ACECLOFENAC LOADED FILM FORMING GELS: *IN VIVO* STUDY

Himani Bajaj[a]*, Mayank Yadav[a], Seema B. Chauhan[a], Anjali Sharma[b], Navin C. Pant[c], Vinod Singh[d] and Mamta F. Singh[e]

(Received 23 January 2023) (Accepted 20 May 2023)

ABSTRACT

For arthritis, there is need of development of a drug delivery system which permits less frequent dosing by maintaining a close contact with the skin for prolonged time period, thereby improving the patient compliance, especially among elderly. Till date, film forming gels of aceclofenac for arthritis are not available in the market. It is a novel approach which can be used as an alternative to conventional topical and transdermal formulations to treat arthritis. Therefore, HPMC and Eudragit RL 100 based film forming gels of aceclofenac were prepared. On the basis of *in vitro* potential, the formulation was further selected and evaluated for their acute skin irritation studies and *in vivo* anti-arthritic activity using primary skin irritation (draize) test and Freund's Complete Adjuvant (FCA) induced arthritis method. The tested formulations were devoid of any irritation potential and no edema formation was observed in any cases Irritation score (primary skin irritation index) for all the formulations was found to be zero, which indicates its safety and acceptability for transdermal administration. The *in vivo* study revealed that there was significant reduction in inflamed paw volume compared to the marketed formulation. The developed film forming gels could be a potential drug delivery platform for the management of arthritis.

Keywords: Aceclofenac, film forming gels, Arthritis, Freund's adjuvant, Transdermal

INTRODUCTION

Rheumatoid arthritis is a crippling disorder with a waxing and waning course. People with rheumatoid arthritis typically have permanent inflammation in several joints. The joints are painful and swollen, and gradually stiffen[1]. Transdermal dosage, film forming gels were prepared, which is a novel approach and which can be used as an alternative to conventional topical and transdermal formulations to treat arthritis. It is defined as non-solid dosage form that produces a film *in situ*, i.e. after application on the skin or any other body surface[2]. These systems contain the drug and film-forming excipients in a vehicle which, upon contact with the skin, leaves behind a film of excipients along with the drug upon solvent evaporation. The marketed dosage forms, i.e. patches, ointments, creams, etc., are associated with several limitations. Patches have various disadvantages, most commonly skin irritation, due to their occlusive possessions causing obstruction of sweat ducts, which in turn prevents loss of water vapor from skin surface, difficulty in applying on the curved surfaces, pain while peeling off and poor aesthetic appeal[3]. Semisolid preparations like creams and ointments overcome some of these drawbacks but have other limitations. These do not ensure persistent contact with the skin surface and can be easily wiped off by the patient's clothes. Hence, repeated application is required in case of chronic diseases like arthritis.

Therefore, there is a need for development of a dosage form for arthritis which permits less frequent dosing by maintaining a close contact with the skin for prolonged time period thereby improving the patient compliance, especially among the elderly. The present paper deals with the skin irritation and *in vivo* studies of the prepared film forming gels of aceclofenac.

[a] Department of Pharmaceutics, Adarsh Vijendra Institute of Pharmaceutical Sciences, Shobhit University, Gangoh, Saharanpur- 247 341, Uttar Pradesh, India

[b] Department of Pharmaceutics, Guru Gobind Singh College of Pharmacy, Yamunanagar-135 001, Haryana, India

[c] Department of Pharmaceutical Sciences, Amarpali Institute of Pharmacy and Sciences, Haldwani, Nainital-263 139, Uttarakhand, India

[d] Department of Pharmaceutical Sciences, Prabha Harjilal College of Pharmacy and Paraclinical Sciences, Jammu 180 013, Jammu & Kashmir, India

[e] Department of Pharmaceutical Sciences, Sardar Bhagwan Singh University, Balawala, Dehradun-248 161, Uttarakhand, India

*For Correspondence: E-mail: himanibajaj@gmail.com

https://doi.org/10.53879/id.60.06.13865

MATERIALS AND METHODS

Materials

Aceclofenac was gifted by Shiva Biogenetic Pharmaceuticals Pvt. Ltd., Solan, India. Freund's adjuvant was purchased from Sigma Aldrich, Mumbai, India. All other chemicals used in the study were of analytical grade.

Development of film forming gel

The polymeric solution of Eudragit RL100[17] was prepared by dispersion method[15] using ethanol as solvent. HPMC was sprinkled over 10 mL of ethanol separately. Both solutions were allowed to swell for 24 h to produce clear solutions. The polymeric solutions were mixed properly with continuous stirring. The ACF drug was dissolved in a specified quantity of ethanol. Solvent ethanol is used as it can dissolve the drug and is rapidly evaporated after topical application, leaving a film on the skin[4]. The drug and polymeric dispersion were mixed properly with continuous stirring. Finally, Tween 80, and polyethylene glycol were added to it and volume was made up to the mark using ethanol and it was stirred until a smooth gel was obtained. The speed of stirrer was maintained in the range of 500-1000 rpm. During the formulation development, care was taken to avoid formation of air bubbles[5].

IN VIVO EVALUATION STUDIES

Skin irritation studies

The skin irritation study procedures and protocols were reviewed and approved by the Institutional Animal Ethical Committee (CPCSEA/IAEC/SBS/2017-18/009), Sardar Bhagwan Singh Post Graduate Institute of Biomedical Sciences and Research, Dehradun, India. This study was performed as per the OCED/OECD guidelines[6] and previous studies[7]. For skin irritation studies, 3 albino rats (300-500 g) of either sex were used[8]. Backs were shaved and care was taken to avoid abrading the skin and only animals with healthy and intact skin were used. The test film forming gel was applied in a single dose 0.5 g to the skin (approximately 6 cm^2) and covered with gauze patch. Untreated skin areas of the test animal serve as control. Skin was observed at 0, 24, 48 and 72 h for any sign of irritability. The mean erythemal scores were recorded and compared with control[9]. The standards score of irritancy studies is depicted in Table I.

In vivo antiarthritic activity[16]

The anti-arthritic activity of the ACF-loaded film forming gel was evaluated in Albino rats by using Freund's complete adjuvant (FCA) induced arthritis model[10].

Animals: Adult inbred Wistar albino rats of either sex weighing between 200-250 g were used for the study. The animals were housed in standard polypropylene cages and maintained under controlled room temperature (22±2°C) and relative humidity (55±5%) with 12h:12 h light and dark cycle[11]. All the animals were provided with commercially available rat normal pellet diet and water *ad libitum*. The guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) of the Govt. of India were followed and prior permission was granted from the Institutional Animals Ethical Committee (CPCSEA/IAEC/SBS/2017-18/009), Sardar Bhagwan Singh Post Graduate Institute of Biomedical Sciences and Research, Dehradun, India for conducting the animal experimental studies.

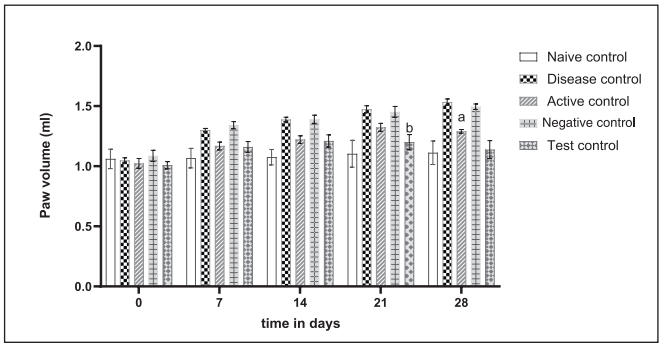

Fig. 1: *In vivo* anti-arthritic activity of film forming gel of aceclofenac using paw volume

Each group (n=6) represents mean ± standard deviation. Two-way ANOVA followed by Tukey's multiple comparison test, F (4, 125) = 132.9, [a]P<0.0001 anti-arthritic effect of active control. F (4, 125) = 159.9, [b]P<0.0001, anti-arthritic F (4, 125) = 159.9 effect of test control.

Table I: Standard score for skin irritation

Sr. No.	Grading of skin reaction	Score
(a)	**Erythema and eschar formation**	
	No erythema	0
	Very slight erythema (barely perceptible)	1
	Well defined erythema	2
	Moderate to severe erythema	3
	Severe erythema (beef redness) to eschar formation preventing grading of erythema	4
(b)	**Oedema formation**	
	No oedema	0
	Very slight oedema (barely perceptible)	1
	Slight oedema (edges of area well defined by definite raising)	2
	Moderate oedema (raised approximately 1mm)	3
	Severe oedema (raised more than 1mm and extending beyond area of exposure)	4

Experimental protocol: The anti-arthritic activity of the ACF-loaded film forming gel was evaluated in Wistar albino rats by using Freund's complete adjuvant (FCA) induced arthritis model[12]. Each rat in the groups was placed in an observation chamber for 10-15 min in order to minimize the stress-related behaviours. Arthritis was induced in all groups of animals excluding naive control with 0.1 mL of Freund's complete adjuvant in the right hind paw[13]. As per the approved protocol, after administration of FCA, active control (marketed gel) and test film forming gel was applied onto the plantar surface of the right hind paw. The inflammatory reactions were rapid and could observed as soon as the FCA was administered.

The animals were divided into five groups, each having six animals. The animals received following treatments:

Naive control: Animals without any interventions were kept under experimental conditions and were provided food and water *ad libitum*.

Arthritis control: Animals were treated with 0.1 mL of Freund's complete adjuvant to induce arthritis.

Active control: Animals were treated with marketed gel of aceclofenac.

Negative control: Animals were treated with film forming gel (HPMC, Eudragit RL 100, Tween 80, polyethylene glycol and ethanol) without aceclofenac.

Test group: Animals were treated with test film forming gel, FG5

As per the protocol, the paw volume of the inflamed albino rats and % inhibition in oedema in each group of animals were measured on the day 0, 7, 14, 21 and 28 days. Data pertaining to analgesic and anti-inflammatory activities are expressed as mean ± SD and were analyzed by the two-way analysis of variance[14]. The per cent inhibition in hind paw oedema was calculated using the following formula,

$$\% \text{ Inhibition in oedema} = \frac{(Vc - Vt}{Vc)} \times 100$$

where Vt - Mean oedema volume of test and Vc - Mean oedema volume of control

RESULTS

The score for each rat at the end of each day were zero for test formulation. Control gel did not cause any viable change daily on any rat. However, no significant difference was found between the erythema score before and after treatment with the gels daily. Furthermore, there was no significant difference between control gel and test gel on any day. Similar results were observed with the marketed preparation. Film forming gel containing aceclofenac was significantly non-irritant and having same safety profile as compared to control gel.

The film-forming gel also exhibited sustained drug release properties. It can be seen during *in vivo* studies that after 13 days, there was a decrease in effectiveness of the marketed gel in reducing the inflammation as shown in Fig. 1.

DISCUSSION

After 72 h observation, the irritation score for film forming gels was found to be zero, which indicated its safety and acceptability for transdermal administration. From these observations, it was concluded that film forming gel containing aceclofenac was significantly non-irritant and have same safety profile as compared to control gel.

The *in vivo* potential was statistically analysed by two-way ANOVA followed by Tukey's multiple comparison test ($P<0.0001$) and was found to be significant compared to the marketed formulation. As per the results, the test formulation showed a significant reduction in FCA induced paw oedema. However, the film-forming system showed a sustained effect in reducing the inflammation even after the thirteenth day. Henceforth, the research described here demonstrates that drug-loaded polymeric films, of acceptable substantivity, flexibility and cosmetic attributes are capable of sustaining release of an active compound over a period of 12 h[21]. Release from a hydrophilic polymeric film i.e. HPMC was greater than that from hydrophobic polymers Eudragit, as expected for the lipophilic drug aceclofenac. Greater substantivity is anticipated for hydrophobic polymer i.e. Eudragit films, for which the "reservoir" of drug may also be held on, as well as within, the skin[18-20]. Consequently, the polymeric film fabricated from combination of hydrophilic and hydrophobic polymer initially provided a burst in release followed by the sustained action due to the reservoir effect.

CONCLUSION

Despite many available approaches for transdermal drug delivery, patient compliance and drug targeting at the desired concentration are still concerns for effective therapies. Precise and efficient film-forming gels provide great potential for controlling drug delivery through the skin with the combined advantages of patch and gels. A novel transdermal system to overcome the major challenges present with the current transdermal dosage form was

successfully developed during this study. Moreover, the developed system prevented the degradation of aceclofenac from aqueous environment and thereby enhanced the stability and efficacy of drug during its therapeutic use for arthritis. The associated disadvantages of both systems (patch and gels) have been surmounted by film-forming gels.

In conclusion, it appears that polymeric film-forming gels FG5 could play a positive role to play in the next generation of transdermal formulations designed to offer sustained drug delivery to and into the skin. Such a formulation can be claimed to decrease duration of therapy, be more accepted by the patients and be a breakthrough in treating chronic diseases such as arthritis. The potentiality of prepared polymeric film forming gels as convenient and aesthetic platform for sustained transdermal drug delivery is comprehensible. Till now, film forming gels of aceclofenac are not available in market. The prepared film forming gel allows the delivery profile to be personalized and optimised in order to get benefit of both a rapid, initial input of drug into the skin and a controlled release over an extended time from the residual film created thereafter. Consequently, the stage is now set for further work to refine the lead formulations and to evaluate their performance in terms of drug delivery into and through the skin for the benefit of humanity.

ACKNOWLEDGEMENT

Authors would like to thank SBSPGI, Balawala, Dehradun, for providing the facilities for the present work.

REFERENCES

1. Kesavanarayanan K.S., Nappinnai M. and Ilavarasan R.: Topical dosage form of valdecoxib: Preparation and pharmacological evaluation, **Acta Pharmac**., 2007, 57(2), 199–209.
2. Kathe K. and Kathpalia H.: Film forming systems for topical and transdermal drug delivery, **Asian J. Pharm. Sci.**, 2017, 12(6), 487-497.
3. Dhiman S., Singh G.T. and Rehni A. K.: Transdermal patches: a recent approach to new drug delivery system**, Int. J. Pharmacy Pharm. Sci.**, 2011,3(5), 26–34.
4. Ranade S., Bajaj A., Londhe V., Kao, and Babul N.: Fabrication of polymeric film forming topical gels, **Int. J. Pharm. Sci. Rev. Res.**, 2014, 26(2), 306–313.
5. Vij N. N. and Saudagar R. B.: Formulation, Development and evaluation of film forming gel for prolonged dermal delivery of terbinafine hydrochloride, **Int. J. Pharm. Sci. Res.**, 2014, 5(9), 537-554.
6. OECD. Guideline for testing of chemicals -Skin Sensitization, Guideline No.404, OECD. Rome. Italy, 2015.
7. Aiyalu R., Govindarjan A. and Ramasamy A.: Formulation and evaluation of topical herbal gel for the treatment of arthritis in animal mode, **Brazilian J. Pharm. Sci.**, 2016, 52(3), 493-508.
8. Prasad V., Kumar N. and Mishra P. R.: Amphiphilic Gels as a Potential Carrier for Topical Drug Delivery, **Drug Deliv.,** 2007,14(2), 75-85.
9. Vogel H.G. Drug discovery and evaluation Pharmacological assays, 2nd edition, Springer, Berlin, Germany, pp 802-203.2002.
10. Mahdi H. J., Khan N. A. K., Asmawi M. Z. B., Mahmud R. and Murugaiyah V.: *In vivo* anti arthritic and anti-nociceptive effects of ethanol extract of *Moringa oleifera* leaves on complete Freund's adjuvant (CFA)- induced arthritis in rats, **Integrated Med. Res.**, 2018,7, 85-94.
11. Dsouza W. Z. and Gude M. R.: Formulation, design, development and evaluation of emulgel for topical delivery of meloxicam in the treatment of rheumatoid arthritis, **Indo. Amer. J. Pharm. Res.,** 2015, 5(3), 5–3.
12. Cui X., Wan R., Bian P., Wu Q., Seshadri V.D.D. and Liu L.: Evaluation of antiarthritic activity of nimbolide against Freund's adjuvant induced arthritis in rats Artificial Cells, **Nanomedicine Biotech.,** 2019, 47(1), 3391–3398.
13. Arellano A., Santoyo S., Martín C. and Ygartua P.: Influence of propylene glycol and isopropyl myristate on the *in vitro* percutaneous penetration of diclofenac sodium from carbopol gels, **European J. Pharm. Sci.,** 1999,7(2),129–135.
14. Padher S. V. and Madgulkar A. R.: Evaluation of topical formulations containing gingerol and piperine in rheumatoid arthritis, **Int. J. Res. Pharm. Pharm. Sci.**, 2017, 2(6), 40-46.
15. Attia M. A. and Badawy H. Y. E.:Film forming gel for treatment of oral mucositis: in vitro studies, **Int. J. Drug Del.**, 2010,2 (3),14–21.
16. Bendele A. M.: Animal models of rheumatoid arthritis, **J. Musculoskel. Neuron Interact.**, 2001, 1(4), 377-385.
17. Bhilegaonkar S. and Parvatkar A.: Eudragit: a versatile and robust platform, **Int. J. Pharm. Sci. Res.,** 2020, 11(6), 2626-2635.
18. Frederiksen K., Guy R. H. and Petersson K.: Formulation considerations in the design of topical, polymeric film-forming systems for sustained drug delivery to the skin, **Europe. J. Pharma. Biopharm.**, 2015, 91, 9-15.
19. Frederiksen K., Guy R.H. and Petersson K.: The potential of polymeric film-forming systems as sustained delivery platforms for topical drugs, **Exp. Opin. Drug. Deliv.**, 2016,13 (3), 349–360.
20. Nimase S. A., Patil P. B. and Saudagar R. B.: A Recent Review on Film Forming Topical Formulation, **J. Drug Deliv. Ther.**, 2019, 9(3-s), 1041-1045.
21. Patwekar M., Patwekar F., Alghamdi S., Kamal M., Allahyani M., Almehmadi M., Kabrah A., Dablool A. S., Alsaiari A. A., Jawaid T., Medikeri A., Samuel K. and Islam F.: Vancomycin as an Antibacterial Agent Capped with Silver Nanoparticles: An Experimental Potential Analysis, **BioMed. Res. Int.**, 2022, 3682757.