers: epidermis and dermis, to be available for systemic absorption. (3)

Transdermal drug delivery system offers several advantages include self-administered and consistent, sustained release of drug, and with steady plasma levels throughout the entire time of administration, hepatic first pass effect and enzymatic degradation of gastrointestinal tract (GIT) is avoided without GIT side effects, and it can be used as an alternate route for patients who are unable to take their pills orally, furthermore, the medication administration terminates with the formula being removed from the surface of skin. (4, 5) Gel is a semi-rigid substance made up of at least two components: a base, which is a smoothly flexible liquid in which the dispersion medium (water) is entirely absorbed. The combination of these two components is what gives the gel its unique properties. (6) Penetration enhancers can improve the drug's penetration through the skin. Permeation enhancers come in a variety of forms, the majority of which are surfactants that can harm cells. Natural enhancers, such as oils and fatty acids, are favored since they are not dangerous to cells like surface active agents and may not interfere with calibration techniques. (7)

Several physical and chemical techniques have been employed to improve MTX skin permeability, like iontophoresis, liposomes, chemical enhancers as well as microneedles. (8) These methods had limited success and some imperfections remain.

**Materials and Methods**

**Materials**

MTX was bought from YIBAI biotechnology (China), Carbopol 940 was purchased from Sigma ch. (USA), Eucalyptus, Peppermint and Olive oils were provided from BAR-SUR-LOUP (France), Triethanolamine (TEA) was bought from Merck (Germany). Franz cell was purchased from SES GmbH (Germany).

**Methods**

**Preparation of methotrexate gel**

The gel was prepared by dissolving the gelling agent 2 (w/v) % of Carbopol 940 in deionized water by stirring until a homogeneous mixture was obtained. Then, the MTX was dissolved in mixture of (1 ml dimethyl sulfoxide and 4ml acetone) and this mixture was left for about 10 min to ensure that completely dissolved in the solution. The MTX solution was added step by step to the homogenous mixture of Carbopol 940. (9)

An enhancer (Eucalyptus, Peppermint and Olive oils) with different concentration was added to six different formulas, for pH adjustment a solution of triethanolamine (TEA) was added in drops and mixed separately with each formula. Eventually, the final volume was made up to 25 ml by adding the sufficient quantity of deionized water, with stirring until a homogeneous gel was made. Different concentration of each component explained in Table 1.

**Table 1. Formulation of MTX 0.1% (w/v) transdermal gel.**

| Formulas | MTX (w/v) % | Carbopol 940 (w/v) % | TEA (v/v) % | Enhancer | Deionized water |
|----------|-------------|----------------------|------------|----------|-----------------|
| F1       | 0.1         | 2                    | 1          | 0        | 0               | Up to 25ml |
| F2       | 0.1         | 2                    | 1          | 2        | 0               | Up to 25ml |
| F3       | 0.1         | 2                    | 1          | 4        | 0               | Up to 25ml |
| F4       | 0.1         | 2                    | 1          | 0        | 2               | Up to 25ml |
| F5       | 0.1         | 2                    | 1          | 0        | 4               | Up to 25ml |
| F6       | 0.1         | 2                    | 1          | 0        | 0               | Up to 25ml |
| F7       | 0.1         | 2                    | 1          | 0        | 4               | Up to 25ml |

**Organoleptic characteristics**

The organoleptic properties of the MTX gel including liquefaction, color, phase separation and homogeneity were investigated by visual inspection at different intervals of 1st, 7th, 14th, 21th, 28th, and 60th days. (9)

**Skin irritation test**

This test was done by applying sufficient quantity of the gel to small region of rats’ skin under supervision (at time of application and after one hour) for any hypersensitivity reaction in the skin (redness, irritation) or any necrosis could be made.
by the gel, the number of rats has been used are seven. (10)

**Changes in pH**

The pH of different formulas was measured using pH meter device at 1\textsuperscript{st}, 7\textsuperscript{th}, 14\textsuperscript{th}, 21\textsuperscript{th}, 28\textsuperscript{th}, and 60\textsuperscript{th} days after formulation. (11)

**Drug content**

A gel amount of 50 mg from each produced formula was taken, then dissolved with 50 ml of pH 7.4 phosphate buffer with stirring until a completely soluble mixture obtained. The mixture then filtered with syringe filter. The concentration of MTX was measured spectrophotometrically by using equation of the drug calibration at 303nm. (12)

**Viscosity**

The viscosity of the formulated gel was determined by using Brookfield Viscometer at 37\degree C. The apparatus spindle was rotated at 10 rpm. (13)

**In vitro drug permeability and release kinetic study**

All formulas permeation were studied by using Franz diffusion cell. The donor compartment was supplied by 1gm of MTX gel and the receptor compartment with 100ml of phosphate buffer PH 7.4. The donor and receptor parts separated by synthetic semipermeable membrane (MW 8000 Da). The media was stirred at 100 rpm and the temperature kept at 37 C. 5ml sample of MTX gel was withdrawn from the receptor which was replaced by the same volume of buffer to maintain the sink condition. The quantity of MTX was determined spectrophotometrically at 303nm by using the phosphate buffer as the blank. The release of the drug determined by applying the result data on korsmeyer peppas model kinetic. (14) In vitro the release mechanism of MTX determined by using the following model equation

\[
\frac{Mt}{M\infty} = K \times t^n
\]

Where \(\frac{Mt}{M\infty}\) is the fractional MTX release from the gel into the receptor media, \(K\) is a drug delivery constant whereas \((n)\) is diffusion coefficient and its value indicates the mechanism of the drug release in the solvent. The \((n)\) value of 0.5 that indicates Quasi-Fickian diffusion mechanism, while if \((n>0.5)\) then anomalous or non-Fickian diffusion mechanism exists and if it is \((=1)\) then the Zero order release one exists. (15)

**Statistical analysis**

One-way analysis of variance (ANOVA) was applied in this research and if \(p<0.05\) the difference was considered statistically significant.

**Results and Discussion**

The general properties of all prepared formulas were good from preparation till two months, so the homogeneity of all formulas was acceptable with no particles was made. The color of all formulas was yellow (attributed to MTX color) and without change in color till two months. (16) The formulas were stable without liquefaction at 2% of Carbopol 940 so this indicates a good percentage of gelling agent. No phase separation was made at all time to the all formulas. The more important character which was skin irritation (Irritancy test), redness and necrosis not observed at rats’ skin after apply sufficient quantity of MTX gel. (17) The drug content data obtained was shown in Table 2. The drug content was between 95.2\% to 97.1\%. This indicates the good content of the drug formulas and good uniformity of the preparation.

The viscosity of the preparations was decreased with increasing the concentration of the enhancer, that are between 17112 cp to 18655 cp. as shown in Table 2 and Figure 1 (18).

| No. | Parameters          | F1   | F2   | F3   | F4   | F5   | F6   | F7   |
|-----|---------------------|------|------|------|------|------|------|------|
| 1   | Color               | yellow | yellow | yellow | yellow | yellow | yellow | yellow |
| 2   | liquefaction        | No   | No   | No   | No   | No   | No   | No   |
| 3   | Phase separation    | No   | No   | No   | No   | No   | No   | No   |
| 4   | Homogeneity         | Good | Good | Good | Good | Good | Good | Good |
| 5   | Skin irritation test| No   | No   | No   | No   | No   | No   | No   |
| 6   | Drug content        | 99.1\% ±1.2 | 95.6\% ±1 | 96.1\% ±2.1 | 96.3\% ±2.1 | 97.1\% ±1.3 | 95.2\% ±1.5 | 95.6\% ±1.9 |
| 7   | Viscosity           | 19775 ±43 | 18655 ±36 | 17323 ±22 | 17745 ±45 | 17112 ±18 | 18221 ±29 | 17342 ±32 |
Changes in pH

The pH of all formulas are within the normal range of skin till 60 days, so no irritation or burning sensation to the skin could be occurred. Figure 2 shows pH as Y axis and duration of storage as X axis.\(^{(19)}\)

In vitro MTX permeability and release kinetic study

The permeability and release of the MTX study by using Franz cell membrane explained in figure 3, according to the results; using the gel without enhancer had poor (20%) permeability after 24 hours, the addition of eucalyptus oil as enhancer at 2% and 4% (F2, F3) respectively, result in significantly (p<0.05) increasing the permeability to 79% and 85% respectively after 24hr, the increasing in permeability due to increasing in enhancer concentration. In formulas with peppermint oil (F4 and F5) the permeability is 92% and 99% with increasing in concentration of enhancer from 2% to 4% respectively. In the F6 and F7 which had olive oil as enhancer with concentration 2% and 4% the permeability reaches 80% and 90% respectively. So, from the data, the permeability of the MTX gel was maximum with peppermint oil due to the solubility of the MTX is higher than in eucalyptus oil and in olive oil. The suggested mechanism for the oils to work as penetration enhancers is either disintegration of the intracellular highly ordered lipid structure or induce conformational modification in the intracellular proteins or might be due to increasing the partitioning of MTX into stratum corium.\(^{(20)}\)
Carbopol 940 was used to control the release of MTX up to 24hr as it acts as a controlling rate polymer. So, according to krosmeyer-peppas model, the kinetic release of drug was obtained. And the mechanism of release was non-Fickain and super case II diffusion which is demonstrated in figure 3. (21)

Table 3. Korsmmeyer-peppas kinetic model for MTX transdermal gel through Synthetic membrane

| formulas | $R^2$ | n-value | Type of release |
|----------|-------|---------|-----------------|
| F1       | 0.9594| 0.74    | Non-fickain diffusion |
| F2       | 0.876 | 2.75    | Super case-II transports |
| F3       | 0.8906| 2.97    | Super case-II transports |
| F4       | 0.9023| 3.57    | Super case-II transports |
| F5       | 0.8879| 3.98    | Super case-II transports |
| F6       | 0.8945| 2.89    | Super case-II transports |
| F7       | 0.9159| 3.26    | Super case-II transports |

Figure 3. Percent of MTX permeated across synthetic semi-permeable membrane at pH 7.4 buffer solution, where n=3.

Conclusion

Methotrexate gel was successfully prepared and evaluated in-vitro with a good permeation across the synthetic membrane. The previous results showed the using of the peppermint oil as permeation enhancer at 4%(v/v) % concentration give worthy transdermal gel of MTX with respectable permeation across semi-permeable membrane consequently for stratum corneum. Moreover, the formula had good drug content, viscosity, pH as an end result suitable for the skin.

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