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PENETRATION ENHANCING EFFECT OF NATURALLY OCCURRING TERPENES ON SKIN PERMEATION OF ROFECOXIB FROM TRANSDERMAL GEL FOR TOPICAL APPLICATION

Abdul Baquee Ahmed[1*], Samir Paul[1], Anupam Sarma[1]
[1]Girijananda Chowdhury Institute of Pharmaceutical Science
Hathkhowapara, Azara, Guwahati-781017, Assam.

ABSTRACT

The objective of this study was to determine the effect of naturally occurring terpenes on the skin permeation of rofecoxib from the transdermal gel prepared with Sodium alginate-Carbopol 940 for topical application. Using *in-vitro* techniques, three different types of naturally occurring terpenes (α-terpineol, + limonene and camphor) were evaluated for the skin permeation of rofecoxib from gel formulations. The effect of concentration of terpenes on the rate of drug permeation from the gel formulations were examined through rat skin mounting on a Keshary-Chien diffusion cell. All the terpenes showed good permeation of rofecoxib from gel formulations. However, α-terpineol at 3% w/w was more effective enhancer with enhancement ratios up to three fold compared with the formulation without terpenes, where as the camphor was much less efficient. The physicochemical properties of the prepared gel formulations were in good agreement with those of a marketed product, indicating feasibility of the topical gel formulation of rofecoxib.

Keywords: Sodium alginate, Carbopol 940, α-terpineol, + limonene and camphor.

1. INTRODUCTION

The primary barrier to dermal and transdermal permeation is the stratum corneum (SC), which is the outermost layer of the skin. The barrier properties of the SC can be

*** Corresponding authors Email: baquee1977@gmail.com**
Phone No. : 09854046526

reduced by the use of enhancers. Terpenes, naturally occurring volatile oils, appear to be promising candidates for clinically acceptable enhancers, (Williams *et al,* 1991). They are reported to have good toxicological profiles, high percutaneous enhancement abilities, and low cutaneous irritancy at low concentrations (1-5%), (El-Kattan *et al*, 2000). Although many studies have investigated the effect of various terpenes on drugs and the responses of various drugs enhanced by terpenes, there are few systemic investigations related to the effects of various terpenes on a variety of permeants.

Rofecoxib, a specific COX2 inhibitor, is one of the most potent non-steroidal anti-inflammatory agents. The drug was approved by FDA in the year 1999, (Jacson *et al*, 2001 and Soniwala *et al*, 2005) for the treatment of pain and inflammation associated with musculoskeletal disorders, primary dysmenorrhea, rheumatoid arthritis and osteoarthritis. The oral bioavailability of rofecoxib is about 93 % and the steady state plasma concentration is reached at 3-4 days with multiple dose oral administration. However, its use has been associated with a number of gastrointestinal disorders, (Layton *et al*, 2002). These potential side effects may be overcome by the topical administration of the drug. The log (P) value of rofecoxib is about 1.705 indicating its lipophilicity for the development of transdermal formulation. Also, rofecoxib having molecular weight of 314.36 Da and melting point in the range of 204 to 208°C can be considered ideal for transport through the skin, (Ahmed, 2006). However, rofecoxib has not been investigated for potential administration via transdermal route except one research paper that reported the enhancement of skin permeation of rofecoxib using topical micro emulsion, (Desai *et al*, 2004).

The purpose of this study was to explore the enhancing effect of terpenes on rofecoxib from transdermal gel prepared from sodium alginate and Carbopol 940. In the present study, three different types of naturally occurring terpenes (α-terpineol, + limonene and camphor) were chosen to evaluate for the skin permeation of rofecoxib from gel formulations. This study utilized Keshary-Chien diffusion cell to explore the influence of terpenes on the *in-vitro* skin permeation of rofecoxib. Further, the gels were also evaluated and compared with a marketed formulation for different *in-vitro* physicochemical properties for feasibility as topical formulation.

2. MATERIALS AND METHODS

2.1 Materials

Rofecoxib was a gift sample from Alembic Pharmaceutical Ltd. (India). Sodium Alginate, Carbopol 940 (Loba Chemie Pvt. Ltd., India), glycerol (Qualigens Fine Chemicals,

India), methyl paraben (Himedia Laboratories Pvt. Ltd., India), triethanolamine (Central Drug House Ltd., India), Polyethylene glycol 400 (Merck Ltd., India), (+)-limonene and cellulose membrane (Sigma Chemical Company, USA), Camphor and α-terpineol (Aldrich Chemical, Germany). Sodium bromide (Loba Chemie Pvt. Ltd., India) and chloroform (Thomas Baker Chemicals Pvt. Ltd., India) were procured and used in this investigation.

2.2 Methods

2.2.1 Preparation of rofecoxib gel

Rofecoxib topical gels were prepared as per the formulations compositions shown in Table1. Gels were prepared by dispersing the rofecoxib and polymers in a mixture of water and glycerol with methylparaben as preservative and varying amount of terpenes. The mixture ware kept under magnetic stirring until homogeneous dispersion was formed. The dispersion was then neutralized and made viscous by the addition of triethanolamine.

2.2.2 *In-vitro* physicochemical evaluation

The physical appearance and homogeneity of the prepared gels were tested by visual observations. The spreadability of the gel formulations was determined at 24 h after permeation, by measuring the spreading diameter of 1 g of gel between two horizontal plates (20 cm × 20 cm) after one min. The standardized weight tied on the upper plate was 125 g, (Vennat *et al*, 1991). The Voveran Emulgel (Novartis Pharma) was considered as reference standard.

The pH of the gel formulations was determined by using a digital pH meter. The measurement was performed at 1, 30 and 60 days after preparation to detect any pH fluctuation with time.

For assay of the drug in gels, rofecoxib was extracted from 1 g of each gel formulations with 20 mL of methanol for 30 min. The resultant mixture was filtered through membrane filter (pore size 0.45 mm). The absorbance of the sample was determined spectrophotometrically at 257 nm (Hitachi U-2001 UV VIS spectrophotometer) after appropriate dilution with aqueous solution of PEG 400 (40% v/v). The concentration of rofecoxib was estimated from the regression equation of the calibration curve (Absorbance = 0.0252 + 0.0117 × concentration, $r^2 = 0.9973$), (Ahmed, 2006).

The viscosity of the gel formulations was determined using Brookfield viscometer with spindle no. 6 at 10 rpm at the temperature of 31°C.

2.2.3 *In-vitro* permeation study

The abdominal hair of Wistar male albino rats, weighing 150 to 200 g, was shaved using an electric razor after sacrificing with excess chloroform inhalation. The abdominal skin was surgically removed and adhering subcutaneous fat was carefully cleaned. The epidermis was then separated from dermis by soaking the full thickness skin in 2 M sodium bromide solution in water for 6 to 8 h, (Higuchi *et al*, 1962). The epidermis was thoroughly washed with water, dried at 25% RH, wrapped in aluminium foil and stored in freeze until further use. The study protocol was approved by Institutional ethical committee of Girijananda Chowdhury Institute of Pharmaceutical Science. For *in vitro* permeation studies, skins were allowed to hydrate for 1 h before being mounted on the Keshary-Chien diffusion cell with the stratum corneum facing the donor compartment. The receptor compartment was filled with aqueous solution of PEG 400 (40% v/v) and receptor phase was maintained at 37 ± 0.5°C. 1 g of the gel was placed on the stratum corneum side in the donor compartment and covered with aluminium foil to prevent drying out. The amount of drug permeated was determined spectrophotometrically at 257 nm by removing 1 mL aliquot through a hypodermic syringe fitted with a 0.22 mm membrane filter, at designated time intervals for 8 h. The volume was replenished with the same volume of prewarmed receiver solution to maintain sink conditions. Blanks are run for each set as described above with placebo gel and calculated accordingly. The above method was used to study the effect of terpenes on rofecoxib permeation from gel in formulations F1 to F9 and without terpenes in formulation F10.

3 RESULTS AND DISCUSSION

3.1 Physicochemical Evaluation

The results of physicochemical properties of the gel formulations were shown in Table 2. From the results it is clearly evident that all the gel formulations showed good homogeneity and spreadability. The drug content of the gel formulations was in the range of 86.25±4.22 to 96.12±5.30%, showing content uniformity. The pH of the gel formulations was in the range of 6.20±0.03 to 6.94±0.02, which lies in the normal pH range of the skin and would not produce any skin irritation. There was no significant change in pH values (varied from 0.02 to 0.05) as a function of time for all formulations. The viscosity of the gel formulations generally reflects its consistency. The consistency of gel formulations lies between viscosity 22000 cps to 32000 cps. The physicochemical properties of the prepared

gel formulations were in good agreement with those of a marketed product namely Voveran Emulgel from Novartis Pharma.

3.2 *In-vitro* Permeation Study

Figure 1 shows the *in-vitro* skin permeation profile of rofecoxib from gels containing 3% w/w terpenes such as α-terpineol, + limonene and camphor in formulations F6, F3 and F9 respectively and without terpenes in formulation F10 across rat epidermis. A linear relationship [(r^2> 0.9 (0.91 to 0.99)] was observed between the cumulative amount permeated and time, indicating zero order permeation kinetics and the permeation of rofecoxib was based on diffusion controlled mechanism. The various permeation parameters are tabulated in Table 3. The steady state flux was observed after a small lag time in the range of 0.33 to 0.47 h.

The cumulative amounts permeated at 8 h from different formulations F1 to F10 were 3833±107.82, 4038±115.23, 4766±176.3, 3355±155.27, 4300±183.22, 5701.16±226.3, 3401±108.33, 3606±111.34, 4101±312.3, 4101±312.3 and 1889.23±114.6 μg/cm^2 respectively (Table 3). The results reveal that there is a significant increase in permeation flux ($p < 0.05$) with addition of different concentration (1-3% w/w) terpenes in the formulation (F1 to F9) with compared to formulation F10 (Without terpenes). The rank order of the various gel formulations based upon their maximum drug permeated is F6 > F3 > F5 > F9 > F2 > F1 > F8 > F7 > F4 > F10. The amount of drug permeated increased with increase in concentration (1-3% w/w) of terpenes in the formulation, further formulation F6 containing 3% w/w of α-terpineol showed enhancement ratios up to three fold compared with the formulation without terpenes F10, whereas formulation F7 to F9 containing camphor showed less permeation of drug with respect to α-terpineol and + limonene at same concentration. According to the result it can be confirmed that addition of terpenes in the formulation was able to increase the permeation of rofecoxib from the gel formulations.

4. CONCLUSION

The goal of this investigation was to explore the enhancing effect of terpenes on rofecoxib from transdermal gel prepared from sodium alginate and Carbopol 940. The results concluded that all terpenes showed good permeation of rofecoxib from gel formulations however; α-terpineol at 3% w/w was more effective enhancer with enhancement ratios up to three fold, whereas the camphor was much less efficient with respect to other terpenes used. The physicochemical properties of the prepared gel formulations were in good agreement with those of a marketed product indicating feasibility

of the topical gel formulation of rofecoxib. In future, compatibility and stability studies need to be carried out.

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Table 1: Compositions of gel formulations

Ingredients	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10
Rofecoxib(mg)	50	50	50	50	50	50	50	50	50	50
Sod.Alginate(mg)	3	3	3	3	3	3	3	3	3	3
Carbopol 940(mg)	1	1	1	1	1	1	1	1	1	1
+ limonene(%w/w)	1	2	3	---	---	---	---	---	---	---
α-terpineol(%w/w)	---	---	---	1	2	3	---	---	---	---
camphor(%w/w)	---	---	---	---	---	---	1	2	3	---
Glycerol(ml)	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Methyl parabene(mg)	10	10	10	10	10	10	10	10	10	10

Table 2: Physicochemical properties of gel formulations

F.N.Code	Spreading diameter After 1 min (mm)	Homogeneity	pH (mean ± SD)	% Rofecoxib Content (mean ± SD)	Viscosity (Centipoises)
F1	66	Homogeneous	6.20±0.03	90.12±6.02	26000
F2	68	Homogeneous	6.66±0.02	94.04±2.16	24000
F3	72	Homogeneous	6.70±0.05	88.03±5.45	23000
F4	64	Homogeneous	6.80±0.03	94.44±1.07	27000
F5	68	Homogeneous	6.94±0.02	90.07±5.12	26500
F6	74	Homogeneous	6.78±0.04	86.25±4.22	23000
F7	65	Homogeneous	6.88±0.05	89.04±3.05	26000
F8	69	Homogeneous	6.60±0.03	91.43±1.56	25500
F9	78	Homogeneous	6.40±0.04	90.27±6.24	22000
F10	60	Homogeneous	6.35±0.04	92.07±3.06	28000
Voveran Emulgel (Novartis Pharma)	58	Homogeneous	6.90±0.05	96.12±5.30	32000

Table-3: Permeation parameters of rofecoxib from various gel formulations across rat epidermis.

F.N. Code	Amount permeated at 8 h($\mu g/cm^2$) (mean± S.D)	J($\mu g/cm^2/h$) (mean±S.D)	T_L(h) (mean±S.D)	Kp($cm^2/S\times10^6$) (mean± S.D)	Best fit regression equation for permeation plot	r^2
F1	3833±107.82	478.45±15.03	0.33±0.04	6.14±0.36	Q=478.45t-45.88	0.9843
F2	4038±115.23	534.72±10.06	0.44±0.02	5.63±0.22	Q=534.72t-87.23	0.9676
F3	4766±176.3	624.17±11.02	0.38±0.05	6.43±0.47	Q=624.17t-33.65	0.9712
F4	3355±155.27	398.68±9.05	0.45±0.03	6.23±0.28	Q=398.68t-112.04	0.9540
F5	4300±183.22	586.38±13.07	0.47±0.02	5.97±0.52	Q=586.38t-72.54	0.9158
F6	5701.16±226.3	735.64±14.26	0.39±0.05	7.13±0.32	Q=735.64t-23.56	0.9985
F7	3401±108.33	402.52±10.11	0.44±0.04	6.52±0.22	Q=402.52t-64.33	0.9722
F8	3606±111.34	426.77±13.22	0.34±0.02	6.02±0.41	Q=426.77t-134.06	0.9640
F9	4101±312.3	565.22±12.23	0.41±0.03	5.98±0.45	Q=565.22t-37.07	0.9467
F10	1889.23±114.6	245.25±9.23	0.40±0.02	7.07±0.42	Q=245.25t-105.21	0.9946

Fig. 1: Effect of terpenes on the permeation of rofecoxib from gel formulations across rat epidermis. Bar represents mean ± S.D (n = 3).